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1 **Pleistocene range shifts, refugia and the origin of**
2 **widespread species in Western Palaeartic water**
3 **beetles**

4

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13

14 **ABSTRACT**

15 Quaternary glacial cycles drove major shifts in both the extent and location of
16 the geographical ranges of many organisms. During glacial maxima, large areas of
17 central and northern Europe were inhospitable to temperate species, and these areas are
18 generally assumed to have been recolonized during interglacials by range expansions
19 from Mediterranean refugia. An alternative is that this recolonization was from non-
20 Mediterranean refugia, in central Europe or western Asia, but data on the origin of
21 widespread central and north European species remain fragmentary, especially for
22 insects. We studied three widely distributed lineages of freshwater beetles (the
23 *Platambus maculatus* complex, the *Hydraena gracilis* complex, and the genus
24 *Oreodytes*), all restricted to running waters and including both narrowly distributed
25 southern endemics and widespread European species, some with distributions spanning
26 the Palearctic. Our main goal was to determine the role of the Pleistocene glaciations in
27 shaping the diversification and current distribution of these lineages. We sequenced four
28 mitochondrial and two nuclear genes in populations drawn from across the ranges of
29 these taxa, and used Bayesian probabilities and Maximum Likelihood to reconstruct
30 their phylogenetic relationships, age and geographical origin. Our results suggest that all
31 extant species in these groups are of Pleistocene origin. In the *H. gracilis* complex, the
32 widespread European *H. gracilis* has experienced a rapid, recent range expansion from
33 northern Anatolia, to occupy almost the whole of Europe. However, in the other two

34 groups widespread central and northern European taxa appear to originate from central
35 Asia, rather than the Mediterranean. These widespread species of eastern origin
36 typically have peripherally isolated forms in the southern Mediterranean peninsulas,
37 which may be remnants of earlier expansion-diversification cycles or result from
38 incipient isolation of populations during the most recent Holocene expansion. The
39 accumulation of narrow endemics of such lineages in the Mediterranean may result
40 from successive cycles of range expansion, with subsequent speciation (and local
41 extinction in glaciated areas) through multiple Pleistocene climatic cycles.

42

43 **Keywords:** glacial refugia, Dytiscidae, Hydraenidae, Quaternary glaciations, range
44 expansion, Mediterranean Peninsulas, Central Asia

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49 **1. Introduction**

50 The Quaternary was a period of drastic cyclical climatic changes, with multiple
51 glacial–interglacial periods, ultimately driven by variations in the earth’s orbit known as
52 Milankovitch cycles. Milankovitch–driven climate oscillations led to large changes in
53 the size and location of the geographic distribution of many species, in some cases
54 resulting in speciation due to the higher probability of isolation of small populations in
55 areas under new selection regimes (Dynesius & Jansson, 2000). These Pleistocene
56 climatic oscillations and the subsequent shifts in ecological conditions, with the
57 repeated fragmentation of populations during glacials and interglacials, have long been
58 hypothesised to have driven the origin of most extant Holarctic species (e.g. Rand,
59 1948; Mayr, 1970).

60 Pleistocene climatic changes were especially drastic in northern latitudes of the
61 Palearctic region, since during the Last Glacial Maximum the European ice sheet
62 covered most areas north of 52°N, with permafrost north of 47°N (Dawson, 1992).
63 Large areas of central and northern Europe therefore became inhospitable to temperate
64 taxa during glacials; in stark contrast to the Mediterranean peninsulas, which retained
65 more temperate climate and vegetation (e.g. Huntley, 1988; Bennett et al., 1991).
66 However, despite the fact that most of central and northern Europe and regions of Asia
67 at similar latitudes were exposed to extremely cold conditions (Dawson, 1992), there
68 were areas on the slopes of mountain ranges and along river valleys where moister
69 conditions prevailed (Soffer, 1990), allowing the local survival of temperate biota in
70 these northern/eastern refugia (e.g. Stewart et al., 2009; Schmitt & Varga, 2012).

71 Two main scenarios could account for the origin of the current central and
72 northern European fauna. Firstly, there is the traditional model of postglacial range
73 expansion from Mediterranean refugia (e.g. Hewitt, 2000), in which central and
74 northern Europe were colonized by northward range expansions mainly from the
75 Iberian, Italian, Balkan and Anatolian peninsulas at the end of the last glaciation.
76 According to this model, populations of most European species were confined to
77 refugial areas in southern Mediterranean peninsulas during glacial maxima, from which
78 they would have re-colonized the continent during interglacials (although Hewitt (2000)
79 also recognised the important role of the Carpathians as providing potential refugia).
80 Whilst such a scenario is well established for some taxa, it is not ubiquitous. A second
81 possibility is that the colonization of central and northern Europe at the end of the Last
82 Glacial was from non-Mediterranean source areas in eastern Europe and Asia (Bilton et

83 al., 1998). According to this view, the isolation of the Mediterranean peninsulas during
84 glacial cycles led to speciation, preventing gene flow with the new colonisers of central
85 and northern Europe during subsequent interglacials. For taxa conforming to this model,
86 southern peninsulas are centres of endemism rather than being a source of colonists
87 (Bilton et al., 1998; Schmitt & Varga, 2012).

88 Such biogeographical isolation of Mediterranean peninsular populations has
89 been suggested previously for small mammals (Bilton et al., 1998) and some insects
90 (e.g. Cooper et al., 1995). Amongst aquatic Coleoptera, the absence of fossil remains of
91 southern species in the abundant central and northern European Quaternary subfossil
92 record (Abellan et al., 2011) supports a view of Mediterranean peninsulas as areas of
93 endemism, rather than significant sources of postglacial colonists. Data from extant
94 species also suggest that current southern endemics have not contributed to the diversity
95 of northern areas (e.g. *Hydrochus* (Hydrochidae), Hidalgo-Galiana & Ribera, 2011; or
96 *Enicocerus* (Hydraenidae), Ribera et al., 2010). Some central and northern European
97 species may have had their origin in Mediterranean peninsulas, but in such cases it
98 appears that the taxa concerned were those whose refugia were located in the
99 northernmost areas of the peninsulas, on the margins of deglaciated areas (e.g. Ribera et
100 al., 2010 for *Enicocerus*, and García-Vázquez & Ribera, 2016 for *Deronectes*),
101 successful expansion possibly being aided by physiological adaptations in such species
102 (Calosi et al., 2010; Cioffi et al., 2016).

103 Despite increased understanding of the evolution of the European insect fauna in
104 recent decades, data on the origin of widespread central and northern European species,
105 which should have necessarily experienced recent expansions of their geographical
106 ranges, remain severely limited. Here we study a suite of such species, using molecular
107 phylogeographic data to clarify their temporal and geographic origin and to better
108 understand the role of the Pleistocene glacial cycles in driving their diversification. We
109 examined species groups from three genera of freshwater beetles, in two different
110 families, whose representatives colonised water independently: 1) the *Hydraena gracilis*
111 complex (“*Haenydra*” lineage, family Hydraenidae); 2) the *Platambus maculatus*
112 complex (family Dytiscidae) and (3) *Oreodytes sanmarkii* (C.R. Sahlberg) and *O.*
113 *davisii* (Curtis) (family Dytiscidae). All taxa concerned are typical of running waters,
114 and include both widespread European and narrowly distributed southern endemic
115 species (Trizzino *et al.*, 2013; Nilsson & Hájek, 2017a,b). They do, however, differ in

116 functional traits and evolutionary histories (see below), facts which contribute to the
117 generality of our conclusions.

118 Using a combination of mitochondrial and nuclear data we reconstruct the
119 phylogenetic relationships, age and geographical origin of the western Palaearctic
120 species of these three widely distributed lineages, to better understand the effects of
121 Quaternary glacial cycles on their diversification and current distributions.

122

123 **2. Material and Methods**

124 *2.1. Taxonomic background and taxon sampling*

125 a) *Hydraena gracilis* complex

126 The genus *Hydraena*, currently with ca. 900 species distributed worldwide
127 (Trizzino et al., 2013) is the largest genus within the family Hydraenidae and probably
128 the most diverse amongst the aquatic Coleoptera (Jäch & Balke, 2008). Within
129 *Hydraena*, the “*Haenydra*” lineage includes ca. 90 species with a north Mediterranean
130 distribution (Trizzino et al., 2013). They are usually found in clean, fast flowing waters,
131 often in mountain streams, from the Iberian Peninsula to Iran and the Urals, but are
132 absent from North Africa (Ribera et al., 2011; Trizzino et al., 2011; Trizzino et al.,
133 2013; Jäch, 2015). Many species of this lineage have very restricted distributions, often
134 limited to a single valley or mountain system, but there are also a few species with very
135 wide geographical ranges.

136 In this work we focus on the most widespread species of “*Haenydra*”, *Hydraena*
137 *gracilis* Germar and its closest relatives in the *H. gracilis* complex *sensu* Jäch (1995),
138 which includes seven recognised species and one subspecies (Trizzino et al., 2013).
139 *Hydraena gracilis* is widely distributed across almost the whole of Europe, ranging
140 from southern France eastwards to Ukraine and northwards to Finland, including the
141 British Isles (Fig. 1). Previous molecular studies, albeit on a limited number of
142 specimens (Ribera et al., 2011), suggested that despite its widespread distribution,
143 genetic differences across its geographic range were minimal. Jäch (1995), however,
144 found morphological differences between specimens from the Balkans and the rest of
145 Europe, supporting the recognition of the subspecies *H. gracilis balcanica*
146 d’Orchymont. *Hydraena gracilis* is absent from the Iberian and Anatolian peninsulas,
147 where it is replaced by different species of the complex (Fig. 1). *Hydraena*
148 *gracilidelphis* Trizzino, Valladares, Garrido & Audisio is the westernmost species of
149 this group, endemic to the Iberian Peninsula (mainly in the north but with some records

150 in the southwest) and the French Pyrenees (Trizzino et al., 2012). The Anatolian
151 Peninsula and adjacent areas are occupied by three species: *H. anatolica* Janssens
152 distributed in northern and eastern Anatolia and parts of the Caucasus and northwestern
153 Iran; *H. graciloides* Jäch in northern Turkey; and *H. crepidoptera* Jäch known only
154 from two northern Turkish provinces (Kastamonu and Sinop). The other two species of
155 the complex, *H. nike* Jäch and *H. elisabethae* Jäch, are endemic to two Aegean islands;
156 Samothraky and Thassos respectively (Trizzino et al., 2013).

157 We studied a total of 48 specimens from five of the seven species of the *H.*
158 *gracilis* complex (we could not obtain fresh specimens of the two Aegean Island
159 endemics) from 37 different localities, covering the full geographical range of the
160 studied species (Fig. 1; Table S1). As outgroups we used three closely related species of
161 the wider *H. gracilis* lineage within “*Haenydra*” (Trizzino et al., 2011; Table S1).

162

163 b) *Platambus maculatus* complex

164 The genus *Platambus* contains 66 recognised species (Nilsson & Hájek, 2017a)
165 and has a wide distribution, being present in the Palearctic, Nearctic, Neotropical and
166 Oriental regions, and is currently divided into eight species-groups (Nilsson & Hájek,
167 2017a). Amongst these the *P. maculatus* group - as defined by Nilsson (2001) - is the
168 largest, with 24 species distributed across Asia and Europe. In a molecular phylogeny of
169 Agabinae Ribera et al. (2004) recovered a paraphyletic *Platambus*, with the *P.*
170 *maculatus* group separated from other Asian and American species.

171 Here we focus on the most widespread species of the group; *P. maculatus*
172 (Linnaeus) and its closest relative, *P. lunulatus* (Fischer von Waldheim), which we refer
173 to as the *Platambus maculatus* complex. *Platambus maculatus* has a wide Palearctic
174 distribution, from western Iberia to northern Iran and Mongolia (including Italy, the
175 Balkans and Anatolia), Scandinavia and the British Isles (Nilsson & Hájek, 2017b; Fig.
176 2). The species has a very variable elytral pattern, which led to the description of many
177 forms all of which are currently considered synonyms of *P. maculatus* (Nilsson &
178 Hájek, 2017a,b). Most conspicuous amongst these is *P. maculatus* “*graellsii*”
179 (Geminger & Harold) from the northwest and centre of the Iberian Peninsula (Millán
180 et al., 2014). The other species of the complex, *P. lunulatus*, is distributed from the
181 Anatolian Peninsula and parts of the Caucasus and Middle East to Egypt (Karaman et
182 al., 2008; Nilsson & Hájek, 2017b) (Fig. 2).

183 We sequenced 106 specimens from 67 different localities of the two recognised
184 species of the *P. maculatus* complex, including the "*graellsii*" form (Fig. 2; Table S1).
185 As outgroups we used seven specimens from different species of the *Platambus*
186 *maculatus* group, as defined in Nilsson & Hájek (2017b).

187

188 c) European species of the genus *Oreodytes*

189 The Holarctic genus *Oreodytes* Seidlitz contains 30 recognised species, four of
190 them split into two subspecies (Fery, 2015; Nilsson & Hájek, 2017a). *Oreodytes* species
191 live in cold streams or lakes margins, generally at high altitude or latitude (Balfour-
192 Browne, 1940; Zack, 1992; Nilsson & Holmen, 1995). The genus is distributed in the
193 Palearctic and Nearctic regions, with six species occurring in Europe. In a previous
194 study, Ribera (2003) recovered a paraphyletic *Oreodytes*, although with low bootstrap
195 support. *Oreodytes* was divided into two lineages corresponding to the main distinction
196 in body size and shape, i.e. larger and more elongate species (including *O. davisii*)
197 versus smaller and rounder ones (including *O. sanmarkii*).

198 Of the six European species of *Oreodytes*, the most widespread is *O. sanmarkii*,
199 distributed over large parts of the Palearctic from the Iberian Peninsula to the Russian
200 Far East, and reaching the Nearctic in northern Canada (Larson et al., 2000; Nilsson &
201 Hájek, 2017a,b) (Fig. 3). In southern Europe, the species is known from the Iberian
202 Peninsula, northern provinces of Italy and the Balkans south to Bulgaria and Macedonia
203 (Fery, 2015; Nilsson & Hájek, 2017b). The species shows a high level of variability in
204 colouration over its large distributional range (e.g. Larson, 1990) but only one
205 subspecies, *O. sanmarkii alienus* (Sharp), endemic to the Iberian Peninsula, is currently
206 recognised (Balke, 1989; Nilsson & Hájek, 2017a,b). Also with a wide Palearctic
207 distribution is *O. davisii*, known from the British Isles to Ukraine and the Caucasus,
208 including Scandinavia, the Mediterranean peninsulas and Turkey (Nilsson & Hájek,
209 2017b) (Fig. 3). In the case of this species an Iberian form has also been recognised as a
210 subspecies on morphological grounds, *O. davisii rhiana* Carr (Carr, 2001; Nilsson &
211 Hájek, 2017a,b). The other European species of *Oreodytes* are *O. septentrionalis*
212 (Gyllenhal), distributed from the Iberian Peninsula to eastern Siberia and Mongolia
213 (Nilsson & Hájek, 2017b); *O. alpinus* (Paykull), with a northern Palearctic distribution,
214 being present from lochs in northern Scotland (Foster, 1992) and Scandinavia to
215 Kamchatka in the Russian far East (Nilsson & Kholin, 1994); *O. meridionalis* Binaghi

216 & Sanfilippo, endemic to the southern Apennines (Rocchi 2007; Nilsson & Hájek,
217 2017b); and the recently described *O. angelinii* Fery from Greece (Fery, 2015).

218 We studied 58 specimens from 35 different localities of all the European species
219 of *Oreodytes* with the exception of the Italian endemic *O. meridionalis* and the newly
220 described *O. angelinii*, with a focus on *O. davisii* and *O. sanmarkii* and the two Iberian
221 subspecies (*O. d. rhianae* and *O. s. alienus* respectively). We also included in the
222 analysis 16 specimens of different Asian and American species of the genus as
223 outgroups (Fig. 3; Table S1).

224

225 2.2. DNA extraction and sequencing

226 Specimens were collected and preserved in absolute ethanol directly in the field.
227 We extracted the DNA non-destructively with commercial kits (mostly "DNeasy Tissue
228 Kit", Qiagen GmbH, Hilden, Germany and "Charge Switch gDNA Tissue Mini Kit",
229 Invitrogen, Carlsbad, CA, USA) following the manufacturers' instructions. Specimens
230 and DNA extractions are deposited in the collections of the Institut de Biología
231 Evolutiva, Barcelona (IBE), Museo Nacional de Ciencias Naturales, Madrid (MNCN)
232 and Natural History Museum, London (NHM). We obtained seven gene fragments from
233 six different genes (four mitochondrial and two nuclear) in five different amplification
234 reactions (see Table S2 for primers and typical sequencing reactions): (1) 5' end of the
235 Cytochrome Oxidase Subunit 1 gene (the barcode fragment; Hebert et al., 2003, COI-
236 5'); (2) 3' end of Cytochrome Oxidase Subunit 1 (COI-3'); (3) 5' end of 16S rRNA plus
237 tRNA transfer of Leucine plus 3' end of NADH subunit 1 (*nad1*) (16S and
238 *nad1* respectively); and internal fragments of the nuclear genes (4) Histone 3 (H3) and
239 (5) Wingless (Wg). Due to their lower variability, the nuclear markers were only
240 sequenced from representative specimens according to geographical and topological
241 criteria. For each amplification reaction we obtained both forward and reverse
242 sequences. In some specimens, due to difficulties in amplification, we used internal
243 primers for the COI-3' sequence, obtaining two fragments of 400 bp each (Table S2).
244 PCR products were purified by standard ethanol precipitation and sent to external
245 facilities for sequencing. DNA sequences were assembled and edited using the
246 Geneious 6 software (Biomatters Ltd, Auckland, New Zealand). Ambiguous calls in the
247 nuclear genes were coded as "N"s. New sequences (561) have been deposited in
248 GenBank with accession numbers LT855666-LT856230 (Table S2).

249

250 2.3. Phylogenetic and divergence time analyses

251 Edited sequences were aligned with MAFFT v.6 using the G-INS algorithm and
252 default values for other parameters (Kato & Toh, 2008). We included sequences
253 obtained from the literature (GenBank and BOLD databases) in some analyses to
254 increase geographical coverage and possible genetic variation not covered by our
255 sampling.

256 In the phylogenetic analyses we employed six partitions, corresponding to the
257 gene fragments COI-5', COI-3', 16S, nad1, H3 and Wg, and used Partition Finder 1.1.1
258 (Lanfear et al., 2012) to estimate the best-fitting models of nucleotide substitution for
259 each partition separately, using AIC (Akaike Information Criterion). We considered the
260 two fragments of the COI gene separately due to the uneven taxonomic coverage (Table
261 S1). To infer the phylogeny of the three groups, and estimate divergence dates amongst
262 species, we used Bayesian methods implemented in Beast 1.8 (Drummond et al., 2012).
263 For the analyses we included both mitochondrial and nuclear markers and implemented
264 the closest available evolutionary model to those selected by Partition Finder. We used
265 a Yule speciation model and ran two analyses to determine which clock model (strict or
266 lognormal relaxed) best fitted the data. As there are no fossils or unambiguous
267 biogeographic events that could be used to calibrate the phylogeny of the studied
268 groups, we applied published estimations of a-priori rates for the same genes in related
269 groups of beetles. For the *Platambus maculatus* complex and the genus *Oreodytes* (both
270 Dytiscidae) we used a rate of 0.013 substitutions/site/MY (SD 0.002) for protein coding
271 genes and 0.0016 substitutions/site/MY (SD 0.0002) for 16S, obtained for the related
272 family Carabidae for the same combination of mitochondrial protein coding and
273 ribosomal genes (Andújar et al., 2012). For the *Hydraena gracilis* complex we used a
274 rate of 0.015 and 0.006 substitutions/site/MY (SDs 0.002 and 0.0002) for the COI-
275 3'+nad1 and 16S partitions respectively, obtained for the related Leiodidae (Cieslak et
276 al., 2014). Clock rates of H3 and Wg were left with uniform priors due to the absence of
277 any suitable estimations of the evolutionary rate for these nuclear genes. We executed
278 two independent analyses with the same settings, running 100 million generations
279 (saving trees every 5,000) or until analyses converged and the number of trees was
280 sufficient according to Effective Sample Size (ESS) values, as measured with Tracer
281 v1.6 (Rambaut et al., 2014). The maximum clade credibility tree of the two runs was
282 compiled with Tree Anotator v1.8 (Drummond et al., 2012) and visualized with FigTree
283 v.1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). For selection of the best molecular

284 clock we used the modified Akaike Information Criterion (AICM) with the moments
285 estimator (Baele et al., 2012), as implemented in Tracer v1.6, with 1,000 bootstrap
286 replicates.

287 To test for potential topological discordances between mitochondrial and nuclear
288 data we analysed the nuclear genes only, applying the best clock and the same settings
289 as in the combined (mitochondrial and nuclear) analysis.

290 We also analysed the combined matrix (mitochondrial and nuclear) using
291 maximum likelihood (ML) methods. ML analyses were performed in RAxML v.7.4.2
292 (Stamatakis et al., 2008) as implemented in RAxML GUI v 1.3.1 (Silvestro &
293 Michalak, 2012). We selected the best tree out of 100 searches using the GTR+G as an
294 evolutionary model with the same six partitions as in the Bayesian analysis. Node
295 support was estimated with 1,000 bootstrap replicates.

296

297 2.4. Demographic analyses

298 To study population history for each of the three groups separately we estimated
299 the population coalescent model that best fitted the data. We used the mitochondrial
300 sequences only and no outgroups. In the analysis for the *H. gracilis* complex we only
301 included specimens of *H. gracilis* and *H. anatolica*, whilst only specimens of *P.*
302 *maculatus* (including the "*graellsii*" form) and *O. sanmarkii* were included in the
303 analyses for *Platambus* and *Oreodytes* respectively. The datasets were divided into four
304 partitions, corresponding to each mitochondrial gene (COI-5', COI-3', 16S and nad1)
305 and the same settings used as in the topological analyses, including evolutionary models
306 selected by Partition Finder and the best molecular clock for each group. To identify the
307 best demographic model we ran four analyses including Constant Size, Exponential
308 Growth, Logistic Growth and Expansion Growth coalescence models. For model
309 selection of the best coalescent analyses we used the modified Akaike Information
310 Criterion (AICM) with the moments estimator as implemented in Tracer v1.6, with
311 1,000 bootstrap replicates. We also computed Bayesian skyline plots (Drummond et al.,
312 2005) for each group, to reconstruct variation in effective population sizes through time.

313

314 3. Results

315 There were no length differences in protein coding genes, and the length of
316 ribosomal genes in the ingroup ranged between 784-786 bp in the *H. gracilis* complex
317 and 796-797 bp in the *P. maculatus* complex and the genus *Oreodytes*. The best

318 evolutionary models, as selected by Partition Finder for each individual partition (Table
319 S3), were implemented in the Bayesian analysis for the *H. gracilis* complex and the
320 species of the genus *Oreodytes*. In the *P. maculatus* complex, when the more complex
321 models were applied the analyses did not converge adequately for both the relaxed
322 lognormal and strict clocks, and in consequence we applied an HKY+I+G model to
323 each partition.

324

325 3.1. *Hydraena gracilis* complex

326 The nucleotide alignment matrix showed no variability in the two nuclear genes,
327 which were therefore excluded from subsequent analyses. The analysis using a
328 lognormal relaxed clock did not converge adequately so we applied a strict molecular
329 clock model.

330 The ultrametric tree obtained with the combined mitochondrial and nuclear data
331 estimated a recent origin and diversification of the *H. gracilis* complex at ca. 0.3 Ma
332 (95% confidence interval (c.i.) 0.4-0.2 Ma) (Fig. 4), with strongly supported
333 monophyly. The complex was divided into two major clades, one included the Iberian
334 *H. gracilidelphis* plus all specimens from the easternmost populations identified as *H.*
335 *anatolica* (eastern Anatolia, the Caucasus and Iran), and the other was formed by two
336 groups, one containing specimens of *H. anatolica* and *H. crepidoptera* from northern
337 Turkey and the other with the widespread European *H. gracilis*, including the
338 subspecies *H. gracilis balcanica* and two Turkish specimens of *H. anatolica* and *H.*
339 *graciloides*. In the latter clade, specimens from the most southeasterly populations
340 occupied basal positions, with the more western and northern populations nested within
341 them (Fig. 4). The lineage with the eastern specimens of *H. anatolica* had a poorly
342 supported position between the two remaining main clades of the complex, which
343 differed between analyses. Bayesian analysis (Fig. 4) recovered the eastern lineage as
344 sister to the Iberian *H. gracilidelphis*, whilst ML analysis (Fig. S1) linked the eastern
345 lineage to the other clade, as sister to *H. gracilis* and the Turkish westernmost
346 populations, but always with low support.

347 In the coalescence analyses a constant size model was preferred over
348 exponential growth (Table 1) (logistic and expansion models did not converge
349 adequately). The Bayesian Skyline Plot showed a continuous, slight increase in
350 population size, with a marked increase at ca. 50,000 years BP and a slight decrease
351 towards the present (Fig. 5A).

352

353 3.2. *Platambus maculatus* complex

354 The relaxed lognormal was significantly better than the strict molecular clock,
355 and was therefore implemented in the Bayesian analyses (Table 1). The temporal origin
356 of the *P. maculatus* complex was estimated to be in the Middle Miocene (ca. 12 Ma)
357 (Fig. 6), although the split between the two extant species (*P. maculatus* and *P.*
358 *lunulatus*) occurred in the Messinian (ca. 6.5 Ma, c.i. 9.3-4.1 Ma). Extant intraspecific
359 variability dates from the Pliocene-Pleistocene boundary (2.5-3.5 Ma). The ultrametric
360 tree obtained from Bayesian analysis using the combined mitochondrial and nuclear
361 matrix strongly supported the monophyly of the two recognised species (Fig. 6).

362 *Platambus maculatus* was divided into three clades, one with specimens with a
363 predominantly western distribution (northern Spain, France and the British Isles,
364 including specimens from some Scottish lochs, these forming a monophyletic lineage);
365 a second clade with specimens from easterly populations (central Europe, Scandinavia,
366 the Balkans, Anatolia and the Middle East); and a third clade including specimens of the
367 Iberian "*graellsii*" form plus *P. maculatus* from northern Italy and the two sampled
368 specimens from one of the localities in the Pyrenees (PIR11, Fig. 6; Table S1). The
369 relationships between these three lineages were not well supported, and varied between
370 analyses. In the Bayesian analysis using only nuclear markers (Fig. S2), all specimens
371 of *P. maculatus* "*graellsii*" plus the northern Italian and PIR11 *P. maculatus* were
372 included within the western clade. In the ML analysis, on the contrary (Fig. S3), we
373 recovered the northern Italian specimens of *P. maculatus* as sister to all other
374 specimens, which were split into three poorly supported groups (western clade, eastern
375 clade and the "*graellsii*" form plus the PIR11 *P. maculatus* referred to above).

376 None of the four coalescence models converged adequately, but the Bayesian
377 Skyline Plot showed a nearly constant effective population size until ca. 15,000 years
378 BP, with a recent increase (Fig. 5B).

379

380 3.3. *European Oreodytes* species

381 A relaxed lognormal clock was preferred over a strict molecular clock, and was
382 implemented in the Bayesian analyses (Table 1). Preliminary results showed the
383 existence of two group of species, one closely related to *O. sanmarkii* (the *O. sanmarkii*
384 group) and the other to *O. davisii* (the *O. davisii* group), the later including the
385 European *O. septentrionalis* and *O. alpinus*. These two clades were constrained as

386 monophyletic in subsequent Bayesian analyses without outgroups. *Oreodytes alpinus*
387 was nested within *O. davisii* in the analysis using the combined mitochondrial and
388 nuclear matrix (Fig. 7), forming a clade with the eastern Palaearctic *O. mongolicus*
389 (Brinck). In the analysis using only nuclear data, *O. alpinus* and *O. mongolicus* were
390 also sisters, but both sister to *O. davisii* (Fig. S4)

391 Divergence time analysis (Fig. 7) dated the separation between the *O. davisii*
392 and *O. sanmarkii* groups to the Oligocene (ca. 27 Ma, c.i. 36-20 Ma) and diversification
393 within them to the Lower Miocene (ca. 22 Ma, c.i. 30-15 Ma) for the *O. sanmarkii*
394 group and the Middle Miocene for the *O. davisii* group (ca. 13 Ma, c.i. 18-10 Ma) (Fig.
395 7). The sister species of the clades *O. davisii*+*O. alpinus*+*O. mongolicus* and *O.*
396 *sanmarkii* were the North American *O. snoqualmie* (Hatch) and *O. obesus* (LeConte),
397 respectively. Intraspecific variation in both *O. davisii* and *O. sanmarkii* was of
398 Pleistocene origin (1.5-2.0 Ma) (Fig. 7).

399 We found no clear phylogeographical signal in *O. davisii*, irrespective of the
400 method of analysis (Bayesian or ML). In contrast, *O. sanmarkii* was divided into two
401 clades with a clear geographical pattern, one including specimens with an eastern
402 distribution, from Mongolia to central Europe, and the other including mainly western
403 specimens, from the Iberian Peninsula, Italy and the British Isles, although also
404 including some individuals from the Carpathians. Within the western clade, Iberian
405 populations from the Pyrenees were separated from those from Portugal and central and
406 northwestern Spain, the latter identified as the subspecies *O. sanmarkii alienus*. This
407 difference was more pronounced in the ML analysis (Fig. S5; although with poor
408 support) and in the analysis using only nuclear data (Fig. S4), which recovered *O. s.*
409 *alienus* as sister to remaining *O. sanmarkii*.

410 In the case of other European species of the group, we found a deep divergence
411 between Mongolian and European specimens of *O. septentrionalis*, estimated to have
412 occurred during the late Miocene (ca. 5.6 Ma, c.i. 8.3-3.5 Ma; Fig. 7).

413 The expansion growth coalescence model performed better than logistic or
414 exponential ones (Table 1); the constant size model failing to converge adequately. The
415 Bayesian Skyline Plot showed that effective population size remained constant until
416 relatively recently, with a sharp increase ca. 10,000 years BP (Fig. 5C).

417

418 **4. Discussion**

419 Our results emphasise the fact that patterns of evolutionary diversification,
420 biogeographical history and range expansion can differ significantly, even when
421 comparing taxa occupying the same broad habitat type in the same region. The three
422 groups of water beetles examined here have all diversified during the Plio-Pleistocene,
423 and been subject to the same historical climatic shifts, but despite this their colonization
424 history and demography differ considerably.

425

426 *4.1. Hydraena gracilis complex*

427 According to our results the *H. gracilis* complex has a recent, Pleistocene origin,
428 with the widespread *H. gracilis* showing extreme genetic homogeneity throughout its
429 range. This suggests a very recent expansion, in agreement with preliminary results for
430 this and other species of the "*Haenydra*" lineage (Ribera et al., 2011). The existence of
431 narrow endemics around the periphery of the range of the complex, both in the far west
432 (Iberian Peninsula) and east (Anatolia, Azerbaijan and Iran) is consistent with
433 successive cycles of range expansion within the complex, followed by local extinctions,
434 most likely during the glacial periods. During such times, remaining populations would
435 have been isolated in refuges, resulting in the divergence of *H. gracilidelphis* in the
436 Iberian Peninsula and *H. anatolica*, *H. graciloides* and *H. crepidoptera* in Turkey and
437 the Middle East - a scenario consistent with the model for diversification of this group
438 proposed by Ribera et al. (2011).

439 On current data it was not possible fully to resolve the relationships of eastern
440 Anatolian and Iranian populations of *H. anatolica*, which may be the remnants of earlier
441 diversification cycles (as is the case for *H. gracilidelphis* in the west) or represent early
442 isolates of the most recent range expansion. The separation between western and eastern
443 populations in *H. anatolica* suggests that the Anatolian Diagonal, a mountain range
444 running from north-eastern to south-western Anatolia, acts as an effective barrier to
445 gene flow between western and eastern regions, a pattern observed in many Turkish
446 taxa (Gündüz et al., 2007). The basal position of northern and central Turkish
447 populations of the *H. gracilis* group suggests a second range expansion from these
448 areas, crossing the straits to the Balkans and expanding to western and northern Europe,
449 resulting in the current widespread European *H. gracilis*. This pattern of expansion has
450 been observed in other taxa, including insects (e.g. grasshoppers, Korkmaz et al., 2014)
451 suggesting that Anatolia was an important glacial refugium, which contributed to the
452 recolonization of Europe in some lineages (Ansell et al., 2011; Hewitt, 1996; Rokas et

453 al., 2003). Such expansion from Anatolia to the Balkans may have been possible
454 through a substantial decrease in sea level during glacial periods (Aksu et al., 1999;
455 Ergin et al., 2007), which resulted in a large part of the Sea of Marmara becoming dry
456 land through which terrestrial and freshwater taxa may have dispersed.

457

458 4.2. *Platambus maculatus* complex

459 Although the precise geographic origin of the *P. maculatus* complex remains
460 uncertain, their closest relatives are distributed in eastern and central Asia (Nilsson,
461 2015; Nilsson & Hájek, 2017b). Subsequent to the western range expansion from
462 central Asia, one lineage differentiated in Asia Minor and Anatolia, resulting in *P.*
463 *lunulatus*, and the other Europe, resulting in *P. maculatus*.

464 Range expansion in *P. maculatus* followed a clear geographical pattern, with
465 western and eastern populations falling into two well-supported clades (Fig. 6). An
466 exception here is the uncertain position of specimens from northern Italy and the Iberian
467 "*graellsii*" form, both of which show appreciable morphological differences in elytral
468 pattern and sculpture. Northern Italian specimens have long been recognised as amongst
469 the largest, most convex and shiny of this species (Sharp, 1882; Balfour-Browne, 1940)
470 (Figs 6, 8), whilst populations from central Iberia were originally described as a distinct
471 species (*Agabus glacialis* Graells, subsequently changed to *A. graellsii*), but then
472 reduced to a variety and finally synonymised with *P. maculatus* (Nilsson & Hájek,
473 2017a,b). They have a more reddish coloration than the typical forms of *P. maculatus*,
474 with a poorly defined colour pattern and a very dense and deep microsculpture, giving
475 their dorsal surface a dull, rough appearance (Sharp, 1882) (Figs 6, 8). Both central
476 Iberian and north Italian populations are at the periphery of the main range of the
477 species, in a situation similar to that for *H. gracilidelphis* and the Iberian *Oreodytes* (see
478 below). In *Platambus* our data are, however, inconclusive regarding the origin of these
479 forms, which could have been isolated in the Iberian and Italian Peninsulas as remnants
480 of an earlier range expansion in the complex, or result from incipient isolation during
481 the most recent expansion event.

482 Within the western clade, specimens from some oligotrophic Scottish lochs, also
483 at the periphery of the species main range, formed a monophyletic lineage. This is most
484 remarkable, as they were, together with *P. maculatus* "*graellsii*" and the northern Italian
485 populations, the only forms highlighted in the monumental revision of Sharp (1882).
486 Scottish loch animals were described as being the smallest of the species, flatter in

487 shape, with a duller surface and reduced yellow markings on the elytra (Sharp, 1882;
488 Balfour-Browne, 1940) (Figs 6, 8). However, three specimens from Loch Eck, with a
489 similar morphology (although with less marked differences) were not placed in this
490 clade, but amongst other lineages within the wider western clade (Fig. 6; Table S1). We
491 performed additional analyses, including sequences obtained from public databases, to
492 identify specimens with similar haplotypes to those of Scottish lochs, and found a single
493 northern Swedish sequence which nested within the Scottish clade (obtained from
494 Bergsten et al., 2012). The external morphology of this specimen was within the typical
495 range of *P. maculatus* however, and other beetles from the same locality had similar
496 morphology, and COI haplotypes which nested within the western continental lineage.
497 The nuclear markers used did not have enough resolution to determine if such
498 incongruences are the result of introgression, but in any case our results suggest that
499 some Scottish populations, and perhaps others in northern Europe, could be remnants of
500 an early northward range expansion in *P. maculatus*.

501

502 4.3. European species of the genus *Oreodytes*

503 Most species of the genus *Oreodytes*, including the sister species of both *O.*
504 *davisii* and *O. sanmarkii* (*O. snoqualmie* and *O. obesus* respectively) are distributed in
505 the western United States and Canada (Larson, 1990; Larson et al., 2000). The
506 geographical origin of European taxa therefore seems to have been via range expansion
507 through Beringia and Asia. In the case of *O. davisii* this expansion has not left any
508 apparent phylogeographical structure in the studied markers. In the combined analyses
509 (largely driven by mitochondrial data) *O. alpinus* and *O. mongolicus* were nested within
510 *O. davisii*, despite considerable morphological differences between these species
511 (Shaverdo & Fery, 2006; Foster & Friday, 2011). In contrast, the analysis using only
512 nuclear data clearly separated both species from *O. davisii*, consistent with past
513 mitochondrial introgression between populations of these closely related species who
514 are likely to have shared the same broad Pleistocene refugia. Such a situation has been
515 reported from a number of other taxa (e.g. Berthier et al., 2006; Nichols et al., 2012)
516 including aquatic beetles (Hidalgo-Galiana et al., 2014, García-Vázquez et al., 2016).

517 In *O. sanmarkii* there is a more clearly defined phylogeographic structure, with
518 extant western and eastern clades dating from the middle Pleistocene. This suggests an
519 early origin of European populations, with subsequent isolation of these in different
520 eastern and western refugia. Both *O. davisii* and *O. sanmarkii* are very cold resistant (in

521 the Pyrenees both can be active in winter in partly frozen streams, I. Ribera unpublished
522 observations), likely able to survive in relatively high northern latitudes during glacial
523 cycles, something which may have favoured local persistence in cryptic northern
524 refugia (see below).

525 Similarly to the *P. maculatus* and *H. gracilis* complexes, the only recognised
526 forms within *O. davisii* and *O. sanmarkii* are the Iberian *O. davisii rhianae* and *O.*
527 *sanmarkii alienus* respectively, both occurring west and south of the Ebro valley (Balke,
528 1989; Carr, 2001). In the analysis of nuclear data, and also ML analysis of the combined
529 dataset, the sequenced specimens of *O. sanmarkii alienus* formed a monophyletic
530 group, although in a relatively unsupported position with respect to other *O. sanmarkii*.
531 Irrespective of its taxonomic status it seems that *O. sanmarkii alienus* is relatively
532 isolated genetically from other European populations, reinforcing the pattern of
533 peripheral isolation across the ranges of widespread European taxa. Although difficult
534 to assess without molecular data, the two missing European species of *Oreodytes* in our
535 study, *O. meridionalis* and *O. angelinii*, are also most likely recent peripheral isolates in
536 Mediterranean peninsulas. Thus, *O. meridionalis*, from the central and southern
537 Apennines (Rocchi, 2007) was considered to be a synonymy of *O. davisii* by
538 Franciscolo (1979) due to their close external morphology. Similarly, *O. angeliini*, from
539 northern Greece, has only been recently recognised as a distinct species by Fery (2015),
540 having been considered within the morphological variability of *O. sanmarkii* by
541 previous authors.

542

543 4.4. Concluding remarks: routes of recolonization

544 Our results show that for some water beetles, as in many other groups, central
545 and northern Europe were recolonized by range expansions from peripheral refugia at
546 the end of the last glaciation. Northern areas of Iberia and Anatolia appear particularly
547 relevant for the taxa studied here, both as sources of recolonists and as cradles for
548 recent, narrow-range endemics. In addition to this classic pattern, however, we also
549 show that some widespread central and northern European species originated not around
550 the Mediterranean basin, but in central Asia, although such taxa still have peripherally
551 isolated forms in the southern Mediterranean peninsulas that in some cases possess
552 divergent haplotypes from those in central and northern Europe. Such species may have
553 colonized northern areas of the continent from cryptic refugia in central/eastern Europe
554 or western Asia during the Holocene. Of particular interest is the possibility of

555 peripheral refugia not only in the Mediterranean region but also in some areas in the
556 north, as suggested by the Scottish form of *P. maculatus*.

557 Glacial refugia during cold episodes in Europe were not restricted to the three
558 southern peninsulas, as shown by multiple examples from the Carpathians (Willis et al.,
559 2000; Deffontaine et al., 2005; Kotlík et al., 2006; Sommer & Nadachowski, 2006), and
560 other areas in north and central Europe (Kullman, 1998; Bilton et al., 1998; Stewart &
561 Lister, 2001). Quaternary deposits suggest that a woodland zone existed in the southern
562 foothills of the Carpathian mountains and in sheltered valleys at mid elevations, even
563 during the LGM (Lozek, 2006; Willis et al., 2000). More easterly areas could have also
564 been involved in the colonization of Europe from Asia, including the Caucasus
565 (Massilani et al., 2016). Other potential refugia may have been situated in the Urals, the
566 northern slopes of the Altai, or the Crimean Peninsula (Grichuk, 1984; Hewitt, 1999;
567 Soffer 1990) as well parts of the Ukraine and European Russia (Tarnowska et al., 2016).

568 In the case of the species studied here, as with other European lotic water beetles
569 (García-Vázquez & Ribera, 2016), interglacial range expansions did not result in the
570 mixing and homogenisation of gene pools, but instead drove the isolation and
571 differentiation of populations at range edges. All the running water beetle lineages
572 studied here are relatively weak dispersers compared to most standing water relatives.
573 All *P. maculatus* examined by Jackson (1952, 1956) had reduced flight muscles and
574 most studied specimens of *O. sanmarkii* have reduced flight muscles, although there is
575 at least one record of a specimen with these fully developed (Foster et al., 2016). *O.*
576 *davisii* and *H. gracilis* are known to fly (Jäch, 1997; Foster et al., 2016) although have
577 been recorded doing so relatively rarely. It is interesting, however, that these two
578 species are the most genetically homogeneous of the species studied here. This
579 relatively poor dispersal ability begs the question as to how some species are able to
580 expand their ranges to continental scales? The relative genetic homogeneity of
581 widespread species, with genetic differentiation only in peripheral isolates, together
582 with our coalescence data, suggest rapid range expansions over short temporal windows
583 which may have provided optimal ecological conditions for movement between habitat
584 patches. Recently deglaciated areas are likely to have supported a high density of lotic
585 environments, something which may have facilitated the expansion of these beetles. In
586 the Massif Central, Ponel et al. (2016) found an increase in fossil remains of lotic water
587 beetle species immediately after the Last Glacial, but also following the Younger Dryas.
588 This abundance of lotic species was associated with an increase in stream flow resulting

589 from snow melt during the rapid warming following these two cold periods. When soil
590 formation and sedimentation transformed the landscape, the abundance of lotic species
591 decreased in parallel with an increase in lentic taxa (Ponel et al., 2016). If the same
592 habitat succession happened at larger geographical scales across the continent, it would
593 have facilitated rapid range expansions in lotic species living close to the margins of
594 deglaciated areas, but only for a short time period. As conditions changed, many
595 populations are likely to have become locally extinct, precipitating the genetic isolation
596 of the remainder and in some their eventual speciation (Ribera et al., 2011). The
597 accumulation of narrowly endemic species in lotic lineages may result from successive
598 cycles of range expansion with subsequent speciation and local extinction in glaciated
599 areas over multiple Pleistocene glacial cycles.

600

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610

611 **References**

612

- 613 Abellán, P., Benetti, C.J., Angus, R.B., Ribera, I., 2011. A review of Quaternary range
614 shifts in European aquatic Coleoptera. *Glob. Ecol. Biogeogr.* 20, 87–100.
- 615 Aksu, A.E., Hiscott, R.N., Yaşar, D., 1999. Oscillating Quaternary water levels of the
616 Marmara Sea and vigorous outflow into the Aegean Sea from the Marmara Sea–
617 Black Sea drainage corridor. *Mar. Geol.* 153, 275–302.
- 618 Andújar, C., Serrano, J., Gómez-Zurita, J., 2012. Winding up the molecular clock in the
619 genus *Carabus* (Coleoptera: Carabidae): assessment of methodological decisions
620 on rate and node age estimation. *BMC Evol. Biol.* 12, 1.
- 621 Ansell, S. W., Stenøien, H. K., Grundmann, M., Russell, S. J., Koch, M. A., Schneider,
622 H., Vogel, J. C., 2011. The importance of Anatolian mountains as the cradle of

623 global diversity in *Arabis alpina*, a key arctic–alpine species. *Ann. Bot.* 108, 241–
624 252.

625 Baele, G., Lemey, P., Bedford, T., Rambaut, A., Suchard, M.A., Alekseyenko, A. V.,
626 2012. Improving the accuracy of demographic and molecular clock model
627 comparison while accommodating phylogenetic uncertainty. *Mol. Biol. Evol.* 29,
628 2157–2167.

629 Balfour-Browne, F., 1940. *British water beetles*. Vol. 2. Ray Society, London.

630 Balke, M., 1989. Zur identität von *Hydroporus alienus* Sharp, 1872 (Coleoptera,
631 Dytiscidae). *Elytron* 3, 113–114.

632 Bennett, K.D., Tzedakis, P.C., Willis, K.J., 1991. Quaternary refugia of north European
633 trees. *J. Biogeogr.* 18, 103–115.

634 Bergsten, J., Bilton, D.T., Fujisawa, T., Elliott, M., Monaghan, M.T., Balke, M.,
635 Hendrich, L., Geijer, J., Herrmann, J., Foster, G.N., Ribera, I., Nilsson, A.N.,
636 Barraclough, T.G., Vogler, A.P. 2012. The effect of geographical scale of
637 sampling on DNA barcoding. *Syst. Biol.* 61, 851–869.

638 Berthier, P., Excoffier, L., Ruedi, M., 2006. Recurrent replacement of mtDNA and
639 cryptic hybridization between two sibling bat species *Myotis myotis* and *Myotis*
640 *blythii*. *Proc. R. Soc. London B Biol. Sci.* 273, 3101–3123.

641 Bilton, D.T., Mirol, P.M., Mascheretti, S., Fredga, K., Zima, J., Searle, J.B., 1998.
642 Mediterranean Europe as an area of endemism for small mammals rather than a
643 source for northwards postglacial colonization. *Proc. R. Soc. London B Biol. Sci.*
644 265, 1219–1226.

645 Calosi, P., Bilton, D.T., Spicer, J.I., Votier, S.C., Atfield, A., 2010. What determines a
646 species' geographical range? Thermal biology and latitudinal range size
647 relationships in European diving beetles (Coleoptera: Dytiscidae). *J. Anim. Ecol.*
648 79, 194–204.

649 Carr, R., 2001. *Oreodytes davisii rhianae* subsp. nov. (Coleoptera: Dytiscidae): an
650 Iberian subspecies distinct from *O. davisii davisii* (Curtis, 1831). *Entomol. Gaz.*
651 52, 183–188.

652 Cieslak, A., Fresneda, J., Ribera, I., 2014. Life-history specialization was not an
653 evolutionary dead-end in Pyrenean cave beetles. *Proc. R. Soc. London B Biol. Sci.*
654 281, 20132978.

655 Cioffi, R., Moody, A.J., Millán, A., Billington, R.A., Bilton, D.T., 2016. Physiological
656 niche and geographical range in European diving beetles (Coleoptera: Dytiscidae).
657 Biol. Lett. 12, 20160130.

658 Cooper, S.J.B., Ibrahim, K.M., Hewitt, G.M., 1995. Postglacial expansion and genome
659 subdivision in the European grasshopper *Chorthippus parallelus*. Mol. Ecol. 4, 49–
660 60.

661 Dawson, A.G., 2013. Ice age Earth: late quaternary geology and climate. Routledge,
662 New York.

663 Deffontaine, V., Libois, R., Kotlík, P., Sommer, R., Nieberding, C., Paradis, E., Searle,
664 J.B., Michaux, J.R., 2005. Beyond the Mediterranean peninsulas: evidence of
665 central European glacial refugia for a temperate forest mammal species, the bank
666 vole (*Clethrionomys glareolus*). Mol. Ecol. 14, 1727–1739.

667 Drummond, A.J., Rambaut, A., Shapiro, B., Pybus, O.G., 2005. Bayesian coalescent
668 inference of past population dynamics from molecular sequences. Mol. Biol. Evol.
669 22, 1185–1192.

670 Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics
671 with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.

672 Dynesius, M., Jansson, R., 2000. Evolutionary consequences of changes in species'
673 geographical distributions driven by Milankovitch climate oscillations. Proc. Natl.
674 Acad. Sci. USA. 97, 9115-9120.

675 Ergin, M., Uluadam, E., Sarikavak, K., Keskin, Ş., Gökaşan, E., Tur, H., 2007. Late
676 Quaternary sedimentation and tectonics in the submarine Şarköy Canyon, western
677 Marmara Sea (Turkey). Geol. Soc. London, Spec. Publ. 291, 231–257.

678 Fery, H., 2015. *Oreodytes angelinii*, a new species from north-eastern Greece
679 (Coleoptera: Dytiscidae: Hydroporinae). Klapalekiana 51, 39–47.

680 Foster, G.N., 1992. Some aquatic Coleoptera from inner Hordaland, Norway. Fauna
681 Norv. Ser. B. 39, 63–67.

682 Foster, G.N., Bilton, D.T., Nelson, B.H. 2016. Atlas of the predaceous water beetles
683 (Hydradephaga) of Britain and Ireland. FSC Publications, Telford, UK.

684 Foster, G.N., Friday, L.E., 2011. Key to adults of the water beetles of Britain and
685 Ireland (Part 1). Handbook for the Identification of British Insects. Vol. 4, Part 5.
686 F. Stud. Counc. R. Entomol. Soc. London, London.

687 Franciscolo, M.E., 1979. Fauna d'Italia, Vol. XIV: Coleoptera. Haliplidae, Hygrobiidae,
688 Gyrinidae, Dytiscidae. Edizioni Calderini, Bologna.

- 689 García - Vázquez, D., Bilton, D.T., Alonso, R., Benetti, C.J., Garrido, J., Valladares,
690 L.F., Ribera, I., 2016. Reconstructing ancient Mediterranean crossroads in
691 *Deronectes* diving beetles. *J. Biogeogr.* 43, 1533–1545.
- 692 García-Vázquez, D., Ribera, I., 2016. The origin of widespread species in a poor
693 dispersing lineage (diving beetle genus *Deronectes*). *PeerJ* 4, e2514.
- 694 Grichuk, V.P., 1984. Late Pleistocene vegetation history. In: Velichko, A.A., Wright Jr,
695 H.E., Barnosky, C.W. (Eds.), *Late Quaternary environments of the Soviet Union*.
696 Longman, London, pp. 155–178..
- 697 Gündüz, İ., Jaarola, M., Tez, C., Yenyurt, C., Polly, P.D., Searle, J.B., 2007.
698 Multigenic and morphometric differentiation of ground squirrels (*Spermophilus*,
699 *Scuiridae*, Rodentia) in Turkey, with a description of a new species. *Mol.*
700 *Phylogenet. Evol.* 43, 916–935.
- 701 Hebert, P.D.N., Ratnasingham, S., de Waard, J.R., 2003. Barcoding animal life:
702 cytochrome c oxidase subunit 1 divergences among closely related species. *Proc.*
703 *R. Soc. London B Biol. Sci.* 270, S96–S99.
- 704 Hewitt, G.M., 1996. Some genetic consequences of ice ages, and their role in
705 divergence and speciation. *Biol. J. Linn. Soc.* 58, 247–276.
- 706 Hewitt, G.M., 1999. Post- glacial re- colonization of European biota. *Biol. J. Linn. Soc.*
707 68, 87–112.
- 708 Hewitt, G., 2000. The genetic legacy of the Quaternary ice ages. *Nature* 405, 907–913.
- 709 Hidalgo-Galiana, A., Ribera, I., 2011. Late Miocene diversification of the genus
710 *Hydrochus* (Coleoptera, Hydrochidae) in the west Mediterranean area. *J Biogeogr*
711 59, 377–385.
- 712 Hidalgo-Galiana, A., Sánchez-Fernández, D., Bilton, D.T., Cieslak, A., Ribera, I., 2014.
713 Thermal niche evolution and geographical range expansion in a species complex of
714 western Mediterranean diving beetles. *BMC Evol. Biol.* 14, 187.
- 715 Huntley, B., 1988. Glacial and Holocene vegetation history: Europe. *Veg. Hist.* 341–
716 383.
- 717 Jäch, M.A., 1995. The *Hydraena* (*Haenydra*) *gracilis* Germar species complex (Insecta:
718 Coleoptera: Hydraenidae). *Ann. Naturhist. Mus. Wien.* 97B, 177–190.
- 719 Jäch, M.A., 1997. Daytime swarming of rheophilic water beetles in Austria (Coleoptera:
720 Elmidae, Hydraenidae, Haliplidae). *Latissimus* 9, 10–11.

- 721 Jäch, M.A., 2015. Hydraenidae. In: Löbl, I., Löbl, D. (Eds.), Catalogue of Palaearctic
722 Coleoptera, 2nd ed. Volume 2/1: Hydrophiloidea - Staphylinoidea.. Brill, Leiden,
723 pp. 130–162.
- 724 Jäch, M.A., Balke, M., 2008. Global diversity of water beetles (Coleoptera) in
725 freshwater. *Hydrobiologia* 595, 419–442.
- 726 Jackson, D.J., 1952. Observations on the capacity for flight of water beetles. *Proc. R.*
727 *Ent. Soc. Lond.* 27, 57–70.
- 728 Jackson, D.J., 1956. The capacity for flight of certain water beetles and its bearing on
729 their origin in the western Scottish isles. *Proc. Linn. Soc. Lond.* 167, 76–96.
- 730 Karaman, B., Kıyak, S., Darılmaz, M.C., 2008. Faunistic study of the aquatic beetles
731 (Coleoptera) of Trabzon province (Turkey). *Mun. Ent. Zool* 3, 437–446.
- 732 Katoh, K., Toh, H., 2008. Recent developments in the MAFFT multiple sequence
733 alignment program. *Brief. Bioinform.* 9, 286–298.
- 734 Korkmaz, E.M., Lunt, D.H., Çıplak, B., Değerli, N., Başbüyük, H.H., 2014. The
735 contribution of Anatolia to European phylogeography: the centre of origin of the
736 meadow grasshopper, *Chorthippus parallelus*. *J. Biogeogr.* 41, 1793–1805.
- 737 Kotlík, P., Deffontaine, V., Mascheretti, S., Zima, J., Michaux, J.R., Searle, J.B., 2006.
738 A northern glacial refugium for bank voles (*Clethrionomys glareolus*). *Proc. Natl.*
739 *Acad. Sci. USA* 103, 14860–14864.
- 740 Kullman, L., 1998. Non- analogous tree flora in the Scandes Mountains, Sweden,
741 during the early Holocene- macrofossil evidence of rapid geographic spread and
742 response to palaeoclimate. *Boreas* 27, 153–161.
- 743 Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. PartitionFinder: combined
744 selection of partitioning schemes and substitution models for phylogenetic
745 analyses. *Mol. Biol. Evol.* 29, 1695–1701.
- 746 Larson, D.J., 1990. *Oreodytes obesus* (Leconte) and *O. sanmarkii* (CR
747 Sahlberg)(Coleoptera: Dytiscidae) in North America. *Coleopt. Bull.* 44, 295–303.
- 748 Larson, D.J., Alarie, Y., Roughley, R.E., 2000. Predaceous diving beetles (Coleoptera:
749 Dytiscidae) of the Nearctic Region, with emphasis on the fauna of Canada and
750 Alaska. NRC Research Press, Ottawa.
- 751 Lozek, V., 2006. Last Glacial paleoenvironments of the West Carpathians in the light of
752 fossil malacofauna. *J. Geol. Sci. Anthropozoic* 26, 73–84.
- 753 Massilani, D., Guimaraes, S., Brugal, J.P., Bennett, E.A., Tokarska, M., Arbogast,
754 R.M., Baryshnikov, G., Boeskorov, G., Castel, J.C., Davydov, S. 2016. Past

755 climate changes, population dynamics and the origin of Bison in Europe. BMC
756 Biol. 14, 93.

757 Mayr, E., 1970. Populations, species, and evolution: an abridgment of animal species
758 and evolution. Harvard University Press, Harvard (MA).

759 Millán, A., Sánchez-Fernández, D., Abellán, P., Picazo, F., Carbonell, J.A., Lobo, J.M.,
760 Ribera, I., 2014. Atlas de los coleópteros acuáticos en la España peninsular.
761 Ministerio de Agricultura, Alimentación y Medio Ambiente, Centro de
762 Publicaciones, Madrid.

763 Nicholls, J.A., Challis, R.J., Mutun, S., Stone, G.N., 2012. Mitochondrial barcodes are
764 diagnostic of shared refugia but not species in hybridizing oak gallwasps. Mol.
765 Ecol. 21, 4051–4062.

766 Nilsson, A.N., 2001. World catalogue of insects. Volume 3: Dytiscidae (Coleoptera).
767 Apollo Books, Stenstrup, DK.

768 Nilsson, A.N., Hájek, J. 2017a. A World Catalogue of the Family Dytiscidae , or the
769 Diving Beetles (Coleoptera , Adepaga). Update distributed as a PDF file via
770 Internet; version 31.I.2017; Available at: <http://www.waterbeetles.eu>

771 Nilsson, A.N., Hájek, J., 2017b. Catalogue of Palearctic Dytiscidae (Coleoptera).
772 Update distributed as a PDF file via Internet; version 1. I. 2017. Available at:
773 <http://www.waterbeetles.eu>

774 Nilsson, A.N., Holmen, M., 1995. Fauna Entomol. Scandinaviaca V. 32. The Aquatic
775 Adepaga (Coleoptera) of Fennoscandia and Denmark. II. Dytiscidae. Brill,
776 Leiden.

777 Nilsson, A.N., Kholin, S., 1994. The diving beetles (Coleoptera, Dytiscidae) of
778 Sakhalin-an annotated checklist. Entomol. Tidskr. 115, 143–156.

779 Ponel, P., Guiter, F., Gandouin, E., Pailles, C., Rioual, P., Djamali, M., Andrieu-Ponel,
780 V., Leydet, M., Van der Putten, N., de Beaulieu, J.L., 2016. Novel insights from
781 coleopteran and pollen evidence into the Lateglacial/Holocene transition in
782 Aubrac, French Massif Central. Palaeogeogr. Palaeoclimatol. Palaeoecol. 463, 83–
783 102.

784 Rambaut, A., Suchard, M.A., Xie, D., Drummond, A.J., 2014. Tracer v1. 6. 2014.
785 Available at <http://beast.bio.ed.ac.uk/Tracer>.

786 Rand, A.L., 1948. Glaciation, an isolating factor in speciation. Evolution 2, 314–321.

787 Ribera, I., 2003. Are Iberian endemics Iberian? A case-study using water beetles of
788 family Dytiscidae (Coleoptera). Graellsia 59, 475–502.

789 Ribera, I., 2008. Chapter 15: Habitat constraints and the generation of diversity in
790 freshwater macroinvertebrates. In: Lancaster, J., Briers, R.A. (Eds.), Aquatic
791 Insects: Challenges to Populations. CAB International, UK, pp. 289–311.

792 Ribera, I., Castro, A., Díaz, J.A., Garrido, J., Izquierdo, A., Jach, M.A., Valladares,
793 L.F., 2011. The geography of speciation in narrow-range endemics of the
794 “*Haenydra*” lineage (Coleoptera, Hydraenidae, *Hydraena*). J. Biogeogr. 38, 502–
795 516.

796 Ribera, I., Castro, A., Hernando, C., 2010. *Ochthebius (Enicocerus) aguilerai* sp. n.
797 from central Spain, with a molecular phylogeny of the Western Palaearctic species
798 of *Enicocerus* (Coleoptera, Hydraenidae). Zootaxa 2351, 1–13.

799 Ribera, I., Nilsson, A.N., Vogler, A.P., 2004. Phylogeny and historical biogeography of
800 Agabinae diving beetles (Coleoptera) inferred from mitochondrial DNA
801 sequences. Mol. Phylogenet. Evol. 30, 545–562.

802 Rocchi, S., 2007. Il genere *Oreodytes* Seidlitz, 1887 negli Appennini. Onychium 5, 29–
803 33.

804 Rokas, A., Atkinson, R.J., Webster, L., Csóka, G., Stone, G.N., 2003. Out of Anatolia:
805 longitudinal gradients in genetic diversity support an eastern origin for a circum -
806 Mediterranean oak gallwasp *Andricus quercustozae*. Mol. Ecol. 12, 2153–2174.

807 Schmitt, T., Varga, Z., 2012. Extra-Mediterranean refugia: The rule and not the
808 exception? Front. Zool. 9, 22.

809 Sharp, D., 1882. On aquatic carnivorous Coleoptera or Dytiscidae. Sci. Trans. R. Dublin
810 Soc. 2, 17–1003.

811 Shaverdo, H.V., Fery, H., 2006. *Oreodytes shorti* sp.n. from Mongolia (Coleoptera,
812 Dytiscidae). Koleopterol. Rundschau 76, 35–42.

813 Silvestro, D., Michalak, I., 2012. RAxMLGUI: a graphical front-end for RAxML. Org.
814 Divers. Evol. 12, 335–337.

815 Soffer, O., 1990. The Russian Plain at the last glacial maximum. In: Soffer, O., Gamble,
816 C. (Eds.), The world at 18000 BP: volume 1, high latitudes. Unwin Hyman,
817 London, pp. 228–252.

818 Sommer, R.S., Nadachowski, A., 2006. Glacial refugia of mammals in Europe:
819 evidence from fossil records. Mamm. Rev. 36, 251–265.

820 Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the
821 RAxML web-servers. Syst. Biol. 57, 758–771.

- 822 Stewart, J.R., Lister, A.M., 2001. Cryptic northern refugia and the origins of the modern
823 biota. *Trends Ecol. Evol.* 16, 608–613.
- 824 Stewart, J.R., Lister, A.M., Barnes, I., Dalén, L., 2009. Refugia revisited: individualistic
825 responses of species in space and time. *Proc. R. Soc. London B Biol. Sci.* 277,
826 661–671.
- 827 Tarnowska, E., Niedziałkowska, M., Gerc, J., Korbut, Z., Górny, M., Jędrzejewska, B.,
828 2016. Spatial distribution of the Carpathian and Eastern mtDNA lineages of the
829 bank vole in their contact zone relates to environmental conditions. *Biol. J. Linn.
830 Soc.* 119, 732–744.
- 831 Trizzino, M., Audisio, P.A., Antonini, G., Mancini, E., Ribera, I., 2011. Molecular
832 phylogeny and diversification of the “*Haenydra*” lineage (Hydraenidae, genus
833 *Hydraena*), a north-Mediterranean endemic-rich group of rheophilic Coleoptera.
834 *Mol. Phylogenet. Evol.* 61, 772–783.
- 835 Trizzino, M., Carnevali, L., De Felici, S., Audisio, P., 2013. A revision of *Hydraena*
836 species of the “*Haenydra*” lineage (Coleoptera, Hydraenidae). *Zootaxa* 3607, 1–
837 173.
- 838 Trizzino, M., Valladares, L.F., Garrido, J., Audisio, P., 2012. Morphological reply to a
839 DNA call: a new cryptic species of *Hydraena* from western Europe, with a
840 complete overview of the *Hydraena gracilis* complex (Coleoptera:
841 Hydraenidae: “*Haenydra*” lineage). *J. Nat. Hist.* 46, 1065–1078.
- 842 Willis, K.J., Rudner, E., Sümegi, P., 2000. The full-glacial forests of central and
843 southeastern Europe. *Quat. Res.* 53, 203–213.
- 844 Zack, R.S., 1992. Notes on the Genus *Oreodytes* (Coleoptera: Dytiscidae) in the Pacific
845 Northwest. *Coleopt. Bull.* 46, 151–154.

846

847

848

849 **Figure legends**

850

851 **Figure 1.** Distribution of studied species of the *Hydraena gracilis* complex. White
852 circles, sampled localities.

853

854 **Figure 2.** Distribution of studied species of the *Platambus maculatus* complex. White
855 circles, sampled localities.

856

857 **Figure 3.** Distribution of *Oreodytes sanmarkii* and *Oreodytes davisii* in the Western
858 Palearctic. Coloured circles, sampled localities for each species, including also
859 *Oreodytes alpinus* and *Oreodytes septentrionalis*. Localities from Mongolia and Siberia
860 for *O. sanmarkii* and *O. alpinus* respectively are not show.

861

862 **Figure 4.** Phylogenetic tree of the *Hydraena gracilis* complex. Ultrametric tree obtained
863 with BEAST with combined nuclear and mitochondrial sequences and a partition by
864 gene. Numbers on nodes represent Bayesian posterior probabilities higher than 0.5. See
865 Table S1 for details of specimens and localities. Habitus photograph, *H. gracilis* (Lech
866 Borowiec).

867

868 **Figure 5.** Coalescence Skyline plots of A) *H. gracilis*; B) *P. maculatus*; C) *Oreodytes*
869 *sanmarkii*. Blue lines represent 95% highest probability density; horizontal axis - time
870 before present (Ma); vertical axis - effective population size (NeT).

871

872 **Figure 6.** Phylogenetic tree of the *Platambus maculatus* complex. Ultrametric tree
873 obtained with BEAST with combined nuclear and mitochondrial sequences and a
874 partition by gene. Numbers on nodes represent Bayesian posterior probabilities higher
875 than 0.5. See Table S1 for details of specimens and localities. Habitus photographs,
876 from base to tip, north Italian form, *P. maculatus* "*graellsii*", north-Scottish form, and
877 typical *P. maculatus* (dotted lines mark the corresponding specimens).

878

879 **Figure 7.** Phylogenetic tree for studied *Oreodytes* species. Ultrametric tree obtained
880 with BEAST with combined nuclear and mitochondrial sequences and a partition by
881 gene. Numbers on nodes represent Bayesian posterior probabilities higher than 0.5. See

882 Table S1 for details of specimens and localities. Habitus photographs, *O. sanmarkii* (L.
883 Borowiec) and *O. davisii* (U. Schmidt).

884

885 **Figure 8.** Habitus of (A) *Platambus maculatus*, standard form (specimen voucher
886 MNCN-AH71); (B) form "*graellsii*" (voucher MNCN-AI733); (C) specimen from north
887 Italy (voucher MNCN-AH191); (D) specimen from the Scottish Lochs (voucher IBE-
888 AI975). See Table S1 for details on the specimens, and Figs 6, S2 and S3 for the
889 phylogenetic relationships of the specimens.

890

891 **Table 1.** Clock and coalescent demographic model comparisons for each group,
 892 including AICM values and standard errors (SE). Best AICM value for each pair shown
 893 in bold; models that failed to converge adequately in brackets or represented by a dash.
 894 Differences < 2 units were not considered significant.

895

group	clock	AICM	SE	coalescence	AICM	SE
<i>H. gracilis</i>	relaxed	[9076.7]	[±0.11]	constant	6705.3	±0.05
	strict	9385.8	±0.24	exponential	6717.2	±0.24
				expansion	[6708.5]	±0.26]
				logistic	-	-
<i>P. maculatus</i>	relaxed	18568.9	±0.52	constant	-	-
	strict	18596.7	±0.44	exponential	[11173.9]	±19.20]
				expansion	[9903.9]	±65.07]
				logistic	-	-
<i>O. sanmarkii</i>	relaxed	23948.4	±0.87	constant	-	-
	strict	23952.8	±0.56	exponential	8204.6	±0.01
				expansion	8200.2	±0.15
				logistic	8202.3	±0.13

896