

Speciation of Iberian diving beetles in Pleistocene refugia (Coleoptera, Dytiscidae)

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Abstract

The Mediterranean basin is an area of high diversity and endemism, but the age and origin of its fauna are still largely unknown. Here we use species-level phylogenies based on \approx 1300 base pairs of the genes 16S rRNA and cytochrome oxidase I to establish the relationships of 27 of the 34 endemic Iberian species of diving beetles in the family Dytiscidae, and to investigate their level of divergence. Using a molecular clock approach, 18–19 of these species were estimated to be of Pleistocene origin, with four to six of them from the Late Pleistocene (\approx 100 000 years). A second, lower speciation frequency peak was assigned to Late Miocene or Early Pliocene. Analysis of the distributional ranges showed that endemic species placed in the tip nodes of the trees are significantly more likely to be allopatric with their sisters than endemic species at lower node levels. Allopatric sister species are also significantly younger than sympatric clades, in agreement with an allopatric mode of speciation and limited subsequent range movement. These results strongly suggest that for some taxa Iberian populations were isolated during the Pleistocene long enough to speciate, and apparently did not expand their ranges to recolonize areas north of the Pyrenees. This is in contradiction to observations from fossil beetles in areas further north, which document large range movements associated with the Pleistocene glacial cycles hypothesized to suppress population isolation and allopatric speciation.

Keywords: biodiversity, Coleoptera, diving beetles, Dytiscidae, endemism, Iberian peninsula, molecular clock, Pleistocene, phylogenies, speciation

Received 31 August 2003; revision received 3 October 2003; accepted 3 October 2003

Introduction

The Mediterranean basin is one of the world's hotspots of biodiversity, with species richness comparable to some tropical areas (Blondel & Aronson 1999; Médail & Quézel 1999; Myers *et al.* 2000). The larger peninsulae have endemism levels of around 20% in many plant and animal groups, especially in mountain areas of Iberia, Italy, the Balkans and Turkey (Baletto & Casale 1991; Greuter 1991; Blondel & Aronson 1999). The origin of this diversity is poorly known, and still a matter of debate. With evidence for the Quaternary glacial cycles emerging in the mid-19th century, these were considered to have played a key role in promoting speciation in the south European biota. The

origin of most extant European species was considered to be a consequence of dramatic changes in ecological conditions or repeated fragmentation of populations during the glacials and interglacials (Rand 1948; Mayr 1970). Elsewhere in the world, glacial cycles and the fragmentation of populations into discrete refugia during the Pleistocene were also viewed as a major engine of speciation (e.g. Haffer 1969; Brown 1982).

More recently, evidence from two different fields, Quaternary entomology and molecular phylogeography, has changed this traditional view in the case of northern temperate taxa. Work on well-preserved beetle remains, mostly from northern and central Europe and North America, has demonstrated that there was little evolutionary change (speciation and extinction) during the Pleistocene, with general morphological stasis, sometimes to an extraordinary degree (Coope 1979, 1994, 1995; Matthews 1979; Angus 1983; Böcher 1990, 1995; Elias 1994; Ashworth 1996; Bennett 1997). Confronted with rapid climatic change,

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species do not seem to acquire novel traits but instead track areas where conditions match their ecological requirements (Jansson & Dynesius 2002).

Parallel to research on fossil remains, molecular data from many north-temperate taxa have revealed a general picture of deep phylogeographic splits within many extant species, which are believed to result from major range shifts during the Pleistocene (Hewitt 1996, 1999, 2000; Avise 2000). Successive contractions into southern refugia during glacials, with subsequent expansions to recolonize newly deglaciated areas in interglacials, have produced a general pattern of high genetic diversity in the south and the presence of fewer selected lineages in the north. Under this model, repeated population isolation during the Pleistocene has reinforced speciation processes that were initiated already during the Pliocene, or produced merely intraspecific population genetic subdivision ('phylogeographic structure') (Avise & Walker 1998; Avise *et al.* 1998; Johns & Avise 1998; see Avise 2000 and Hewitt 2000 for reviews). The high frequency of range expansion and consequent introgression between populations from different refugial areas would have prevented the differentiation of species-level taxa. Under such a scenario, most extant north-temperate species had their origin during the Pliocene or Late Miocene (Zink & Slowinski 1995; Klicka & Zink 1997; although see Arbogast & Slowinski 1998 for a critique; Klicka & Zink 1999; Voelker 1999). Outside the northern latitudes genetic studies have also refuted the Pleistocene speciation model, suggesting a much older divergence of extant species (e.g. Patton & DaSilva 1998; Schneider *et al.* 1998; Loughheed *et al.* 1999). The generality of these conclusions is, however, unclear, particularly for narrow-range endemic taxa occurring at lower latitudes.

Here we investigate the origin of the endemic fauna of diving beetles in the Iberian peninsula, one of the richest Mediterranean areas. Diving beetles (Dytiscidae) are a moderately diverse group of ≈ 4000 species world-wide (Nilsson 2001), with more than 300 species in Western Europe, of which about half are found in the Iberian peninsula (Ribera 2000; Millán *et al.* 2002). The level of endemism in the Iberian peninsula is the highest in Europe, with 34 species restricted to Spain and Portugal, in some cases also extending into a small area of southern France. Knowledge about their sister relationships within and outside the Iberian peninsula, and their level of divergence in mitochondrial DNA (mtDNA) gene sequences, could clarify the time of separation from their common ancestors and hence solve the question about stasis during the Pleistocene. In addition, analysis of distributional ranges of sister species could provide further details about the geography of speciation and the relevance of postulated range movements. Here we establish the relationships of 27 of the Iberian endemic diving beetles based on two mitochondrial genes for a reconstruction of their geographical and temporal origin.

Materials and methods

Taxon sampling

There are 33 recognized endemic species of Dytiscidae in the Iberian peninsula, including eight which extend into southern France along the north face of the Pyrenees (Ribera 2000; Millán *et al.* 2002). One additional taxon was included (*Scarodytes cf. halensis*), as it was suspected that it may constitute an unrecognized species. All endemic species have been recognized based on morphological differences, with the exception of *Nebrioporus croceus*, which was described on the basis of its deviating karyotype (Angus *et al.* 1992). Representatives of 27 of these endemic taxa were sampled, covering all genera with endemic species except *Hygrotus*, for which *H. fresnedai* could not be obtained. The species studied here belong to five major clades of Dytiscidae: (i) *Ilybius* (two Iberian endemic species in the *I. chalconatus-erichsoni* clade, Nilsson 2000; Ribera *et al.* 2003b); (ii) the *Graptodytes* group of genera (five species in *Graptodytes*, *Iberoporus* and *Stictonectes*); (iii) *Hydroporus* (five endemic species in three of the main clades within the genus, I. Ribera, unpublished data); (iv) *Deronectes* (nine species); and (v) the *Nebrioporus* group of genera (six species in *Nebrioporus*, *Scarodytes* and the *Stictotarsus duodecimpustulatus* species group, Nilsson & Angus 1992; Ribera 2003) (Table 1; see Fig. 1 for the geographical origin of the specimens studied). Both classification and nomenclature of all Dytiscidae species follows Nilsson (2001).

The seven missing endemic taxa include complexes of sibling species likely to be of very recent origin, as well as species which are morphologically more distinct and

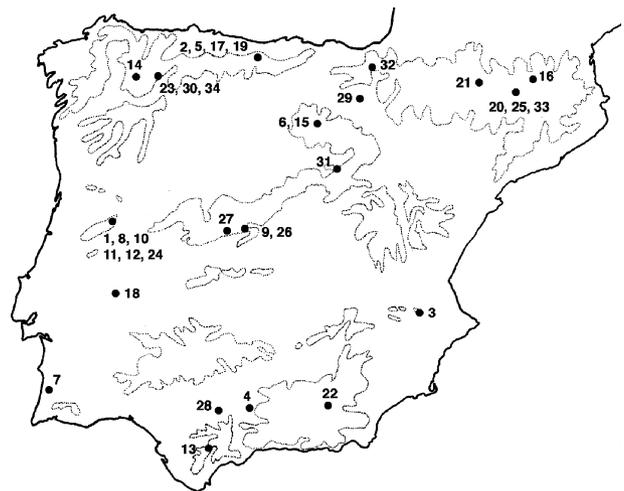


Fig. 1 Map of the Iberian peninsula, with the geographical origin of the specimens of the Iberian endemic species of Dytiscidae used in the study. Dotted areas, 1000 m above sea level contour lines. See Table 1 for the reference number of the specimens.

Table 1 List of the specimens of Iberian endemic species used in the study, with country of origin, locality, collector and GenBank accession numbers

Species	Locality	Collector	16Sr RNA	COI
ILYBIUS				
1 <i>Ilybius albarracinensis</i>	P, Guarda, Sa. Estrela	I. Ribera	AF309277	AF309334
2 <i>Ilybius dettneri</i>	S, León, Puerto de San Glorio	D.T. Bilton	AY138659	AY138746
GRAPTODYTES group				
3 <i>Graptodytes castilianus</i>	S, Albacete, Fte. La Higuera	A. Millán	AY250912	—
4 <i>Iberoporus cermenius</i>	S, Córdoba, Priego de Córdoba	A. Castro	AY250918	AY250958
5 <i>Rhithrodytes bimaculatus</i>	S, León, Puerto de San Glorio	D.T. Bilton	AY250934	AY250974
6 <i>Stictonectes epipleuricus</i>	S, Burgos, Pineda de la Sierra	I. Ribera	AF518255	AF518285
7 <i>Stictonectes occidentalis</i>	P, Algarve	P. Aguilera	AY250942	AY250980
HYDROPORUS				
8 <i>Hydroporus brancoi</i>	P, Guarda, Sa. Estrela	I. Ribera	AF518265	AF518295
9 <i>Hydroporus nevadensis</i>	S, Ávila, Sa. de Gredos	H. Fery	AF518265	AF518295
10 <i>Hydroporus paganettianus</i>	P, Guarda, Sa. Estrela	D.T. Bilton	AY365290	AY365324
11 <i>Hydroporus vagepictus</i>	P, Guarda, Sa. Estrela	I. Ribera	AF518281	AF518312
12 <i>Hydroporus vespertinus</i>	P, Guarda, Sa. Estrela	D.T. Bilton	AY365291	AY365325
DERONECTES group				
13 <i>Deronectes algibensis</i>	S, Cádiz, Puerto de Galis	I. Ribera	AF309261	AF309318
14 <i>Deronectes angusi</i>	S, Lugo, Quintá	I. Ribera	AF309253	AF309310
15 <i>Deronectes angusi</i> 2	S, Burgos, Pineda de la Sierra	I. Ribera	AF309253	—
16 <i>Deronectes aubei sanfilippoi</i>	F, Pyr. Or., NW Prats de Mollo	H. Fery	AY250907	AY250949
17 <i>Deronectes aubei sanfilippoi</i> 2	S, León, Puerto de San Glorio	D.T. Bilton	AY250908	AY250950
18 <i>Deronectes bicostatus</i>	P, Sa. Sao Mamede, Portalegre	I. Ribera	AF309263	AF309320
19 <i>Deronectes costipennis</i>	S, León, Puerto de San Glorio	D.T. Bilton	AY250951	AF309324
20 <i>Deronectes delarouzei</i>	S, Barcelona, Saldes	P. Aguilera	AF309254	AF309311
21 <i>Deronectes delarouzei</i> 2	S, Lleida, Llesp	H. Fery	AY250909	AY250952
22 <i>Deronectes depressicollis</i>	S, Granada, Puerto de la Ragua	I. Ribera	AF309264	AF309321
23 <i>Deronectes ferrugineus</i>	S, León, Puente del Rey	I. Ribera	AF309265	AF309322
24 <i>Deronectes ferrugineus</i> 2	P, Guarda, Sa. Estrela	D.T. Bilton	AF309265	AF309322
25 <i>Deronectes fosteri</i>	S, Barcelona, Saldes	P. Aguilera	AF309260	AF309317
26 <i>Deronectes wewalkai</i>	S, Ávila, Sa. de Gredos	H. Fery	AF309266	AF309323
27 <i>Deronectes wewalkai</i> 2	S, Ávila, Sa. de Gredos	D.T. Bilton	AF309266	AF309323
28 <i>Nebrioporus baeticus</i>	S, Sevilla, Osuna	I. Ribera	AF309245	AF309302
29 <i>Nebrioporus baeticus</i> 2	S, Navarra, Finca la Eguara	I. Ribera	AF309245	—
30 <i>Nebrioporus carinatus</i>	S, León, Puente del Rey	I. Ribera	AF309246	AF309303
31 <i>Nebrioporus croceus</i>	S, Soria, Muriel de la Fuente	H. Fery	AY250925	AY250965
32 <i>Nebrioporus fabressei</i>	S, Navarra, Barindano	I. Ribera	AY250926	AY250966
33 <i>Scarodytes</i> cf. <i>halensis</i>	S, Barcelona, Saldes	H. Fery	AF309248	AF309305
34 <i>Stictotarsus bertrandi</i>	S, León, Puente del Rey	I. Ribera	AY250946	AY250984

See Fig. 1 for the geographical origin of the specimens. Species names follow Nilsson (2001) (see this reference for species' authors). S, Spain; P, Portugal; F, France.

probably older (Ribera 2003). There is therefore no reason to suspect that their inclusion would modify the overall conclusions of this paper, because their degree of morphological divergence is typical of the range in taxa included in our analyses. The dytiscid fauna of the Iberian peninsula is taxonomically well known (Ribera 2000), and new species are not very likely to be discovered (with the possible exception of species living in underground waters).

For some taxa more than one specimen was included, as a crude assessment of species monophyly and their degree of intraspecific variation (see Discussion). In two cases species

were found to be not monophyletic: the Iberian *Scarodytes* cf. *halensis* was very divergent from the specimen from Finland and sister to *S. nigriventris*, suggesting that it may be an unrecognized, morphologically cryptic and possibly endemic species; and the two subspecies of *Deronectes aubei* were paraphyletic with respect to *D. delarouzei* (Fig. 1). For the purpose of this paper, *D. delarouzei* plus *D. aubei sanfilippoi* were conservatively considered a single taxon endemic to the Iberian peninsula (Fery & Brancucci 1997) (see Discussion). The two specimens of *Deronectes wewalkai* and *D. ferrugineus* had, respectively, identical sequences

for both genes, and the 16S rRNA sequence was identical in each of two specimens of *D. angusi* and *Nebrioporus baeticus* (the cytochrome oxidase I sequence of one of the specimens of each could not be obtained). In total, 34 specimens of endemic taxa were included in the analyses (Table 1), plus 110 additional nonendemic taxa (see Appendix).

DNA extraction, gene sequencing

Extraction and sequencing followed standard procedures outlined in previous works on Dytiscidae (e.g. Ribera *et al.* 2001, 2003b), using two fragments of mtDNA: a single fragment of ≈ 500 base pairs (bp) of 16S rRNA using primers 16Sa–16Sb; and a single fragment of ≈ 800 bp of cytochrome oxidase I (COI), using primers 'Jerry' and 'Pat' (Simon *et al.* 1994) (see Ribera *et al.* 2001; 2003b; for details). New sequences were submitted to GenBank under accession numbers AY250906–AY250985 and AY365258–AY365326.

Phylogenetic analysis

Species-level phylogenies were constructed for as many species as possible from each genus that included Iberian endemics. In the case of widespread, Holarctic genera (*Ilybius*, *Hydroporus*), existing species-level trees (Ribera *et al.* 2003b; I. Ribera, unpublished data) were used to determine the closest relatives of the Iberian endemics, with only the relevant subclades included here. For the other three lineages two species of *Laccornis* (tribe Laccornini) and two species of *Laccornellus* (a Neotropical Hydroporini, Nilsson 2001) were used as outgroups. COI sequences were not length variable, and alignment of the 16S rRNA fragment was performed in a two-step procedure (Phillips *et al.* 2000). Sequences were aligned in CLUSTALW (Higgins *et al.* 1992; default values) followed by adjustments of obvious mismatches, and tree searches were performed on the aligned matrix using parsimony analysis in PAUP4.0b8a (Swofford 2000), with gaps coded as a fifth character (Giribet & Wheeler 1999). PAUP searches consisted of 10 000 heuristic tree bisection reconnection (TBR) replicates. When the resolution obtained under equal weighting was low, characters were reweighted *a posteriori* according to the rescaled consistency index on the most parsimonious trees (Farris 1969) for a secondary heuristic search. The significance of the congruence among genes was tested with the Partition Homogeneity Test (Farris *et al.* 1994; Swofford 2000) as implemented in PAUP. Node support was measured with nonparametric bootstrap (Felsenstein 1985), with 1000 iterations of 100 TBR replicates.

Rate of evolution and geographical analysis

To estimate branch lengths, sequences of the in-group taxa were fitted using maximum likelihood (ML) models estim-

ated on the topology of the parsimony trees. Optimal ML models for the combined data were selected using MODELTEST 3.06 (Posada & Crandall 1998). To estimate relative node ages the ML branch lengths were fitted on the parsimony-derived topology assuming a molecular clock and the likelihood was compared to that obtained assuming no clock (Felsenstein 1981). If the likelihood ratio was not significant, branch lengths were obtained directly in PAUP enforcing a molecular clock. If the likelihood ratio was significant the data set was split into subtrees, which were tested for a molecular clock separately. New parsimony searches were performed on these subsets, rooting the tree according to the topology obtained with outgroups, and the same test for clock-like variation was repeated for each subtree. If evolutionary rates were still significantly different from a clock, the nonparametric-rate-smoothing (NPRS) method of Sanderson (1997), as implemented in TREEEDIT v1.0 (Rambaut & Charleston 2001) was used for determining branch length. The NPRS method tends to 'stretch' the branches so that they are usually longer in comparison to the direct enforcement of a molecular clock in PAUP, even when applied to a tree which does follow a molecular clock (Barraclough & Vogler 2002; Sanderson 2002; unpublished observations). As we use an external, fixed calibration this produces older divergence estimations, which are conservative for our conclusion of recent speciation. Estimations based on a direct enforcement of a General Time Reversible (GTR) model in PAUP were also obtained for comparison.

All species considered to be morphologically very close to the Iberian endemics were included in the analyses, with the exception of *Graptodytes pietrii* (postulated sister to *G. castilianus*) and *Rhithrodytes dorsoplagiatus* (postulated sister to *R. bimaculatus*) (Fery 1995; Bilton & Fery 1996). For these species pairs, it was arbitrarily assumed that the sister would diverge at the midpoint of the branch leading to the first node (Table 2). On three occasions an Iberian endemic was sister to a species for which one of the two gene sequences (COI or 16SrRNA) could not be obtained. To estimate sequence divergence in these cases, the estimated branch lengths of the combined ultrametric tree (excluding the species with a missing sequence) were regressed to those obtained using only the gene for which one sequence was available. The regression equation was then used to estimate the length of the combined branch from the length of the branch for the available gene (Fig. 2). Estimated values were (Table 2): *Graptodytes castilianus*–*G. aequalis*, total divergence (TD) = $1.232 \times D16S$ (divergence16S), $r^2 = 0.75$, $n = 30$; *Deronectes platynotus*–*D. costipennis*, TD = $2.4844 \times D16S$, $r^2 = 0.97$, $n = 14$; and *Hydroporus longulus*–(*H. nevadensis* + *H. brancoi*), TD = $0.883 \times DCOI$, $r^2 = 0.96$, $n = 124$.

For an absolute calibration of branch lengths we used the standard approximately 2% divergence per million years (Myr) for insect mtDNA (Brown *et al.* 1979; Brower 1994),

Table 2 Sister species, estimated divergence and degree of allopatry of the studied Iberian endemic species of Dytiscidae

Endemic species	Sister	Distribution of the sister	Sympatry	Divergence	NPRS
<i>Ilybius albarracinensis</i>	<i>I. satunini</i>	E Mediterranean	0	0.004	—
<i>I. dettneri</i>	<i>I. hozgargantae</i>	S Spain, N Morocco	0	0.031	—
<i>Graptodytes castilianus</i>	<i>G. aequalis</i> (<i>G. pietrii</i>)	(Tunisia, Algeria)	0	(< 0.002)* (0.001)† 0.0015	—
<i>Iberoporus cermenius</i>	<i>Metaporus</i> + <i>Graptodytes</i>	W Palaeartic	100	0.122	—
<i>Rhithrodytes bimaculatus</i>	<i>R. numidicus</i> + <i>sexgutattus</i> (<i>R. dorsoplagiatus</i>)	(Algeria)	0	(< 0.085)* 0.043	—
<i>Stictionectes epipleuricus</i>	<i>S. optatus</i> + <i>samai</i> + <i>occidentalis</i>	W Palaeartic	100	0.007	—
<i>S. occidentalis</i>	<i>S. optatus</i> (partim)	W Palaeartic	100	0.007	—
<i>Hydroporus brancoi</i>	<i>H. nevadensis</i>	Iberian endemic	100	0.000	—
<i>H. nevadensis</i>	<i>H. brancoi</i>	Iberian endemic	[100]	[0.000]	—
<i>H. nevadensis-brancoi</i>	<i>H. longulus</i>	European (excl. Iberia?)	0	(0.002)† 0.002	—
<i>H. paganettianus</i>	<i>H. obscurus</i>	European (excl. Iberia)	0	0.011	—
<i>H. vagepictus</i>	<i>H. palustris</i>	W Europe (excl. Iberia)	5	0.007	—
<i>H. vespertinus</i>	<i>H. erythrocephalus</i>	European (excl. Iberia)	0	0.002	—
<i>Deronectes algibensis</i>	<i>D. hispanicus</i> + <i>opatrinus</i> + <i>bicostatus</i>	S France, Iberia, N Morocco	100	0.045	0.062
<i>D. aubei sanfilippoi</i> + <i>D. delarouzei</i>	<i>D. aubei aubei</i>	Alps	0	0.005	0.011
<i>D. angusi</i>	<i>D. latus</i>	European (excl. Iberia)	0	0.002	—
<i>D. bicostatus</i>	<i>D. hispanicus</i> + <i>opatrinus</i>	S France, Iberia, N Morocco	100	0.045	0.061
<i>D. costipennis</i>	<i>D. platynotus</i>	C Europe	0	(0.003)† 0.005	0.011
<i>D. depressicollis</i>	<i>D. fosteri</i>	Iberian endemic	0	0.045	0.054
<i>D. fosteri</i>	<i>D. depressicollis</i>	Iberian endemic	[0]	[0.045]	[0.054]
<i>D. ferrugineus</i>	<i>D. wewalkai</i>	Iberian endemic	0	0.000	0.001
<i>D. wewalkai</i>	<i>D. ferrugineus</i>	Iberian endemic	[0]	[0.000]	[0.001]
<i>D. ferrugineus-wewalkai</i>	<i>D. depressicollis</i> + <i>fosteri</i>	Iberian endemic	0	0.050	0.064
<i>Nebrioporus baeticus</i>	<i>N. nemethi</i>	Morocco	0	0.004	0.009
<i>N. carinatus</i>	<i>N. croceus</i>	Iberian endemic	0	0.001	0.003
<i>N. croceus</i>	<i>N. carinatus</i>	Iberian endemic	[0]	[0.001]	[0.003]
<i>N. fabressei</i>	<i>N. croceus</i> + <i>carinatus</i>	Iberian endemic	2	0.002	0.006
<i>N. carinatus</i> cplx	<i>N. assimilis</i>	W Europe (excl. Iberia)	0	0.041	0.075
<i>Scarodytes</i> cf. <i>halensis</i>	<i>S. nigriventris</i>	Corsica, Sardinia	0	0.007	0.021
<i>Stictotarsus bertrandi</i>	<i>S.12-pustulatus</i> + <i>maghrebinus</i>	W Palaeartic	100	0.062	0.114

Sister, sister species (or clade); in parenthesis, species assumed to be the sister according to morphology (see text). Sympatry, percentage of overlap between the range of sister taxa (measured with respect to the smaller range). Divergence, estimated divergence by directly enforcing a molecular clock, in substitutions/site.

*Maximum estimate for the species which assumed true sister was not included (the assumed divergence is half of this maximum estimate). †Species for which one of the sequences of the species pair was missing (see Methods and Fig. 2 for the method used to estimate total divergence).

Square brackets indicate duplicate data points not included in the analyses (i.e. species pairs in which both are Iberian endemics). NPRS, estimated divergences according to the Non-Parametric Rate Smoothing of Sanderson (1997), for the lineages with rates significantly different from a clock.

corresponding to a rate of nucleotide change (per branch) of 0.01 substitutions/site/Myr.

Geographical data on the distribution of the endemic species were obtained from published sources (Ribera 2000 and references therein). For an estimation of the degree of overlap between ranges, approximate distributions were represented by shaded areas on a map of the Iberian peninsula, and the area size was estimated using image analysis software (National Institutes of Health (NIH) image 1.62, available at the NIH web page). Distribution maps are available from the first author. Overlap of geographical distributions

between sister taxa was calculated as the percentage of the smaller range that is occupied by the range in common to both (Lynch 1989). All sympatry values were either 0% (both species completely allopatric) or 100% (smaller-range species fully sympatric with their sister), with the exception of two cases with very low sympatry (less than 5%). The parameter was thus treated as a dichotomous variable for the statistical analysis ('allopatric': overlap 0–5%, 'sympatric': overlap 100%). We used standard statistical tests to analyse the numerical data, specified in the corresponding sections of the text. Geological ages follow Harland *et al.* (1990).

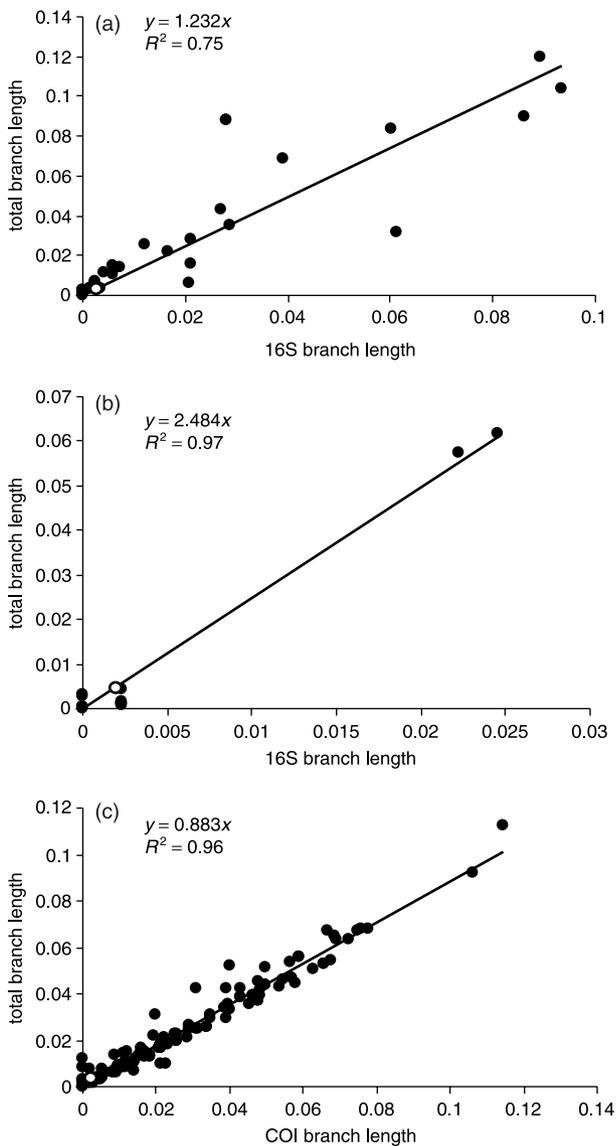


Fig. 2 Estimation of the total divergence for species with one sequence missing (see Materials and methods), using the regression between the estimated branch lengths of the combined ultrametric tree (excluding the species with a missing sequence) and the branch lengths obtained using a single gene (that available for all specimens). (a) *Graptodytes* ($n = 30$); (b) *Deronectes* ($n = 14$); (c) *Hydroporus* ($n = 124$). Estimated values represented by open circles.

Results

Phylogenies and rates of evolution

Phylogenetic relationships for the five major dytiscid lineages comprising Iberian endemics and their nonendemic relatives are represented in Fig. 3. Details of the tree statistics are given in Table 3.

For branch-length calculations in *Ilybius*, *Hydroporus* and the *Graptodytes* group the rates of evolution were not

significantly different from a clock (Table 3) and an ultrametric tree was enforced directly under a GTR model. As the rate of variation in *Deronectes* was not consistent with a clock, they were further split into three monophyletic lineages: (i) the *D. latus* + *abnormicollis* group; (ii) the *D. bicostatus* + *opatrinus* group; and (iii) the *D. aubei* + *platynotus* group (Fig. 3) (species groups follow Fery & Brancucci 1997). The first lineage did not deviate significantly from a clock, and an ultrametric tree was enforced under a GTR model. The two other lineages deviated significantly ($P = 0.032$ and $P < 0.0001$, respectively), and the NPRS correction was applied. For the *Nebrioporus* group, rates were significantly heterogeneous even when *Nebrioporus* and *Scarodytes* plus *Stictotarsus* were considered separately, and branch lengths were also estimated using NPRS (Table 3).

Level of divergence of the endemic species

Most of the Iberian endemics were placed near the tips of the tree, being sister to a single species, or a species pair (Fig. 3). In only two cases was there evidence for diversification of a lineage within the Iberian peninsula, in a section of the Iberian *Deronectes* and the *Nebrioporus carinatus* complex. Similarly, in only three cases was an Iberian endemic sister to a larger clade: *Iberoporus cermenius* (sister to the genera *Metaporus* plus *Graptodytes*, with a total of 21 species, although with low support), *Stictotarsus bertrandi* (sister to the *S. duodecimpustulatus* group, with eight species) and *Deronectes algibensis* (sister to three species of *Deronectes*) (Fig. 1; number of species according to Nilsson 2001).

Levels of pairwise sequence divergence between sister species ranged from zero for *Hydroporus nevadensis* and *H. brancoi*, for which the specimens studied had identical sequences, to more than 24% for *Iberoporus cermenius* (Table 2). The distribution of the levels of divergence was clearly skewed. Of the 23 analysed species pairs, between 12 and 15 (for calculations under NPRS and with the direct enforcement of a molecular clock, respectively) showed a per branch divergence of less than 0.01 substitutions/site, i.e. a total divergence of less than 2% (Table 2, Fig. 4). Among the tail of larger divergences, a smaller frequency peak was observed between 0.035 and 0.053 substitutions/site for the direct enforcement of a clock, and between 0.053 and 0.075 for NPRS (Fig. 4).

A one-factor ANOVA was used to test the relationship of sequence divergence and degree of allopatry (Sokal & Rohlf 1995). When species were divided in two categories, 'allopatric' or 'sympatric' (see Methods), divergence differences were significant for both the ultrametric trees directly enforced under a GTR model and obtained with NPRS ($P < 0.05$). The average divergence for allopatric taxa was 0.011/0.014 substitutions/site (for the trees directly enforcing a molecular clock and those obtained with NPRS, respectively), whereas the average divergence for sympatric

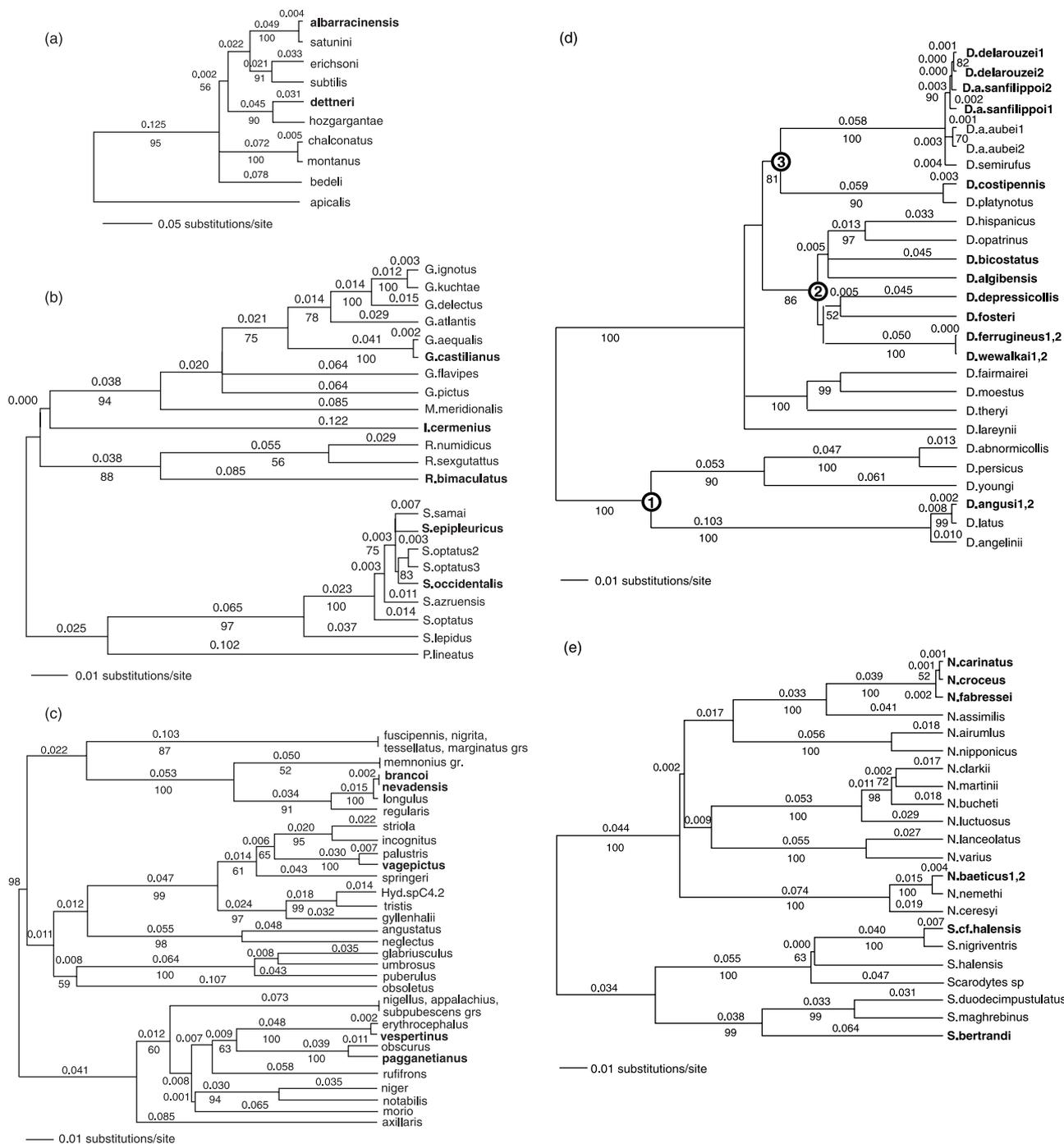


Fig. 3 Ultrametric trees used in the estimation of divergence times. (a) *Ilybius*, (b) *Graptodytes* group, (c) *Hydroporus*, (d) *Deronectes*, (e) *Nebrioporus* group. In bold, Iberian endemics. Numbers above branches are branch lengths; below branches, bootstrap support values of the corresponding node. Circled numbers in (d) refer to the three clades estimated separately (see Appendix for the origin of the specimens). See Nilsson (2001) for the composition of the species groups of *Hydroporus*.

taxa was 0.041/0.053 substitutions/site (Fig. 5). These results were also obtained when the species pairs which are morphologically more similar (i.e. *Deronectes ferrugineus*–*D. wewalkai*, *Hydroporus nevadensis*–*H. brancoi* and the *Nebrioporus*

carinatus complex) were treated as a single terminal in the analysis of sister groups. The relationship between node level and degree of allopatry was also tested with a 2 × 2 contingency table (with a G-test of independence

Table 3 Tree statistics and maximum likelihood models for the phylogenies used in the analyses

	No. taxa (ingroup)	PHT	No. trees	Length	Total char.	Info. char.	CI	ML model	Gamma	<i>P</i> clock
Ilybius	10	1.00	3	342	1284	141	0.78	GTR + I	—	0.24
Graptodytes	23	0.42	30 (5)	1135	1271	352	0.52	GTR + I + G	1.90	0.06
Hydroporus	66	0.04	9	2232	1280	348	0.27	GTR + I + G	0.74	0.16
Deronectes	27	0.99	59 (1)	865	1263	277	0.51	GTR + I + G	1.00	< 0.001
Nebrioporus	24	0.67	2	866	1274	290	0.52	GTR + I + G	1.66	< 0.001

No. taxa (ingroup), number of taxa in the ingroup; PHT, partition homogeneity test; No. trees, number of most parsimonious trees (in brackets, number of trees after reweighting); Total char., length of the aligned sequence; Info. char., number of informative characters; CI, consistency index (with equally weighted data); ML, optimal maximum likelihood model (of the reweighted sequence in *Graptodytes* and *Deronectes*); Gamma, value of the gamma shape parameter; *P* clock, probability that the sequences follow a molecular clock, as measured with a likelihood ratio (see Materials and methods).

Table 4 2 × 2 contingency table testing the association between degree of sympatry and node level for the Iberian endemic species of Dytiscidae

	Node level		Totals
	tip (species)	lower (clade)	
Sympatry			
0–5%	15	1	16
100%	1	6	7
Totals	16	7	23

G-test = 15.04; Williams correction = 1.10; adjusted $G_a = 13.67$; $P = 0.0002$ (see Table 2 for primary data).

For species pairs in which both sisters are Iberian endemics only one data point is included.

and the Williams correction; Sokal & Rohlf 1995). Iberian endemics which are sisters to a single species (i.e. at the highest node levels in the tree) have an allopatric distributional range with their sister more frequently than species which are sister to a larger clade (i.e. at lower node levels) (Table 4; $P < 0.001$).

Calibration of molecular clocks

Using the standard calibration for insect mtDNA of 2%/Myr (Brown *et al.* 1979; Brower 1994), most of the Iberian endemic species considered were of very recent origin, having diverged less than 1 Myr ago in the Early to Middle Pleistocene (Fig. 3). Indeed, some species pairs were at the resolution limit of the genes studied, with a single base change in 1300, corresponding to $\approx 80\,000$ years given a rate of 2% change per Myr (*D. ferrugineus*–*D. weiwalkai*, with identical COI sequence, and only one change in 16S rRNA), or even younger (*H. nevadensis*–*H. brancoi*, with identical sequences for both genes).

The second frequency peak in divergence times corresponds to an age of between 3.5 and 5.3 Myr (Early Pliocene) for the branch lengths estimated directly enforcing a GTR model; and between 5.3 and 7.5 Myr (Late Miocene) for the estimation using NPRS for the clades that do not follow a clock (Fig. 4). Species in this group included several of the endemic *Deronectes* and *Rhithrodytes bimaculatus*, although the likely sister of the latter, *R. dorsoplagiatus*, was missing from the analysis, and hence its divergence is probably an overestimate. Finally, *Stictotarus bertrandi* and the monotypic *Iberoporus cermenius* had divergence times > 5 Myr, with an origin from Middle to Late Miocene.

The conclusion of a Pleistocene origin of most species is robust even under greatly reduced evolutionary rates for mtDNA substitution, up to an approximately eight-fold reduction of the standard 2% suggested by most studies of insects. At a rate of 0.25% divergence per Myr (0.00125 substitutions/branch/site), only between five and seven of the species pairs would have an estimated divergence of fewer than 0.002 substitutions/branch/site, corresponding to an age of 1.64 Myr (the start of the Pleistocene, Harland *et al.* 1990) (Table 2).

Discussion

Contrary to the view of the Pleistocene as a time of little, if any, diversification at the species level (Elias 1994; Bennet 1997; Klicka & Zink 1997, 1999; Jansson & Dynesius 2002) this study shows that most of the Iberian endemic species of Dytiscidae are of middle to upper Pleistocene origin. Our findings are in agreement with a number of other phylogenetic analyses which provide evidence for fast Pleistocene radiation in Northern Hemisphere insects and other groups (e.g. Vogler *et al.* 1998; Clarke *et al.* 2001; Veith *et al.* 2003). In contrast, Pleistocene subfossil beetle data strongly support the notion that species track suitable habitat when climatic conditions change, and that these range

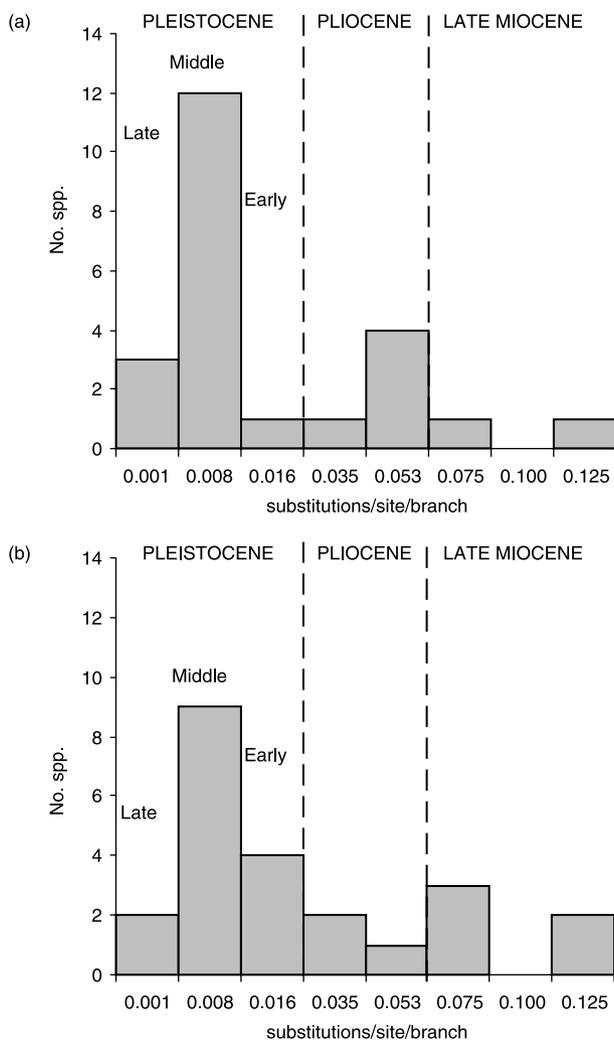


Fig. 4 Frequency histogram of the divergence level of the Iberian endemic species of Dytiscidae. (a) directly enforcing a GTR model; (b) with the nonparametric rate smoothing correction of Sanderson (1997). For species pairs in which both sisters are Iberian endemics only one data point is included. Note that the intervals of frequencies were adjusted to fit main geological periods (see Table 2 for primary data). Calibration corresponds to a standard mitochondrial clock of 2% divergence per Myr.

movements generally suppress speciation (Coope 1994, 1995; Elias 1994; Ashworth 1996). The retraction of species ranges at the height of the glacial cycles into Mediterranean refugia would lead to differentiation between these areas, followed by range expansions and the confluence of divergent genotypes in areas further in the north, or the formation of hybrid zones (Hewitt 1996, 1999).

Our results for the Iberian endemic Dytiscidae challenge this scenario on two accounts, as neither the geographical distribution nor the inferred time of divergence are consistent with the expectations from the hypothesis of major Pleistocene range movements and species stability. If spe-

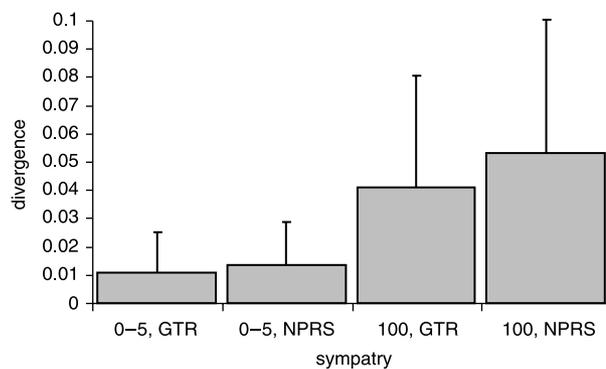


Fig. 5 Average estimated divergence of the Iberian endemic species of Dytiscidae according to the degree of sympatry with their sisters. 0-5, less than 5% sympatry; 100, 100% sympatry; GTR, divergences estimated by a direct enforcement of a GTR model; NPRS, divergences estimated with the nonparametric rate smoothing method of Sanderson (1997) (see Materials and methods). Bars represent standard deviations. For species pairs in which both sisters are Iberian endemics only one data point is included. Differences are significant for both the GTR and NPRS estimates, as measured with a one-factor ANOVA ($P < 0.05$).

ciation is allopatric (Mayr 1963), distributional ranges of sister species are expected to be nonoverlapping following the speciation event, and only over time will range movements produce areas of sympatry (Lynch 1989; Berlocher 1998; Barraclough & Vogler 2000; Barraclough & Nee 2001). In agreement with this scenario, we found that Iberian endemics at the tip nodes were generally distributed in allopatry with their sister, whereas endemics placed deeper in the tree showed a greater degree of sympatry with the sister clade. Furthermore, a detailed analysis of the geographical ranges of the endemic species and their sisters showed that in most pairs with nonoverlapping ranges these occupy closely adjacent areas, separated by physical barriers (e.g. the Ebro valley or the Pyrenees; Ribera 2003). If after speciation geographical ranges of the new sister species vary independently, and present ranges are a random (or quasi-random) portion of the past range (Roy *et al.* 1996; Losos & Glor 2003), the expectation would be similar degrees of sympatry among sister and non-sister species (i.e. at different node levels of the phylogeny), and no geographical proximity among the range of the sisters. The pattern revealed by the Iberian endemic species is not consistent with this scenario. Instead, the analysis of species distributions supports the general occurrence of allopatric speciation with minimal subsequent range movement, i.e. speciation *in situ*.

Second, the age of these species is very recent, well within the time window in which major range movements have been documented among European beetles (e.g. Elias 1994) or postulated to occur under the scenario of contraction and expansion cycles (Hewitt 1996). Our results

strongly suggest that most endemic Iberian species of Dytiscidae are not only of recent origin, they are also likely to have remained within the limits of the Iberian peninsula for all of their evolutionary history.

Our conclusion about the recent origin of the Iberian endemic dytiscids is dependent on three assumptions: (i) the accuracy of the current taxonomy; (ii) the accuracy of the phylogenetic reconstruction; and (iii) the accuracy of the molecular clock calibration. For most species we accept the current taxonomy at face value, as there are several recent morphological revisions of the respective genera or species groups which fulfil the generally accepted criteria for species recognition in insects (Fery & Hendrich 1988; Fresneda & Fery 1990; Angus *et al.* 1992; Nilsson & Angus 1992; Fery & Nilsson 1993; Fery 1995; Bilton & Fery 1996; Fery *et al.* 1996; Fery & Brancucci 1997; Fery 1999; Castro & Delgado 2001). For the majority of cases it seemed appropriate to represent each species by a single exemplar, as there is no indication that these individuals would not represent groups ('species') which are well separated from other such groups by clearly recognizable diagnostic differences. However, we included more than one specimen if species recognition was questionable, e.g. where species were very close morphologically (*Deronectes wewalkai* and *D. ferrugineus*); where morphological boundaries within a species complex were difficult to recognize (*D. aubei*–*D. delarouzei* complex, Fery & Brancucci 1997); where isolated populations could represent cryptic species (*Nebrioporus baeticus*); or where morphology suggested that Iberian specimens represented unrecognized species (*Scarodytes halensis*). More comprehensive sampling of populations would undoubtedly help in the precise delimitation of the species boundaries, and could lead to the recognition of further (more recent) cryptic species and hence to a refinement of our conclusions, in particular for the very recent divergences. However, the wider conclusions of our study are unlikely to be affected by these problematic cases of species delimitation. When some of the morphologically closest species pairs were pooled in a single taxon (*Deronectes wewalkai* and *D. ferrugineus*, *Hydroporus nevadensis* and *H. brancoi*, and the *Nebrioporus carinatus* complex) this had little effect on the conclusions of predominantly recent speciation.

As for the accuracy of the phylogenies, the placement of the Iberian endemic species is in general very robust. Of the 23 nodes placing an Iberian endemic, 16 had bootstrap values of 75–100%. Low bootstrap support was generally found at nodes deeper in the tree, specifying relationships of e.g. some Iberian *Deronectes* or *Iberoporus cermenius*; or within some complexes of very recent species (e.g. *Stictonectes* or the *Deronectes aubei* group). The fact that in all cases when a sister relationship was strongly suggested by morphology our trees were in perfect agreement (see the taxonomic revisions mentioned above) contributes to in-

creased confidence in our results. In any case, alternative placements of the species in nodes with low support do not change substantially the overall estimated divergence, and thus do not affect our conclusions. The trees presented here for the genus *Deronectes* differ slightly from a previous analysis based on a subset of the sequence data (Ribera *et al.* 2001), but these differences affect mostly species with high divergences and low nodal support. In other cases, differences may be the result of missing data, as in the species pair *D. costipennis*–*D. platynotus*, considered to be sisters in the revision of Fery & Brancucci (1997) and recovered with strong support here, but not in Ribera *et al.* (2001), where the 16S sequence of *D. costipennis* was not available.

Finally, applying a standard 2% divergence per Myr is a gross oversimplification, as molecular rates differ between genes and between taxonomic groups (Arbogast *et al.* 2002; Bromham & Penny 2003). However, our conclusion of a mostly Pleistocene origin of the Iberian endemic species is robust to a wide range of molecular clock calibrations, and would only be controversial under rates of evolution below approximately 0.25% divergence per Myr, i.e. slower than any rate reported in the literature for any group of insects. The standard 2% is often considered a conservative estimate (see Arbogast & Slowinski 1998 and references therein), and published estimates for beetles range from \approx 5–6% divergence per Myr for recent species (Juan *et al.* 1996; Clarke *et al.* 2001), through those around the standard 2% (Caccone & Sbordoni 2001; Farrell 2001; Barraclough & Vogler 2002), to about one-third of this value (Gómez-Zurita *et al.* 2000 for 16S rRNA alone).

In conclusion, the results from this study seem to be based on well-founded taxonomic and phylogenetic data. They are in clear contradiction to the view derived from subfossil data of Quaternary beetles in western Europe, in particular Britain, and similar fossil data for the Late Tertiary of the Arctic, which support species constancy (Matthews 1979; Böcher 1990, 1995). We believe that this discrepancy has to be evaluated in light of the geographical bias of studies on European insect fossils, which have been conducted almost exclusively in northern latitudes (see Elias 1994 for a comprehensive review of fossil locations). These areas were affected in a very different way by the Pleistocene climatic changes and now have few, if any, narrow-range endemic species of diving beetles (Ribera *et al.* 2003a). The virtual lack of fossil data from the areas of high endemism, among them the Iberian peninsula, makes it difficult to draw general conclusions about the origins of the Palaearctic fauna as a whole. The only known Pleistocene fossil of an extant Iberian 'endemic', *Ochthebius figueroi*, a species of the unrelated aquatic beetle family Hydraenidae that was originally described from the headwaters of the Ebro river (northern Spain), was found in southern Britain (Angus 1993, 1997). This finding would seem to contradict

our conclusions about constancy of ranges for the Iberian endemics. However, *O. figueroi* belongs to a group within subgenus *Asiobates* including several species with very reduced and isolated distributions, sometimes known only from the type locality, and with unknown sister relationships (Ribera 2000). They are likely to represent true relicts of formerly wider, still poorly known distributions, as supported by the recent discovery of *O. figueroi* in Morocco (Ribera 2000). This is in contrast to the species included in this study, with geographically well-structured distributions whose sisters occupy adjacent, but nonoverlapping ranges (Ribera 2003), consistent with allopatric speciation.

Our study suggests that extrapolating from what we know of widespread, northern taxa may be misleading. The extent to which the conclusions of our work are applicable to other species-rich Mediterranean groups remains to be investigated but it is conceivable that speciation in many southern groups with abundant narrow-range endemics was less affected by major range movements during times of changing climatic conditions. There is increasing evidence that Mediterranean populations of some taxa are long-term allopatric isolates, and hence the postulated refugia represent areas of endemism rather than undergoing dynamic exchange driven by climatic cycles (see, e.g. Bilton *et al.* 1998; Kropf *et al.* 2002; Crespi *et al.* 2003; Michaux *et al.* 2003; Veith *et al.* 2003 for a similar example in North America). For these lineages, speciation may have taken place in a comparatively stable geographical setting, where populations could persist and differentiate without being affected by the merging and extinction of gene pools prevalent in northern areas with greater amplitude of climatic shifts (Jansson & Dynesius 2002; Jansson 2003).

Acknowledgements

We thank all those listed in the Appendix for sending specimens for study, Ali Cieslak for help during collecting trips, Hans Fery and Anders Nilsson for taxonomic advice, and David Bilton, Garth Foster, two anonymous referees and the Associate Editor for comments. This work was supported by The Leverhulme Trust, a Marie Curie Fellowship of the EU, and NERC.

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Appendix

List of the specimens used to establish the relationships of the Iberian endemic species of Dytiscidae, with geographical origin, collector and GenBank accession numbers. Species names follow Nilsson (2001) (see this reference for species' authors).

Species	Region	Collector	16S rRNA	COI
ILYBIUS				
<i>Ilybius apicalis</i>	Kuriles	N. Minakawa	AF309279	—
<i>Ilybius bedeli</i>	Tunisia	I. Ribera & A. Cieslak	AY138658	—
<i>Ilybius chalconatus</i>	Morocco	P. Aguilera	AF309278	AF309335
<i>Ilybius erichsoni</i>	Sweden	A.N. Nilsson	AY138661	AY138748
<i>Ilybius hozgargantae</i>	Spain (Cádiz)	I. Ribera	AY138664	AY138751
<i>Ilybius montanus</i>	UK	I. Ribera	AY138666	AY138753
<i>Ilybius satunini</i>	Russia	A.N. Nilsson	AY138670	AY138757
<i>Ilybius subtilis</i>	Sweden	A.N. Nilsson	AF309276	AF309333
GRAPTODYTES group of genera				
<i>Graptodytes aequalis</i>	Spain (Albacete)	A. Millan	AY250910	AY250953
<i>Graptodytes atlantis</i>	Morocco	I. Ribera	AY250911	—
<i>Graptodytes delectus</i>	Gran Canaria	I. Ribera & A. Cieslak	AY250913	AY250954
<i>Graptodytes flavipes</i>	Spain (Huelva)	I. Ribera	AY250914	AY250955
<i>Graptodytes ignotus</i>	Spain (Girona)	P. Aguilera	AY250915	AY250956
<i>Graptodytes kuchtae</i>	Mallorca	I. Ribera & A. Cieslak	AY250916	AY250957
<i>Graptodytes pictus</i>	UK	I. Ribera	AY250917	—
<i>Metaporus meridionalis</i>	Spain (Albacete)	I. Ribera	AY250919	AY250959
<i>Porhydrus lineatus</i>	UK	I. Ribera	AY250933	AY250973
<i>Rhithrodytes numidicus</i>	Tunisia	I. Ribera & A. Cieslak	AY250935	—
<i>Rhithrodytes sexguttatus</i>	Corsica	I. Ribera & A. Cieslak	AY250936	AY250975
<i>Stictionectes azruensis</i>	Morocco	Pellicchia & Pizzetti	AY250940	AY250979
<i>Stictionectes lepidus</i>	Spain (Ávila)	H. Fery	AY250941	—
<i>Stictionectes optatus</i>	Corsica	I. Ribera & A. Cieslak	AY250943	AY250981
<i>Stictionectes optatus2</i>	Morocco	I. Ribera	AY250944	AY250982
<i>Stictionectes optatus3</i>	Mallorca	I. Ribera & A. Cieslak	AY250944	AY250983
<i>Stictionectes samai</i>	Tunisia	I. Ribera & A. Cieslak	AY250945	—
HYDROPORUS				
<i>Hydroporus</i> sp. 23.2	Canada	I. Ribera & A. Cieslak	AY365258	AY365292
<i>Hydroporus</i> sp. 32.4	Canada	I. Ribera & A. Cieslak	AY365259	AY365293
<i>Hydroporus</i> sp. 32.5	Canada	I. Ribera & A. Cieslak	AY365260	AY365294
<i>Hydroporus</i> sp. 33.4	Canada	I. Ribera & A. Cieslak	AY365261	AY365295
<i>Hydroporus</i> sp. C3.6	US	C. Hernando	AY365262	AY365296
<i>Hydroporus</i> sp. C3.7	US	C. Hernando	AY365263	AY365297
<i>Hydroporus</i> sp. C4.2	US	C. Hernando	AY365264	AY365298
<i>Hydroporus</i> sp. TW1	US	T. Berendok	AY365265	AY365299
<i>Hydroporus acutangulus</i>	Sweden	A.N. Nilsson	AF518256	AF518286
<i>Hydroporus analis</i>	Spain (Ávila)	H. Fery	AF518257	AF518287
<i>Hydroporus angustatus</i>	UK	I. Ribera	AY365266	AY365300
<i>Hydroporus axillarar</i>	US	I. Ribera & A. Cieslak	AY365267	AY365301
<i>Hydroporus basinotatus</i>	Spain (Cádiz)	I. Ribera	AF518258	AF518288
<i>Hydroporus compunctus</i>	Gomera	D.T. Bilton	AF518259	AF518289
<i>Hydroporus despectus</i>	Canada	I. Ribera & A. Cieslak	AY365268	AY365302
<i>Hydroporus discretus</i>	Spain (Albacete)	I. Ribera	AF518260	AF518290
<i>Hydroporus erythrocephalus</i>	UK	I. Ribera	AF518261	AF518291
<i>Hydroporus fuscipennis</i>	Sweden	A.N. Nilsson	AF518262	AF518292
<i>Hydroporus geniculatus</i>	Sweden	A.N. Nilsson	AY365269	AY365303
<i>Hydroporus glabriusculus</i>	UK	I. Ribera	AY365270	AY365304
<i>Hydroporus gyllenhalii</i>	UK	I. Ribera	AF518263	AF518293
<i>Hydroporus hirtellus</i>	US	I. Ribera & A. Cieslak	AY365271	AY365305
<i>Hydroporus incognitus</i>	Czech Republic	J. Statszny	AY365272	AY365306
<i>Hydroporus limbatus</i>	Morocco	I. Ribera	AF518264	AF518294
<i>Hydroporus lluci</i>	Mallorca	I. Ribera & A. Cieslak	AY365273	AY365307
<i>Hydroporus longulus</i>	UK	G.N. Foster	—	AY365326
<i>Hydroporus longulus2</i>	Spain (Santander)	H. Fery	AF518265	AF518295
<i>Hydroporus lucasi</i>	Spain (Granada)	I. Ribera	AF518266	AF518296
<i>Hydroporus marginatus</i>	Spain (Burgos)	I. Ribera	AF518267	AF518298

Appendix Continued

Species	Region	Collector	16S rRNA	COI
<i>Hydroporus melanarius</i>	Sweden	A.N. Nilsson	AF518268	—
<i>Hydroporus memnonius</i>	UK	I. Ribera	AF518269	AF518300
<i>Hydroporus morio</i>	Sweden	A.N. Nilsson	AY365274	AY365308
<i>Hydroporus necopinatus necopinatus</i>	Portugal	D.T. Bilton	AY365275	AY365309
<i>Hydroporus neglectus</i>	UK	I. Ribera	AY365276	AY365310
<i>Hydroporus nigellus</i>	Sweden	B. Andren	AY365277	AY365311
<i>Hydroporus niger</i>	US	K.B. Miller	AF518270	AF518301
<i>Hydroporus nigrita</i>	Spain (Burgos)	I. Ribera	AF518271	AF518302
<i>Hydroporus normandi</i>	Spain (Zaragoza)	P. Aguilera	AY365278	AY365312
<i>Hydroporus notabilis</i>	Sweden	A.N. Nilsson	AY365279	AY365313
<i>Hydroporus obscurus</i>	Sweden	A.N. Nilsson	AF518272	AF518303
<i>Hydroporus obsoletus</i>	Portugal	I. Ribera	AF518273	AF518304
<i>Hydroporus occidentalis</i>	Canada	I. Ribera & A. Cieslak	AY365280	AY365314
<i>Hydroporus palustris</i>	Italy	M. Toledo	AY365281	AY365315
<i>Hydroporus percivinus</i>	US	I. Ribera & A. Cieslak	AY365282	AY365316
<i>Hydroporus pilosus</i>	Tenerife	D.T. Bilton	AF518274	AF518305
<i>Hydroporus planus</i>	Russia	A.N. Nilsson	AF518275	AF518306
<i>Hydroporus puberulus</i>	Sweden	A.N. Nilsson	AY365283	AY365317
<i>Hydroporus pubescens</i>	UK	I. Ribera	AF518276	AF518307
<i>Hydroporus regularis</i>	Corsica	I. Ribera & A. Cieslak	AY365284	AY365318
<i>Hydroporus rufifrons</i>	UK	G.N. Foster	AF518277	AF518308
<i>Hydroporus springeri</i>	Italy	M. Toledo	AY365285	AY365319
<i>Hydroporus striola</i>	UK	I. Ribera	AY365286	AY365320
<i>Hydroporus submuticus</i>	Sweden	A.N. Nilsson	AY365287	AY365321
<i>Hydroporus subpubescens</i>	US	I. Ribera & A. Cieslak	AY365288	AY365322
<i>Hydroporus tessellatus</i>	Portugal	I. Ribera	AF518279	AF518310
<i>Hydroporus tristis</i>	Sweden	A.N. Nilsson	AY365289	AY365323
<i>Hydroporus umbrosus</i>	Finland	T. Berendok	AF518280	AF518311
DERONECTES group of genera				
<i>Deronectes abnormicollis</i>	Russia	J. Cooter	AF309250	AF309307
<i>Deronectes angelinii</i>	Italy	I. Ribera & A. Cieslak	AF309268	AF309325
<i>Deronectes aubei aubei</i>	France	I. Ribera & A. Cieslak	AF309269	AF309326
<i>Deronectes aubei aubei2</i>	Switzerland	I. Ribera & A. Cieslak	AY250906	AY250948
<i>Deronectes fairmairi</i>	Morocco	I. Ribera	AF309255	AF309312
<i>Deronectes hispanicus</i>	Spain (Albacete)	I. Ribera	AF309258	AF309315
<i>Deronectes lareynii</i>	Corsica	I. Ribera & A. Cieslak	AF309259	AF309316
<i>Deronectes latus</i>	UK	I. Ribera	AF309252	AF309309
<i>Deronectes moestus</i>	Corsica	I. Ribera & A. Cieslak	AF309256	AF309313
<i>Deronectes opatrinus</i>	Spain (Barcelona)	P. Aguilera	AF309257	AF309314
<i>Deronectes persicus</i>	Iran	H. Fery	AF309251	AF309308
<i>Deronectes platynotus</i>	Czech Republic	J. Statszny	AF309267	—
<i>Deronectes semirufus</i>	France	I. Ribera & A. Cieslak	AF309270	AF309327
<i>Deronectes theryi</i>	Morocco	I. Ribera	AF309262	AF309319
<i>Deronectes youngi</i>	Iran	H. Fery	AF309249	AF309306
<i>Nebrioporus airumilus</i>	China	J. Bergsten	AY250920	AY250960
<i>Nebrioporus assimilis</i>	UK	G.N. Foster	AY250921	AY250961
<i>Nebrioporus bucheti cazorlensis</i>	Spain (Málaga)	P. Aguilera	AY250922	AY250962
<i>Nebrioporus ceresyi</i>	Morocco	I. Ribera	AY250923	AY250963
<i>Nebrioporus clarkii</i>	Spain (Sevilla)	I. Ribera	AY250924	AY250964
<i>Nebrioporus lanceolatus</i>	Iran	K. Elmi & H. Fery	AY250927	AY250967
<i>Nebrioporus luctuosus</i>	Italy	I. Ribera & A. Cieslak	AY250928	AY250968
<i>Nebrioporus martinii</i>	Corsica	I. Ribera & A. Cieslak	AY250929	AY250969
<i>Nebrioporus nemethi</i>	Morocco	P. Aguilera	AY250930	AY250970
<i>Nebrioporus nipponicus</i>	Japan	J. Bergsten	AY250931	AY250971
<i>Nebrioporus variegatus</i>	Iran	K. Elmi & H. Fery	AY250932	AY250972
<i>Scarodytes halensis</i>	Finland	T. Berendok	AY250937	AY250976
<i>Scarodytes nigriventris</i>	Corsica	I. Ribera & A. Cieslak	AY250938	AY250977
<i>Scarodytes sp.</i>	Iran	K. Elmi & H. Fery	AY250939	AY250978
<i>Stictotarsus duodecimpustulatus</i>	Spain (Cádiz)	I. Ribera	AF309247	AF309304
<i>Stictotarsus maghrebinus</i>	Morocco	I. Ribera	AY250947	AY250985