

ARE IBERIAN ENDEMICS IBERIAN? A CASE-STUDY USING WATER BEETLES OF FAMILY DYTISCIDAE (COLEOPTERA)

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ABSTRACT

The phylogenetic relationships and the geographical origin of 27 of the 34 species and of 3 of the 9 subspecies of Iberian endemic Dytiscidae are studied, based on species level phylogenies constructed with two mitochondrial gene fragments (16S rRNA and Cytochrome Oxidase I). All Iberian endemic species for which more than one specimen was included were monophyletic with the exception of the complex *Deronectes aubei sanfilippo* Fery & Brancucci, 1997-*D. delarouzei* (Jac. Du Val, 1857). The genus *Stictotarsus* as presently defined is polyphyletic, containing three different lineages: the *S. duodecimpustulatus* group—including the Iberian endemic *S. bertrandi* (Legros, 1956)—, *Trichonectes otini* (Guignot, 1941) (new combination) and the *S. griseostriatus* and *S. roffii* groups, which are in need of a new generic name. The genus *Oreodytes* is found to be paraphyletic, although with low bootstrap support. The species *Nebrioporus (Nebrioporus) martinii* (Fairmaire, 1858) (new combination) is transferred from the subgenus *Zimmermannius* to *Nebrioporus*. The Iberian populations of *Stictotarsus griseostriatus* (De Geer, 1774) and the endemic subspecies *Oreodytes davisii rhianae* Carr, 2001, *O. sanmarkii alienus* (Sharp, 1872) and *Hydroporus normandi normandi* Régimbart, 1903 do not form well characterised lineages, as measured with the mitochondrial markers used in this study.

The Iberian endemic species of Dytiscidae are divided in three groups according to the type of vicariant origin: 1) within-Iberian species, when the sister species (or clade) of the Iberian endemic is also Iberian endemic; 2) Iberian/European, when the sister occurs in Europe north of the Pyrenees; and 3) Iberian/North African, when the sister occurs in North Africa. Within-Iberian endemics are found to be on average older than Iberian/European and Iberian/North African species, they have more restricted distributions within the Iberian peninsula (they occur typically in only one of the main biogeographical regions), and tend to occur exclusively in running waters. The within-Iberian species are best represented by the “Iberian” clade of the genus *Deronectes*, formed by six endemic species plus two species with wider distributions. Most species in this group originated in rapid succession in the Late Miocene-Early Pliocene boundary by repeated vicariant events in the three main mountain massifs in the Iberian peninsula: the Pyrenees, the Baetic ranges, and the Sistema Central plus mountain massifs of the NW. On the contrary, most of the Iberian/European species seem to be the recent (Pleistocene) vicariants of a species with a widespread distribution encompassing the Iberian peninsula, at present restricted to south and west of the Ebro valley. The results of these analyses suggest that the Iberian peninsula was an isolated refuge during the Quaternary glaciations, in where allopatric speciation was frequent among some lineages of Dytiscidae diving beetles.

Key words: 16S rRNA, Cytochrome Oxidase I, phylogenies, molecular clocks, endemism, speciation, Pleistocene glaciations, Iberian peninsula, Coleoptera, Dytiscidae.

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RESUMEN

¿Son los endemismos ibéricos realmente ibéricos? El caso de los Coleópteros acuáticos de la familia Dytiscidae (Coleoptera)

Se estudian las relaciones filogenéticas y el origen geográfico de 27 de las 34 especies, y de 3 de las 9 subespecies, de endemismos ibéricos de la familia Dytiscidae, en base a filogenias de las especies construidas con dos fragmentos de genes mitocondriales (16S rRNA y Citocromo Oxidasa I). Todas las especies ibéricas de las que se pudo estudiar más de un ejemplar son monofiléticas, con la excepción del complejo *Deronectes aubei sanfilippoï* Fery & Brancucci, 1997-*D. delarouzei* (Jac. Du Val, 1857). El género *Stictotarsus* tal y como está definido en la actualidad es polifilético, al estar compuesto de tres linajes distintos: el grupo de *S. duodecimpustulatus* —que incluye el endemismo ibérico *S. bertrandi* (Legros, 1956)—, *Trichonectes otini* (Guignot, 1941) (**nueva combinación**) y los grupos de *S. griseostriatus* y *S. roffii*, que precisan un nuevo nombre genérico. El género *Oreodytes* es parafilético, aunque con poco soporte de bootstrap. La especie *Nebrioporus (Nebrioporus) martinii* (Fairmaire, 1858) (**nueva combinación**) se transfiere del subgénero *Zimmermannius* a *Nebrioporus*. Las poblaciones ibéricas de *Stictotarsus griseostriatus* (De Geer, 1774) y las subespecies endémicas *Oreodytes davisii rhianae* Carr, 2001, *O. sanmarkii alienus* (Sharp, 1872) e *Hydroporus normandi normandi* Régimbart, 1903 no forman linajes bien caracterizados en base a los marcadores mitocondriales utilizados en este estudio.

Los endemismos ibéricos de Dytiscidae se dividen en tres grupos en función del tipo de origen vicariante: 1) especies intra-ibéricas, cuando la especie o el clado hermano de un endemismo ibérico es otro endemismo ibérico; 2) ibero-europeas, cuando el grupo hermano se distribuye por Europa al norte de los Pirineos; y 3) ibero-norteafricanas, cuando el grupo hermano se distribuye en el norte de África. Los endemismos intra-ibéricos son en general más antiguos que los ibero-europeos o los ibero-norteafricanos, tienen distribuciones más restringidas (típicamente sólo en una de las principales regiones biogeográficas ibéricas), y tienden a ocupar exclusivamente aguas corrientes. El mejor ejemplo de especies intra-ibéricas lo constituye el clado ibérico del género *Deronectes*, formado por seis endemismos y dos especies con distribuciones algo más amplias. La mayoría de las especies del grupo se originaron en rápida sucesión en la transición del Mioceno tardío al Plioceno, en una serie de fragmentaciones vicariantes en los tres principales macizos montañosos de la península Ibérica: los Pirineos, las cordilleras Béticas, y el macizo Central más los sistemas del noroeste de la Península. Por el contrario, la mayoría de las especies ibero-europeas parecen ser las vicariantes recientes (del Pleistoceno) de especies con una distribución amplia, y están normalmente restringidas al sur y el oeste del valle del Ebro. Los resultados de este estudio sugieren que la península Ibérica fue un refugio aislado durante las glaciaciones del Cuaternario, en el que la especiación alopátrica en alguno de los linajes de coleópteros acuáticos Dytiscidae fue relativamente frecuente.

Palabras clave: 16S rRNA, Citocromo Oxidasa I, filogenias, relojes moleculares, endemismo, especiación, Pleistoceno, glaciaciones, península Ibérica, Coleoptera, Dytiscidae.

Introduction

The Mediterranean basin, and within it the Iberian peninsula, are among the world's most diverse areas (Mittermeier *et al.*, 1998; Blondel & Aronson, 1999; Médail & Quézel, 1999; Myers *et al.*, 2000), with up to 20% of endemic taxa in several groups of plants and animals in mountain areas of Spain and Portugal (Baletto & Casale, 1991; Greuter, 1991; Domínguez Lozano *et al.*, 1996; Blanca *et al.*, 1998; Blondel & Aronson, 1999). There have been considerable advances in the understanding of this diversity in recent years, both in documenting biogeographical patterns (e.g. Doadrio, 1988; Dennis *et al.*, 1998; Puente *et al.*, 1998; Vargas *et al.*, 1998; Ribera, 2000; García-

Barros *et al.*, 2002) and in establishing the origin of selected Iberian taxa and their phylogenetic and phylogeographic relationships, mainly with molecular methods (e.g. Ferris *et al.*, 1998; Frati *et al.*, 1998; Arntzen & Wallis, 1999; García-París & Jockusch, 1999; Lenk *et al.*, 1999; Alexandrino *et al.*, 2000; Gómez-Zurita *et al.*, 2000; Machordom *et al.*, 2000; Oliverio *et al.*, 2000; Seddon *et al.*, 2001; Branco *et al.*, 2002; Ribera *et al.*, 2003a; see Hewitt, 1999, 2000 for reviews). There is, however, a paucity of studies bringing together these two approaches, that is, searching for general patterns in the origin and the phylogenetic and phylogeographic relationships in relatively rich groups of organisms with Iberian representation (but see e.g. Zardoya & Doadrio, 1998 for an example with cyprinid fishes).

Table 1.— Distribution of the Iberian endemic species and subspecies of Dytiscidae. Classification and nomenclature follows Nilsson (2001). Areas: geographic distribution within Iberia (see Fig. 7a): 1, Pyrenean; 2, Cantabrian; 3, Hercinian; 4, Baetic; 5, S West. a, Present in the north side of the Pyrenees.

Table 1.— Distribución de las especies y subespecies de Dytiscidae endémicas de la península Ibérica. Clasificación y nomenclatura según Nilsson (2001). Áreas: distribución geográfica en la península Ibérica (ver Fig. 7a): 1, Pirenaica; 2, Cantábrica; 3, Herciniana; 4, Bética; 5, Suroeste. a, Presente en la cara norte de los Pirineos.

Species	Areas				
	1	2	3	4	5
Agabinae					
<i>Agabus picotae</i> Foster & Bilton, 1997					+
<i>Ilybius albarracinensis</i> Fery, 1986	+a	+	+		
<i>I. dettneri</i> Fery, 1986			+		
Graptodytes group					
<i>Graptodytes castilianus</i> Fery, 1995	+		+		
<i>Iberoporus cermenius</i> Castro & Delgado, 2001				+	
<i>Rhithrodytes agnus agnus</i> Foster, 1993			+		
<i>R. agnus argaensis</i> Bilton & Fery, 1996			+		
<i>R. bimaculatus</i> (Dufour, 1852)	+a	+			
<i>Stictonectes epipleuricus</i> (Seidlitz, 1887)	+a	+	+	+	+
<i>S. occidentalis</i> Fresneda & Fery, 1990					+
Hydroporus					
<i>Hydroporus brancoi</i> Rocchi, 1981			+		
<i>H. brancuccii</i> Fery, 1987			+		
<i>H. cantabricus</i> Sharp, 1882					
<i>H. constantini</i> Hernando & Fresneda, 1996			+		
<i>H. decipiens</i> Sharp, 1877				+	+
<i>H. necopinatus necopinatus</i> Fery, 1999					
<i>H. nevadensis</i> Sharp, 1882		+	+	+	+
<i>H. normandi alhambrae</i> Fery, 1999					
<i>H. normandi normandi</i> Régimbart, 1903	+a	+	+	+	+
<i>H. paganettianus</i> Scholz, 1923		+	+		
<i>H. vagepictus</i> Fairmaire & Laboulbène, 1854	+a	+	+		
<i>H. vespertinus</i> Fery & Heindrich, 1988		+	+		
Deronectes group					
<i>Deronectes algibensis</i> Fery & Fresneda, 1988				+	
<i>D. angusi</i> Fery & Brancucci, 1990			+		
<i>D. aubei sanfilippoi</i> Fery & Brancucci, 1997	+a	+			
<i>D. bicostatus</i> (Schaum, 1864)			+		
<i>D. costipennis costipennis</i> Brancucci, 1983			+		
<i>D. costipennis gignouxii</i> Fery & Brancucci, 1989		+			
<i>D. delarouzei</i> (Jac. du Val, 1857)	+a	+			
<i>D. depressicollis</i> (Rosenhauer, 1856)				+	
<i>D. ferrugineus</i> Fery & Brancucci, 1987			+		
<i>D. fosteri</i> Aguilera & Ribera, 1996	+				
<i>D. wewalkai</i> Fery & Fresneda, 1988			+		
<i>Nebrioporus (Zimmermannius) baeticus</i> (Schaum, 1864)	+		+	+	
<i>N. (Nebrioporus) bucheti cazorlensis</i> (Lagar, Fresneda & Hernando, 1987)	+			+	
<i>N. (N.) carinatus</i> (Aubé, 1836)		+	+		
<i>N. (N.) croceus</i> Angus, Fresneda & Fery, 1992			+		
<i>N. (N.) fabressei</i> (Régimbart, 1901)	+a		+		
<i>Oreodytes davisii rihanae</i> Carr, 2001					
<i>O. sanmarkii alienus</i> (Sharp, 1872)					
<i>Scarodytes cf. halensis</i> (Fabricius, 1787)	+?	+	+	+	+
<i>Stictotarsus bertrandi</i> (Legros, 1956)			+		
Hygrotini					
<i>Hygrotus fresnedai</i> (Fery, 1992)			+		

Here I present some results of an ongoing investigation on the evolutionary and geographical origins of the Western Mediterranean diving beetles of the family Dytiscidae, centred on the Iberian endemic species. Dytiscidae are a moderately species rich family of Coleoptera Adephaga (ca. 4,000 species worldwide, Nilsson, 2001), with more than 300 species in Western Europe. About half of the European fauna can be found in the Iberian peninsula, which has also the highest number of endemics (Ribera, 2000; Millán *et al.*, 2002). In a separate work it is shown that most of these species are of very recent, Pleistocene origin, although some of them date back to the Late Miocene-Early Pliocene boundary (Ribera & Vogler, in press). Using species level phylogenies of most of the endemic species and subspecies constructed with two mitochondrial genes (16S rRNA and Cytochrome Oxidase I), I here investigate their geographical origin, the patterns of vicariant speciation, and the interrelationships among habitat, range area and age.

Material and Methods

TAXON SAMPLING

There are at present 33 recognised species and nine subspecies of endemic Dytiscidae in the Iberian peninsula, including eight which occur in some areas in south France, on the north side of the Pyrenees (Ribera, 2000; Millán *et al.*, 2002, Table 1). There is evidence that Iberian populations of two additional taxa, *Scarodytes halensis* (Fabricius, 1787) and *Stictotarsus griseostriatus* (De Geer, 1774), may constitute unrecognised species (Nilsson & Angus, 1992; Nilsson & Holmen, 1995; Ribera & Vogler, in press). Genetic data of specimens of 34 of these endemic taxa, covering all genera except for the only endemic species of *Hygrotus* (*H. fresnedai*) (see Table 1 for the authors of the Iberian endemic Dytiscidae mentioned in the text), were obtained and used in the phylogenetic analyses (Table 2). For some taxa more than one specimen were included, as a crude test of monophyly as well as substantial intraspecific variability, with a total of 44 specimens of endemic taxa studied in total (Fig. 1; Appendix 1; Table 2). Of two of the endemic subspecies (*H. necopinatus necopinatus* and *N. bucheti cazorlensis*, Table 1) only Iberian specimens could be obtained, and as their level of divergence could not be assessed they are not further considered here. Some of the analyses only required data on the distribution and ecology of the species (see below), and in them all Iberian endemic Dytiscidae were included.

Following partly Ribera & Vogler (in press), the studied Iberian endemic taxa were divided in four lineages: the *Deronectes* group of genera (Nilsson & Angus, 1992; Alarie & Nilsson, 1996) (genera *Deronectes*, *Nebrioporus*, *Stictotarsus*, *Oreodytes* and *Scarodytes*); the *Graptodytes* group of genera (*sensu* Ribera *et al.*, 2002) (*Graptodytes*, *Metaporus*, *Iberoporus*, *Stictonectes* and *Porhydrus*); *Hydroporus* (six taxa included in three main clades within the genus); and *Ilybius* (two species included in the *I. chalconatus-erichsoni* clade, Nilsson 2000; Ribera *et al.*, 2003c). The total number of taxa included in the combined phylogenies was 187 (Table 2; Appendix). Classification and nomenclature of all taxa mentioned follow Nilsson (2001), unless otherwise stated.

DNA EXTRACTION, GENE SEQUENCING

Specimens were collected in absolute ethanol, and muscular tissue used for DNA isolation via a standard Phenol-Chloroform extraction or by extraction columns (Quiagen). Sequences of 16S rRNA (16S onwards) were amplified as a single fragment of ca. 500 bp, using primers 16Sa (5'ATGTTTTTGTAAACAGGCG) for the 5' end of the gene, and 16Sb (5'CCGGTCTGAACTCAGATCATGT) for the 3' end (Simon *et al.*, 1994). A single fragment of ca. 800 bp of COI (from the middle of region E3 to the 3' end, Lunt *et al.*, 1996) was amplified using primers "Jerry" (5'CAACATT-TATTTTGATTTTTTGG) and "Pat" (5'TCCAATG-CACTAATCTGCCATATTA) (Simon *et al.*, 1994). It was not possible to obtain PCR products for the 16S of two specimens (*Hydroporus normandi ifransensis* Fery, 1999 and *H. longulus* Mulsant & Rey, 1861, Appendix), and the COI fragment of a number of species (Table 2, Appendix).

The following general PCR cycling conditions were used for DNA amplification: 1 to 2 min at 95°C, 30 seconds at 94°C, 30 seconds at 47-50°C (depending on the melting temperatures of the primer pair used), 1-2 min at 72°C (repeated for 35 to 40 cycles), and 10 min at 72°C. Amplification products were purified using a GeneClean II kit (Bio 101, Inc.). Automated DNA sequencing reagents were supplied by Perkin Elmer Applied BioSystems Ltd. (ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit). Sequencing reactions were purified by ethanol precipitation and were electrophoresed on an ABI3700 sequencer. Sequencing errors/ambiguities were edited using the Sequencher 3.0 software package (Gene Codes Corporation). Some sequences were obtained from Ribera *et al.* (2001, 2003c) and Ribera & Vogler (in

Table 2.— Sister taxa, genetic divergences, range area, type of vicariant origin and habitat of the Iberian endemic species of Dytiscidae.
 Tabla 2.— Taxones hermanos, divergencias genéticas, área de distribución, tipo de origen vicariante y hábitat de las especies de Dytiscidae endémicas de la península Ibérica.

endemic	sister	sister distribution	% over	direct	NPRS	area km ²	type	hab.
<i>Agabus picotae</i>	? (<i>A. heydeni</i>)	(Iberia, N Africa)	(0)	?	?	1500	(1)	0
<i>Ilybius albaracinensis</i>	<i>I. satunini</i>	E Mediterranean	0	0.004	-	200000	2	1
<i>Ilybius detneri</i>	<i>I. hozgargantae</i>	S Spain, N Morocco	0	0.031	-	60000	3	2
<i>Graptodytes castilianus</i>	<i>G. aequalis</i> (<i>G. pierii</i>)	(Tunisia, Algeria)	0	0.002	-	170000	3	1
<i>Iberoporus cermentius</i>	<i>Metaporus</i> + <i>Graptodytes</i>	W Palaearctic	100	0.122	-	50	1	s
<i>Rhithrodytes agnus</i>	?	?	?	?	?	4500	?	0
<i>R. bimaculatus</i>	<i>R. numidicus</i> + <i>sexguttatus</i>	(Algeria)	0	0.043	-	55000	3	0
	(<i>R. dorsoplagiatus</i>)							
<i>Stictonectes epipleuricus</i>	<i>S. optatus</i> + <i>samati</i> + <i>occidentalis</i>	W Palaearctic	100	0.007	-	650000	?	0
<i>S. occidentalis</i>	<i>S. optatus</i> (partim)	W Palaearctic	100	0.007	-	10000	?	0
<i>Hydroporus brancoi</i>	<i>H. nevadensis</i>	Iberian endemic	100	0.000	-	100000	1	1
<i>H. nevadensis</i>	<i>H. brancoi</i>	Iberian endemic	[100]	[0.000]	-	350000	1	1
<i>H. nevadensis-brancoi</i>	<i>H. longulus</i>	European (excl. Iberia?)	0	0.002	-	350000	2	1
<i>Hydroporus brancoi</i>	? (<i>H. discretus</i>)	(W Palaearctic)	(100)	?	?	20000	?	0
<i>H. cantabricus</i>	? (<i>H. brancoi</i>)	(Iberian endemic)	(100)	?	?	100	(1)	0
<i>H. constantini</i>	?	?	?	?	?	5000	?	0
<i>H. decipiens</i>	? (<i>H. analis</i>)	(W Mediterranean)	?	?	?	135000	?	1
<i>H. paganettianus</i>	<i>H. obscurus</i>	W Europe (excl. Iberia)	0	0.011	-	70000	2	1
<i>H. vagepictus</i>	<i>H. palustris</i>	W Europe (excl. Iberia)	5	0.007	-	450000	2	1
<i>H. vesperinus</i>	<i>H. erythrocephalus</i>	W Europe (excl. Iberia)	0	0.002	-	75000	2	2
<i>Deronectes alpehensis</i>	<i>D. hispanicus</i> + <i>oparinus</i> + <i>bicostatus</i>	Iberian endemic?	100	0.045	0.062	8500	1	0
<i>D. aubei sanfilippo</i> + <i>D. delarouzei</i>	<i>D. a. aubei</i>	Alps	0	0.005	0.011	90000	2	0
<i>D. angusi</i>	<i>D. latus</i>	W Europe (excl. Iberia)	0	0.002	-	80000	2	0
<i>D. bicostatus</i>	<i>D. hispanicus</i> + <i>oparinus</i>	Iberian endemic?	100	0.045	0.061	125000	1	0
<i>D. costipennis</i>	<i>D. platynotus</i>	C & N Europe	0	0.005	0.011	75000	2	0
<i>D. depressicollis</i>	<i>D. fosteri</i>	Iberian endemic	0	0.045	0.054	40000	1	0
<i>D. fosteri</i>	<i>D. depressicollis</i>	Iberian endemic	[0]	[0.045]	[0.054]	15000	1	0
<i>D. ferrugineus</i>	<i>D. wewalkai</i>	Iberian endemic	0	0.000	0.001	65000	1	0
<i>D. wewalkai</i>	<i>D. ferrugineus</i>	Iberian endemic	[0]	[0.000]	0.001	45000	1	0
<i>D. ferrugineus-wewalkai</i>	<i>D. depressicollis</i> + <i>fosteri</i>	Iberian endemic	0	0.050	0.064	87000	1	0
<i>Nebrioporus carinatus</i>	<i>N. croceus</i>	Iberian endemic	[0]	[0.001]	0.003	68000	1	0
<i>N. fabressi</i>	<i>N. carinatus</i>	Iberian endemic	0	0.001	0.003	1100	1	0
<i>N. baeticus</i>	<i>N. croceus</i> + <i>carinatus</i>	Iberian endemic	2	0.002	0.006	180000	1	0
<i>Scarodytes cf. halensis</i>	<i>N. assimilis</i>	W Europe (excl. Iberia)	0	0.041	0.075	250000	2	0
<i>Stictotarsus bertrandii</i>	<i>N. nemethi</i>	Morocco	0	0.004	0.009	150000	3	0
<i>Hygrotes fresnedai</i>	<i>S. nigriventris</i>	Corsica, Sardinia	0	0.007	0.021	650000	2	1
	<i>S. 12-pustulatus</i> + <i>maghrebinus</i>	W Palaearctic	100	0.062	0.114	50000	?	0
	? (<i>H. saginatus</i>)	(C&E Mediterranean)	(0)	?	?	80000	?	2

Sister, sister species (or clade) according to the phylogenies in this study; in parenthesis, species assumed to be the true sister according to morphology (see text). %over, percentage of the range of the Iberian endemic species which overlap with the range of the sister (measured with respect to the smaller range). Direct, estimated divergence in substitutions/site, estimated by directly enforcing a molecular clock in PAUP; NPRS, estimated divergences according to the Non Parametric Rate Smoothing of Sanderson (1997), for the lineages which rates were significantly different from a clock (all divergences from Ribera & Vogler, in press); area, estimated surface of the present distribution of the species (see Methods); type, type of vicariant origin: 1, within Iberia, 2, Iberian/European, 3, Iberian/North African; hab., habitat: 0, running, 1, standing and running, 2, standing; s, subterranean (data from Ribera et al., 2003). In square brackets, duplicate data points not used in the analyses (i.e. species pairs of which both are Iberian endemics). / Sister, especes (o clado) hermano según las filogenias de este estudio; entre paréntesis, especies que se supone son los auténticos hermanos de acuerdo a la morfología (ver texto). %over, porcentaje del rango de la especie endémica que se solapa con el rango del taxon hermano (medido respecto al rango menor). Direct, divergencia estimada forzando directamente un reloj molecular en PAUP, en sustituciones/posición; NPRS, divergencia estimada de acuerdo al método Non Parametric Rate Smoothing de Sanderson (1997), para los linajes en los que la tasa de evolución difiere significativamente de un reloj molecular (todas las divergencias tomadas de Ribera & Vogler, en prensa); área, superficie estimada de la distribución actual de la especie (ver Métodos); tipo, tipo de origen vicariante: 1, intra-ibérico, 2, ibero-europeo, 3, ibero-norteafricano; hab., hábitat: 0, corriente, 1, estanco y corriente, 2, estanco, s, subterráneo (datos tomados de Ribera et al., 2003). Entre corchetes, datos duplicados no utilizados en el análisis (pares de especies en los que las dos son endemismos ibéricos).

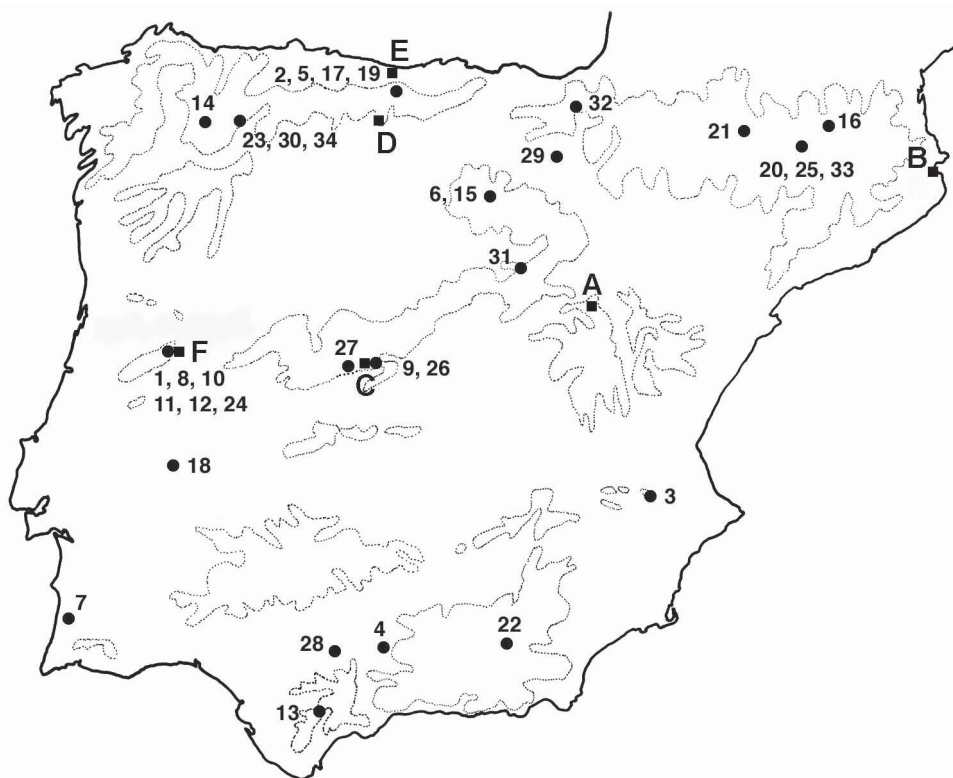


Fig. 1.— Geographical origin of the specimens of Iberian endemic species and subspecies included in the study (see Appendix 1 for specimens' identities).

Fig. 1.— Origen geográfico de los ejemplares de especies y subespecies ibéricas incluidas en el estudio (ver Apéndice 1 para la identidad de los ejemplares).

press), new sequences will be deposited in GenBank.

PHYLOGENETIC ANALYSIS

Species-level phylogenies were constructed with as many taxa as possible from the genera in which the Iberian endemics were included. Phylogenies of *Ilybius* and the *Graptodytes* gr. were taken from Ribera & Vogler (in press). For *Hydroporus*, the same data set as Ribera & Vogler (in press) was used, with the addition of several specimens of two of the subspecies of *H. normandi*. For the *Deronectes* group of genera, species of *Oreodytes* and *Stictotarsus* were added to those used in Ribera & Vogler (in press), to establish the relationships of the Iberian subspecies or populations.

The analyses of the new sequences was performed in a two step procedure (Phillips *et al.*, 2000), aligning sequences in ClustalW (Higgins *et al.*, 1992, default values) followed by hand correction

of obvious mistakes, and tree searches on the aligned matrix using parsimony analysis in PAUP4.0b6 (Swofford, 2000), with gaps coded as a fifth character (Giribet & Wheeler, 1999). PAUP searches consisted of TBR heuristic explorations of 10,000 replicates.

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 non-parametric bootstrap (Felsenstein, 1985), with 1000 iterations of 100 TBR replicates.

RATE OF EVOLUTION AND GEOGRAPHICAL ANALYSIS

Estimated branch lengths for the Iberian species of Dytiscidae were taken from Ribera & Vogler (in press). Sequences of the ingroups only were fitted using maximum likelihood (ML) to the topology of the optimal tree obtained with parsimony. Optimum ML models for the combined data were selected

using Modeltest 3.06 (Posada & Crandall, 1998). To estimate relative node ages, ML branch lengths were fitted assuming a molecular clock and the likelihood compared to that obtained assuming no clock (Felsenstein, 1981). When the ratio was not significant, an ultrametric tree could be directly obtained in PAUP by the enforcement of a molecular clock. If the sequences could not be said to follow a molecular clock, the tree was split in different lineages, and a new topology estimated through parsimony to try to maximise sequence homogeneity, rooting the ingroup trees according to the topology of the more inclusive phylogeny (Ribera & Vogler, in press). If after this procedure differences with a clock were still significant, the Non-Parametric Rate Smoothing (NPRS) method of Sanderson (1997), as implemented in TreeEdit v1.0 (Rambaut & Charleston, 2001), was used. However, the method introduced a large deformation in the resulting tree, with a total divergence ca. double that obtained when a clock was directly enforced in PAUP. For that reason, the ultrametric tree obtained by the direct enforcement of a GTR model in PAUP was retained for comparison (see Results).

To have an approximate calibration of the branch lengths we used the standard 2% divergence per million years (Myr) for insect mitochondrial DNA (Brown *et al.*, 1979; Brower, 1994), corresponding to a base rate (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), and habitat data from Ribera *et al.* (2003b). To have an estimate of the range size, approximate distributions were represented by shaded areas in a map of the Iberian peninsula, and the area estimated using image analysis software (NIH image 1.62, freely available at the NIH web page) (distributional maps available from the author). Statistical methods to analyse the numerical data are standard tests, specified in the text in the corresponding sections. In the statistical analyses of the genetic divergences (i.e. the length of the terminal branches leading to Iberian endemics in the estimated ultrametric trees) only one data point was used when the two sister species were Iberian endemics. Geological ages follow Harland *et al.* (1990).

Results

PHYLOGENIES AND RATES OF EVOLUTION

Ilybius: The topology of the tree and the branch lengths (Fig. 2a) were taken from Ribera & Vogler

(in press). The two studied endemic species of *Ilybius* (*I. albarracinensis* and *I. dettneri*, Table 1) were included in the *I. chalconatus-erichsoni* clade (Nilsson, 2001; Ribera *et al.*, 2003c), and respectively sisters to *I. satunini* (Zaitsev, 1913) (from the middle East and the Caucasus) and *I. hozgargantae* (Burmeister, 1983) (North Morocco, extreme south of Spain) (Fery & Nilsson, 1993; Nilsson, 2000) (Fig. 2a, Table 2).

Graptodytes group of genera: The topology of the tree and the branch lengths (Fig. 2b) were taken from Ribera & Vogler (in press). *Stictonectes* and *Porhydrus* are sisters, and sister to *Rhithrodytes*, *Iberoporus*, *Metaporus* and *Graptodytes*. The only Iberian endemic genus of Dytiscidae, *Iberoporus*, is sister to *Metaporus* plus *Graptodytes*, although with low support (Fig. 2b). In the tree obtained with equally weighted characters the three genera form a basal polytomy including also *Rhithrodytes*, and under some parameter combinations *Iberoporus* is sister to *Rhithrodytes*. The two topologies (*Iberoporus* sister to *Rhithrodytes* or to *Metaporus* plus *Graptodytes*) are not significantly different, as measured with the non-parametric Templeton (1983) Wilcoxon signed-ranks statistic ($p = 0.78$), or the Shimodaira & Hasegawa (1999) test using RELI with 10,000 bootstrap replicates ($p = 0.13$), using the ML model implemented for the calibration of the branch lengths. Among the species of *Stictonectes*, *S. optatus* (Seidlitz, 1887) is found to be polyphyletic, with the specimens of Corsica in a basal position with respect to a clade including specimens from Morocco and Mallorca (Fig. 2b).

Hydroporus group of genera: The topology of the tree is identical to that of Ribera & Vogler (in press), with the addition of several specimens of *H. normandi* (Fig. 2c). The endemic Iberian species were widespread within three large clades of *Hydroporus*, one including the *H. memnonius* and longulus groups, another including the *H. striola* and *tristis* groups, and a third including most of the Nearctic species, but also the *H. erythrocephalus* and *obscurus* groups (all species groups follow Nilsson, 2001).

The specimen from Cantabria identified as *H. longulus* according to morphology (H. Fery, personal communication, 2001) had identical sequences for both genes to those of *H. nevadensis* and *H. brancoi*, but different from the British specimen of *H. longulus* (Fig. 2c). With the limited data presented here it is not possible to delimit species boundaries within the complex of species around *H. longulus*, and this specimen is provisionally interpreted as belonging to the complex of Iberian *H. nevadensis*-*H. brancoi*.

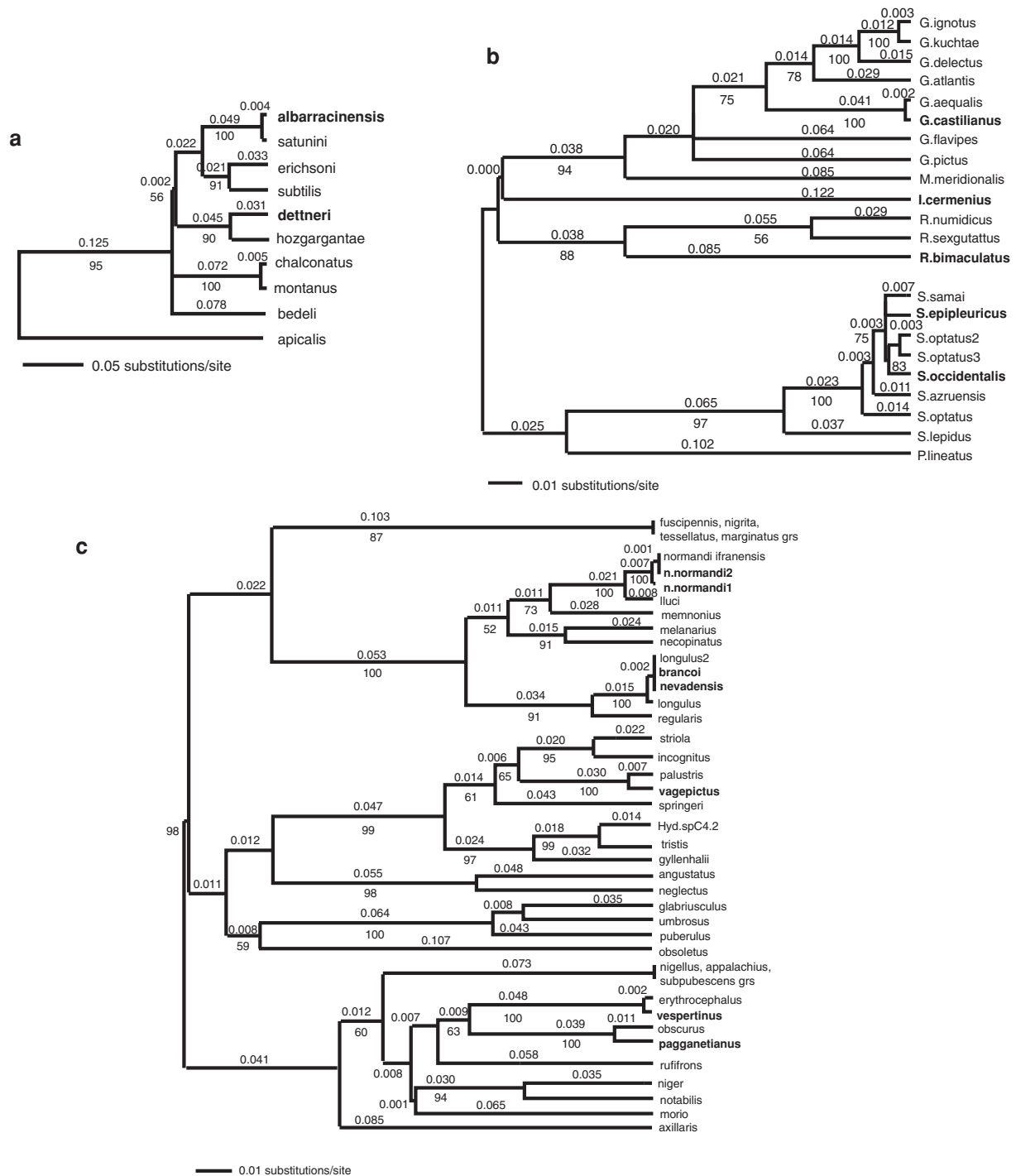


Fig. 2.— Ultrametric trees used in the estimation of divergence times. a, *Ilybius*; b, *Graptodytes* gr. of genera; c, *Hydroporus*. In bold, Iberian endemics. Numbers above branches are branch lengths; below branches, non-parametric bootstrap support values of the corresponding node (modified from Ribera & Vogler, in press).

Fig. 2.— Árboles ultramétricos utilizados en la estimación de las edades de divergencia. a, *Ilybius*; b, grupo de géneros afines a *Graptodytes*; c, *Hydroporus*. En negrita, endemismos ibéricos. Los números sobre las ramas son su longitud; bajo las ramas, valores de soporte del bootstrap no paramétrico del nodo correspondiente (modificado de Ribera & Vogler, en prensa).

Within *H. normandi*, the specimen from Morocco was sister to that from Girona (NE Spain), and both sister to the one from Gallocanta (Zaragoza, E Spain) (Fig. 2c). In Fery (1999) the two known populations of *H. normandi* in Morocco (High Atlas and Moyen Atlas) were described as distinct subspecies (*H. n. ifnii* Fery, 1999 and *H. n. ifranensis* respectively). The specimen from Azrou included in this study, at only 20 km of the locus typicus of *H. n. ifranensis*, is considered to belong to his subspecies (as was considered in Fery, 1999: 240). According to the phylogeny obtained here *H. n. normandi* is not monophyletic. This does not have any implication for its taxonomic status, as it is expected that subspecies (or even species) may be paraphyletic due to lack of coalescence or to restricted gene flow (Avise, 2000), but precludes an analysis based on the limited number of sequences available. More specimens and molecular markers would be necessary to assess the taxonomic status of the subspecies of *H. normandi*.

Deronectes group of genera: Cytochrome Oxidase I sequences were of equal length, and length variability in the 16S rRNA fragment within the ingroup ranged from 506 bp (*D. algibensis*) to 513 bp (*S. roffii*). A heuristic search using gaps as 5th character resulted in >1,000 equally parsimonious trees (not shown) of length 3,417 and consistency index 0.24. Both genes were not significantly incongruent, as measured with the Partition Homogeneity Test implemented in PAUP ($p=0.47$). Data were reweighted a posteriori according to the rescaled consistency index (RC), and a new heuristic search conducted on the set of equally parsimonious trees. This new search resulted in a single tree represented in Fig. 3.

The most remarkable result is the polyphyly of the genus *Stictotarsus*, as defined by Nilsson & Angus (1992), which is formed by three distinct lineages: the species of the *S. duodecimpustulatus* gr., the species of the *S. griseostriatus* and *S. roffii* groups, and *S. otini* (Guignot, 1981), which stands in a very isolated position (Fig. 3). The species of the *S. griseostriatus* and *S. roffii* groups are included together with the genera *Deronectes* and *Oreodytes* in a monophyletic clade, while *S. otini* and the species of the *S. duodecimpustulatus* gr. are included together with *Nebrioporus* and *Scarodytes* in its sister lineage.

Within the *Deronectes* clade, the species of *Deronectes* are monophyletic and sister to a clade formed by *Oreodytes* and the species of the *Stictotarsus griseostriatus* and *S. roffii* groups (Fig.

3). *Oreodytes* is divided in two lineages, which are paraphyletic with respect to the species of *Stictotarsus*: one including the larger and more elongated species —*O. davisii* (Curtis, 1831) and related taxa—, and the other the round, smaller species —*O. sanmarkii* (C.R. Sahlberg, 1826) and related taxa, Fig. 3—. The monophyly of the genera *Deronectes* and the *S. griseostriatus* and *S. roffii* groups is on the contrary strongly supported (Fig. 3).

Among the species of the *Deronectes aubei* gr. (*sensu* Fery & Brancucci, 1997), the two western taxa (*D. delarouzei* and *D. aubei sanfilippoi*) are monophyletic and sister to one of the two eastern taxa —*D. aubei aubei* (Mulsant, 1843)—, making the two subspecies of *D. aubei* polyphyletic. Moreover, the two specimens sampled of *D. aubei sanfilippoi* are paraphyletic with respect to the two specimens of *D. delarouzei*.

The four specimens of *O. davisii* sampled (including two *O. d. rhianae*) have identical 16S rRNA sequence. Only the COI sequence of two specimens could be obtained (Appendix), but when these were included in the analysis, *O. alpinus* (Paykull, 1798) was subordinated within *O. davisii*, and *O. d. davisii* became paraphyletic with respect to *O. d. rhianae* and *O. alpinus*. More data will be necessary to solve the possible monophyly of *O. davisii* and its subspecies (especially of specimens from the Pyrenees).

A similar situation occurs with the specimens of *O. sanmarkii*, of which no COI sequence could be obtained. Based on the 16S rRNA sequence, *O. s. sanmarkii* would be paraphyletic with respect to *O. s. alienus*, as one of the specimens (*O. sanmarkii* 2, from Switzerland) has an identical 16S sequence to that of *O. s. alienus*.

The studied specimens identified as *S. griseostriatus* fall in two well defined clades, one formed by specimens from Canada and California, and the other from Morocco, Portugal and Switzerland, but also one from California. More specimens are needed to solve the apparent geographical incongruence of these two clades, but what seems certain is that the European *S. griseostriatus* —and probably its related species, *S. multilineatus* (Falkenström, 1922) and *S. macedonicus* (Guéorguiev, 1959), Nilsson & Angus, 1992— are members of a lineage of Nearctic origin, which has expanded its range to the Palaearctic only relatively recently. With the molecular information available there is nothing to support the distinctiveness of the Iberian populations of *S. griseostriatus*, and in consequence is not further considered here.

The phylogeny of the species of *Deronectes* is largely congruent with that of Ribera *et al.* (2001).

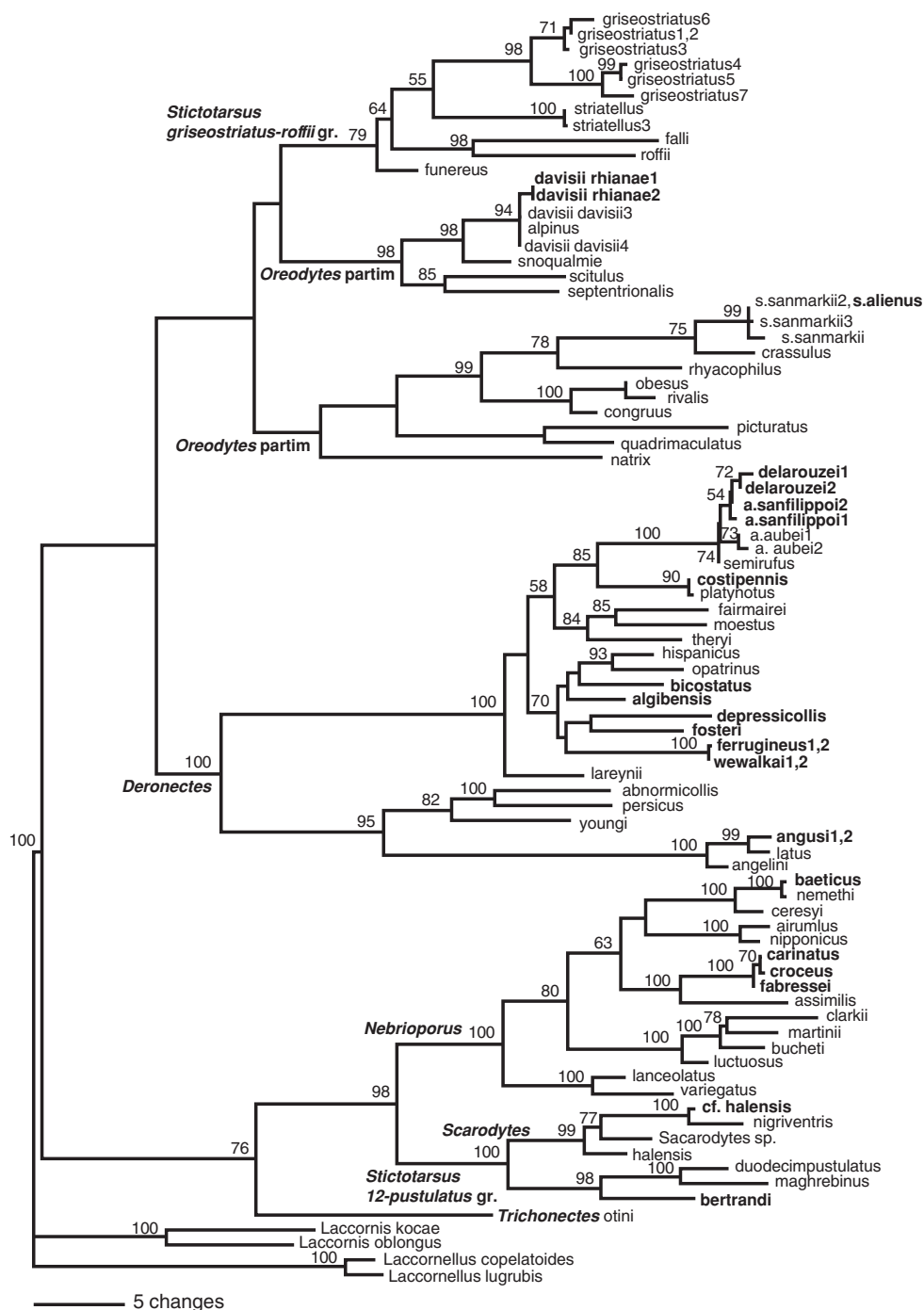


Fig. 3.— Phylogram of the single most parsimony tree of the *Deronectes* group of genera, obtained after reweighting the characters according to the rescaled consistency index. Numbers by nodes, non-parametric bootstrap support values (obtained with reweighted characters). In bold, Iberian endemic species and subspecies (see Appendixes for the origin of the specimens).

Fig. 3.— Filograma del árbol más parsimonioso del grupo de géneros afines a *Deronectes*, obtenido tras recalibrar los caracteres de acuerdo al índice de consistencia recalibrado. Números junto a los nodos, valores de soporte bootstrap no paramétrico (obtenidos con los caracteres recalibrados). En negrita, especies y subspecies endémicas de la península Ibérica (ver los Apéndices para el origen de los ejemplares).

The genus has two main lineages, one formed by the *D. latus* and *D. parvicollis* groups (*sensu* Fery & Brancucci, 1997), and the second by the rest of the species. Within the later, *D. lareynii* (Fairmaire, 1858) (from Corsica) is basal and sister to two main clades: one formed for what can be called the “Iberian” *Deronectes*, including most of the Iberian endemics, and a second including the *D. aubei* and *D. platynotus* groups, plus *D. fairmairei* (Leprieur, 1876), *D. moestus* (Fairmaire, 1858) and *D. theryi* (Peyerimhoff, 1925) (Fig. 3). The sister relationship between *D. platynotus* (Germar, 1834) and *D. costipennis* (the *D. platynotus* group *sensu* Fery & Brancucci, 1997) was not recovered in the phylogeny of Ribera *et al.* (2001), probably due to missing data. The two specimens of *D. angusi*, *D. wewalkai* and *D. ferrugineus* had identical sequences each (Ribera & Vogler, in press).

Within the *Nebrioporus* lineage, *Stictotarsus otini* is basal and sister to all remaining species. *Stictotarsus bertrandi* is sister to *S. duodecimpustulatus* (Fabricius, 1792) plus *S. maghrebinus* Mazzoldi & Toledo, 1998, and the three species sister to *Scarodytes*. The Iberian specimen of *Scarodytes halensis* was not sister to the specimen from Finland, suggesting that it is an unrecognised, possible endemic species, sister to *S. nigriventris* (Zimmermann, 1919) (from Corsica and Sardinia) (Ribera & Vogler, in press). The two specimens of *Nebrioporus baeticus* (from Murcia and Navarra) had identical sequences for 16S rRNA (the COI sequence of the specimen from Navarra could not be obtained).

Based on the available molecular information none of the subspecies of *Hydroporus* or *Oreodytes* could be considered to be a distinctive lineage, either because of their non-monophyly (e.g. *H. normandi normandi*), or for being identical to non-Iberian lineages (*Oreodytes*). It must be stressed that this does not preclude their taxonomic distinctiveness (see comments to *H. normandi* above). Similarly, based on the studied sequences there is no evidence for the distinctiveness of the Iberian *Stictotarsus griseostriatus*, and in consequence further analyses were restricted to the recognised endemic species plus *Scarodytes cf. halensis* (Table 2). Estimated divergences for the Iberian endemic species within these groups were taken from Ribera & Vogler (in press). To maximise rate homogeneity in the cases where sequences could not be said to follow a molecular clock a new tree topology was found using only the ingroup taxa, and an ultrametric tree estimated using an optimal ML model both directly in PAUP and using Sanderson’s (1997) NPRS (see Methods, Fig. 3).

The topology of the genus *Deronectes* using the ingroup only was identical to that obtained using outgroups, with the only difference that the position of the lineage of *D. moestus*-*D. fairmairei*-*D. theryi* was unresolved, and placed in a basal polytomy (Figs 3, 4a). The topology of the *Nebrioporus* gr. obtained using only the ingroup taxa was also very similar to that obtained using outgroups, with only one exception: while in the tree including outgroups the species of the subgenus *Zimmermannius* —*N. ceresyi* (Aubé, 1838), *N. baeticus* and *N. nemethi* (Guignot, 1950)— were subordinated within *Nebrioporus* s.str., as sister to the species-pair *N. airumilus* (Kolenati, 1845) and *N. nipponicus* (Takizawa, 1933) (Figs 3, 4b), in the tree estimated without outgroups the two subgenera are sisters and respectively monophyletic (Fig. 4b). The two topologies do not differ significantly, as measured with the non-parametric Templeton (1983) Wilcoxon signed-ranks statistic ($p = 0.37$), or the Shimodaira & Hasegawa (1999) test using REL with 10,000 bootstrap replicates ($p = 0.37$), using the ML model implemented for the calibration of the branch lengths. The species *N. martinii* (Fairmaire, 1858) (from Corsica and Sardinia), which in Nilsson & Angus (1992) and Nilsson (2001) is included in the subgenus *Zimmermannius*, appears to be sister to *N. clarkii* (Wollaston, 1862), within *Nebrioporus* s. str.

PATTERNS OF VICARIANCE AND DIVERGENCE

The geographical origin of the Iberian endemic species of Dytiscidae can be broadly categorised in three groups, according to their distribution and the distribution of their sisters: 1) within-Iberian, if their sister is also an Iberian endemic; 2) Iberian/European, when the sister is distributed in Europe north of the Pyrenees, and 3) Iberian/North African, when the sister is distributed in North Africa. In three cases the sister taxa included widespread species present in Iberian as well as in other areas, and thus it was not possible to include them in any of these three categories (Table 2). The distribution of *I. hozgargantae* is hypothetically assumed to be primarily North African, as the species is present in north Morocco from the Rif to the extreme NE of the Moyen Atlas (Ribera *et al.*, 1999 and unpublished data), but only in the extreme south of Spain (Burmeister, 1983; Fery & Nilsson, 1993). Similarly, the distribution of *D. opatrinus* (Germar, 1824) and *D. hispanicus* (Rosenhauer, 1856) is assumed to be primarily Iberian, as the most parsimony reconstructed ancestral distribution of the whole clade in which they are included is Iberian (Figs 3, 4a). Both species are currently

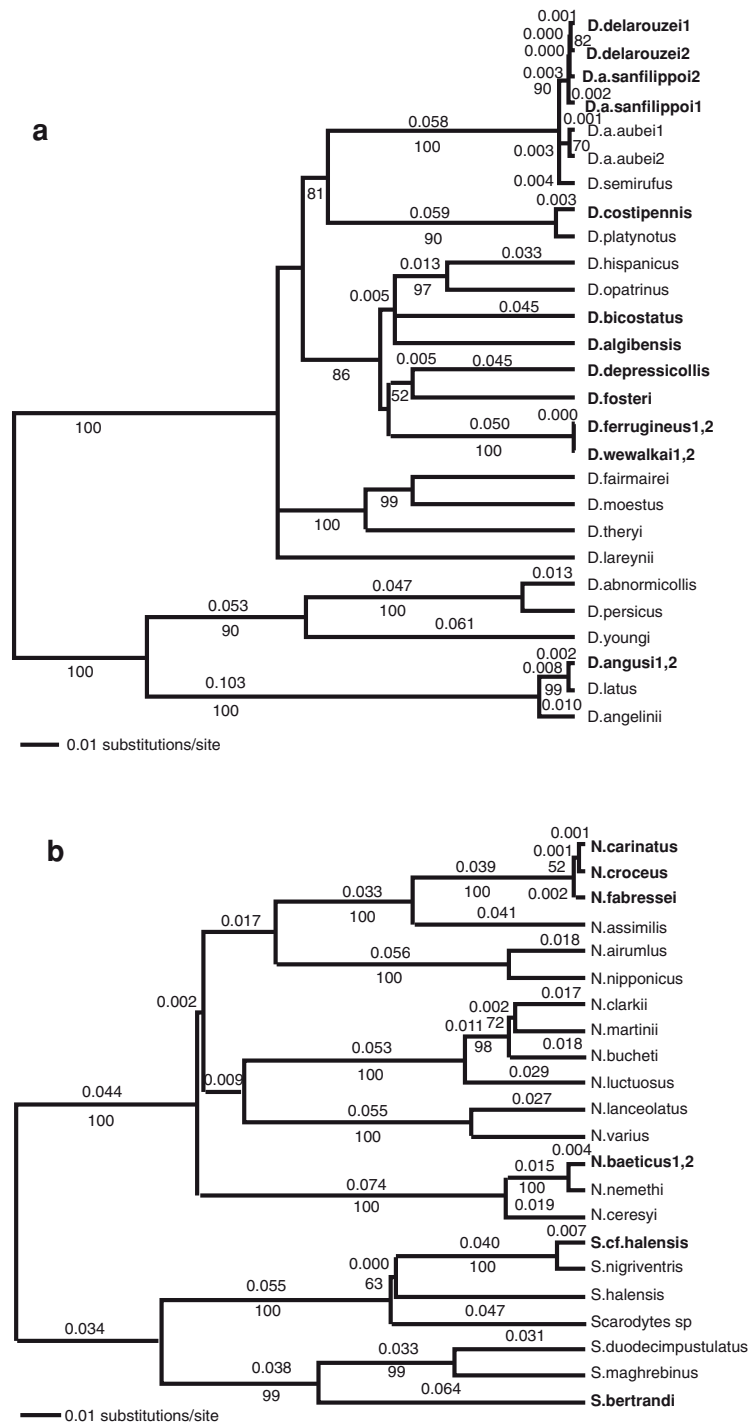


Fig. 4.— Ultrametric trees used in the estimation of divergence times. a, *Deronectes*; b, *Nebrioporus* gr. of genera. In bold, Iberian endemics. Numbers above branches are branch lengths; below branches, non-parametric bootstrap support values of the corresponding node (modified from Ribera & Vogler, in press).

Fig. 4.— Árboles ultramétricos utilizados en la estimación de las edades de divergencia. a, *Deronectes*; b, grupo de géneros afines a *Nebrioporus*. En negrita, endemismos ibéricos. Los números sobre las ramas son sus longitudes, bajo las ramas, valores de soporte bootstrap no paramétrico del nodo correspondiente (modificado de Ribera & Vogler, en prensa).

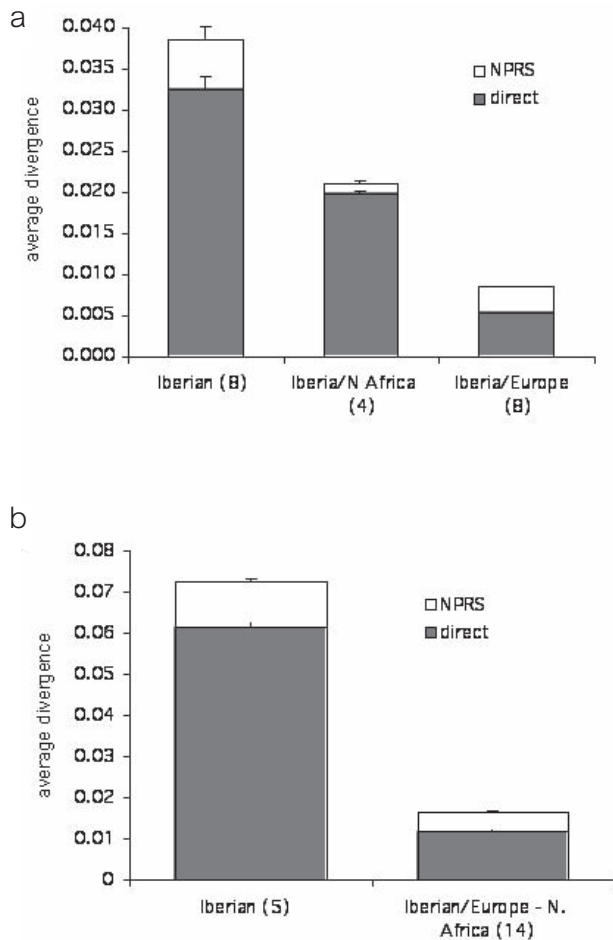


Fig. 5.— Average divergence of the Iberian endemic species of Dytiscidae according to their vicariant origin. a, all species, three vicariant types; b, species complexes pooled together (*D. ferrugineus*-*D. wewalkai*, *H. nevadensis*-*H. brancoi*, *N. carinatus* cplx), two vicariant types. Direct, estimates directly enforcing a molecular clock in PAUP; NPRS, estimates using the Non-Parametric Rate Smoothing of Sanderson (1997). Divergences in substitutions/site/ branch. In parentheses, number of species in each of the groups.

Fig. 5.— Divergencia media de los endemismos ibéricos de Dytiscidae de acuerdo al tipo de su origen vicariante. a, todas las especies, tres tipos de origen vicariante; b, complejos de especies combinados en un solo punto (*D. ferrugineus*-*D. wewalkai*, *H. nevadensis*-*H. brancoi*, *N. carinatus* cplx), dos tipos de origen vicariante. Direct, estimas forzando directamente un reloj molecular en PAUP; NPRS, estimas utilizando el método Non-Parametric Rate Smoothing de Sanderson (1997). Divergencias en sustituciones/posición/rama. En paréntesis, número de especies en cada uno de los grupos.

present in Iberia and south France, and *D. hispanicus* also in the extreme north of Morocco (Fery & Brancucci, 1997).

The species with an Iberian/European type of origin had the lowest average divergences, followed by the Iberian/North African type, with the within-Iberian splits having the largest differences (Fig. 5a). However, differences among the three groups were not significant, either as measured with ANOVA or with pairwise double-tailed t-tests (Sokal & Rohlf, 1995). When the endemic species that form complexes with very similar morphologies were pooled in single data-points (i.e. *D. ferrugineus*-*D. wewalkai*, *H. nevadensis*-*H. brancoi*, and the species of the *N. carinatus* cplx), differences between the within-Iberian and the Iberian/European or Iberian/North African vicariants became highly significant (ANOVA, $p < 0.001$, both using direct enforcement of a clock in PAUP or the NPRS) (Fig. 5b). Two of these complexes have an Iberian/European type of sister relationship: *H. nevadensis*-*H. brancoi* with *H. longulus*, and the *N. carinatus* complex with *N. assimilis* (Paykull, 1798), present only north of the Pyrenees (the other very close species complex, *D. ferrugineus*-*D. wewalkai*, is sister to an Iberian clade, Table 2).

Within the endemic species with an Iberian/European pattern, two subgroups could be made: those for which the Iberian species does not reach the Pyrenean region (as defined in Ribera, 2000, i.e., including the Ebro valley, Fig. 7a), and those for which the endemic species reaches the Pyrenees and, in some cases, southern France (Tables 1, 2). In only two cases the vicariant European species reaches the north side of the Pyrenees: *Hydroporus palustris* (Linnaeus, 1761) (present in Val d'Aran and in the French Cerdanya, Ribera *et al.*, 1995), and *H. longulus* (although the taxonomic identity of the Iberian populations is uncertain). There are no significant differences between the average divergence of these two groups.

Among the endemic species with a within-Iberian origin, most of the vicariant splits seem to have occurred within the Hercinian region, which is also the largest and geologically more heterogeneous (Tables 1, 2; Fig. 7a; see Ribera, 2000). Only four of the within-Iberian species do not occur in the Hercinian region: *D. fosteri* (Pyrenean), and *D. depressicollis*, *D. algibensis* and *I. cermenius* (South East) (Table 1).

The species with an Iberian/European origin are distributed in the three northern regions of the Iberian peninsula, in particular in the Cantabrian region, where all of them except *D. angusi* occur.

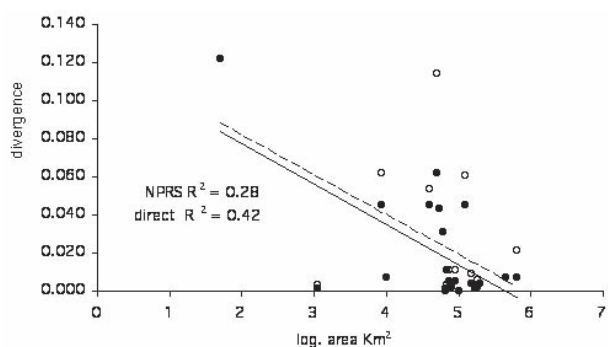


Fig. 6.— Regression between divergence (substitutions / site / branch) and the area of distribution of the Iberian endemics of Dytiscidae. The upper left point corresponds to *Iberoporus cermenius* (see text). Direct (solid circles, continuous line), estimates directly enforcing a molecular clock in PAUP; NPRS (empty circles, dashed line), estimates using Non-Parametric Rate Smoothing of Sanderson (1997).

Fig. 6.— Regresión entre divergencia (sustituciones/posición/rama) y el área de distribución de los endemismos ibéricos de Dytiscidae. El punto de la parte superior izquierda corresponde a *Iberoporus cermenius* (ver texto). Direct (círculos negros, línea continua), estimas forzando directamente un reloj molecular en PAUP; NPRS (círculos blancos, línea de puntos), estimas utilizando el método Non-Parametric Rate Smoothing de Sanderson (1997).

The only species of this group present in the south is the Iberian *Scarodytes* cf. *halensis*, which presumably has a widespread (but still unknown) distribution. Of the four species with a Iberian/North African vicariant origin, two are present in the south east (*N. baeticus* and *G. castilianus*, Millán *et al.*, 2002), but two of them are exclusive of the north: *I. detneri* and *R. bimaculatus* (Tables 1, 2).

RELATIONSHIPS BETWEEN AGE AND RANGE

The regression between divergence and logarithm of range size for all Iberian endemic Dytiscidae is negative and highly significant ($p < 0.001$, for both the direct enforcement and using NPRS; Fig. 6). However, a large part of this effect is due to the very small range of the stygobiont species *Iberoporus cermenius* (known only from one locality in Córdoba, Castro & Delgado, 2001), which is also the endemic taxa with the highest divergence with respect to its sister. When this species is removed, the relationship is still negative, although not significant ($p > 0.1$ for both the direct enforcement and using NPRS, and equally when the species complexes are treated as single points or not).

To reduce the effect of the uncertainty in the estimated range size of the species, a broad categorisation can be made according to their presence/absence in biogeographically distinct areas. In Ribera (2000) the Iberian peninsula was divided in five major biogeographical regions according to the distribution of the main tertiary basins (Fig. 7a), and it was shown that the fauna of water beetles, including that of the Dytiscidae, differ significantly among them. The range extension of a species can be quantified as the number of regions they occupy, as it is reasonable to assume that dispersion within a region is more frequent, and of more stochastic nature, than dispersion across regions. When thus considered, the regression between number of areas in which the species is present and divergence becomes partially significant ($p < 0.1$, both for the direct estimation and the NPRS; Fig. 7b). The species with a within-Iberian type of origin tend to occupy only one geographical area, while those with an Iberian/European or Iberian/North African origin tend to be more widespread within the Iberian peninsula, occupying two or more areas (Tables 1, 2; as measured with a 2x2 Contingency table with the G-test and the Williams correction, $P < 0.05$, Sokal & Rohlf, 1995).

TYPE OF HABITAT, DISTRIBUTION AND DIVERGENCE

The Iberian endemic species living in standing water or in both types of habitat (standing and running) had on average a significantly larger range, as measured with a one factor ANOVA ($n = 33$, $p < 0.05$, both using the untransformed surface or the logArea; average range of standing and both, 212,727 km²; average range of running, 79,186 km²; including species for which no phylogenies were available).

Endemic species of running water seem also to be more likely to have a within-Iberian type of origin, while species of standing water or both were more likely to have an Iberian/European or an Iberian/North African origin (Table 2; 2x2 contingency table, $p = 0.05$).

The average divergence of the endemic species of running water from their sisters was not significantly different from that of the species living in standing water or in both types of habitats ($p > 0.1$, both for the direct enforcement of a clock in PAUP and the estimation using NPRS). However, when the species complexes *D. ferrugineus-wewalkai*, *H. nevadensis-brancoi* and the *N. carinatus* complex are included in substitution of the individual species, these differences become highly significant ($p < 0.05$, both for the direct estimation and that obtai-

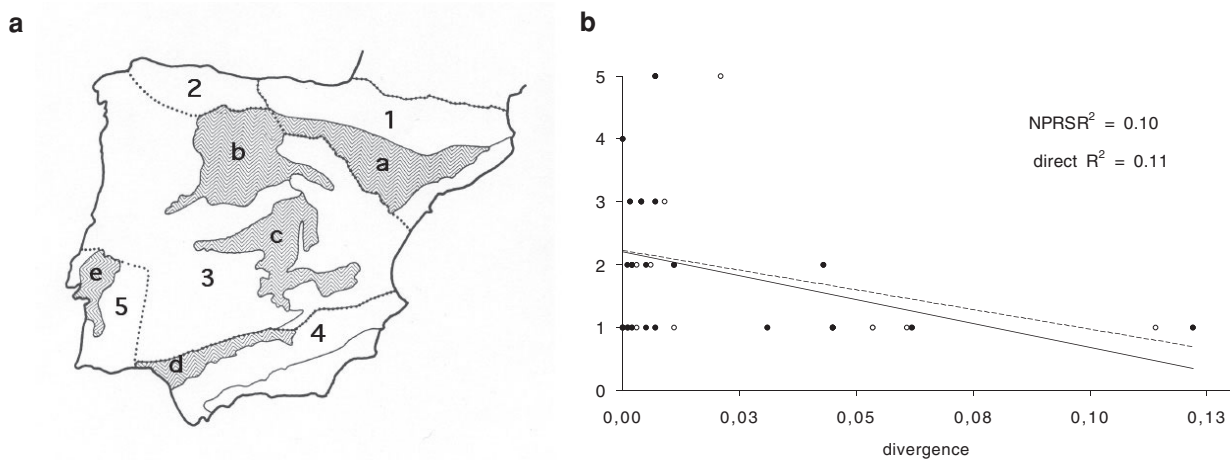


Fig. 7.— a, Biogeographical regions within the Iberian peninsula defined by the tertiary basins (modified from Ribera, 2000; tertiary basins follow Friend & Dabrio, 1996); b, regression between divergence (substitutions/site/branch) and the number of biogeographical regions in which the Iberian endemics of Dytiscidae occur. Biogeographical areas: 1, Pyrenean; 2, Cantabrian; 3, Hercinian; 4, South East (Baetic region); 5, South West. Tertiary basins: a, Ebro; b, Duero; c, upper Tajo and Guadiana; d, Guadalquivir; e, lower Tajo (see Ribera, 2000 for a justification and delimitation of the areas). Direct (solid circles, continuous line), estimates directly enforcing a molecular clock in PAUP; NPRS (empty circles, dashed line), estimates using Non-Parametric Rate Smoothing of Sanderson (1997).

Fig. 7.— a, Regiones biogeográficas de la península Ibérica, definidas de acuerdo a las cuencas terciarias (modificado de Ribera, 2000; cuencas terciarias según Friend & Dabrio, 1996); b, regresión entre divergencia (sustituciones/posición/rama) y el número de regiones biogeográficas en las que se encuentran los endemismos ibéricos de Dytiscidae. Regiones biogeográficas: 1, Pirenaica; 2, Cantábrica; 3, Herciniana; 4, Sureste (región Bética); 5, Suroeste. Cuencas terciarias: a, Ebro; b, Duero; c, alto Tajo y Guadiana; d, Guadalquivir; e, bajo Tajo (ver Ribera, 2000 para una justificación y delimitación de las regiones). Direct (círculos negros, línea continua), estimas forzando directamente un reloj molecular en PAUP; NPRS (círculos blancos, línea de puntos), estimas utilizando el método Non-Parametric Rate Smoothing de Sanderson (1997).

ned using NPRS, average divergence of the running water species 0.029-0.041 substitutions/site, average divergence of the standing water species 0.008-0.010 substitutions/site).

Discussion

TAXONOMIC ISSUES

The molecular data available is compatible with the monophyly of all the Iberian endemic species of Dytiscidae with the exception of the species in the *Deronectes aubei* group (for the complex *H. nevadensis-H. brancoi* the resolution of the sequenced fragment is not enough to appreciate differences). The lack of monophyly in the mitochondrial markers studied does not imply that the species in question are not distinct (as they seem to be morphologically well characterised, Fery & Brancucci, 1997), but, if distinct, they are of very recent origin (late Pleistocene) (Ribera & Vogler, in press). There

is also the possibility that the mitochondrial haplotype of some of the specimens studied is the result of genetic introgression (hybridisation).

From the taxonomic point of view, the most transcendental result of this study is the necessity to split the genus *Stictotarsus* in three lineages: *Stictotarsus* proper (the *S. duodecimpustulatus* gr. *sensu* Nilsson & Angus, 1992, including the Iberian *S. bertrandi*), the *S. griseostriatus* and *S. roffii* groups, and *Stictotarsus otini*. The later species is never sister to any other species of *Stictotarsus*, irrespective of the parameter combination or the set of taxa included in the analyses. Its morphological distinctiveness was already noted by Guignot (1959: 446), who attributed it a “position systématique encore un peu incertaine”. *Stictotarsus otini* was originally described as a *Potamonectes*, within its own subgenus, *Trichonectes* (Guignot, 1941). Subsequently *Trichonectes* was synonymised with *Stictotarsus*, as part of the species of “*Potamonectes*” were transferred to this genus

(Nilsson & Angus, 1992; Nilsson, 2001). According to the results of the phylogenetic analysis, I reinstate *Trichonectes* as a valid taxon, as a monotypic genus of Hydroporini for the single species *Trichonectes otini* (Guignot, 1941) (new combination).

Stictotarsus was first used for the species *S. duodecimpustulatus*, and in consequence the species in the *S. duodecimpustulatus* group (*sensu* Nilsson & Angus, 1992 and Nilsson, 2001) are the only ones to be included in a strict sense of the genus *Stictotarsus*. The species of the *S. griseostriatus* and *S. roffii* groups were variously described under the genera *Hydroporus*, *Deronectes*, *Dytiscus*, *Hyphydrus* and *Potamonectes*, the type species of all of which fall outside *Stictotarsus* (as defined in Nilsson & Angus, 1992) (Nilsson, 2001). A new name would thus be necessary for the genus including the species of the *S. griseostriatus* and *S. roffii* groups, although a complete morphological description is out of the scope of this work and needs a separate treatment.

Within the remaining genera of the *Deronectes* gr., *Nebrioporus* is sister to the clade formed by *Scarodytes* and the species of the *Stictotarsus duodecimpustulatus* group, a relationship already suggested on the basis of the study of the female reproductive system by Burmeister (1976) and, partly, Nilsson & Angus (1992) (these authors considered *Stictotarsus* monophyletic and sister to *Nebrioporus* plus *Scarodytes*). Within the genus *Nebrioporus*, the species of the *N. ceresyi* gr. (*N. ceresyi*, *N. baeticus* and *N. nemethi*) are sister to the rest in the tree without outgroups. This clade (together with *N. martinii* among the sampled species) is currently included in the subgenus *Zimmermannius*, characterised by the absence of spines in the elytra (Nilsson & Angus, 1992). However, *Nebrioporus martinii* is found to be sister to *N. clarkii*, in agreement with recent morphological work demonstrating its close proximity (Pederzani & Schizzerotto, 2000). The same authors show that the character of the elytral spine is highly labile, with frequent intra-populational polymorphism. *Nebrioporus martinii* is thus here removed from the subgenus *Zimmermannius* and placed in *Nebrioporus* s.str., as *Nebrioporus (Nebrioporus) martinii* (Fairmaire, 1858) (new combination). The type species of *Zimmermannius* is *N. canariensis* (Bedel, 1881). At present there is no available information on the phylogenetic position of this species (as well as other species in the subgenus, see Nilsson & Angus, 1992), and thus the exact composition of the *N. ceresyi* clade is unknown —as

well as the availability of the name *Zimmermannius* for it, in case it is confirmed as sister to *Nebrioporus* s.str.

The paraphyly of *Oreodytes* is not well supported, and changes in nomenclature are not advisable until more comprehensive analyses are available. It is however interesting to note that the distinction among the two lineages correspond to the distinction of main size and shape within the genus (i.e. species larger and more elongated, such as e.g. *O. davisii*, and species smaller and more round, such as e.g. *O. sanmarkii*). In Larson *et al.* (2000) this distinction in two lineages is noted, although because of the existence of intermediate forms it is not given any taxonomic relevance. The somewhat deviating *O. picturatus* (Horn, 1883), with a peculiar morphology (Larson *et al.*, 2000), and *O. natrix* (Sharp, 1884), formerly included in the genus *Neonectes* (now considered a synonymy of *Oreodytes*, Nilsson, 2001), are basal to the “small and round” clade, in agreement to their general morphology, although with low bootstrap support.

Within the *Graptodytes* group of genera (*sensu* Ribera *et al.*, 2002), *Iberoporus cermenius* seems to have an isolated position, although still uncertain. Results presented here place it sister to *Graptodytes* plus *Metaporus*, but under some other combinations of taxa and outgroups it seems to be sister to *Rhithrodytes* (unpublished results). The eventual inclusion of more species of the genus (in particular the Iberian *R. agnus*), and of the French *Siettitia* (also stygobiontic, Guignot, 1933), would contribute to resolve the relationships of the only endemic genus of Iberian Dytiscidae.

The lack of Iberian specimens of *Stictoneectes optatus* in the analyses does not allow to draw any conclusion about their taxonomic status, but results suggest that what is currently recognised as “*S. optatus*” could be a complex of cryptic, very similar species.

EFFECT OF MISSING SPECIES AND PHYLOGENETIC UNCERTAINTY

The inclusion of the missing endemic species of Dytiscidae in the analyses is not likely to modify substantially the main conclusions of this work. Some of the missing species are morphologically distinct and likely to be of old origin (*Hydroporus constantini*, *Rhithrodytes agnus*), but others are complexes of species morphologically very similar, which are very likely to be of recent origin (*Agabus picotae-A. heydeni* Wehncke, 1872; *Hydroporus decipiens-H. analis* Aubé, 1838, or the complex of species around *H. cantabricus-necopinatus-bran-*

coi; Foster & Bilton, 1997; Fery, 1999). The water beetles of the Iberian peninsula, and in particular the Dytiscidae, are relatively well known (Ribera, 2000). Although it is always possible that new species have remained undetected, their number is likely to be low, with the possible exception of underground water species, of which new taxa within the *Iberoporus-Rhithrodytes* (and perhaps *Siئتitia!*) lineages are expected to occur.

Poor taxonomy is also not likely to be a strong confounding factor. All endemic species have been recognised based on morphological differences, with the exception of *N. croceus*, described on the base of its deviating karyotype (Angus *et al.*, 1992). To have an at least preliminary test for the monophyly of the endemic species and subspecies, more than one specimen from different localities were sampled whenever possible. As discussed above, at least for the species our results are compatible with their monophyly, with the only exception of the *D. aubei* complex, where *D. aubei sanfilippo* and *D. delarouzei* were treated as a single, endemic species. Most of the conclusions are robust to the consideration of three of the closes species complexes as single data points (*D. ferrugineus-wewalkai*, *H. nevadensis-brancoi*, and the *N. carinatus* cplx). The delimitation of species depends ultimately on the species concept applied to the data available (see e.g. Sites & Marshall, 2003 for a recent review), but if a monophyletic distinct lineage (as defined with molecular data) has also some morphological diagnostic characters, there is no reason for not consider it a valid species.

The phylogenetic position of the Iberian endemics is very well supported, with most of the nodes having bootstrap support values above 85%. Most of the nodes with lower support affect the position of the species with larger divergences, such as *Iberoporus cermenius*, or some of the species of the Iberian lineage of *Deronectes*, but alternative placements do not change substantially the estimation of the divergences, or the type of origin (see below). Another indication of the robustness of the phylogenies presented here is the general agreement with the species proposed as sisters according to morphology (when the proposed sister was included) (see Fery & Hendrich, 1988; Fresneda & Fery, 1990; Angus *et al.*, 1992; Nilsson & Angus, 1992; Fery & Nilsson, 1993; Fery *et al.*, 1996; Fery & Brancucci, 1997; Fery, 1999; Castro & Delgado, 2001 for morphological discussion of the affinities of the Iberian endemic Dytiscidae).

GEOGRAPHICAL ORIGIN OF THE IBERIAN ENDEMICS

In Ribera & Vogler (in press) it was shown that the most frequent mode of speciation of the Iberian endemic Dytiscidae is likely to be allopatry, as there is a strong tendency for the recent sister species to have perfect allopatric distributions, while older species, or species at a lower level in the phylogeny (sister to more than one species) tend to largely overlap with their sisters. The three types of geographical distribution of the Iberian endemics and their sisters can thus be assumed to be the result of three different types of vicariant events: within Iberia, between Iberia and Europe north of the Pyrenean region, and between Iberia and North Africa. The most frequent type of vicariant origin of the Iberian endemics are either within Iberia or Iberian/European, with only four cases of Iberian/North African vicariance (one of them uncertain, as the sister —*I. hozgargantae*— is also present in South Spain). Of the three endemic species with unknown origin, *S. occidentalis* and *S. epiplericus* are very recent (Middle Pleistocene, Ribera & Vogler, in press) and not likely to have suffered major shifts in their distributional ranges. This would suggest that they are of within Iberia (*S. occidentalis*) and Iberian/European origin (*S. epiplericus*), although at present other possibilities cannot be excluded. *Stictotarsus bertrandi* is an isolated species of older origin (late Miocene, Ribera & Vogler, in press), and, although at present restricted to NW of Spain, its geographical origin is at present impossible to estimate.

Most of the endemic species have allopatric, but contiguous, or almost contiguous, distributions with their respective sisters. Among the Iberian/European vicariants, only in the species pair *I. albarracinensis*-*I. satunini* there is a wide geographical gap in between their respective distributions (Greece, Turkey and SW Russia for *I. satunini*, north Iberia for *I. albarracinensis*). The distribution limits of most species pairs agree with major geographical barriers, either the Ebro valley (known to have retained a semi-arid steppe-like environment for most of the Pleistocene, Ribera & Blasco-Zumeta, 1998) or the Pyrenees. However, it is uncertain whether the vicariance Iberia/Europe is defined by the Pyrenees or the Ebro Valley, as in most cases neither the Iberian endemic or its sister occur in the whole Pyrenean region (Tables 1, 2). When they do, as in e.g. the species pairs *H. vagepictus*-*H. palustris*, the Iberian endemic also occurs elsewhere other than the Pyrenean region, and thus it is not possible to reject an origin outside this area with a subsequent range expansion. Based on the

general affinities between the fauna of the Pyrenees and that of southern France, and on the absence of most Iberian endemics from the southern Pyrenean mountain ranges and the rest of the Pyrenean region (Ribera, 2000), the Ebro valley could be speculated to have been the geographical barrier promoting the isolation and speciation by vicariance of part of the Iberian endemic Dytiscidae fauna.

Species with an origin in vicariant events within the Hercinian region are generally restricted to one or more mountain massifs, delimited by some of the Tertiary basins (Friend & Dabrio, 1996; Ribera, 2000). A special case are the species of the "Iberian" lineage of *Deronectes*. The low support of the basal relationship within the clade, with very short branches in comparison to those leading to the species, suggests a rapid diversification. With the exception of the very recent species pair *D. ferrugineus*-*D. wewalkai*, all the species of this lineage are of relative old origin (late Miocene or Early Pliocene, Ribera & Vogler, in press), and distributed in mountain massifs: *D. fosteri* in the Pyrenees, *D. depressicollis* in the eastern Baetic ranges, *D. algibensis* in the western Baetic ranges, and *D. bicostatus* and the complex *D. ferrugineus*-*D. wewalkai* overlapping almost perfectly in the Sistema Central and the north-western mountain systems in the Hercinian region. Within the clade there are two more widespread species, *D. opatrinus* and *D. hispanicus*, assumed to be of Iberian origin, although their ancestral distribution is difficult to estimate. This phylogenetic and geographic pattern is compatible with a scenario in which two ancestral sister lineages of Iberian *Deronectes* with a widespread distribution experienced simultaneous and congruent vicariance events among the tree main mountain areas in the Iberian peninsula. Thus, each of the lineages (see Figs 3, 4a) has a vicariant in the south East (*D. algibensis* and *D. depressicollis* respectively), the Hercinian region (*D. bicostatus* and *D. ferrugineus*-*D. wewalkai*), and the Pyrenean (*D. hispanicus*-*D. opatrinus* and *D. fosteri*) (Fig. 8). This would require a Pyrenean origin for the ancestor of *D. hispanicus*-*D. opatrinus*, which would subsequently speciate and expand their range to most of the Iberian peninsula, south France and (for *D. hispanicus*) the extreme north of Morocco (Fery & Brancucci, 1997). The estimated divergence time for the basal two *Deronectes* lineages is between 5 and 6.5 Myr (Ribera & Vogler, in press), i.e. at the Late Miocene-Early Pliocene boundary. This was a time of global ecological and climatic change (e.g. Cerling *et al.*, 1997; Krijgsman *et al.*, 1999). However, very few ende-

mic Iberian species seem to have originated during the period in which the Mediterranean climate time was established, or at the start of the glaciations (3.2-2.3 Myr ago, Suc, 1984) (Table 2; Ribera & Vogler, in press).

The above discussion is based on the assumption that, under some circumstances, the present range of a species retains some information on its geographical origin and, indirectly, its mode of speciation (Lynch, 1989; Berlocher, 1998; Barraclough & Vogler, 2000; Barraclough & Nee, 2001). It is known that species of beetles in central and northern Europe experienced dramatic distributional shifts during the Pleistocene (see Elias, 1994 for a review), and that in many cases the present range of the species is only a random or quasi-random subset of what could have been its historical range, thus erasing any possibility of inferring its geographical origin or mode of speciation (Roy *et al.* 1996; Losos & Glor 2003). However, under this scenario the expected distribution of sister species would also be random, i.e. with similar degrees of overlap than with other, not directly related species. As shown in Ribera & Vogler (in press), the range of the Iberian endemic species in the tip of the phylogenies (i.e. sister to another species) do overlap with their sisters significantly less than species which are sister to a clade (i.e. sister to more than one species). In addition to that, sister species have often non-overlapping but contiguous ranges, separated only by geographical barriers (e.g. the Ebro Valley or the Pyrenees in the case of the species pairs with an Iberian/European distributional pattern, see above), strongly suggesting that their ranges have remained more or less stable since their vicariant speciation. In some cases sister species have widely disjoint distributions (e.g. *Ilybius albaracinensis*-*I. satunini*, *Rhithrodytes bimaculatus*-*R. dorsoplagiatus*, or *Graptodytes castilianus*-*G. pietrii*, although in the later two cases the sister relationship could not be established), and inferences about the ancestral distribution are much less well founded. A similar case are species which sister is either a clade or a species with a widespread distribution. In these cases, any attempt to reconstruct the ancestral area remains highly speculative.

DIVERGENCE, AREA RANGE AND HABITAT

One of the most interesting results of the analysis of the Iberian endemics of Dytiscidae is their apparent very recent origin (Ribera & Vogler, in press). Most of the species seem to differ less than 2% from their sisters, which at the standard rates for insect mitochondrial DNA means they are less

than 1 Myr old, i.e. from Middle to Late Pleistocene. The Pleistocene origin of the majority of the species is very robust with respect to uncertainties in the rate of the molecular clock, or with respect to the method used for estimating the ultrametric tree (direct enforcement in PAUP or the NPRS method of Sanderson, 1997). These results are against the view of the Pleistocene as a time of little evolutionary change at the species level (Coope, 1979; 1994; Elias, 1994; Bennet, 1997; Klicka & Zink, 1997, 1999; Janson & Dynesius, 2002). On the contrary, at least for diving beetles the Pyrenees and the Ebro valley seem to have been almost insurmountable geographical barriers during most of the Pleistocene, isolating Iberian populations from those in the rest of Europe long enough for them to speciate in allopatry. This will be in agreement with recent work on other taxa, for which during the Pleistocene glaciations Iberia (and other peninsulas in southern Europe) could have remained isolated rather than being a refuge from where to re-colonise northern areas during the interglacials (Bilton *et al.*, 1998; Kropf *et al.*, 2002; Michaux *et al.*, 2003). For these taxa, northern refuges could be the source of most present-day central and north European populations (e.g. Stewart & Lister, 2003).

The negative relationship between divergence and area of the distributional range suggests that, for the Iberian species of Dytiscidae, the range of the species tends to decrease with time, although the significance of this relationship depends completely on the inclusion of *Iberoporus cermenius*, the older of the Iberian endemics, and known only from a single well (Castro & Delgado, 2001). When the distributions were categorised in a semi-quantitative scale (number of biogeographical regions in which they are present), the relationships became marginally significant. This effect is mostly due to the species with restricted Iberian distributions (and with a within-Iberian vicariant origin), which are on average older than the more widespread species with Iberian/North African or Iberian/European vicariant origin (although differences are not fully significant unless the species complexes are treated as single data-points, see above). Thus, there seem to be two heterogeneous groups of Iberian endemics: one group of very recent species, product of the fragmentation of a species widespread in Iberia and Europe or North Africa during the Pleistocene, and another of species with more restricted distributions, product of within-Iberian older vicariant events as well as some more recent splits. Species of the first group may be experiencing a range con-

traction, although data presented here is not enough to detect significant trends. The negative relationship of Figs 6 and 7a can be explained by the juxtaposition of these two heterogeneous groups. The fact that older species have more restricted ranges has to be interpreted with caution, as it may not necessarily imply that the range of the more recent species tends to decrease with time. These two groups have different origins and are likely to have different historical trajectories.

The heterogeneity of these two groups of endemics (within-Iberian and Iberian/European or North African vicariant species) is further manifest when the relationships with habitat are explored. Within-Iberian species tend to occupy running habitats, and Iberian/European or North African vicariant species tend to be in both running or standing, or in standing waters. The smaller range of the species in running water is in agreement with the results of Ribera & Vogler (2000) and Ribera *et al.* (2003b), who found the same pattern to be general among the western European water beetles. In these works, an explanation based on the different constraints that the two habitats impose is proposed. Due to differences in long-term stability of these two types of habitat (small standing water bodies are generally of much shorter geological duration than streams or small rivers), species living in them would develop different dispersal strategies. Thus, diving beetles living in the more stable running water habitat would be expected to lose dispersal ability, and therefore have more differentiated geographical genetic structure, reduced range sizes, a higher geographical species turnover, and higher rates of speciation and extinction. In contrast, beetles living in standing water, with the short geological life-span of such habitats, would be forced to migrate more frequently in search of suitable places to live, with a consequent increase in dispersal ability, and the opposite macroecological and macroevolutionary strategies (Ribera & Vogler, 2000; Ribera *et al.*, 2003b; Vogler & Ribera, 2003; see also Hubbell, 2001 for the wider ecological and evolutionary consequences of different dispersal capabilities).

The lack of significant differences in the average divergence between the species in running waters (which tend to have smaller ranges and a within-Iberian vicariant origin) and those in standing water or in both habitats (with larger ranges and an Iberian/European or North African origin) seems contrary to the predictions of Ribera & Vogler (2000), who hypothesised that running water species, due to the higher evolutionary turno-

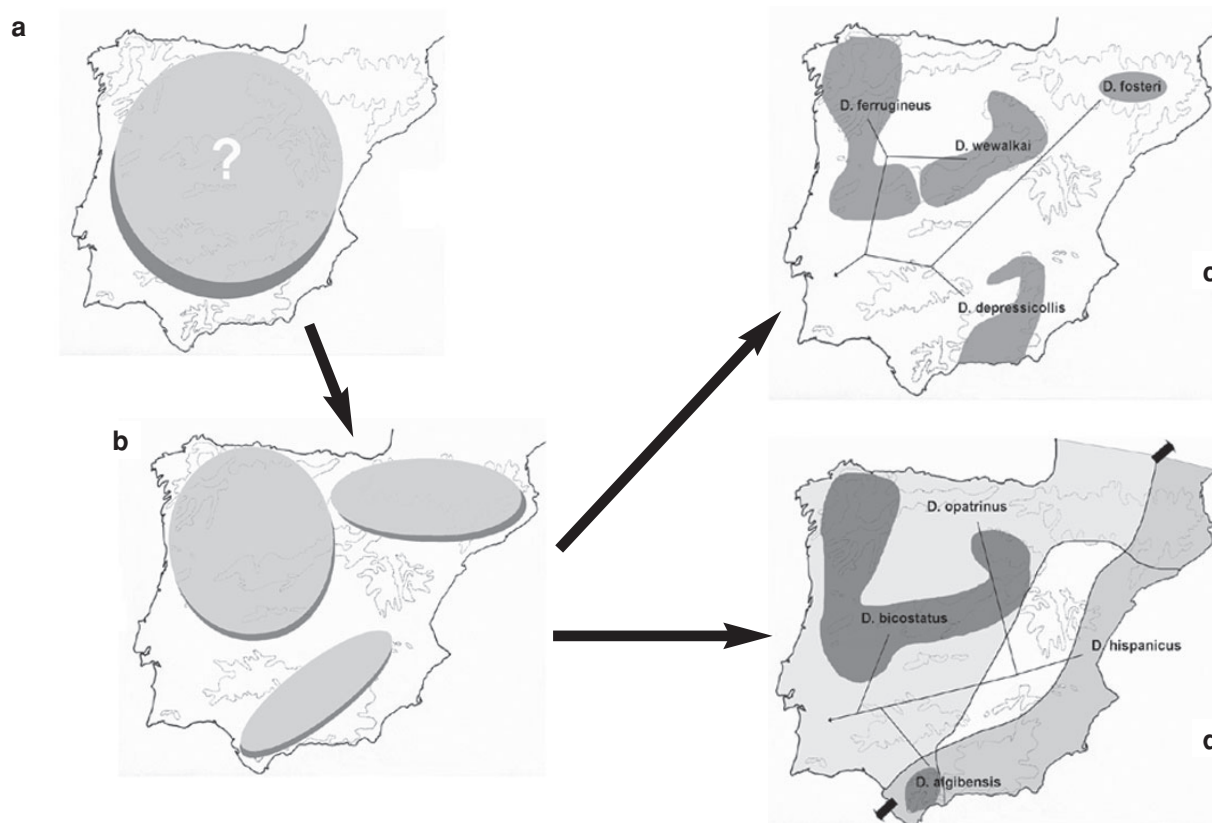


Fig. 8.— Hypothetical reconstruction of the vicariant speciation events in the evolution of the Iberian clade of *Deronectes* (see text for details, and Fig. 4a for the phylogeny of the species and the estimated divergences). a, the two ancestor (sister) species have a hypothetical widespread Iberian distribution during Late Miocene; b, in rapid succession (less than ca. 1 Myr) two vicariant events result in separated lineages in the Baetic ranges, the Pyrenees, and central and NW Iberia, leading one of the lineages to c; *D. depressicollis* (Baetic ranges), *D. fosteri* (Pyrenees) and *D. ferrugineus* and *D. wewalkai* (C & NW Iberia) (the last two of Late Pliocene origin); and the second to d, *D. algibensis* (Baetic ranges), *D. hispanicus* and *D. opatrinus* (with present distributions reaching S France and N Morocco (*D. hispanicus*), but assumed to be Pyrenean in origin, dating ca. Late Pliocene), and *D. bicostatus* (C & NW Iberia).

Fig. 8.— Reconstrucción hipotética de la especiación vicariante en la evolución del clado ibérico de *Deronectes* (ver texto para detalles, y Fig. 4a para la filogenia de las especies y la estima de las divergencias). a, las dos especies (hermanas) ancestrales tienen una hipotética distribución extendida en la península durante el Mioceno tardío; b, en rápida sucesión (menos de ca. 1 Myr) dos sucesos de vicarianza resultan en líneas aisladas en las cordilleras Béticas, los Pirineos e Iberia central y del noroeste, resultando uno de los linajes en c; *D. depressicollis* (cordilleras Béticas), *D. fosteri* (Pirineos) y *D. ferrugineus* y *D. wewalkai* (Iberia central y del noroeste) (las dos últimas especies originadas en el Pleistoceno tardío); y el segundo a d, *D. algibensis* (cordilleras Béticas), *D. hispanicus* y *D. opatrinus* (con distribuciones actuales que alcanzan el sur de Francia y el norte de Marruecos —*D. hispanicus*—, pero que se asume son originalmente Pirenaicas, de aproximadamente el Plioceno tardío), y *D. bicostatus* (Iberia central y del noroeste).

ver, would be on average younger than standing water species. Several factors have to be considered though. The species studied are not representative, as a subset of species with reduced range (Iberian endemics) was selected a priori (see above), among which only two were exclusive of standing water (*I. dettneri* and *H. vespertinus*, Ribera *et al.*, 2003b). Species found in both types of habitats (standing

and running) rather than having mixed characteristics seem to be more similar to one of the two main types, probably depending on their phylogenetic origin (Ribera *et al.*, 2003b and unpublished results). It is also possible that the high extinction rate that the running water lineages are supposed to suffer (Ribera & Vogler, 2000) results in a very heterogeneous tree topology, with some isolated species with

long branches (i.e. those for which all closest relatives became extinct) and others with very shallow divergences (as, for example, the *Nebrioporus carinatus* complex, or the species pair *D. ferrugineus*-*D. wewalkai*). A final option is that lineages of the two types of species (in standing and in running waters) may have very similar macroevolutionary patterns, but resulting from processes operating at different spatial scales (Ribera *et al.*, 2001).

ACKNOWLEDGEMENTS

This work has benefited from financial help from the EU (Marie Curie Fellowships), the Leverhulme Trust and NERC (grants to Alfred P. Vogler). I specially thank all the people who contributed with specimens for molecular studies (listed in the Appendix), in particular P. Aguilera, D. T. Bilton, A. Castro, H. Fery, G. N. Foster, C. Hernando and A. Millán, who by doing that made this work possible, and to Elisa Ribera, Marta Albarrán and James Hogan for help with some of the sequencing. The expertise of Ali Cieslak was of invaluable help in our collecting trips. Robert Angus, David Bilton, Garth Foster and Mario Toledo are thanked for their comments and discussion.

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Appendix 1.— List of the specimens of Iberian endemic species used in the study, with locality of origin (F, France, P, Portugal, S, Spain), collector and GenBank accession numbers (when taken from previous works). Species names follow Nilsson (2001). 1 to 34, Iberian endemic species; A to F, Iberian endemic subspecies, or Iberian populations of widespread species (see Fig. 1 for the geographical origin of the specimens).

Apéndice 1.— Lista de los ejemplares de endemismos ibéricos de Dytiscidae utilizados en el estudio, con localidad de origen (F, Francia, P, Portugal, S, España), recolector y número de referencia de GenBank (cuando la secuencia se ha tomado de trabajos anteriores). Los nombres de las especies siguen a Nilsson (2001). 1 a 34, especies endémicas de la península Ibérica; A a F, subspecies endémicas, o poblaciones ibéricas de especies con distribución más amplia (ver la Fig. 1 para el origen geográfico de los ejemplares).

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 GR					
3	<i>Graptodytes castilianus</i>	S, Albacete, Fte. La Higuera	A.Millan	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
A	<i>Hydroporus n. normandi</i>	S, Zaragoza, Santed	P.Aguilera		
B	<i>Hydroporus n. normandi</i> 2	S, Girona, Sant Pere Pescador	M.Martinoy		
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 GR					
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, São 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 crocerus</i>	S, Soria, Muriel de la Fuente	H.Fery	AY250925	AY250965
32	<i>Nebrioporus fabressei</i>	S, Navarra, Barindano	I.Ribera	AY250926	AY250966
C	<i>Oreodytes davisii rhianae</i>	S, Ávila, Sa. De Gredos	H.Fery	AF309244	AF309301
D	<i>Oreodytes davisii rhianae</i> 2	S, León, r. Yuso	R.Carr		-
E	<i>Oreodytes sanmarkii alienus</i>	S, Oviedo, S Llanes	H.Fery		-
33	<i>Scarodytes cf. halensis</i>	S, Barcelona, Saldes	H.Fery	AF309248	AF309305
34	<i>Stictotarsus bertrandi</i>	S, León, Puente del Rey	I.Ribera	AY250946	AY250984
F	<i>Stictotarsus griseostriatus</i>	P, Guarda, Sa. Estrela	I.Ribera		

Appendix 2.— List of the specimens used in the phylogenies to establish the relationships of the Iberian endemic species of Dytiscidae, with area of origin, collector and GenBank accession numbers (when taken from previous works). Species names follow Nilsson (2001).

Appendix 2.— Lista de los ejemplares utilizados en la construcción de filogenias para establecer las relaciones de los endemismos ibéricos, con su origen geográfico, recolector y número de referencia de GenBank (cuando la secuencia se ha tomado de trabajos anteriores). Los nombres de las especies siguen a Nilsson (2001).

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.Millán	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>Stictonectes azruensis</i>	Morocco	Pellecchia & Pizzetti	AY250940	AY250979
<i>Stictonectes lepidus</i>	Spain (Ávila)	H.Fery	AY250941	-
<i>Stictonectes optatus</i>	Corsica	I.Ribera & A.Cieslak	AY250943	AY250981
<i>Stictonectes optatus 2</i>	Morocco	I.Ribera	AY250944	AY250982
<i>Stictonectes optatus 3</i>	Mallorca	I.Ribera & A.Cieslak	AY250944	AY250983
<i>Stictonectes 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 axillaris</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

Species	Region	Collector	16S rRNA	COI
<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 longulus 2</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
<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 n. 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 ifranensis</i>	Morocco	R.Gerecke	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 aubei 2</i>	Switzerland	I.Ribera & A.Cieslak	AY250906	AY250948
<i>Deronectes fairmairei</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

Species	Region	Collector	16S rRNA	COI
<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>Oreodytes alpinus</i>	UK	I.Ribera		
<i>Oreodytes congruus</i>	Canada	I.Ribera & A.Cieslak		
<i>Oreodytes crassulus</i>	Canada	I.Ribera & A.Cieslak		
<i>Oreodytes davisii davisii</i> 3	UK	R.Carr		
<i>Oreodytes davisii davisii</i> 4	UK	G.N.Foster		
<i>Oreodytes natrix</i>	Japan	J.Bergsten		
<i>Oreodytes</i> cf. <i>obesus</i>	US	I.Ribera & A.Cieslak		
<i>Oreodytes picturatus</i>	US	I.Ribera & A.Cieslak		
<i>Oreodytes quadrimaculatus</i>	US	I.Ribera & A.Cieslak		
<i>Oreodytes rhyacophilus</i>	US	I.Ribera & A.Cieslak		
<i>Oreodytes rivalis</i>	US	I.Ribera & A.Cieslak		
<i>Oreodytes sanmarkii</i>	Spain (Burgos)	I.Ribera		-
<i>Oreodytes sanmarkii</i> 2	France (Alps)	I.Ribera & A.Cieslak		
<i>Oreodytes sanmarkii</i> 3	UK	I.Ribera		
<i>Oreodytes sanmarkii</i> 4	France (Pyr.Or.)	H.Fery		-
<i>Oreodytes</i> cf. <i>scitulus</i>	Canada	I.Ribera & A.Cieslak		
<i>Oreodytes septentrionalis</i>	UK	G.N.Foster		
<i>Oreodytes snoqualmie</i>	Canada	I.Ribera & A.Cieslak		
<i>Scarodytes halensis</i> 3	Finland	T.Berendok	AY250937	AY250976
<i>Scarodytes nigriventris</i>	Corsica	I.Ribera & A.Cieslak	AY250938	AY250977
<i>Scarodytes</i> sp	Iran	K.Elmi & H.Fery	AY250939	AY250978
<i>Stictotarsus falli</i>	US	Y.Alarie		
<i>Stictotarsus duodecimpustulatus</i>	Spain (Cádiz)	I.Ribera	AF309247	AF309304
<i>Stictotarsus funereus</i>	US	Y.Alarie		
<i>Stictotarsus griseostriatus</i> 2	Switzerland	I.Ribera & A.Cieslak		
<i>Stictotarsus griseostriatus</i> 3	Morocco	I.Ribera		
<i>Stictotarsus griseostriatus</i> 4	Canada	I.Ribera & A.Cieslak		
<i>Stictotarsus griseostriatus</i> 5	Canada	I.Ribera & A.Cieslak		
<i>Stictotarsus griseostriatus</i> 6	US	I.Ribera & A.Cieslak		
<i>Stictotarsus griseostriatus</i> 7	US	I.Ribera & A.Cieslak		
<i>Stictotarsus maghrebinus</i>	Morocco	I.Ribera	AY250947	AY250985
<i>Stictotarsus roffii</i>	US	Y.Alarie		
<i>Stictotarsus striatellus</i>	US	I.Ribera & A.Cieslak		
<i>Stictotarsus striatellus</i> 3	US	I.Ribera & A.Cieslak		
<i>Trichonectes otini</i>	Spain (Jaén)	I.Ribera		
OUTGROUPS				
<i>Laccornellus copelatooides</i>	Chile	I.Ribera		
<i>Laccornellus lugubris</i>	Argentina	L.Fernandez		
<i>Laccornis kocae</i>	Russia	A.N. Nilsson		
<i>Laccornis oblongus</i>	UK	D.T.Bilton	AF309241	AF309298