Integrative taxonomy of Iberian *Merodon* species (Diptera, Syrphidae)

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Abstract

The genus *Merodon* Meigen, 1803 (Syrphidae, Diptera), with more than 50 European species, is primarily distributed in the Mediterranean region, there being 34 species that occur in the Iberian Peninsula. The morphological variation found within some species from the Iberian Peninsula prompted us to test their taxonomic status by integrating morphological and molecular data. We generated partial sequences of the mitochondrial, protein-coding gene cytochrome *c* oxidase subunit I (COI), the nuclear, internal transcribed spacer (ITS2) region, and the D2 region of the nuclear 28S rRNA gene. COI and ITS2 sequences were obtained for most included taxa.

The variability of the COI sequences showed great differences between the studied species groups, exhibiting an interspecific range from 0.29% to 12.5% between ingroup taxa. Closely related taxa of the *aureus* complex (e.g. *M. quercetorum* and *M. legionensis*) presented identical COI sequences. The obtained ITS2 sequences showed low intraspecific variability, and only a few taxa presented more than one genotype. Species status and delimitation were discussed for all taxa in the light of available morphological and molecular character information. Using the obtained sequence data for COI and 28S we inferred the phylogenetic relationships of the included taxa, using parsimony analysis. Separate analysis of the COI sequences identified four, quite well-supported clades within *Merodon*, the *desuturinus, albifrons, nigritarsis* and *aureus* groups. Combined analysis of the COI and 28S genes produced a topology similar to the COI topology.

Key words: *Merodon*, Syrphidae, integrative taxonomy, molecular data, phylogenetic relationships, intraspecific variation.

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Introduction

The genus *Merodon* Meigen, 1803 (Syrphidae, Diptera), represented in Europe by more than 50 species, is a diverse genus of hoverflies, with most species occurring in the Mediterranean region. From the Iberian peninsula 34 species of *Merodon* are now recorded (Marcos-García *et al.* 2002; Marcos-García *et al.* in press). Of these ca 50% are identified as taxa endemic to the Iberian Peninsula (Marcos-García *et al.* in press). Data about adult habitats, flowers visited, flight periods and Spanish distribution are provided for particular species by Marcos-García (1985a; 1985b; 1989; 1990a; 1990b). The revision by Marcos-García *et al.* (in press) covers all taxa occurring on the Iberian Peninsula (including type studies), and provides a key for species identification and a zoogeographical discussion. Vujic *et al.* (in prep.) have studied the subgeneric relationships of *Merodon*, and provide additional data on morphological variability of taxa, based on analysis of species and specimens from a broad geographical area. These comprehensive studies are the platform for the present text.

Intraspecific morphological variability is well known in some *Merodon* species, e.g. *Merodon equestris* (Fabricius, 1794) that exhibits distinctive colour morphs, and *M. aeneus* Megerle *in* Meigen, 1822 that exhibits a wide range of colour varieties (Sack 1932). *M. tricinctus* Sack, 1913 presents a high intraspecific variability in the shape of the anterior lobe of the surstyli of the male genitalia (Popov, 2000). Marcos-García *et al.* (in press) describe intraspecific morphological variability for nine species from the Iberian Peninsula.

Analysis of mtDNA sequence data has been used extensively to study the evolutionary relationships both within and among species. Since mitochondrial DNA sequences frequently evolve faster than do nuclear sequences (e.g. Simon *et al.* 1994), the number of variable and informative sites is often greater for mtDNA than for nuclear loci. MtDNA is particularly useful for species-level and genus-level analyses, as demonstrated in a large number of studies of animal evolutionary relationships (e.g. Caterino & Sperling 1999; Scheffer & Wiegmann 2000; Caterino *et al.* 2001; Ståhls *et al.* 2003, 2004; Arevalo *et al.* 2004; Ståhls 2006).

DNA sequence information, from mitochondrial or nuclear genes, might not always correspond with species recognised through application of traditional morphological and ecological criteria. The sole use of the barcoding-sequence, a 650-bp fragment of the 5´ end of the mitochondrial cytochrome *c* oxidase subunit I gene (COI), has been suggested as a species identification system for most of life (Hebert *et al.* 2003, 2004a,b, Savolainen *et al.* 2005). Multiple recent papers summarize the pros and cons of DNA barcoding and review and discuss the issue from many points of view (e.g. Barrett & Hebert 2005, DeSalle *et al.* 2005, Ebach & Holdrege 2005, Hebert & Gregory 2005, Prendini 2005, Rubinoff & Holland 2005, Wheeler 2005, Will *et al.* 2005, Rubinoff 2006). The present study has produced COI sequences that constitute a contribution towards a 'barcode' database for the genus *Merodon*. The COI-3´ region here employed is, however, not the

'barcoding fragment' (COI-5´ region, the "Folmer fragment") employed in the barcoding framework. For the COI gene a comprehensive set of universally conserved primers was published by Simon *et al.* in 1994, and the primers working well for various genera of Syrphidae typically excluded the COI-5´ region. Studies on phylogenetic relationships of taxa of Syrphidae have included a 560 bp COI-3´-fragment to 1128 nt (constituting a large part of the 1540 bp in total) of the COI gene, analysed in conjunction with other datasets (e.g. Pérez-Bañón *et al.* 2003, Ståhls *et al.* 2004, Milankov *et al.* 2005, Rojo *et al.* 2006).

Dayrat (2005) coined and defined the term "Integrative Taxonomy" as the science that aims to delimit the units of life's diversity from multiple and complementary perspectives. In short, morphologists have traditionally described hypothetical morphospecies based on the observed morphological variation of the taxa under study. These morphospecies are then subjected to reappraisal using other approaches and additional data (molecular, ecological, etc.). Using this integrative approach, it is implied that the resulting species hypotheses will be better founded, because they are based on more comprehensive data from multiple sources. The study of intra- and interspecific variation will always remain the core of integrative taxonomy, but the practise of integrative taxonomy be dependent upon practicalities such as whether taxa available for morphological study are available for molecular study. Will *et al.* (2005) used the term in a way consistent with this view, but expressed somewhat different views in respect of implementation strategy. As suggested by Rubinoff (2006), an integrated approach, using mtDNA and nuclear DNA in conjunction with morphology and ecology, is better able to access different avenues of inheritance, producing more accurate results that are essential when assessing and managing biodiversity.

"Integrative Taxonomy" is used in the process of species delimitation. This term is clearly related to the term "total evidence" (Kluge 1989) that is used for phylogenetic inference, since the key element in both concepts is to use *all* of the available evidence and thus obtain a better supported hypothesis for either species delimitation or the relationships suggested in a phylogenetic tree. Thus, they are applied in different scientific fields for two different purposes.

The aim of the study reported on here was to employ the concept of integrative taxonomy, interpreted as the use of DNA data from multiple sources in conjunction with morphological characters and available distribution information, to engender species concepts for comparison with previously established species boundaries based on morphological taxonomy only. We employed mitochondrial COI sequences of the 3´ region (hereafter COI) in conjunction with DNA characters from two additional gene regions, the D2 expansion region of the nuclear ribosomal 28S rRNA gene (28S) and the nuclear, internal transcribed spacer two-region (ITS2). These were used in addition to morphological characters, for a sample of taxa from a geographically restricted region, the Iberian Peninsula. We were particularly interested in contrasting the observed morphological variation in particular taxa with molecular variability. We discuss our

results in light of all the molecular data (particularly for the 3′ fragment of the COI gene) and morphological data that were available. We also estimated the phylogenetic relationships among the included taxa based on the information available for COI and 28S gene sequences. This is not intended as a description of new species as demanded by article 16.1 (ICZN 1999).

Material and methods

Taxon sampling

Most specimens used for molecular analysis (Table 1) were derived from the Alicante region (SE Spain), while others originated in elsewhere in Spain, Greece (two specimens) or Andorra (one specimen). For species identification we used the key of Marcos-García *et al*. (in press).

Obtaining good quality DNA sequences requires high quality (large sized) DNA, but this requirement cannot always be met. Out of the total of 34 species occurring in Spain, fresh specimens of only 17 could be obtained for molecular work, thus limiting our analysis. Multiple specimens (up to nine) for each species were used when possible (Table 1). DNA voucher specimens were deposited in CEUA (University of Alicante, Spain), MZH (Zoological Museum of the Finnish Museum of Natural History, Helsinki, Finland) or NS (University of Novi Sad, Serbia & Montenegro) (Table 1).

Molecular characters

Information about the three gene regions was generated for closely-related purposes. The mitochondrial COI and the nuclear ITS2 region have been used for species delimitation, having been shown to be taxonomically informative for measuring the levels of intraspecific variability in Syrphidae and the relationships among closely related species (e.g. Ståhls & Nyblom 2000, Ståhls *et al*. 2004, Milankov *et al*. 2005, Massetti *et al*. 2006), while the combined sequence data of COI and nuclear ribosomal 28S have been informative for interspecific and generic level comparisons (e.g. Ståhls *et al*. 2003, Ståhls 2006).

Laboratory methods

DNA was extracted from 1–3 legs or other parts of single individuals of dry, pinned specimens, using the NucleoSpin Tissue DNA Extraction kit (Machery–Nagel) according to the manufacturer's protocol and re-suspended in 50 μl ultrapure water.

PCR amplifications were carried out in 25 μl reactions containing 2–5 μl of DNA extraction, 0.25 μl of Taq DNA polymerase (5U/μl), 1 μl of each dNTP 200 mM, 1 μl of each primer (10 pmol/μl), 2.5 μl PCR buffer 10x without $MgCl_2$, 2 μl $MgCl_2$ 25 mM and ultrapure water. Primers used for PCR and sequencing are listed in Table 2. PCR products were purified using the GFX PCR Purification kit (Amersham Biotech). Sequences were

TABLE 1. Data on included specimens and GenBank accession numbers for obtained sequences.

Taxon	Collecting locality	Sex	Laboratory Code	Deposition	GenBank accession number COI	GenBank accession number 28S	GenBank accession number ITS2
Merodon albifrons Meigen, 1822	SPAIN, Alicante, Alcoi, El Menetjador, 1352 m. 24-05-2002. Leg.: X. Mengual.	Male	X1	CEUA 00002091	DQ386317		DQ386376
Merodon albifrons Meigen, 1822	SPAIN, Alicante, Alcoi, El Menetjador, 1352 m. 24-05-2002. Leg.: X. Mengual.	Male	X2	CEUA 00002088	DQ386318		DQ386377
Merodon albifrons Meigen, 1822	SPAIN, Alicante, Agres, Foia Ampla, 1060 m. 14-08-2001/30-08-2001. Leg.: X. Mengual.	Female	X3	CEUA 00001652	DQ386319		DQ386378
Merodon albifrons Meigen, 1822	SPAIN, Alicante, Agres, Foia Ampla, 1060 m. 10-09-2002/24-09-2002. Leg.: X. Mengual.	Female	X4	CEUA 00001653	DQ386320	DQ386356	DQ386379
Merodon albifrons Meigen, 1822	GREECE, Lesvos, 1.25 km S Pili. 140 m. 30°10'14N 26°25'11E. Olive grove complex landscape. Aegean University 0004112.	Male	S534	MZH	DQ386312	DQ386354	
Merodon antonioi Marcos-García, Vujić et Mengual, *in litt.*	SPAIN, Ciudad Real, P.N. Cabañeros. 11-IX-2004. Leg. A. Ricarte.	Male	S545	MZH CEUA 00002079	DQ386314		DQ386375
Merodon arundanus Marcos-García, Vujić et Mengual, *in litt.*	SPAIN, Cádiz, Sierra de Grazalema, Coros, 1331 m. 20-IV-2003. N36°47' W5°22'. Leg.: W. van Steenis & E. S. Bakker.	Male	X33	CEUA 00002082	DQ386346	DQ386370	DQ386403
Merodon avidus B *sensu* Milankov *et al.*, 2001	SPAIN, Ávila, Piedralaves, 18-07-2001. Leg.: J.R. Verdú.	Male	X14	CEUA 00002086	DQ386329	DQ386361	DQ386388

to be continued.

TABLE 1 (continued) .

Taxon	Collecting locality	Sex	Laboratory Code	Deposition	GenBank accession number COI	GenBank accession number 28S	GenBank accession number ITS2
Merodon cabanerensis Marcos-García, Vujić et Mengual, *in litt.*	SPAIN, Ciudad Real, P.N. Cabañeros. 18-03-2005. Leg.: A. Ricarte.	Male	Y175	MZH	DQ386316	DQ386355	
Merodon chalybeus Wiedemann *in* Meigen, 1822	SPAIN, Cáceres, Embalse de Guadiloba. N39° 29' W6° 17'. 16-IV-2002. Leg.: W. van Steenis.	Male	X32	CEUA 00002081	DQ386345	DQ386369	DQ386402
Merodon chalybeus Wiedemann *in* Meigen, 1822	SPAIN, Cádiz, Tarifa, Río Jara. 08-04-2002. Leg.: W. van Steenis & E.S. Bakker.	Female	X70	W. van Steenis collection	DQ386351		
Merodon elegans Hurkmans, 1993	SPAIN, Alicante, Xixona, Mas de Cano, 940 m. 21-05-2001. Leg.: X. Mengual.	Female	X11	CEUA 00002099	DQ778055		DQ386386
Merodon elegans Hurkmans, 1993	SPAIN, Alicante, Alcoi, El Menetjador, 1352 m. 02-07-2002/16-07-2002. Leg.: X. Mengual.	Male	X13	CEUA 00002092	DQ386328	DQ386360	DQ386387
Merodon funestus (Fabricius, 1794)	SPAIN, Alicante, Agres, Caveta del Buitre, 1200 m. 29-05-2001. Leg.: X. Mengual.	Male	X28	CEUA 00002101	DQ386343	DQ386367	DQ386400
Merodon funestus (Fabricius, 1794)	SPAIN, Ciudad Real, P.N. Cabañeros. 2005. Leg.: A. Ricarte.	Male	S544	MZH	DQ386313		
Merodon geniculatus Strobl *in* Czerny & Strobl, 1909	SPAIN, Valencia, Bocairent, Mas del Parral, 900 m. 04-06-2002/17-06-2002. Leg.: X. Mengual.	Male	X5	CEUA 00002084	DQ386321	DQ386357	DQ386380
Merodon geniculatus Strobl *in* Czerny & Strobl, 1909	SPAIN, Alicante, Agres, Foia Ampla, 1060 m. 16-07-2002/30-07-2002. Leg.: X. Mengual.	Male	X34	CEUA 00002072	DQ386347	DQ386371	DQ386404

to be continued.

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TABLE 1 (continued) .

Taxon	Collecting locality	Sex	Laboratory Code	Deposition	GenBank accession number COI	GenBank accession number 28S	GenBank accession number ITS2
Merodon geniculatus Strobl *in* Czerny & Strobl, 1909	SPAIN, Ciudad Real, P.N. Cabañeros. 29-V-2004. Leg. A. Ricarte.	Male	S546	MZH CEUA 00002080	DQ386315		
Merodon legionensis Marcos-García, Vujić et Mengual, *in litt.*	SPAIN, Alicante, Agres, Caveta del Buitre, 1200 m. 27-08-2002/10-09-2002. Leg.: X. Mengual.	Female	X55	NS	DQ386350		
Merodon nigritarsis Rondani, 1845	SPAIN, Alicante, Xixona, Mas de Cano, 940 m. 05-06-2001/20-06-2001. Leg.: X. Mengual.	Male	X7	CEUA 00002094	DQ386323	DQ386359	DQ386382
Merodon nigritarsis Rondani, 1845	SPAIN, Alicante, Xixona, Mas de Cano, 940 m. 15-05-2002/04-06-2002. Leg.: X. Mengual.	Male	X8	CEUA 00002096	DQ386324		DQ386383
Merodon nigritarsis Rondani, 1845	SPAIN, Alicante, Alcoi, El Menetjador, 1352 m. 03-06-2002/17-06-2002. Leg.: X. Mengual.	Female	X9	CEUA 00002093	DQ386325		DQ386384
Merodon nigritarsis Rondani, 1845	SPAIN, Alicante, Xixona, Mas de Cano, 940 m. 23-05-2001/05-06-2001. Leg.: X. Mengual.	Female	X10	CEUA 00002095	DQ386326		DQ386385
Merodon nigritarsis Rondani, 1845	SPAIN, Alicante, Xixona, Mas de Sant Ignaci, 1020 m. 15-05-2002/04-06-2002. Leg.: X. Mengual.	Male	X12	CEUA 00002097	DQ386327		
Merodon obscuritarsis Strobl *in* Czerny & Strobl, 1909	SPAIN, Alicante, Agres, Foia Ampla, 1060 m. 15-05-2002/04-06-2002. Leg.: X. Mengual.	Female	X6	CEUA 00001377	DQ386322	DQ386358	DQ386381

to be continued.

TABLE 1 (continued) .

Taxon	Collecting locality	Sex	Laboratory Code	Deposition	GenBank accession number COI	GenBank accession number 28S	GenBank accession number ITS2
Merodon obscuritarsis Strobl in Czerny & Strobl, 1909	SPAIN, Alicante, Agres, Foia Ampla, 1060 m. 15-05-2002/04-06-2002. Leg.: X. Mengual.	Male	X29	CEUA 00001376	DQ386344	DQ386368	DQ386401
Merodon obscuritarsis Strobl in Czerny & Strobl, 1909	SPAIN, Alicante, Agres, Foia Ampla, 1060 m. 27-08-2002/10-09-2002. Leg.: X. Mengual.	Female	X36	CEUA 00001372	DQ386348	DQ386372	DQ386405
Merodon obscuritarsis Strobl in Czerny & Strobl, 1909	SPAIN, Alicante, Xixona, Mas de Sant Ignaci, 1020 m. 07-05-2001/23-05-2001. Leg.: X. Mengual.	Female	X37	CEUA 00001371	DQ386349	DQ386373	DQ386406
Merodon obscuritarsis Strobl in Czerny & Strobl, 1909	SPAIN, Alicante, Agres, Foia Ampla, 1060 m. 29-05-2001. Leg.: X. Mengual.	Male	X42	CEUA 00001375			DQ386411
Merodon obscuritarsis Strobl in Czerny & Strobl, 1909	SPAIN, Alicante, Agres, Foia Ampla, 1060 m. 05-06-2001/19-06-2001. Leg.: X. Mengual.	Male	X43	CEUA 00001373			DQ386412
Merodon obscuritarsis Strobl in Czerny & Strobl, 1909	SPAIN, Alicante, Agres, Foia Ampla, 1060 m. 08-05-2001/23-05-2001. Leg.: X. Mengual.	Male	X44	CEUA 00001374			DQ386413
Merodon obscuritarsis Strobl in Czerny & Strobl, 1909	SPAIN, Alicante, Alcoi, El Menetjador, 1352 m. 24-05-2002. Leg.: X. Mengual.	Male	X45	CEUA 00001369		DQ386374	DQ386414

to be continued.

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TABLE 1 (continued) .

Taxon	Collecting locality	Sex	Laboratory Code	Deposition	GenBank accession number COI	GenBank accession number 28S	GenBank accession number ITS2
Merodon obscuritarsis Strobl in Czerny & Strobl, 1909	SPAIN, Alicante, Alcoi, El Menetjador, 1352 m. 24-05-2002. Leg.: X. Mengual.	Male	X46=X77	CEUA 00001370	DQ386352		DQ386415
Merodon ottomanus Hurkmans, 1993	SPAIN, Alicante, Agres, Caveta del Buitre, 1200 m. 08-05-2001/23-05-2001. Leg.: X. Mengual.	Male	X27	CEUA 00002077	DQ386342	DQ386366	
Merodon quercetorum Marcos–García, Vujić et Mengual, *in litt.*	SPAIN, Alicante, Agres, Caveta del Buitre, 1200 m. 14-08-2001/30-08-2001. Agres. Alicante. SPAIN. Leg.: X. Mengual.	Female	X19	CEUA 00002074	DQ386334	DQ386363	DQ386393
Merodon quercetorum Marcos–García, Vujić et Mengual, *in litt.*	SPAIN, Alicante, Agres, Caveta del Buitre, 1200 m. 14-08-2001/30-08-2001. Leg.: X. Mengual.	Male	X20	CEUA 00002075	DQ386335		DQ386394
Merodon quercetorum Marcos–García, Vujić et Mengual, *in litt.*	SPAIN, Alicante, Agres, Caveta del Buitre, 1200 m. 30-08-2001/11-09-2001. Leg.: X. Mengual.	Female	X21	NS	DQ386336	DQ386364	DQ386395
Merodon serrulatus Wiedemann *in* Meigen, 1822	SPAIN, Alicante, Agres, Caveta del Buitre, 1200 m. 23-05-2001/05-06-2001. Leg.: X. Mengual.	Male	X23	CEUA 00001361	DQ386338	DQ386365	DQ386396

to be continued.

TABLE 1 (continued).

Taxon	Collecting locality	Sex	Laboratory Code	Deposition	GenBank accession number COI	GenBank accession number 28S	GenBank accession number ITS2
Merodon serrulatus Wiedemann *in* Meigen, 1822	SPAIN, Alicante, Agres, Caveta del Buitre, 1200 m. 23-05-2001/05-06-2001. Leg.: X. Mengual.	Male	X24	CEUA 00001362	DQ386339		DQ386397
Merodon serrulatus Wiedemann *in* Meigen, 1822	SPAIN, Alicante, Agres, Foia Ampla, 1060 m. 23-05-2001/05-06-2001. Leg.: X. Mengual.	Female	X25	CEUA 00001365	DQ386340		DQ386398
Merodon serrulatus Wiedemann *in* Meigen, 1822	SPAIN, Alicante, Agres, Caveta del Buitre, 1200 m. 05-06-2001. Leg.: X. Mengual.	Male	X26	CEUA 00001363	DQ386341		DQ386399
Merodon serrulatus Wiedemann *in* Meigen, 1822	SPAIN, Alicante, Alcoi, El Menetjador, 1352 m. 24-05-2002. Leg.: X. Mengual.	Male	X38	CEUA 00001366			DQ386407
Merodon serrulatus Wiedemann *in* Meigen, 1822	SPAIN, Alicante, Alcoi, El Menetjador, 1352 m. 07-06-2001. Leg.: X. Mengual.	Male	X39	CEUA 00001368			DQ386408
Merodon serrulatus Wiedemann *in* Meigen, 1822	SPAIN, Alicante, Alcoi, El Menetjador, 1352 m. 23-05-2001/05-06-2001. Leg.: X. Mengual.	Male	X40	CEUA 00001364			DQ386409
Merodon serrulatus Wiedemann *in* Meigen, 1822	SPAIN, Alicante, Alcoi, El Menetjador, 1352 m. 24-05-2002. Leg.: X. Mengual.	Male	X41	CEUA 00001367			DQ386410
Merodon serrulatus Wiedemann *in* Meigen, 1822	GREECE, Lesvos, Agiasos. Site 2, locality 6. 6-VI-2005.	Male	S531	MZH	DQ386311	DQ386353	
Merodon unguicornis Strobl *in* Czerny & Strobl, 1909	SPAIN, Alicante, Xixona, Venta de la Carrasqueta, 980 m. 23-04-2002/15-05-2002. Leg.: X. Mengual.	Male	X15	CEUA 00002104	DQ386330		DQ386389

to be continued.

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TABLE 1 (continued) .

Taxon	Collecting locality	Sex	Laboratory Code	Deposition	GenBank accession number COI	GenBank accession number 28S	GenBank accession number ITS2
Merodon unguicornis Strobl *in* Czerny & Strobl, 1909	SPAIN, Alicante, Agres, Caveta del Buitre 1200 m. 23-05-2001/05-06-2001. Leg.: X. Mengual.	Male	X16	CEUA 00001655	DQ386331	DQ386362	DQ386390
Merodon unguicornis Strobl *in* Czerny & Strobl, 1909	SPAIN, Alicante, Agres, Caveta del Buitre, 1200 m. 23-05-2001/05-06-2001. Leg.: X. Mengual.	Female	X17	CEUA 00001654	DQ386332		DQ386391
Merodon unguicornis Strobl *in* Czerny & Strobl, 1909	SPAIN, Alicante, Alcoi, El Menetjador, 1352 m. 15-05-2002/04-06-2002. Leg.: X. Mengual.	Female	X18	CEUA 00002087	DQ386333		DQ386392
Merodon unicolor Strobl *in* Czerny & Strobl, 1909	SPAIN, Valencia, Chelva. 10-04-1994/24-04-1994. Leg.: C. Pérez-Bañón.	Male	X22	CEUA 00002220	DQ386337		
Merodon unicolor Strobl *in* Czerny & Strobl, 1909	ANDORRA, Andorra. 10 km NE city. 17-05-2003. Leg: Halada.	Male	S477	MZH	DQ386309		

generated with an ABI 377 automated DNA sequencer (Applied Biosystems) using the BigDye Terminator Cycle Sequencing kit v1.1 (Applied Biosystems). Electropherograms were inspected and forward and reverse sequences were assembled and edited for each DNA region, using Sequence Navigator™ 1.0 (Applied Biosystems). All sequences were deposited in GenBank (see Table 1 for accession numbers).

TABLE 2. Primers used for amplifying and sequencing the COI, 28S, and ITS2 fragments.

	PRIMER	Sequence	Source
COI	C1-J-2183 (alias Jerry)	5'-CAACATTTATTTTGATTTTTTGG-3'	Simon *et al.*, 1994
	TL2-N-3014 (alias Pat)	5'-TCCAATGCACTAATCTGCCATATTA-3'	Simon *et al.*, 1994
28S	28S(F2)	5-AGAGAGAGTTCAAGAGTACGTG-3'	Belshaw *et al.* 2001
	28S(3DR)	5'-TAGTTCACCATCTTTCGGGTC-3'	Belshaw *et al.* 2001
ITS2	ITS2A (f)	5'-TGTGAACTGCAGGACACAT-3'	Beebe & Saul, 1995
	ITS2B (r)	5'-TATGCTTAAATTCAGGGGGT-3'	Beebe & Saul, 1995

Parsimony analyses

Parsimony analyses were performed separately for the COI dataset and the combined COI+28S data. If obtained COI sequences were identical between samples of a particular taxon, only one sequence was included in the parsimony analysis. For the 28S gene we sequenced only one specimen per species in most cases. The 28S sequences data was mainly used for the combined parsimony analysis. As the sequence length variation among ingroup taxa was low (4 nucleotides), we were confident to align the 28S sequences by eye.

We used the program NONA v2.0 (Goloboff 1993) for the parsimony analyses (command line: hold 100000; mult*500; hold/200). All characters were equally weighted, and gaps were treated as missing data. NONA spawn from Winclada (Nixon 2002) was used for calculating evidential support for different clades, using Bremer support values (branch support) and bootstrap support values (1000 replications; mult*20; hold/2). The Bremer support value for a particular clade indicates the number of extra steps from the most parsimonious tree at which the clade fails to be resolved as successively longer trees are examined. A high numerical value indicates good support. Non-parametric bootstrapping involves resampling data with replacement, and was calculated using 1000 replicates. *Eumerus etnensis* van der Goot, 1964 (Eumerini) was used as outgroup (GenBank accession number AY533315 for COI and AY540907 for 28S).

Results

Molecular markers

COI: For the COI we obtained 784 nucleotide characters for 44 samples representing 17 putative ingroup taxa. The mean AT content was 71.6%. Uncorrected pairwise divergences between ingroup taxa were calculated for the COI gene, and ranged between 0.29% (samples X33*M. arundanus* Marcos-García, Vujic *et* Mengual, *in litt.* and X77*M. obscuritarsis* Strobl *in* Czerny & Strobl, 1909) and 12.5% (samples X11*M. elegans* Hurkmans, 1993 and X15–18*M. unguicornis* Strobl *in* Czerny & Strobl, 1909). Uncorrected pairwise divergence between the outgroup and all ingroup taxa was highest for *M. unguicornis* (14.9%). These levels are similar to the divergences between species found in other genera of Syrphidae (Ståhls et al. 2004, Ståhls 2006) for the same gene.

ITS2: For the ITS2 region we obtained 333–496 nucleotides for 41 samples representing 13 ingroup taxa. In five taxa we obtained the ITS2 sequence for a single specimen. The ITS2 was only used for intraspecific comparisons in all taxa with multiple sequences, with the aim of surveying and scoring the number of intraspecific genotypes. The highest number of intraspecific genotypes was found for *M. albifrons*, while five taxa showed identical ITS2 sequences. For this highly variable gene region alignment between both ingroup taxa and between ingroup and outgroup was an ambiguous and difficult task.

28S: For the 28S rRNA gene we pruned the obtained sequences to 375–379 nucleotides length for 22 samples representing 14 ingroup taxa. The aligned matrix consisted of 396 nucleotides of which 21 were parsimony informative.

Parsimony analyses

Separate analysis of COI

No insertions or deletions occurred in the COI dataset so alignment was unambiguous. Of the obtained 784 nucleotides, 194 sites were parsimony-informative. Parsimony analysis produced 63 equally parsimonious trees of 577 steps in length, with a consistency index (CI) of 0.53 and a retention index (RI) of 0.83. The strict consensus is shown in Fig. 1. The COI gene identified four, well-defined clades within the analysed Iberian *Merodon* species, the *desuturinus, albifrons, nigritarsis* and *aureus* groups.

Combined analysis

The 28S sequences were manually aligned as sequences varied with only 4 nucleotides between ingroup taxa. For this analysis, specimens were used for which sequences of both COI and 28S were obtained. Three species lacking 28S sequence, *M. antonioi*, *M. legionensis* or *M. unicolor*, were also included, using only COI.

Parsimony analysis of the combined COI and 28S data produced 9 equally parsimonious trees of 682 steps in length (CI = 0.56, RI = 0.66), with a topology resolving the same groups as in the separate analysis of COI. Fig. 2 shows the strict consensus tree.
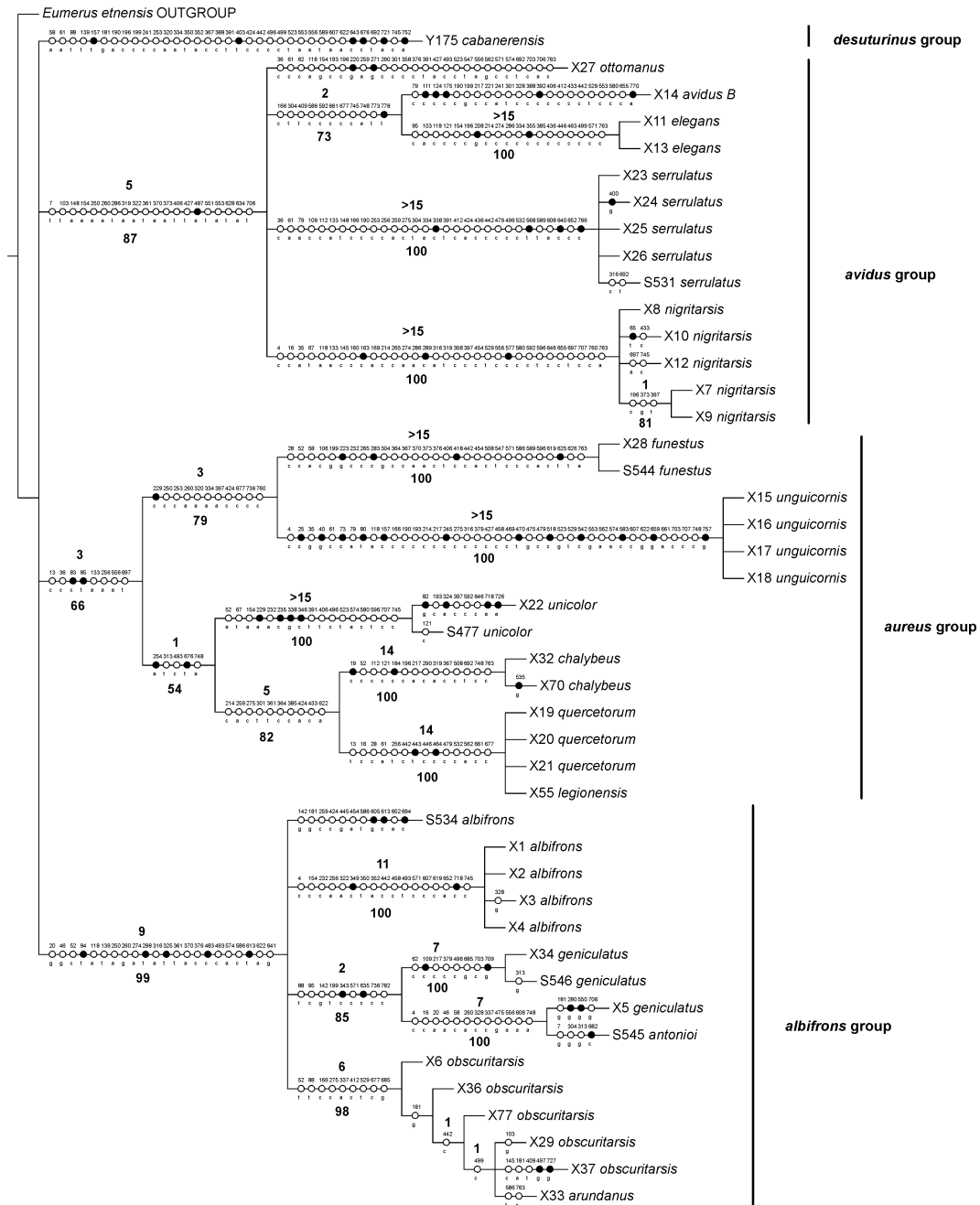
FIGURE 1. Parsimony analysis of COI sequences. Strict consensus of 63 equally parsimonious trees, L =577, CI = 0.53, RI = 0.83. Open circles denote homoplasious characters and filled circles denote nonhomoplasious characters. Bremer support values are indicated above branches, bootstrap values below.

FIGURE 2. Combined analysis of COI and 28S sequences. Strict consensus of 9 equally parsimonious trees, L = 682, CI = 0.56, RI = 0.66. Open circles denote homoplasious characters and filled circles denote nonhomoplasious characters. Bremer support values are indicated above branches, bootstrap values below.
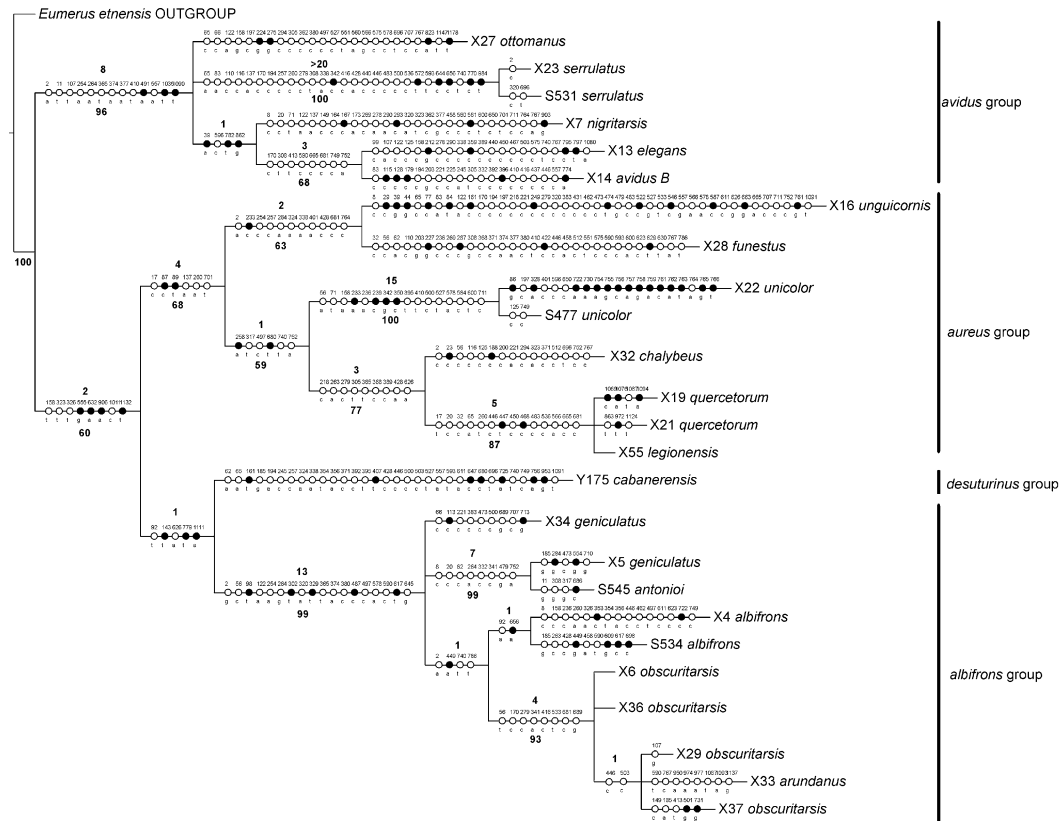
Integrative taxonomy of *Merodon* spp.

desuturinus group

This group comprises three members in the Palaearctic area (Vujic *et al*. in prep). Of these only one taxon, *M. cabanerensis* Marcos-García, Vujic *et* Mengual, *in litt.* occurs on the Iberian Peninsula. Uncorrected pairwise divergences of COI, between *M. cabanerensis* and included members of the *albifrons* group, range from 7.91 to 9.06%. Parsimony analysis resolved this taxon as the sister group to the *albifrons* group, a placement that is in agreement with similarity of some morphological characters (Vujic *et al*. in prep.).

albifrons group

In Iberia the *M. albifrons* group is the most diverse and contains 14 species (Marcos-García *et al*. in press). Five of these are included in the study reported on here.

We obtained three male and two female specimens that by morphological characters were identified as *M. albifrons* Meigen, 1822 (samples X1–X4 from Spain: Alicante and S534 from Greece: Lesvos). The COI sequences were identical among the four specimens from Alicante. These were obtained from two locations 12 km apart (Foia Ampla in Agres and El Menetjador in Alcoi). The uncorrected pairwise divergence between the Spanish *albifrons* and the *albifrons* from Greece was 3.32%. The 28S sequences obtained for one specimen from Spain and the one from Greece were identical. The ITS2 fragment of four *M. albifrons* specimens from Spain produced three different genotypes, with variability in a dinucleotide repeat region, $AT_{(1-5)}$ (Fig. 3). This variability we interpret as intraspecific. The divergence of the COI in the Spanish and Greek *albifrons* (3.32%) is in conflict with their identical morphology and 28S sequences. The 28S gene is more conservative and accumulates change more slowly, which is generally also the case with morphological characters. This could explain our results.

M. albifrons is widely distributed in the Mediterranean area, and several hundred specimens have been studied, but the observed slight morphological variability was not so striking as to suggest the presence of cryptic taxa, except for *M. hurkmansi* Marcos-García, Vujic *et* Mengual, *in litt.* from Algeria (Marcos-García *et al*. in press). Although DNA sequence data was obtained from 2–5 specimens (28S vs. COI + ITS2, respectively), we conclude that the samples from Spain and Greece probably represent different taxa. To confirm this result, additional samples from the Mediterranean area will have to be collected for both molecular and morphological study.

```
X1 M. albifrons      ...TATATATATATATATATAT----------TAAAAAAATTTA...
X2 M. albifrons      ...TATATATATATATATATATATATATATATTAAAAAAATTTA...
X3 M. albifrons      ...TATATATATATATATATAT---------TAAAAAAATTTA...
X4 M. albifrons      ...TATATATATATATATATATATATAT--TAAAAAAATTTA...
```

FIGURE 3. *M. albifrons* from Spain produced three different genotypes for ITS2 with variability in a dinucleotide repeat region, $AT_{(1-5)}$.

The study included three specimens of *M. geniculatus* Strobl *in* Czerny & Strobl, 1909 and one specimen of *M. antonioi* Marcos-García, Vujic *et* Mengual, *in litt.,* all from Spain. The recent taxonomic study of Iberian *Merodon* (Marcos-García *et al*. in press) discovered and described two cryptic taxa close to *M. geniculatus*, one of them being *M. antonioi*. All three *M. geniculatus* specimens used for DNA sequencing share the same morphological characters, and agree with the holotype of *M. geniculatus*.

The parsimony analysis resolved samples S546 + X34 as the sister group to X5 + S545 (Figs. 1 and 2). Two *geniculatus* specimens differed in only one nucleotide change (0.13%) (samples S564 and X34), and the third specimen (X5) was resolved as the sister taxon of *M. antonioi* (S545), with an uncorrected pairwise divergence of COI which was

1.15%, while it was 3.06% between X5 and X34. The 28S sequences were obtained for samples X5 and X34 and differ by 2 nucleotide changes. The ITS2 sequences were obtained for samples X34, X5 and S545. These sequences are all distinct (e.g. 18 gaps were required to manually align X5 and X34 ITS2 sequences, while comparisons of these with sample S545 require at least 30 gaps + some nucleotide changes). The magnitude of the differences observed between the *M. geniculatus* and *M. antonioi* samples for the different gene regions is consistent with the general description of 28S as being the more conservative, ITS2 being fast evolving and the evolutionary rate of COI being intermediate between 28S and ITS2. The apparent molecular divergence of the COI, 28S and ITS2 sequences of the *geniculatus* samples that were resolved in two different lineages suggests the presence of an additional morphologically cryptic taxon (not agreeing with any of the recently described taxa), while *M. antonioi* is distinct in both morphological and molecular characters from its sister "taxon" (X5). Morphological differences between *Merodon geniculatus* and *M. antonioi* include the length of basoflagellomere, the form of the hind legs and the shape of cerci of the male terminalia. These species have sympatric populations in Cabañeros National Park, Spain (our samples S546 and S545).

For *M. obscuritarsis* we obtained COI sequences for five specimens, 28S sequences for three specimens and ITS2 sequences for nine specimens. The intraspecific,uncorrected divergence between these specimens ranged from 0.14 to 0.89% for the COI gene, while the sequence samples for 28S and ITS2 were identical for the respective gene region. This taxon is morphologically variable. The recently described taxon *M. arundanus,* that parsimony analysis resolved as sister to *M. obscuritarsis* (specimen X29), is clearly different from *M. obscuritarsis* morphologically. Marcos-García *et al*. (in press) described differences e.g. in male genitalia, colour and length of body hairs and structure of integument. Uncorrected pairwise divergences of the COI ranged from 0.28 to 0.89% between the *M. obscuritarsis* samples and *M. arundanus*. *M. arundanus* differs from *M. obscuritarsis* by five nucleotide changes for the 28S, and by two indels and three nucleotide changes for the ITS2. The intraspecific divergences of COI for samples of *M. obscuritarsis*, and the interspecific divergences between *M. arundanus* and *M. obscuritarsis,* were completely overlapping. The levels of divergence exhibited between the different gene regions are surprising and not in agreement with conclusions relating to the *M. geniculatus* samples (previous section).

nigritarsis group

This is a very diverse group, especially in the eastern Mediterranean area. Many taxa belonging to this group were revised by Hurkmans (1993). Only 9 are reported from the Iberian Peninsula (Marcos-García *et al*. in press).

Both morphological and molecular characters supported all taxa recognised in this group as distinct species. Interspecific divergences of the COI ranged from 5.89 to 8.47% between the included taxa.

For *M. nigritarsis* Rondani, 1845 we obtained COI sequences for five specimens, from two separate localities 6.5 km apart, and all were identical. The ITS2 sequences were obtained from four specimens and these were also identical. This taxon is widely distributed in southern and central Europe.

For *M. elegans* we obtained COI and ITS2 sequences for two specimens, and these were identical for the respective gene regions. The distributional range for *M. elegans* is Spain and Northwest Africa.

Milankov *et al*. (2001) studied populations on the Balkan Peninsula and separated two taxa, *M. avidus* A and *M. avidus* B, based on allozyme data combined with morphological data. The single specimen included in the present analysis agrees with the morphological concept of *M. avidus* B of Milankov *et al*. (2001). The uncorrected pairwise divergence of COI between sampled specimens of *avidus* A and *avidus* B was 6.16% (unpublished data). Taxonomic status and species delimitation of all taxa of the *M. avidus* complex will be presented separately (Milankov *et al*., in prep.).

M. serrulatus Wiedemann *in* Meigen, 1822 is the most widespread taxon of the genus *Merodon*, with a range that includes Russia (Altai Mountains), the Ukraine (Black Sea) and all of the Mediterranean area (Milankov *et al*., in prep.). We obtained COI sequences for four samples (three localities, Table 1), and these were identical. The intraspecific COI divergence of three *M. serrulatus* specimens from Russia, Spain and Greece ranged from 0.0 to 0.37% (unpublished data). ITS2 sequences were obtained for eight samples from two localities, and were identical. The 28S sequences from two samples needed one indel to be aligned.

aureus group

This species group includes a large number of taxa occurring around the Mediterranean, with many locally endemic species. Five of seven Iberian species belonging to the *aureus* group are endemics, the exceptions being *M. funestu*s and *M. chalybeus* (Marcos-García *et al*. in press).

M. funestus (Fabricius, 1794) is morphologically the most distinct taxon in the group. The uncorrected pairwise divergences between *M. funestus* and other taxa of the *aureus* group ranged from 8.94 to 11.34%. The two COI sequences obtained for *M. funestus* didn't show differences. Parsimony analysis resolved *M. unguicornis* and *M. funestus* as sister taxa. The uncorrected pairwise sequence divergence of COI between these taxa was 10.11%. *M. funestus* is widely distributed in the Mediterranean area.

The two specimens of *M. unicolor* Strobl in Czerny & Strobl, 1909 studied showed a COI divergence of 1.25%. Although the morphology of these specimens is similar, the COI divergence indicated the presence of morphologically cryptic species. We await additional material derived from a broader geographical area to further explore this hypothesis. We could not obtain sequences from other loci for this taxon. *M. unicolor* is known from western parts of the Mediterranean area.

The COI sequences of the two specimens of *M. chalybeus* Wiedemann *in* Meigen, 1822 incorporated into this analysis showed one nucleotide difference (28S and ITS2 sequences were obtained for one specimen only). *M. chalybeus* is known from Spain, southern France and the former Yugoslavia.

Marcos-García *et al*. (in press) described *M. quercetorum* and *M. legionensis*, with type localities in Puerto Honduras (Hervás, Cáceres, Spain) and Murias de Paredes (León, Spain). The COI sequences of the three *M. quercetorum* and the single *M. legionensis* sample studied were identical. The manual alignment of obtained ITS2 sequence between samples X19+X20 vs. X21 of *M. quercetorum* required only one indel. The ITS2 sequence was not obtained for *M. legionensis*. The two taxa can be separated morphologically, e.g. using characters of the pilosity of the abdomen and hind legs. Further morphological and molecular study of additional specimens will hopefully shed more light on the status of these taxa.

Discussion

A subgeneric classification of the genus *Merodon* is in preparation (Vujic *et al*.). From the study reported on here we can recognise four monophyletic groups that are consistent with the morphological data: *desuturinus*, *albifrons*, *nigritarsis* and *aureus* groups. The 28S rRNA gene has proved to be of limited value in providing phylogenetic insights, due to its conservative nature, since this dataset exhibited only 21 variable and parsimony-informative sites. Hence, combined analysis of COI and 28S sequences produced topology similar to that derived from separate analysis of COI (Figs. 2 and 1, respectively).

DNA Barcoding vs Integrative taxonomy

Several studies have demonstrated the utility of DNA barcodes (sequences) to diagnose species, reveal cryptic species, link different life stages of local faunas, identify parasites and their invertebrate disease vectors, and in forensics and pest management (e.g. Palumbi & Cipriano 1998; Symondson 2002; Baker *et al*. 2003; Besansky *et al*. 2003; Whiteman *et al*. 2004; Miller *et al*. 2005; Smith *et al*. 2005; Smith *et al*. 2006). But problems using DNA barcodes have also been revealed, e.g. mitochondrial introgression between taxa, recent speciation followed by incomplete lineage sorting or interbreeding (Palumbi & Cipriano 1998; Scheffer & Wiegmann 2000; Croucher *et al*. 2004; Bachtrog *et al*. 2006; Kaila & Ståhls 2006).

The need for use of an integrative taxonomic approach to species delimitation was pointed out by Dayrat (2005), Rubinoff & Holland (2005), and Will *et al*. (2005).

The results we have obtained in this integrative taxonomic study of species of the genus *Merodon* exemplify three different situations that can be encountered, in which datasets can give contradictory or congruent signals. These are presented and discussed below.

Morphology and DNA in concordance

Species for which specimens sequenced for one or several loci showed concordance between the "taxonomic signal" derived from both morphology and sequences were *M. nigritarsis*, *M. elegans*, *M. serrulatus*, *M. unguicornis*, *M. funestus*, *M. chalybeus* and *M. quercetorum*.

These are cases where DNA barcoding would be applicable, at least for samples collected on the Iberian Peninsula, where molecular COI barcodes with high probability would in each case identify only one species. However, we agree with DeSalle *et al.* (2005) that the COI sequences of a small number of specimens may not be (or are not likely to be) representative of the possible variability of the species as a whole, especially for a taxon with a large geographic range. The species listed above presented uncorrected pairwise distances ranging between 6.80% (*M. otttomanus* vs. *M. serrulatus*) and 12.50% (*M. elegans* vs. *M. unguicornis*), magnitudes of difference that are highly indicative.

Different morphology with identical DNA sequences

The second situation we encountered involved support for two different taxa morphologically, but COI sequences that are identical or almost identical. This is the case of *M. legionensis* and *M. quercetorum*, two recently described species (Marcos-García *et al*. in press) that have sympatric populations and are morphologically very similar, yet discernible using some diagnostic characters. This could indicate mitochondrial introgression between the taxa, or speciation followed by incomplete lineage sorting. Statistically supported introgression between animal species has recently been, shown to occur in widely different taxonomic groups and far more commonly than previously recognised (see review by Funk & Omland, 2003). There are multiple examples of introgression in insects (see Funk & Omland, 2003; Shaw, 2002; Bachtrog *et al*. 2006) and in other arthropods like spiders (Croucher *et al*. 2004).

In this particular case COI barcodes would fail in the identification or delimitation of species. In the absence of additional sources of information on species limits, mitochondrial barcoding necessarily relies on some combination of mitochondrial monophyly and genetic distance to indicate probable species (Scheffer *et al*. 2006). In cases of mitochondrial introgression or incomplete lineage sorting, the species-level tree will show no resolution for the closest species and DNA barcoding will fail in the delimitation of species. Cryptic taxa are defined by molecular characters comparing them with well-known and well-studied taxa that are based on morphological, ecological and biogeographical characters, since they can only be recognised through comparison with already studied taxa.

Species with intraspecific variability of DNA sequences but similar morphology

In the present study species that show great intraspecific variability in their mitochondrial DNA, but that are morphologically indistinguishable are represented by *M. geniculatus* and *M. albifrons*.

The information we derived from the DNA sequence data for three samples of *M.*

geniculatus is in conflict with the observed morphological similarity. The apparent molecular divergence of the COI, 28S and ITS2 sequences of the *geniculatus* samples (that were resolved in two different lineages, Figs. 1 and 2) suggests the presence of an additional, morphologically cryptic taxon (not agreeing with any of the recently described taxa). Funk and Omland (2003) stated that "If other described species are more closely related to such "cryptic species" than the cryptic specie are to each other, a mitochondrial gene tree might hint at cryptic taxa by revealing polyphyly in the form of two phylogenetically separated clades", and "Such cryptic species might reflect the retention of ancestral morphology". *M. geniculatus* agrees perfectly with these predictions. Our results indicate that additional sampling of specimens from a broad geographic range will be necessary if intraspecific and interspecific variability are to be distinguished.

M. albifrons specimens from Spain have no differences in their COI sequences. The variability of the ITS2 can be addressed as intraspecific due to the nature of the dinucleotide repeat (loop) region (Fig. 3). COI for the included Spanish *albifrons* and the *albifrons* from Greece shows a divergence of 3.32%, being in conflict with their apparently identical morphology, and can indicate the presence of morphologically cryptic taxa.

Discovery of cryptic species is one of the goals that can be achieved with DNA barcodes (e.g. Hebert *et al*. 2004a; Kaila & Ståhls 2006; Smith *et al*. 2006). Thus, DNA barcoding has a potential utility to reveal taxonomic information and help to bring to light differences that are not expressed by morphology.

A special case in this study of species with intraspecific differences of DNA sequences is *M. obscuritarsis,* because in this instance observed intraspecific variability and interspecific variability overlap completely. The recently described taxon *M. arundanus,* that parsimony analysis resolved as sister to *M. obscuritarsis* (specimen X29), is morphologically clearly different from *M. obscuritarsis,* but uncorrected pairwise divergences of the COI ranged from 0.28 to 0.89% between the *M. obscuritarsis* samples and *M. arundanus*, and were overlapping with the intraspecific divergences for the *M. obscuritarsis* sequences (0.12 to 0.89%). Marcos-García *et al*. (in press) described differences in some morphological characters clearly allowing separation of the two taxa. *M. arundanus* is described from the Natural Park of Sierra de Grazalema (Andalucia), a UNESCO Biosphere Reserve dating from 1975, where the highest precipitation values for Iberia are recorded and the endemic spruce *Abies pinsapo* Boiss occurs, that is otherwise known only as a Tertiary fossil. Because *M. arundanus* is morphologically distinct, Marcos-García *et al*. (in press) recognise it as a taxon endemic to Grazalema. This taxon agrees with a common pattern for endemicity in the Iberian Peninsula: the areas with highest concentrations of endemic species occur in mountain areas (Martín *et al*. 2000; Castro-Parga *et al*. 1996). Thus, we hypothesize that the low divergence between *M.arundanus* and *M. obscuritarsis*, exhibited for both the COI and the ITS2 regions, indicates that these taxa have speciated recently, despite the reality that the presence of distinctive morphological diagnostic characters and the level of 28S divergence both

suggest the opposite. This could represent a case of incomplete lineage sorting following recent speciation. The results for the COI gene are ambiguous with the available data.

DNA barcoding rules for species delimitation don't work in these cases. A problematic issue in the use of a DNA barcoding approach on a single mitochondrial gene is the adoption of quite simplistic and arbitrary criteria for determining species limits using percentage of uncorrected pairwise distances (Hebert *et al.* 2003). Many authors have discussed species delimitation boundaries in a DNA barcoding context (Sperling 2003; Will & Rubinoff 2004; Prendini 2005; Rubinoff 2006), concluding that the 3% divergence rule for insects would only conceal incongruent character distributions without solving the underlying biological problems (Sperling 2003) and that the ranges for intra- and interspecific variation are still mostly unknown and will vary among groups and across gene loci (e.g. Prendini 2005). But DNA barcoding generates information, not knowledge (Ebach & Holdrege 2005). The use of a short sequence from a single marker cannot by itself define species, but can help in the recognition.

Results from recent studies discourage delimitation of closely related species using COI barcodes (Scheffer *et al.* 2005; Kaila & Ståhls 2006; Rubinoff 2006) and suggest that evidence from different sources, such as morphological and ecological data, and molecular evidence from more than one molecular locus, should be used for species delimitation and identification (Sperling 2003; Dayrat 2005). DNA sequences observed in closely related, recently diverged, hybrid, or polyploid species, the very cases for which identification may be most crucial (Sperling 2003; Will and Rubinoff 2004), will often be too similar to allow their discrimination.

The present study based on DNA sequences observed in a set of taxa sampled from a geographically restricted region revealed both conflict and congruence in the taxonomic information derived from morphological and molecular characters. If we had used only morphological characters, we would have failed to recognize 3 possible cryptic species (15.8%; X5*M. geniculatus*, X22*M. unicolor* and S534*M. albifrons*). If only COI sequences were used, 2 species would not have been identified (10.5%; X33*M. arundanus* and X55*M. legionensis*).

We are convinced that a more comprehensive sampling of taxa and specimens would have resulted in both a higher number of conflicting cases and potential resolution of existing ones. Although the obtained sequence data set (COI, ITS2, 28S) was not complete for all taxa, it showed that delimitation and identification of species is potentially better achieved if based on information from multiple loci, used in conjunction with morphology. We conclude that congruency in the taxonomic signals derived from morphological and molecular data does result in the best support for species hypotheses. However, it is also apparent that a more comprehensive data set, supported by geographically more diverse taxon sampling would have enhanced the findings of this study. This study has, however, encouraged us to continue using the integrative approach in future studies on the taxonomy of Syrphidae.

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References

Arevalo, E., Zhu, Y., Carpenter, J.M. & Strassmann, J.E. (2004) The phylogeny of the social wasp subfamily Polistinae: evidence from microsatellite flanking sequences, mitochondrial COI sequence, and morphological characters. *BMC Evolutionary Biology*, 4, 8. Available from: http://www.biomedcentral.com/1471–2148/4/8 (accessed 6 February 2006).

Bachtrog, D., Thornton, K., Clark, A. & Andolfatto, P. (2006) Extensive introgression of mitochondrial DNA relative to nuclear genes in the *Drosophila yakuba* species group. *Evolution*, 60(2), 292–302.

Baker, C.S., Dalebout, M.L., Lavery, S. & Ross, H.A. (2003) www.DNA-surveillance: applied molecular taxonomy for species conservation and discovery. *Trends in Ecology and Evolution*, 18, 271–272.

Barrett, R.D.H. & Hebert, P.D.N. (2005) Identifying spiders through DNA barcodes. *Canadian Journal of Zoology*, 83, 481–491.

Beebe, N.W. & Saul, A. (1995) Discrimination of all members of the *Anopheles punctulatus* complex by polymerase chain reaction-restriction fragment length polymorphism analysis. *The American Journal of Tropical Medicine and Hygiene*, 53, 478–481.

Belshaw, R., Lopez-Vaamonde, C., Degerli, N. & Quicke, D.L.J. (2001) Paraphyletic taxa and taxonomic chaining: evaluating the classification of braconine wasps (Hymenoptera: Braconidae) using 28S D2-3 rDNA sequences and morphological characters. *Biological Journal of the Linnean Society*, 73, 411–424.

Besansky, N.J., Severson, D.W. & Ferdig, M.T. (2003) DNA barcoding of parasites and invertebrate disease vectors: what you don't know can hurt you. *Trends in Parasitology*, 19(12), 545–546.

Castro-Parga, I., Moreno-Saiz, J.C., Humphries, C.J. & Williams, P.H. (1996) Strengthening the natural and national park system of Iberia to conserve vascular plants. *Botanical Journal of the Linnean Society*, 121, 189–206.

Caterino, M.S. & Sperling, F.A.H. (1999) *Papilio* phylogeny based on mitochondrial cytochrome oxidase I and II genes. *Molecular Phylogenetics and Evolution*, 11, 122–137.

Caterino, M.S., Reed, R., Kuo, M. & Sperling, F.A.H. (2001) A partioned likelihood analysis of swallowtail butterfly phylogeny. *Systematic Biology*, 50, 106–127.

Croucher, P.J.P., Oxford, G.S. & Searle, J.B. (2004) Mitochondrial differentiation, introgression of species in the *Tegenaria atrica* group (Araneae: Agelenidae). *Biological Journal of the Linnean Society*, 81, 79–89.

Czerny, L. & Strobl, P.G. (1909) Spanische Dipteren. III. *Verhandlungen der Zoologisch-Botanischen Gesellschast*, 59, 121–301.

Dayrat, B. (2005) Towards integrative taxonomy. *Biological Journal of the Linnean Society*, 85, 407–415.

DeSalle, R., Egan, M.G. & Siddall, M. (2005) The unholy trinity: taxonomy, species delimitation, and DNA barcoding. *Philosophical Transactions of the Royal Society of London B Biological Sciences*, 360(1462), 1905–1916

Ebach, M.C. & Holdrege, C. (2005) DNA barcoding is no substitute for taxonomy. *Nature*, 434, 697.

Fabricius, J.C. (1794) *Entomologia systematica emendata et aucta. Secundum classes, ordines, genera, species adjectis synonymis, locis, observationibus, descriptionibus.* Vol. 4. Hafniae [= Copenhagen], 472 pp.

Funk, D.J. & Omland, K.E. (2003) The frequency, causes and consequences of species level paraphyly and polyphyly: insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution and Systematics*, 34, 397–423.

Goloboff, P.A. (1993) NONA. Noname (a bastard son of Pee-wee), version 2.0 (32 bit version). Available from: http://www.cladistics.com/aboutNona.htm (accessed 6 February 2006).

Goot, V.S. van der (1964) Summer records of Syrphidae (Diptera) from Sicily, with field notes and descriptions of new species. *Zoologisch Medelingen Rijksmuseum van Natuurlijke Historie*, 39, 414–432.

Hebert, P.D.N., & Gregory, T.R. (2005) The promise of DNA barcoding for taxonomy. *Systematic Biology*, 54, 852–859.

Hebert, P.D.N., Cywinska, A., Ball, S.L. & deWaard, J.R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London B*, 270, 313–322.

Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H., & Hallwachs, W. (2004a) Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astraptes fulgerator. Proceedings of the National Academy of Sciences of the United States of America*, 101, 14812–14817.

Hebert, P.D.N., Stoeckle, M.V., Zemiak, T.S. & Francis, C.M. (2004b) Identification of birds through DNA barcodes. *PloS Biology*, 2, e312, 1657–1663.

Hurkmans, W. (1993) A monograph of *Merodon* (Diptera: Syrphidae). Part I. *Tijdschrift voor Entomologie*, 136, 147–234.

International Commission on Zoological Nomenclature. (1999) *International Code of Zoological Nomenclature* (4th edition), The International Trust for Zoological Nomenclature, London, 306 pp.

Kaila, L. & Ståhls, G. (2006) DNA barcodes: Evaluating the potential of COI to differentiate closely related species of *Elachista* (Lepidoptera: Gelechioidea: Elachistidae) from Australia. *Zootaxa*, 1170, 1–26.

Kluge, A.G. (1989) A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). *Systematic Zoology*, 38(1), 7–25.

Marcos-García, M.A. (1985a) Contribución al conocimiento de la sirfidofauna del Pirineo del Alto Aragón. II (Diptera, Syrphidae). *Boletim da Sociedade Portuguesa de Entomologia*, 1, 521–532.

Marcos-García, M.A. (1985b) Los Syrphidae (Diptera) de las sierras occidentales del Sistema Central español. Subfamilias: *Eristalinae, Lampetiinae, Microdontinae, Milesiinae* y *Cerianinae. Boletín de la Asociación española de Entomología*, 9, 187–210.

Marcos-García, M.A. (1989) *Merodon escorialensis* Strobl, 1909, *stat. nov.* (Diptera, Syrphidae). *Annales Societé Entomologique Française*, 25, 243–247.

Marcos-García, M.A. (1990a) Nuevos datos sobre las especies ibéricas del género *Merodon* Meigen, 1822 y descripción de la genitalia de *Merodon unguicornis* Strobl,1909 (*Diptera, Syr-*

phidae). *Anales de Biología*, 16, 49–51.

Marcos-García, M.A. (1990b) Catálogo preliminar de los Syrphidae (Diptera) de la Cordillera Cantábrica (España). *Eos*, 66, 81–235.

Marcos-García, M.A., Rojo, S. & Pérez-Bañón, C. (2002) Family Syrphidae. *In*: Sociedad Entomológica Aragonesa (Ed.), *Catálogo de los Dípteros de España, Portugal y Andorra (Insecta)*. Sociedad Entomológica Aragonesa, Zaragoza, Spain, 132–136.

Marcos-García, M.A., Vujić, A. & Mengual, X. (2007) Revision of Iberian species of the genus *Merodon* Meigen, 1803 (Diptera: Syrphidae). *European Journal of Entomology*, in press.

Martín, J., García-Barros, E., Gurrea, P., Luciañez, M.J., Munguira, M.L., Sanz M.J. & Simón, J.C. (2000) High endemism areas in the Iberian Peninsula. *Belgian Journal Entomologie*, 2, 47–57.

Masetti A., Luchetti A., Sommaggio D., Burgio G. & Mantovani B. (2006) Phylogeny of *Chrysotoxum* species (Diptera: Syrphidae) inferred from morphological and molecular characters *European Journal of Entomology,* 103, 459–467.

Meigen, J.W. (1803) Versuch einer neuen Gattungseinteilung der europäischen zweiflügeligen Insekten. *Magazin für Insektenkunde*, 2, 259–281.

Meigen, J.W. (1822) *Systematische Beschreibung der bekannten europäischen zweiflügeligen Insekten*. Dritter Theil.Schulz-Wundermann, Hamm. x + 416 pp.

Milankov V., Vujić A. & Ludoški J. (2001) Genetic divergence among cryptic taxa of *Merodon avidus* (Rossi,1790) (Diptera: Syrphidae). *International Journal of Dipterological Research*, 12(1), 15–24.

Milankov, V., Stamenković, J., Ludoški, J., Ståhls, G. & Vujić, A. (2005) Diagnostic molecular markers and genetic relationships among three species from the *Cheilosia canicularis* group (Diptera: Syrphidae). *European Journal of Entomology*, 102, 125–131.

Miller, K.B., Alaire, Y., Wolfe, G.W. & Whiting, M.F. (2005) Association of insect life stages using DNA sequences: the larvae of *Phylodytes umbrinus* (Motschulsky) (Coleoptera: Dytiscidae). *Systematic Entomology*, 30, 499–509.

Nixon, K. C. (2002) WinClada ver. 1.00.08. Published by the author, Ithaca, New York. Available from: http://www.cladistics.com/Winclada_agree_no_buttons.htm. (accessed 6 February 2006).

Palumbi, S. R. & Cipriano, F. (1998) Species identification using genetic tools: the value of nuclear and mitochondrial gene sequences in whale conservation. *The Journal of Heredity*, 89(5), 459–464.

Pérez-Bañón, C., Rojo, S., Ståhls , G. & Marcos-García, M.A. (2003) Taxonomy of European *Eristalinus* Fabricius, 1805 (Diptera: Syrphidae) using larval morphology and molecular data. *European Journal of Entomology*, 100, 417–428.

Popov, G.V. (2000) A new synonym of *Merodon tricinctus* (Diptera, Syrphidae). *Vestnik Zoology*, 34, 99–102.

Prendini, L. (2005) Comment on "Identifying spiders through DNA barcodes". *Canadian Journal of Zoology*, 83, 481–491.

Rojo, S., Ståhls, G., Pérez-Bañón, C. & Marcos-García, M.A. (2006) Testing molecular barcodes: Invariant mitochondrial DNA sequences vs the larval and adult morphology of West Palaearctic *Pandasyopthalmus* species (Diptera: Syrphidae: Paragini). *European Journal of Entomology,* 103, 443–458.

Rondani, C. (1845) Sulle specie Italiane del genere *Merodon*. Memoria decimoquarta per servire alla ditterologia Italiana. *Nuovi Annali delle Scienze Naturali (Bologna)*, 4, 254–267.

Rubinoff, D. & Holland, B.S. (2005) Between the two extremes: Mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and systematic inference. *Systematic Biology*, 54, 952–961.

Rubinoff, D. (2006) Utility of Mitochondrial DNA Barcodes in Species Conservation. *Conservation Biology*, doi:10.1111/j.1523-1739.2006.00372.x

Sack, P. (1913) Die Gattung *Merodon* Meigen (*Lampetia* Meig. olim.). *Abhandlungen der Sencken-bergischen Naturforschenden Gesellschaft*, 31, 427–462.

Sack, P. (1932) Syrphidae. *In*: Linder, E. (Ed.): *Die Fliegen der Paläarktischen Region. Band IV / 6.* E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, pp. 1–451.

Savolainen, V., Cowan, R.S., Vogler, A.P., Roderick, G.K. & Lane, R. (2005) Towards writing the encyclopaedia of life: an introduction to DNA barcoding. *Philosophical Transactions of the Royal Society of London B Biological Sciences*, 360, 1805–1811.

Scheffer, S.J. & Wiegmann, B. M. (2000) Molecular Phylogenetics of the Holly Leafminers (Diptera: Agromyzidae: Phytomyza): Species Limits, Speciation, and Dietary Specialization. *Molecular Phylogenetics and Evolution*, 17(2), 244–255.

Scheffer, S.J., Lewis, M.L. & Joshi, R.C. (2006) DNA barcoding applied to invasive leafminers (Diptera: Agromyzidae) in the Philipines. *Annals of the Entomological Society of America*, 99 (2), 204–210.

Shaw, K.L. (2002) Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: What mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 16122–16127.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. (1994) Evolution, weighting and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain-reaction primers. *Annals of the Entomological Society of America*, 87, 651–701.

Smith, M.A., Fisher, B.L. and Hebert, P.D.N. (2005) DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar. *Philosophical Transactions of the Royal Society of London B Biological Sciences*, 360, 1825–1834.

Smith, M.A., Woodley, N.E. Janzen, D.H., Hallwachs, W. & Hebert, P.D.N. (2006) DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *Proceedings of the National Academy of Sciences of the United States of America*, 103(10), 3657–3662.

Sperling, F. (2003) DNA barcoding: Deux et machina. *Newsletter of the Biological Survey of Canada (Terrestrial Arthropods)*, 22 (2), 50–53.

Ståhls, G. & Nyblom, K. (2000) Phylogenetic analysis of the genus *Cheilosia* (Diptera, Syrphidae) using mitochondrial COI sequence data. *Molecular Phylogenetics and Evolution*, 15, 235–241.

Ståhls, G. (2006) Placement of *Cacoceria* and phylogenetic relationships of the xylotine genera od the tribe Milesiini (Diptera, Syrphidae: Eristalinae) based on molecular characters. *Zootaxa*, 1171, 17–29.

Ståhls, G., Hippa, H., Rotheray, G., Muona, J. & Gilbert, F. (2003) Phylogeny of Syrphidae (Diptera) inferred from combined analysis of molecular and morphological characters. *Systematic Entomology*, 28, 433–450.

Ståhls, G., Vujic, A., Stuke, J.-H., Doczkal, D. & Muona, J. (2004) Phylogeny of the genus *Cheilosia* and the tribe Rhingiini (Diptera, Syrphidae) based on molecular and morphological characters. *Cladistics,* 4, 1–17.

Symondson, W.O.C. (2002) Molecular identification of prey in predator diets. Molecular Ecology, 83, 137–147.

Wheeler, Q.D. (2005) Losing the plot: DNA "barcodes" and taxonomy. *Cladistics*, 21, 405–407.

Whiteman, N.K., Santiago-Alarcón, D., Johnson, K.P. & Parker, P.G. (2004) Differences in straggling rates between two genera of dove lice (Insecta: Phthiraptera) reinforce population genetic and cophylogenetic patterns. *International Journal of Parasitology*, 34, 1113–1119.

Will, K. & Rubinoff, D. (2004) Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics*, 20, 47–55.

Will, K.W., Mishler, B.D. & Wheeler, Q.D. (2005) The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biol*ogy, 54, 844–851.