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DNA BARCODING

COI barcodes for identification of *Merodon* hoverflies (Diptera, Syrphidae) of Lesvos Island, Greece

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Abstract

DNA barcoding has become a useful system for linking different biological life stages, and for identification of species within a known taxonomic framework. In this study, we generated mitochondrial DNA COI barcodes using adult specimens of all 22 species of the hoverfly genus *Merodon* (Diptera, Syrphidae) occurring on Lesvos island (Greece). The generated COI barcodes could well discriminate between all *Merodon* taxa of Lesvos, except for *M. loewi* and *M. papillus* that shared the same haplotype, despite their clear morphological differences. In addition, the barcodes revealed two cases of hitherto unknown morphologically cryptic species close to *M. avidus* and *M. nigritarsis*, respectively. Because only few successful rearings of immature stages of *Merodon* hoverflies are available, the larval host plant remains unknown for these phytophagous taxa. The obtained COI barcode library for the *Merodon* spp. of Lesvos will constitute a tool to link any unknown immature stages with already known species, and thus provide important life-history information and promise for ecological studies.

Keywords: COI, DNA barcoding, geophytes, Lesvos, *Merodon*

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Introduction

The hoverfly genus *Merodon* Meigen 1803 (Syrphidae, Diptera) is distributed over the Palaearctic and Afrotropical biogeographical regions. More than 50 species show a distribution restricted to the Mediterranean region alone. A still higher species number is known from the steppes of Eastern Europe and beyond, with over 60 species known from Turkey (Marcos-Garcia *et al*. 2007 and references therein). A taxonomic monograph of the genus that is under preparation (Vujić *et al*. in prep. 1) confirms that the total number of *Merodon* species is presently 160.

Merodon belongs to the subfamily Eristalinae, and together with its sister group genus *Eumerus* Meigen, 1822 constitute the tribe Eumerini. The adults of the genus *Merodon* are medium- to large-sized hoverflies (7–23 mm), mostly with dark ground colour (sometimes with metallic lustre) and reddish or pale abdominal colour pattern and/or pilosity of thorax and abdomen. Distinctive characteristics of adult *Merodon* flies include a triangular projection beneath the distal part of the hind femora and vein R_{4+5} curving deeply into cell R5. If the above-mentioned diagnostic characters are overlooked, large *Merodon* species may be confused with syrphid bumblebee mimics of various genera, e.g. species genus *Eristalis* Latreille, 1804, whereas small and slender species could be confused with *Eumerus* spp. The adult flies are thermophilous, usually found flying through the vegetation, often close to the ground, or resting on stones and bare soil (Hurkmans 1993). The recent study of the Iberian *Merodon* by Marcos-Garcia *et al*. (2007) described eight species new to science and showed that the Iberian Peninsula hosts many endemic species of this genus.

The hoverflies stand next to bees in importance as pollinators of wild flowers and crops (Free 1993; Proctor *et al*. 1996). *Merodon* species are particularly associated with the pollination of bulbous plants, both wild and cultivated (Petanidou 1991). A recent study exploring gut contents of different taxa of the *Merodon ruficornis* group and other co-existing syrphid groups, confirmed the exclusive preference

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Fig. 1 The Island of Lesvos in the Eastern Aegean Sea, Greece.

for pollen of *Ornithogalum* spp. (Liliaceae) by several taxa of the *M. ruficornis* group, as compared with the other studied syrphid groups (P. Radisic, personal communication).

The immature stages of very few *Merodon* species have been described so far (< 10). All of them are known to develop in underground bulbs or rhizomes of geophytes belonging to the families Liliaceae, Amaryllidaeceae and Hyacinthaceae (Hurkmans 1993; Rotheray 1993; Stepanenko & Popov 1997). Stepanenko & Popov (1997) described the biology of the immature stages of *Merodon nigritarsis* Rondani, 1845 on specimens reared and studied under laboratory conditions, but they also did careful observations in the field. They found that *M. nigritarsis* females deposit their eggs in the ground next to *Hyachinthella pallasiana* (Hyacinthaceae), and first instar larvae enter the plant's bulbs to feed on the fresh bulb tissue. Furthermore, there are very few published observations of oviposition of *Merodon* spp. They include Hurkmans' (1988) observations on female *Merodon loewi* van der Goot, 1964 exhibiting egg-laying behaviour on *Ornithogalum* plants, and a *Merodon avidus* (Rossi, 1790) specimen ovipositing in the rosette of a flowering plant of *Muscari* sp. (Liliaceae) (Reemer & Goudsmits 2004). These two *Merodon* species together with the mentioned plant genera are abundant on Lesvos. Observational data on the larval host plant exists only for the *Merodon nigritarsis* species occurring on the island of Lesvos, but based on the above-cited information it seems likely that bulb plants are the hosts for all *Merodon* spp.

Merodon of Lesvos

Lesvos is the biggest island in the north Aegean, Greece and the third largest in the whole Aegean after Crete and Evvoia (size 1632.8 km^2; Fig. 1). The landscape is quite diverse, consisting of Mediterranean habitats, mainly pine forests (dominated by *Pinus brutia* Ten. and, to a lesser extent, by *P. nigra* Arnold), scrub (dominated by evergreen shrubs such as *Quercus coccifera* L., *Pistacia lentiscus* L. and *Olea europaea* L. var. *sylvestris*, with regular occurrence of low scrub or phrygana dominated by *Cistus* spp., *Thymus capitatus* (L.) Hofmanns & Link., and *Sarcopoterium spinosum* (L.) Spach), managed and semi-abandoned olive groves, oak woodlands (mixed communities of deciduous oaks) and a chestnut forest (*Castanea sativa* Miller). The climate is typical Mediterranean with hot and dry summers and cool and wet winters. The island, as well as the entire Aegean area, is very rich in geophytes. In a single phryganic plant community near Athens, geophytes constituted 20.3% of the 133 plant species with conspicuous flowers (Petanidou *et al*. 1995). A study of the flora of Lesvos showed that geophytes *sensu lato* constituted 14.8% of the entire flora of the island (Bazos 2005). This fits well with the known distribution and diversity of *Merodon* in the Mediterranean area (Dirickx 1994; Mengual *et al*. 2006; Marcos-Garcia *et al*. 2007).

Systematic faunistic surveys of Syrphidae on the island of Lesvos through different field campaigns using different collecting methods were carried out during the years 2001–2005 (described in Vujić *et al*. 2007), and found 106 species of hoverflies occurring on the island. Additional collecting trips were made by the authors in different seasons between 2006 and 2008 when mostly hand-netting was used. As a result, the total number of *Merodon* species of the island is, thus, presently 22 including two species new to science recently published from the island (Vujić *et al*. 2007), and five species identified as species new to science and awaiting description (Vujić *et al*. in prep. 1 & 2). Given the large collecting effort, i.e. employing various methods repeated during different years and seasons and the large size of the flies facilitating easy observation both in the field and when sorting insect catches, we expect few additions to the species list.

A 650-bp fragment of the 5′-end of the mitochondrial COI gene is proposed as a universal barcode. Using a generated barcode library, the COI barcode sequences is estimated to eventually facilitate the identification of all Metazoan species (Hebert *et al*. 2003; Hebert *et al*. 2004). The aim of the present study was therefore to generate barcodes for all *Merodon* species occurring on Lesvos based on morphologically identified adult specimens. Extracting DNA from eggs or 1–3 instar larvae of *Merodon* that could be obtained from plant bulbs thus facilitates the association of the unknown immature sample with the adult fly, by comparison of their COI barcode sequences with the barcodes here generated. Thus, as unknown immature stages of these taxa most certainly will become available to us with our ongoing increased efforts to search for eggs and larvae in plant bulbs, our aim was to evaluate if COI barcodes will demonstrate their utility to discern the *Merodon* species of the island of Lesvos.

Materials and methods

Merodon sampling

The *Merodon* material obtained from all the collecting undertaken on the island of Lesvos during years 2001–2008 was identified using a morphological identification key of the *Merodon* species Lesvos (see Supporting Information). Specimens were deposited in the insect collections of CEUA (University of Alicante, Spain) and of the Melissotheque of the Aegean (University of the Aegean, Mytilene, Greece).

Out of the 22 species occurring on Lesvos, specimens for sequencing were available for 21 species, whereas for the 22nd species, i.e. *Merodon pruni*, we used an individual collected in the Turkish mainland. Multiple specimens for each species were used when possible (Table 1). DNA voucher species occurring on Lesvos and were used for molecular specimens were deposited in CEUA (University of Alicante, Spain) and MZH (Zoological Museum of the Finnish Museum of Natural History, Helsinki, Finland).

Molecular analysis

DNA was typically extracted from two legs of the adult fly (sometimes including also part of the abdomen) using the Nucleospin Tissue DNA extraction kit (Machery-Nagel) following manufacturer's protocols and resuspended in 50 μL of ultra-pure water. The Folmer fragment or 'barcode fragment' of the 5′ region of COI was amplified using standard polymerase chain reaction protocols with forward primer LCO (5′-GCTCAACAAATCATAAAGATATTGG-3′) and reverse primer HCO (5′-TAAACTTCAGGGTGACC AAAAAATCA-3′) (Folmer *et al*. 1994). The fragments were sequenced using the same primers and the Big Dye Terminator version 1.1 cycle sequencing kit (Applied Biosystems). All sequences were submitted to EMBL as COI barcodes, for accession numbers see Table 1.

The COI barcode sequences were clustered using both parsimony and neighbour-joining analyses. Alignment of COI sequences was trivial due to lack of insertions or deletions and was done by eye. *Cheilosia scutellata* (Fallen, 1817) (tribe Rhingiini), was used as outgroup. Parsimony analysis was performed using NONA (Goloboff 1999) spawn with the aid of Winclada (Nixon 2002), using the heuristic search algorithm with 1000 random addition replicates (mult*1000), holding 100 trees per round (hold/100), maxtrees set to 100 000 and applying tree-bisection–reconnection branch swapping. All base positions were treated as equally weighted characters. The NJ tree was calculated using PAUP version 4.0b8 (Swofford 2001) using the Kimura 2-parameter model (Kimura 1980), with stepwise random addition and 1000 replicates. Non-parametric bootstrap resampling values were calculated for each tree using 1000 replicates and 10 random addition replicates per bootstrap replicate.

Results

DNA sequences

We obtained a 650-bp sequence from all *Merodon* taxa except for *M. hamifer*, for which we could only obtain a shorter barcode of 445 nucleotides. In average, 1–5 specimens/taxon were sequenced. The observed intraspecific variability was low, i.e. 1–3 haplotypes/species differing by 0–4 nucleotide changes (Fig. 2). The AT content was 69.4%. Uncorrected interspecific pairwise divergences ranged from 1.54% between *M. nigritarsis* and *M. femoratoides*, as well as between *M. avidus* and *M.* sp. nova 2, to 12.77% between *M. erivanicus* and *M. spinitarsis*. Kimura 2-parameter distances were 2.68% and 13.94% for the same comparisons, respectively.

Analyses

The COI 5′-fragment comprised 650 nucleotides for 44 ingroup specimens (21 taxa), and a partial barcode for one species (see above). The number of parsimony-informative characters was 182. The parsimony analysis resulted in two equally parsimonious trees of 603 steps length with a consistency index of 0.48 and retention index of 0.74, and the result is shown in a strict consensus tree (Fig. 2). The resulting neighbour-joining tree is shown in Fig. 3.

Species identification vs. species delimitation

The COI barcodes produced for the identification of the *Merodon* species were successful in the identification of almost all taxa presently known to occur on Lesvos Island. We found only one conflicting case, i.e. in the species *M. papillus* and *M. loewi*, where the COI barcodes were identical for both species, despite the taxa being morphologically well defined. Yet, the COI sequences revealed the presence of one morphologically cryptic taxon (*Merodon* sp. nova 2, Fig. 2) within the *M. avidus* complex. A more detailed study of the samples showed some morphological characters that separated the taxa (See Supporting Information). Uncorrected pairwise difference was 2.77% between *M. avidus* and the *M.* sp. nova 2. Two other *Merodon* taxa were identified as new to science during this study (*M.* sp. nova 3 and 4, Fig. 2), and these are both morphologically (see Supporting Information) and molecularly (Figs 2 and 3) well corroborated. *Merodon* sp. nova 5 was identified as new to science already previously based on specimens collected

Table 1 List of specimens used for molecular work including GenBank Accession numbers. NS = not submitted

Species	Lab code, sex	Collecting locality ('m' values are metres above sea level)	GenBank Accession no. COI -5′	Collecting method
Merodon albifrons Meigen, 1822	MZH_S534 male	Greece, Lesvos, 1.25 km S Pigi, 140 m, 39°10′14N, 26°25′11E, 9–11.V.2004, Dahm, Lamborn, Messinger leg. Aegean university 0004112	FM206495	Yellow pantrap
Merodon albifrons Meigen, 1822	MZH_Y706 female	Greece, Lesvos, 4 km SSE Agiassos, chestnut forest area, 8.V.2007, G. Ståhls leg.	FM206510	Hand-netted
Merodon avidus (Rossi, 1790), var. A *sensu* Milankov *et al*. 2001	MZH_S409 male	Greece, Lesvos, Vatoussa, 20–28.IV.2001, Rojo & Pérez-Bañon leg.	FM206488	Malaise trap
Merodon avidus (Rossi, 1790), var. A *sensu* Milankov *et al*. 2001	MZH_Y560 male	Greece, Lesvos, 4 km SSE Agiassos, chestnut forest area, 9.V.2007, G. Ståhls leg.	FM206517	Hand-netted
Merodon avidus (Rossi, 1790), var. A *sensu* Milankov *et al*. 2001	MZH_Y561 male	Greece, Lesvos, 4 km SSE Agiassos, chestnut forest area, 9.V.2007, G. Ståhls leg.	FM206518	Hand-netted
Merodon clunipes Sack, 1913	MZH_S583 male	Greece, Lesvos, Agiassos, 10.VI.2005, Pérez-Bañon-Bañon, Rojo & Ståhls leg.	FM206519	Hand-netted
Merodon erivanicus Paramonov, 1925	MZH_S408 male	Greece, Lesvos, Vatoussa, 5–12.V.2001, Rojo & Pérez-Bañon	FM206487	Malaise trap
Merodon erivanicus Paramonov, 1925	MZH_S411 female	Greece, Lesvos, Sigri, 30.IV.-10.V.2001, Rojo & Pérez-Bañon	FM206512	Malaise trap
Merodon femoratoides Paramonov, 1925	CEUA_A521 male	Greece, Lesvos, 2005	NS	Hand-netted
Merodon hamifer Sack, 1913	MZH_S523 male	Greece, Lesvos, 9.1 km S Mytilene, 130 m, 39°02′19″N, 26°35′58″E, 11–13.V.2004, O. Messinger leg. Aegean university 0006981	NS	Hand-netted
Merodon hamifer Sack, 1913	CEUA_A517 female	Greece, Lesvos, 4.6 km N Plomari; 600 m, 39°01′17″ N, 26°22′28″E, 10–15 year burn. Transect #8, 12.VI.2004, M. Greenwell. Aegean university 0012905	FM206520	Hand-netted
Merodon loewi van der Goot, 1964	MZH_S540 male	Greece, Lesvos, 9.1 km S Mytilene, 130 m, 39°02′19″N, 26°35′58″E, 05.IV.2004, O. Messinger leg. Aegean university 0012716	FM206516	Hand-netted
Merodon longicornis Sack, 1913	MZH_S520 male	Greece, Lesvos, Agiassos 10.VI.2005, Pérez-Bañon, Rojo & Ståhls leg.	FM206497	Hand-netted
Merodon nanus Sack, 1931	MZH_S458 male	Greece, Lesvos, 10.0 km S Mytilene, 100 m, 39°′00′51N, 26°35′26E, 9–11.V.2004, Dahm, Lamborn, Messinger leg. Aegean university 0004287	FM206490	Hand-netted
Merodon nanus Sack, 1931	MZH_S541 male	Greece, Lesvos, 2 km NE Achladeri, alt. 70 m, 39°10′05″N, 26°17′40″E, 9.V.2004, Leg. O. Messinger. Aegean university 0012870	NS	Hand netted
Merodon nanus Sack, 1931	MZH_S542 female	Greece, Lesvos, 10.0 km S Mytilene, 100 m, 39°00′51N, 26°35′26E, 9–11.V.2004, Dahm, Lamborn, Messinger leg., Aegean university 0004285	FM206515	Hand-netted
Merodon nigritarsis Rondani, 1845	MZH_S533	Greece, Lesvos, Agiassos, 10.VI.2005, stream valley, Pérez-Bañon, Rojo & Ståhls leg.	FM206494	Hand-netted
Merodon papillus Vujić *et al*. 2007	MZH_S407 male	Greece, Lesvos, Vatoussa, 20–28.IV.2001, Rojo & Pérez-Bañon leg.	FM206486	Malaise trap
Merodon papillus Vujić *et al*. 2007	CEUA_S412 female	Greece, Lesvos, Sikaminia, 24.IV.-12.V.2001,Rojo & Pérez-Bañon leg.	FM206513	Malaise trap
Merodon pruni (Rossi, 1790)	MZH_S577	Turkey, Erzurum Askale-Bayburt, 2500 m, 40°02N, 40°28E, 20.7.2005. Stuke leg; 1017	FM206498	Hand-netted
Merodon sapphous Vujić *et al*. 2007	MZH_S462 female	Greece, Lesvos, 3.8 km SSE Agiassos; 760 m, 39°3′17N 26°23′50E; chestnut forest. 12:00–13:00, 26.IX.2004, C. Pérez-Bañon leg., Aegean university 0013038	FM206491	Hand-netted
Merodon serrulatus Wiedemann in Meigen, 1822	MZH_S531 male	Greece, Lesvos, Agiassos, 6.VI.2005, Pérez-Bañon, Rojo & Ståhls leg.	FM206493	Hand-netted
Merodon serrulatus Wiedemann in Meigen, 1822	MZH_S575 male	Greece, Lesvos, Agiassos, X.2006, C. Pérez-Bañon leg.	FM206504	Hand-netted
Merodon serrulatus Wiedemann in Meigen, 1822	MZH_Y701 male	Greece, Lesvos, 4 km SSE Agiassos, chestnut forest area, 8.V.2007, G. Ståhls leg.	FM206502	Hand-netted

Table 1 *Continued*

Species	Lab code, sex	Collecting locality ('m' values are metres above sea level)	GenBank Accession no. COI -5′	Collecting method
Merodon serrulatus Wiedemann in Meigen, 1822	MZH_Y702 female	Greece, Lesvos, 4 km SSE Agiassos, chestnut forest area, 8.V.2007, G. Ståhls leg.	FM206503	Hand-netted
Merodon species nova	CEUA_S519	Greece, Lesvos, 4.6 km N, Plomari, 600 m, 39°01′17″N, 26°22′28″E, 14.07.2004.	NS	Hand-netted
Merodon species nova 1	CEUA_S522 male	Greece, Lesvos, Sikaminia, 24.VII-2.VIII.2001, Rojo & Pérez-Bañon leg.	NS	Malaise trap
Merodon species nova 1	MZH_S536 male	Greece, Lesvos, Agiassos, 10.VI.2005, stream valley, Pérez-Bañon, Rojo & Ståhls leg.	NS	Hand-netted
Merodon species nova 2	CEUA_S532 male	Greece, Lesvos, 4.6 km N Plomari; 600 m, 39°01′17″N, 26°22′28″E, 10–15 year burn. Transect #8, 14.Vii.2004, H. Dahm. Aegean university 0012781	NS	Hand-netted
Merodon species nova 3	CEUA_S567 male	Greece, Lesvos, 5.4 km SSE Agiassos; 760 m, 39°3′17″N, 26°23′50″E, mixed maquis, 06.X.2005, C. Pérez-Bañon leg., Aegean university 0023670	NS	Hand-netted
Merodon species nova 3	CEUA_S568	Greece, Lesvos, 5.4 km SSE Agiassos; 760 m, 39°3′17″N, 26°23′50″E, mixed maquis, 06.X.2005, C. Pérez-Bañon leg., Aegean university 0023650	NS	Hand-netted
Merodon species nova 4	CEUA_S569 male	Greece, Lesvos, 5.4 km SSE Agiassos; 760 m, 39°3′17″N, 26°23′50″E, mixed maquis, 06.X.2005, C. Pérez-Bañon leg., Aegean university 0023647	NS	Hand netted
Merodon species nova 4	MZH_S574 female	Greece, Lesvos, Agiassos, VI.2005, Pérez-Bañon, Rojo & Ståhls leg.	NS	Hand-netted
Merodon species nova 5	MZH_Y789 female	Greece, Lesvos, Haramida Beach, 22.10.2008, A. Vujic leg.	NS	Hand-netted
Merodon species nova 5	MZH_Y791 female	Greece, Lesvos, Pyrgi Thermi, 23.10.2008, G. Ståhls leg.	NS	Hand-netted
Merodon species nova 5	MZH_Y792	Greece, Lesvos, Lisvorio hot springs area, 24.10.2008, G. Ståhls leg.	NS	Hand-netted
Merodon spinitarsis Paramonov, 1929	MZH_S410 male	Greece, Lesvos, Vatoussa, 20–28.IV.2001, Rojo & Pérez-Bañon	FM206489	Malaise trap
Merodon spinitarsis Paramonov, 1929	MZH_Y703 male	Greece, Lesvos, 4 km SSE Agiassos, chestnut forest area, 8.V.2007, G. Ståhls leg.	FM206505	Hand-netted
Merodon testaceus Sack, 1913	MZH_S406 male	Greece, Lesvos, Sikaminia, 10-17.V.2001, Rojo & Pérez-Bañon	FM206511	Malaise trap
Merodon testaceus Sack, 1913	CEUA_S518 female	Greece Lesvos, SSE Agiassos, alt. 600 m, 39°4′5″N 26°23′17″ E, 15.05.2004.	FM206500	Hand-netted
Merodon testaceus Sack, 1913	MZH_S521 male	Greece, Lesvos, Sikaminia, 24.IV-2.V.2001, Rojo & Pérez-Bañon leg.	FM206501	Malaise trap
Merodon testaceus Sack, 1913	MZH_Y700 male	Greece, Lesvos, 4 km SSE Agiassos, chestnut forest area, 9.V.2007, G. Ståhls leg.	FM206499	Hand-netted
Merodon velox Loew, 1869	MZH_S537 male	Greece, Lesvos, 2.0 km SSE Agiassos, 600 m, 39°4′9″N, 26°23′17″E, open pine forest, 15.V.2004, T. Petanidou leg. Aegean university 0008668	FM206496	Hand-netted
Merodon velox Loew, 1869	MZH_Y704 female	Greece, Lesvos, 4 km SSE Agiassos, chestnut forest area, 9.V.2007, G. Ståhls leg.	FM206506	Hand-netted
Merodon velox Loew, 1869	MZH_Y705 male	Greece, Lesvos, 4 km SSE Agiassos, chestnut forest area, 8.V.2007, G. Ståhls leg.	FM206507	Hand-netted
Merodon velox Loew, 1869	MZH_Y732 female	Greece, Lesvos, Sifneos, 30.4.2008, G. Ståhls leg.	FM206508	Hand-netted
Merodon velox Loew, 1869	MZH_Y733 female	Greece, Lesvos, Sifneos, 30.4.2008, G. Ståhls leg.	FM206509	Hand-netted

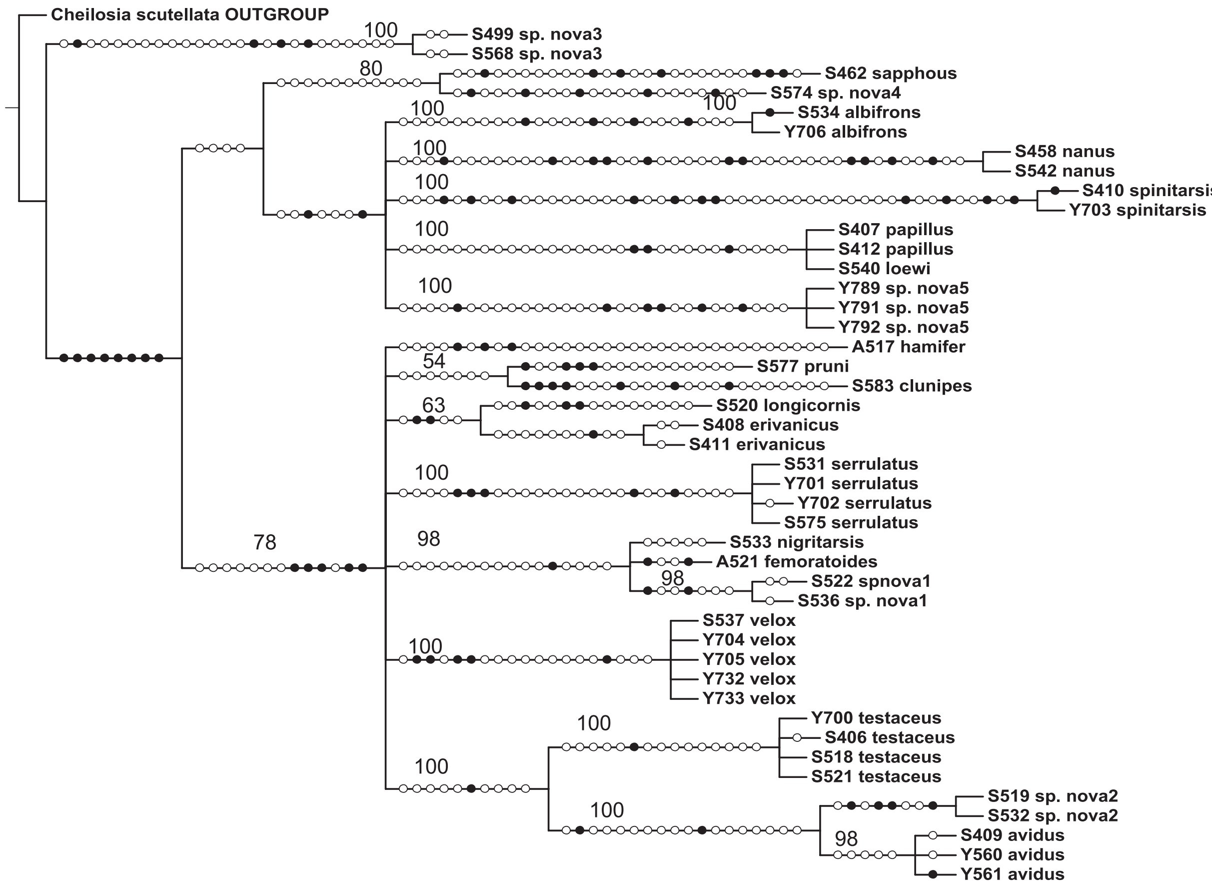

Fig. 2 Strict consensus tree of 18 equally parsimonious trees resulting from parsimony analysis of the barcode fragment (650 nt). Length 634 steps, Consistency index (CI) = 0.46, retention inxex (RI) = 0.75. Bootstrap values are indicated above branches. Filled circles denote unique changes, open circles non-unique.

on different Mediterranean islands and in Turkey (Vujić *et al.* in prep. 2). It differs from its sister species *M. geniculatus* by 3.51%.

Discussion

Performance of the COI barcodes in identification

Discovery of cryptic species is one of the goals that can be achieved with DNA barcodes. The short sequence of a single marker cannot alone define species, but it can help in their discovery. The present study revealed high taxonomic congruence between information obtained from the molecular characters and that based on morphological characters. Barcodes readily identified the taxa (Figs 2 and 3), but also revealed potential problems for, e.g. *Merodon loewi*, a taxon widespread in the Eastern Mediterranean area. Previous studies in the Balkan Peninsula showed that *M. loewi* shared the same COI haplotype with *Merodon armipes*, although morphologically these taxa are well defined (Milankov *et al*. 2008). On the island of Lesvos, on the other hand, barcodes of *M. loewi* are identical to *Merodon papillus* that is also morphologically easily distinguished from *M. loewi* (see morphological identification key, Supporting Information). All taxa belong to the *ruficornis*-group of taxa comprising eight species in the Mediterranean area. In this case, the COI barcode fails to discriminate the taxa in question, and species identification must be verified using morphology and/or additional gene regions that express species specific genotypes. The suggested reasons for identical haplotypes between taxa are retained ancestral polymorphism or mitochondrial introgression between the taxa. This particular species group requires a separate study, as a few sequences of a widespread species are not likely to be representative of the molecular variability of the species as a whole. Relating the feeding ecology of the *ruficornis* group of species with the expressed COI diversity could provide interesting results.

Utility of COI for discovery of hidden diversity

Smith *et al.*'s (2006) test of the barcoding concept in Diptera applied COI data to a diverse assemblage of *Belvosia* Robineau-Desvoidy, 1830 parasitic flies (Tachinidae). Their

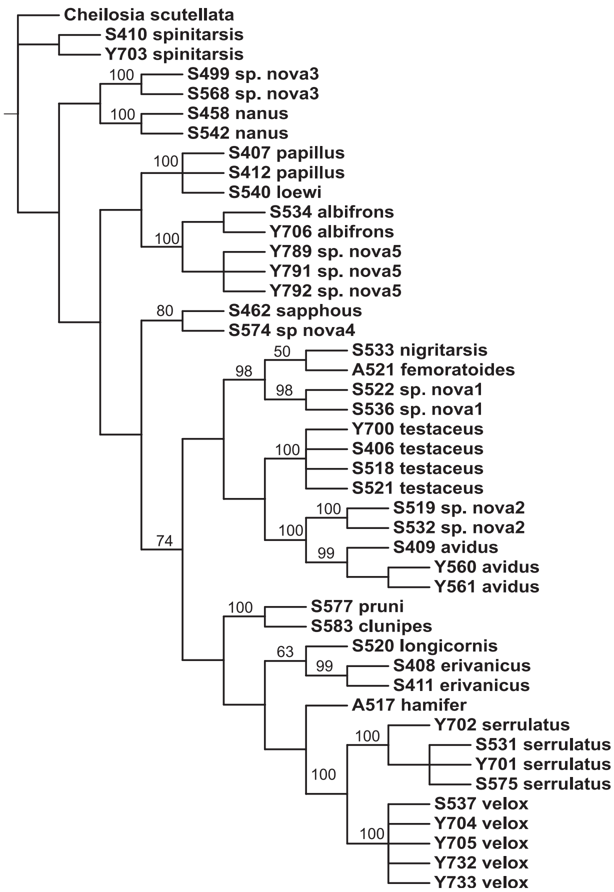

Fig. 3 Neighbour-joining tree reconstructed based on Kimura 2-parameter model. Bootstrap values are indicated above branches.

conclusion was that morphological concepts were largely supported by molecular data, but that the morphology alone could not unravel the true diversity hidden within some species groups. A previous study of *Merodon* spp. of the Iberian Peninsula showed both conflict and congruence between taxonomic information from molecular and morphological characters, and similarly revealed hidden diversity within a few species groups (Mengual *et al*. 2006). In this study, barcodes also revealed morphologically cryptic taxa. *Merodon nigritarsis*, *M. femoratoides* and *M.* sp. nova 1 as well as *M. avidus* and *M.* sp. nova 2 showed different COI sequences (Figs 2 and 3), but taxa within these two clusters are morphologically very close to almost inseparable, particularly in the female sex. In the present study, DNA barcoding showed its utility in revealing taxonomic information and helped to bring to light differences that were not expressed by morphology. But we stress the importance of using character evidence from different sources, such as morphological and ecological data, and molecular evidence from more than one molecular locus for taxonomic work aiming at species delimitation (e.g. Sperling 2003; Dayrat 2005).

Molecular barcodes can be useful for speeding up rates of discovery, especially in circumstances where linking different developmental stages through larval rearing is time-consuming or coupled with low success rate. Thus, DNA barcoding can represent a particularly useful tool in this specialized setting of insect–plant relationships on the geographically restricted area of Lesvos and the Aegean in general. The utility of barcodes may indeed be the highest in already established and restricted taxonomic and/or geographical settings, as demonstrated in the present study (see also, e.g. Mikkelsen *et al*. 2007).

Biodiversity and conservation issues

Merodon species can be important pollinators for plants, sometimes even as specialists as was shown by Peterson *et al*. (2007), and particularly for geophytes. On the island of Lesvos with a very high diversity and densities of geophytes, the presence of similarly specialized *Merodon* pollinators interlinked with larval–host plant specialized relationships is highly probable, as the *Merodon* species constitute a striking 20% of the hoverfly fauna of the island. In comparison, the fifth largest Greek island of Chios (Fig. 1) harbours 15 species of *Merodon* (Taylor 2003), and from the island of Sardinia (Italy), 14 species of *Merodon* (15% of total Syrphidae) were recorded (Mason *et al*. 2006; Speight 2006). Turkey is also rich in both geophyte plants and *Merodon* species (*c*. 19% of Syrphidae) (Marcos-Garcia *et al*. 2007). Barcoding can therefore bring part of the information necessary to study questions that have high value for biodiversity and conservation studies. Integrating COI barcoding into morphological and ecological studies of *Merodon* spp. throughout the Mediterranean region will eventually enable identification of samples of all the life stages using molecular and/or morphological characters. The recently described *Merodon sapphous* Vujic, Radenkovic & Pérez-Bañon and *M.* sp. nova 4 of this study belong to the *aureus* group of species for which a very high number of localized endemic species are known from around the Mediterranean Basin (Vujić *et al*. 2007, in prep. 2). A high level of endemism has important implications for conservation, which further prompts us to carry out studies on the ecology of the *Merodon* species of Lesvos, in addition to survey the *Merodon* fauna and its genetic variation of nearby islands and the mainland of Turkey.

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Supporting Information

The following supporting information is available for this article:

Key for Lesvos species of genus *Merodon*

Figs S1–8 Right antenna, inner view (basoflagellomere and pedicel); 1. *Merodon longicornis*; 2. *M. erivanicus*; 3. *M. testaceus*; 4–5. *M. pruni*; 6. *M. albifrons*; 7–8. *M.* sp. nova 3; 1, 4, 6, 7 males; 2, 3, 5, 8 females.

Figs S9–13 Left hind femur and tibia, lateral view; 9. *Merodon serrulatus*; 10. *M. clunipes*; 11. *M. loewi*; 12. *M. papillus*; 13. *M.* sp. nova 5; 9, 10 females; 11–13 males.

Figs S14–16 Male genitalia, left surstylus, lateral view; 14. *Merodon serrulatus*; 15. *M. clunipes*; 16. *M. erivanicus*; x, posterior surstyle lobe; y, anterior surstyle lobe; c, lateral protuberance.

Figs S17–24 Male genitalia; 17–20. left surstylus, lateral view; 21–24. theca; 17, 21. *Merodon testaceus*; 18, 22. *M. nigritarsis*; 19, 23. *M. femoratoides*; 20, 24. *M.* sp. nova 1; d, basal projection of theca; e, lingula.

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