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Identification of the most common predatory hoverflies of Mediterranean vegetable crops and their parasitism using multiplex PCR

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Abstract The larvae of many hoverflies (Diptera: Syrphidae) are important polyphagous predators used in integrated pest management programs. Because the accurate identification of preimaginal stages by morphological characters is difficult, we have developed a multiplex PCR to identify the immature and/or adult stages of the most common syrphid species in Mediterranean vegetable crops: *Episyrphus balteatus*, *Scaeva pyrastri*, *Eupeodes corollae*, *Meliscaeva auricollis*, *Sphaerophoria scripta,* and *Sphaerophoria rueppellii*. The latter two species were amplified by the same primer pair due to the high similarity of their cytochrome oxidase subunit I sequences. Additionally, the assay included a primer pair targeting *Diplazon laetatorius*, a common koinobiont ichneumonid endoparasitoid of predatory syrphid larvae. The multiplex PCR assay proved to be highly specific and sensitive, and it was used to study the assemblage of hoverfly species in larval stage in two Mediterranean lettuce crops in two consecutive years. The molecular analysis revealed that *Eu. corollae*, *Ep. balteatus*, and *Sph. scripta/Sph. rueppellii* were the species present in the investigated fields. Species composition differed depending on sampling date and whether the larvae were collected on the plants or on the ground. The parasitoid *D. laetatorius* was not detected in any of the analyzed hoverfly larvae, suggesting low-parasitism pressure in the studied syrphid populations. The wide distribution of most of these syrphid species makes this multiplex PCR assay an ideal tool to deepen our knowledge on the ecology of these polyphagous hoverfly species in preimaginal stages and to improve the use of hoverflies to control insect pests.

Keywords Syrphidae · COI · *Diplazon laetatorius* · Molecular species identification · Lettuce crops · Diagnostic PCR

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Introduction

Hoverflies (Diptera: Syrphidae) are an abundant group of insects present in natural and agriculture related ecosystems. With about 750 species recorded in Europe (Speight 2011), at least 355 species are recorded from Spain (Marcos-García et al. 2002). Their adults provide crucial ecosystem services as important pollinators, obtaining their energy requirements by feeding on nectar and pollen (Haslett 1983; Branquart and Hemptinne 2000; Jauker et al. 2012). The larvae of about 35 % of the species of the family of syrphids are polyphagous predators of a broad range of soft-bodied arthropods, including coleopteran and lepidopteran larvae, mites, thrips, and hemipterans (e.g.,

coccids, psyllids, whiteflies and particularly aphids) being the preferred prey (Rojo et al. 2003; Rotheray and Gilbert 2011). Predatory larvae of many syrphid species hide under bark or underneath soil particles during the day and are mostly active at dawn and dusk. This behavior makes them less conspicuous than other natural enemies (Hagen et al. 1999). In Spain, 124 syrphid species with predaceous larvae have been reported by Marcos-García et al. (1998), most of them commonly found in the Mediterranean basin (Speight 2011). Some predatory hoverflies are abundant in different agroecosystems such as fruit orchards, woodlands, grasslands, scrublands, as well as in arable and vegetable crops (Ghahari et al. 2008; Haenke et al. 2009; Hopper et al. 2011). Less than 30 syrphid predaceous species had been related to herbaceous plants at the Iberian Peninsula (Rojo and Marcos-García 1998; Rojo et al. 2003). Six of these hoverfly species are commonly found in Mediterranean vegetable crops such as lettuce (Rojo 1995; Pascual-Villalobos et al. 2006; Morales et al. 2007): *Episyrphus balteatus* (De Geer), *Scaeva pyrastri* (Linnaeus), *Eupeodes corollae* (Fabricius), *Meliscaeva auricollis* (Meigen), *Sphaerophoria scripta* (Linnaeus), and *Sphaerophoria rueppellii* (Wiedemann).

Syrphid larvae may be attacked by a wide range of hymenopteran parasitoids belonging to the families Ichneumonidae, Encyrtidae, Pteromalidae, Megaspilidae, and Figitidae (Scott 1939; Rotheray and Gilbert 2011). However, the most common endoparasitoids of predatory species belong to the family Ichneumonidae and the subfamily Diplazontinae (Bordera et al. 2000, 2001). Particularly *Diplazon laetatorius* (Fabricius) has been reported as the most important natural enemy of hoverflies in terms of abundance of the taxon around the world (Greco 1997; Jankowska 2004). This species is a koinobiont endoparasitoid that oviposits into the syrphid eggs or first instars larvae, with the imago emerging from the syrphid puparium (Mayadunnage et al. 2009).

Although the larvae of many hoverfly species are important biocontrol agents (Rojo et al. 2003; Hopper et al. 2011), it is quite difficult to obtain accurate identification of preimaginal stages, particularly in the first larval stages, using exclusively morphological characters (Bastian 1986; Laska et al. 2006). Moreover, larvae of many species are unknown and the color pattern of common species it is not retained after preserving them in ethanol (Rotheray 1993). Rearing field-collected larvae to the adult stage is recommended for a correct morphological identification (Gilbert 1993), however, this is a time-consuming process which can be accompanied by a high mortality (Jankowska 2004). At the same time, it is also difficult to discriminate between parasitized and nonparasitized hoverfly larvae to assess how parasitoids may impact hoverfly larval populations and their biocontrol success (Hazell et al. 2005). Hence, an alternative technique is needed which allows identifying hoverflies in their larval stage and to detect parasitism by *D. laetatorius*.

To date, two DNA-based approaches are most widely used for species identification: (i) DNA barcoding (Hebert et al. 2003), where species-specific sequences are generated and identified via a reference database (e.g., Mengual et al. 2008; Stahls et al. 2009; Benefer et al. 2013), or (ii) diagnostic PCR where species-specific primers may be used either individually in one PCR amplifying just one target species (singleplex PCR) or simultaneously in a multiplex PCR which enables the parallel identification of several species (King et al. 2011; Staudacher et al. 2011). While the former approach can be limited by the sequence barcode information available in databases such as GenBank or Bold to identify the sequence, the latter technique, is particularly useful once species-specific primers have been developed and when large sample numbers have to be screened because it is cost-effective and quick. Results obtained by multiplex PCR are usually not corrupted by the presence of endoparasitoid DNA, which can be a problem when using the barcoding approach because the mixture of different sequences may foil species identification (Traugott et al. 2013). On the other hand, multiplex PCR can only identify those taxa for which primers have been developed, which means that this approach needs to be carefully checked for cross-reactivity to ensure accurate results.

The aims of this study were: (1) to design species-specific primers for the six most common hoverfly species found in Mediterranean vegetable crops, as well as the parasitoid *D. laetatorius*; (2) to embed these primers in a multiplex PCR assay to easily and rapidly identify these syrphid species including the detection of parasitoid DNA; and (3) to use this molecular tool to identify which of these hoverflies species are present in larval stage in two Mediterranean lettuce crops in two consecutive years as well as to assess the levels of parasitism by *D. laetatorius*.

Materials and methods

Insects

Twenty hoverfly species commonly present in European agricultural environments (Table 1) were used for designing species-specific primers targeting the most common hoverfly species found in Mediterranean vegetable crops: *Ep. balteatus*, *Sc. pyrastri*, *Eu. corollae, M. auricollis, Sph. scripta*, and *Sph. rueppellii*. These specimens were collected in several locations of Spain and Germany (Table 1).

Table 1 Syrphid, potential prey, and parasitoid species tested in the specificity test with the hoverfly- and parasitoid-specific primers described in Table 2

Order	Family	Species	Location (country)
Diptera	Syrphidae	*Dasysyrphus albostriatus* (Fallén)	Butenbock (G)
		Epistrophe nitidicollis (Meigen)	Butenbock(G)
		Episyrphus balteatus (De Geer)	Ruthe (G), Cabrils, lab rearing (S)
		Eupeodes corollae (Fabricius)	Ruthe (G), Alicante (S)
		Eupeodes lucasi (Marcos-García&Laska)	Alicante (S)
		Eupeodes luniger (Meigen)	Niedernwöhren (G)
		Melanostoma mellinum (Linnaeus)	Ruthe (G)
		Melanostoma scalare (Fabricius)	Ruthe (G)
		Meliscaeva auricollis (Meigen)	Alicante (S)
		Meliscaeva cinctella (Zetterstedt)	Ruthe (G)
		Paragus tibialis (Fallén)	Alicante (S)
		Platycheirus albimanus (Fabricius)	Ruthe (G)
		Platycheirus clypeatus (Meigen)	Ruthe (G)
		Scaeva albomaculata (Macquart)	Niedernwöhren (G)
		Scaeva pyrastri (Linnaeus)	Niedernwöhren (G), Alicante (S)
		Scaeva selenitica (Meigen)	Niedernwöhren (G)
		Sphaerophoria rueppellii (Wiedemann)	Valencia (S)
		Sphaerophoria scripta (Linnaeus)	Ruthe (G),Valencia (S)
		Syrphus ribesii (Linnaeus)	Valencia (S)
		Xanthandrus comtus (Harris)	Valencia (S)
Hemiptera	Aphididae	*Nasonovia ribisnigri* (Mosley)	Madrid, lab rearing (S)
		Aphis gossypii (Glover)	Madrid, lab rearing (S)
		Aulacorthum solani (Kaltenbach)	Madrid, lab rearing (S)
		Hyperomyzus lactucae (Linnaeus)	Madrid, lab rearing (S)
		Macrosiphum euphorbiae (Thomas)	Madrid, lab rearing (S)
		Myzus persicae (Sulz.)	Madrid, lab rearing (S)
Thysanoptera	Thripidae	*Frankliniella occidentalis* (Pergande)	Cabrils, lab rearing (S)
		Thrips tabaci Lindeman	Cabrils, lab rearing (S)
Collembola	Entomobryidae	*Entomobrya* sp.	Cabrils (S)
Hymenoptera	Ichneumonidae	***Diplazon laetatorius*** (Fabricius)	Alicante (S)

Target species are highlighted in bold

G Germany, *S* Spain

Sequencing and primer design

A nondestructive DNA extraction method was used to avoid morphological damage to the adult syrphid samples (Staudacher et al. 2011), and a minimum of one adult specimen per species was sequenced. The adult hoverflies were incubated overnight at 58 °C with 180 µl of buffer ATL and 20 µl of Proteinase K (10 mg ml^{-1}, AppliChem, Darmstadt, Germany). DNA was extracted from this solution using the DNeasy Tissue Kit (Qiagen, Hilden, Germany; protocol for animal tissues) following the manufacturer's protocol and stored at −20 °C. One negative extraction control was included in each batch of 30 samples. All syrphids were amplified using the universal primers LC01490/HC02198 described in Folmer et al. (1994), obtaining fragments of the cytochrome *c* oxidase subunit I (COI) gene of approximately 700 bp in length. Each 10 µl PCR contained 1.5 µl of DNA extract, 5 µl of 2× Multiplex PCR Master Mix (Qiagen), 1 µM of each primer, and 1.5 µl of PCR-grade RNase-free water (Qiagen). Thermocycling was done using Mastercycler Gradient PCR machines (Eppendorf, Hamburg, Germany); the thermocycling program consisted of an initial denaturation step of 15 min at 95 °C, followed by 35 cycles of 20 s at 94 °C, 30 s at 52 °C, 45 s at 72 °C, and a 3 min final extension at 72 °C. PCR products were electrophoresed on 1.5 % agarose gels stained with GelRed™ (Biotium, Hayward, USA) and visualized under UV light. PCR products were purified with ExoSAP®-IT (GE Helthcare, Little Chalfont, UK) following the manufacturer's

recommendation and sequenced according to the dideoxychain-termination method. Sequences were aligned and edited manually using Bioedit Sequence Alignment Editor v. 7.0.9.0 (Hall 1999). The obtained sequences were submitted to GenBank database (see Table s1 for accession numbers). These sequences were also aligned with other sequences from the GenBank database (Table s1) using CLUSTALW2 (www.ebi.ac.uk/Tools/msa/clustalw2) and checked for species-specific primer-binding sites. All primer pairs (five for the six hoverfly target species and one for the parasitoid *D. laetatorius*) were designed using Primer Premier 5 (Premier Biosoft International, CA, USA).

Multiplex PCR and specificity assay

All field-collected larval syrphid specimens tested by multiplex PCR were also DNA extracted using the DNeasy Tissue Kit (QIAGEN; protocol for animal tissues). Total DNA was eluted in 100 µl of AE buffer provided by the manufacturer and stored at −20 °C. Two negative extraction controls were added to each set of 28 samples. Multiplex PCR was optimized testing different concentrations of primers and thermocycling conditions. The final reaction volumes (10 µl) contained 1.5 µl of DNA extract, 5 µl of 2× Multiplex PCR Master Mix (Qiagen), 1 µl of 10× primer mix, 1 µl of 5× Q-solution, and 1.5 µl of PCR-grade RNase-free water (Qiagen). Primer concentrations in the primer mix were different depending on the species (see Table 2). In a 2720 thermocycler (Applied Biosystems, CA, USA), the DNA extracts were subjected to 95 °C for 15 min, followed by 35 cycles of 94 °C for 30 s, 64 °C for 90 s, and 72 °C for 60 s and a final extension of 72 °C for 10 min. Target DNA and water were always included as positive and negative controls, respectively. PCR products were separated by electrophoresis in 3.6 % agarose gels stained with ethidium bromide and visualized under UV light.

Primer specificity was evaluated not only by testing the six target hoverfly species, but also the other 14 nontarget hoverfly species (1–4 individuals/species) used for primer design. Additionally, nine potential hoverfly prey species which are commonly found in Mediterranean lettuce crops, including aphids, thrips, and collembolans, as well as the hoverfly parasitoid *D. laetatorius*, were tested (3 individuals/species) (Table 1).

Different concentrations of *D. laetatorius* DNA were analyzed to characterize the sensitivity of the primer pair targeting the parasitoid. The initial DNA concentration tested with the multiplex PCR protocol described above was 0.4 ng/µl which was twofold diluted down to 2.5 pg/µl. DNA concentrations were measured in a Qubit Fluorometer (Invitrogen, CA, USA) using the Quant-iT^{TM} dsDNA HS assay kit (Invitrogen).

Analysis of field-collected hoverfly larvae

Two lettuce fields (var. Maravilla) located in El Maresme area (Barcelona, Spain) were sampled. One was an experimental field at IRTA (41°31′4.33″N, 2°22′37.87″E) and the other one was a commercial field in 50-km distance (41°28′26.07″N, 1°57′34.52″E).

In the experimental field, two consecutive lettuce crops were planted: one from beginning of April until end of May and another from beginning of June until beginning of August, both in 2009 and 2010. Twenty to thirty lettuces were collected on May18th and 19th 2009; July 7th 2009; and May 11th, 18th, and 25th 2010. All lettuces were brought individually in plastic bags to the laboratory, where all syrphid larvae were collected. On May 12th 2009, the experimental field was also manually sampled

Table 2 Syrphid- and parasitoid-specific primer pairs. Columns show target species, primer names (F and R denotes forward and reverse primers, respectively), sequences, product sizes, and the primer concentrations used in the multiplex PCR

Target species	Primer name	Sequence (5′–3′)	Size (bp)	Con. (µM)
Meliscaeva auricollis	Mel-aur-F1	TGAACAGTTTATCCTCCTCTTTCTT	96	0.4
	Mel-aur-R2	TGATGATATACCTGCTAAATGTAAAGAG		
Sphaerophoria scripta/*Sphaerophoria rueppellii*	Sph-rue-scr-F2	GATTATTACCTCCTTCTYTAACATTACTT	165	0.4
	Sph-rue-scr-R1	TTGATGATATTCCTGCTAAATGTAAT		
Scaeva pyrastri	Sca-pyr-F3	TATTTTTTCTCTACATTTAGCTGGTATG	314	0.3
	Sca-pyr-R1	TGGATCTCCTCCTCCTGCA		
Eupeodes corollae	Eup-cor-F2	TGATTATTACCTCCATCTTTAACTCTT	395	0.2
	Eup-cor-R2	GATGATATTCCAGCTAAATGAAGG		
Episyrphus balteatus	Epi-bal-F1	GCAGAACTTGGTCATCCTGGT	754	0.2
	Epi-bal-R1	GGTATTCGATCATAAGTAATTCCATG		
Diplazon laetatorius	Dip-F2	CTGTATATCCCCCTTTATCTTCTAATT	220	0.8
	Dip-R3	GGGAACTGCTAATAATAATAAAATTGT		

once for syrphid larvae found on the ground. In the commercial field, also twenty to thirty lettuces were sampled once on April 22th 2009. All collected larvae were stored at −20 °C until molecular analyses.

All syrphid larvae were individually analyzed by multiplex PCR to study parasitism by *D. laetatorius* and the syrphid larval species composition depending on the sampled season (spring and summer), year (2009 and 2010) and substrate (lettuce or ground). Species percentages were calculated and compared in order to determine whether they were influenced by the season, year, substrate and sample location.

Results

Multiplex PCR and specificity assay

COI sequences of 21 hoverfly species were generated and submitted to GenBank (accession numbers are shown in Table s1). Six specific primer pairs were designed for the hoverflies *Ep. balteatus*, *Eu. corollae*, *M. auricollis*, *Sc. pyrastri, Sph. scripta/Sph. rueppellii* and the parasitoid *D. laetatorius* (Table 2). *Sphaerophoria scripta* and *Sph. rueppellii* were covered by one primer pair as their sequences were very similar (97.6 % sequence identity for a 570 bp long stretch of COI sequence). The hoverfly primers generated DNA fragments ranging from 96 to 754 bp depending on the species (Fig. 1; Table 2). The parasitoid *D. laetatorius* was also detected with the parasitoid primers, amplifying a specific 220 bp fragment. Detection of the parasitoid was possible down to a DNA concentration of 0.4 pg/µl PCR.

When these primers were tested in the multiplex PCR for cross-amplification against the other hoverfly species and potential prey of hoverfly larvae (Table 1), all non-target samples were negative, demonstrating the specificity of the assay.

Analysis of field-collected hoverfly larvae

Diagnostic PCR allowed identifying 169 field-collected syrphid larvae from both fields and years. Only three taxa (*Eu. corollae, Ep. balteatus* and *Sph. scripta/Sph. rueppellii*) were found. Overall, *Eu. corollae* dominated the catches (74 % of all collected larvae), followed by *Sph. scripta/Sph. rueppellii* (14 %) and *Ep. balteatus* (12 %). On the lettuce plants in spring 2009, the species assemblage in the experimental field was very similar to that in the commercial field (Fig. 2). On the ground however, *Eu. corollae* was found almost exclusively when searching for hoverfly larvae on the soil surface. In spring 2010, only *Eu. corollae* and *Ep. balteatus* were captured while in summer 2009, *Sph. scripta/Sph. rueppellii* were the taxa with the highest representation, followed by *Eu. corollae*, whereas, *Ep. balteatus* was only occasionally found (Fig. 2). None of the syrphid larvae tested positive for DNA of the parasitoid *D. laetatorius*.

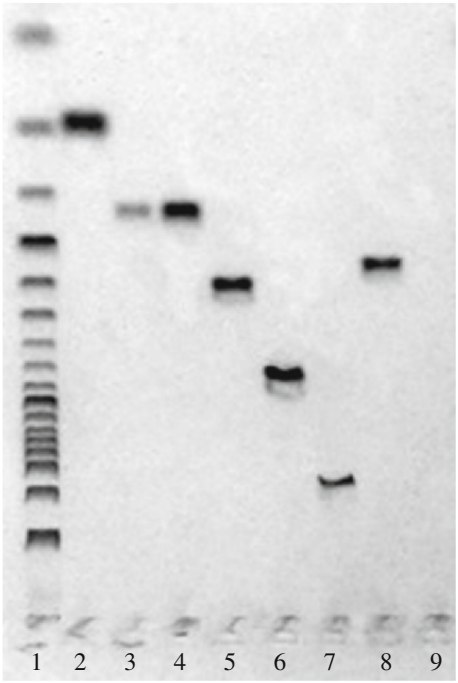

Fig. 1 DNA fragments obtained by multiplex PCR amplification using the specific syrphid- and parasitoid-specific primers. *Lane 1* DNA size marker (50 bp ladder), L2: *Meliscaeva auricollis* (96 bp), *L3 Sphaerophoria scripta* (165 bp), *L4 Sphaerophoria rueppellii* (165 bp), *L5 Scaeva pyrastri* (314 bp), *L6 Eupeodes corollae* (395 bp), *L7 Episyrphus balteatus* (754 bp), *L8 Diplazon laetatorius* (220 bp), and *L9* negative control

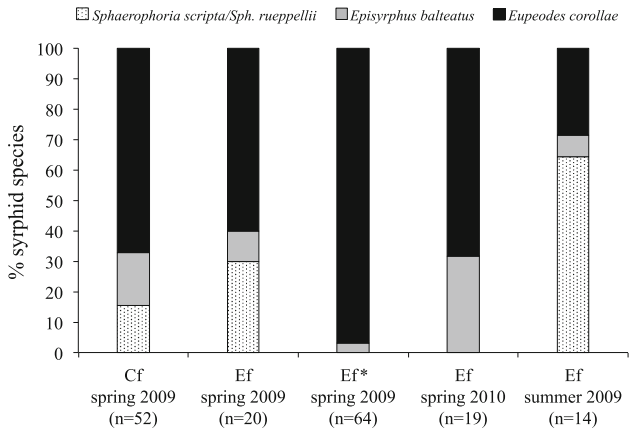

Fig. 2 Syrphid species composition found in two fields (*Cf* commercial field, *Ef* experimental field), two seasons (spring and summer), 2 years (2009 and 2010) and collected either on lettuce plants or on the ground (*asterisk*)

Discussion

The multiplex PCR assay developed in this study allows unambiguous identification of the five most common predatory hoverfly taxa present in Mediterranean vegetable crops. Moreover, the assay includes a primer pair for the parasitoid *D. laetatorius*, a common ichneumon-parasitoid of hoverfly larvae. A primer pair for the parasitoid has been included in the assay because parasitoid eggs and larvae are easily missed when inspecting the hoverfly larvae under a dissecting microscope which can lead to an underestimation of the real parasitism rate (Moreno-Ripoll et al. 2012). Compared to an identification of the larvae via a DNA barcode (Jinbo et al. 2011), the current approach has the advantage that whole body DNA extracts which might also contain DNA of prey and/or parasitoids can be tested. This nonsyrphid DNA would cause problems for DNA barcoding if general invertebrate/metazoan primers are used to generate the COI fragment used as the barcode DNA region. Using Sanger sequencing, sequence-based identification of one type of DNA in the sample is preferable. A mixture of syrphid, prey and/or parasitoid DNAs can lead to unreadable sequences or preferential amplification of parasitoid DNA (Lee and Lee 2012) and prohibit species identification. This could be avoided by using Next Generation Sequencing technologies, which have been also recently used to identify a wide range of insect prey items present in the gut of predaceous syrphid larvae (author's unpublished results).

When the designed primers were tested for specificity, none of the other syrphid species potentially present in Mediterranean vegetable crops nor any other potential prey species yielded false positives. The latter were tested because of the possibility of amplifying prey remains from the gut content of the hoverflies. The lack of amplification demonstrated that the PCR products were exclusive from the syrphid taxa. The assay developed here can be used to identify all developmental stages, and even parts or remains of the targeted species, which makes its possibility of application manifold. In the case of the primers that produce a band smaller than 400 bp [i. e., *M. auricollis* (96 bp), *Sphaerophoria* spp. (165 bp), *Sc. pyrastri* (314 bp), and *Eu. corollae* (395 bp)], they could also be used to test other predators for consumption of these hoverfly species.

When the multiplex PCR assay designed here was used to study the composition of hoverfly larvae communities in Mediterranean lettuce fields, only three syrphid taxa were found: *Eu. corollae*, *Ep. balteatus*, and *Sph. scripta/Sph. rueppellii*. Previous studies conducted also in lettuce crops in Spain confirm these results (Pascual-Villalobos et al. 2006; Morales et al. 2007), being also the main syrphid species found. Other species, such as *M. auricollis* have also been observed in lettuce crops in Spain, but in much less proportion (Rojo and Marcos-García 1998).

The multiplex PCR assay also detected temporal differences in the hoverfly species assemblages. *Eupeodes corollae* and *Ep. balteatus* were more abundant in spring whereas *Sph. scripta/Sph. rueppellii* densities peaked in summer. The same temporal pattern (*Eu. corollae/Ep. balteatus/Sph. rueppellii*) was found in a previous study on aphidophagous syrphid population dynamics in pepper greenhouses in the southeast Spain (Pineda and Marcos-García 2008). *Eupeodes corollae*, *Ep. balteatus*, and *Sph. scripta* are highly migratory species (Speight 2011) that move to Central Europe during summer and the mated females returning to South Europe in autumn (Rotheray and Gilbert 2011). On the other hand, *Sph. rueppellii* is a resident Mediterranean species which is well adapted to high-ambient temperatures (Pineda and Marcos-García 2008; Amorós-Jiménez et al. 2012). In relation with these biological traits, larvae of both species of the genus *Sphaerophoria* were found in Spanish lettuce crops during spring, but only *Sph. scripta* was found in autumn (Morales et al. 2007). For this reason, those syrphid larvae which were collected in summer 2009 in this study and which were assigned by the multiplex PCR approach to the two molecularly indistinguishable species *Sph. scripta/Sph. rueppellii* probably belong to *Sph. rueppellii.*

When analyzing the syrphid larvae collected on the ground, we did not find a complex of syrphid species like on the lettuce plants. Instead, *Eu. corollae* was the most abundant species. *Episyrphus balteatus* and *Sph. scripta/Sph. rueppellii* were hardly and not found on the ground, respectively. This behavior is also related with the preference of these hoverfly species (like most Syrphinae) to pupate on the plant on which their prey occur. However, according to Dusek and Laska (1961), *Eu. corollae* overwinters as pupa, which is unusual for aphidophagous hoverflies (Stubbs and Falk 1983).

From all syrphid larvae analyzed here, none was found to be parasitized by *D. laetatorius,* suggesting that the syrphid populations in the investigated fields did not experience top-down pressure by this endoparasitoid. Note, however, that the current result could also be explained by the comparably low number of syrphid larvae analyzed, as parasitism rates are usually not very high in hoverfly larvae. For example, in lettuce crops, Smith and Chaney (2007) found less than 5 % of parasitism by *D. laetatorius* after analyzing 1,087 syrphid larvae collected in Californian crops. Krawczyk et al. (2011) reported that 3 % of the syrphid pupae inspected ($n = 538$) were parasitized in maize fields in Poland, where the dominant syrphid parasitoid was *Pachyneuron grande* (Hymemoptera: Pteromalidae). In cabbage fields, also in Poland, parasitism by *D. laetatorius* was found as high as 22 % when 410 syrphid

larvae and pupae were analyzed (Jankowska 2004). Lacking parasitoid DNA detection in diagnostic PCRs could also be ascribed to a low sensitivity of the assay (Traugott and Symondson 2008).The sensitivity of the current multiplex PCR for detecting parasitoid DNA, however, is highly comparable to previous assays which allowed detection of eggs and early instar larvae of parasitoids (e.g., Traugott et al. 2006). Therefore, we think that the current results are not due to a methodological artifact but represent a nonexisting/very low level of parasitism of these hoverfly larvae by *D. laetatorius.*

The multiplex PCR approach described here is an efficient tool for the rapid identification of the main hoverfly species present in Mediterranean vegetable crops. Because the larvae of these hoverfly species are known to be important predators of several insect pests, and the species studied in the present study have been identified in other agroecosystems (Jansen 2000; Marshall and West 2007; Sajjad et al. 2008) or forest ecosystems (Kehlmaier and Martínez de Murguía 2004), this molecular method will be particularly useful for further studies on population dynamics, distribution, and abundances of these syrphid species. A molecular tool for detecting *D. laetatorius* parasitism within syrphid larvae has also been described here, allowing to further examine which effect this parasitoid has on syrphid populations and their ability to control pest populations. A better understanding of the identity of the predators and their feeding activities would allow to better conserve key predators in conservation biological programs in vegetable crops.

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