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#### New information about genetic structure of *Bactrocera oleae* species revealed by ISSR markers

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**Abstract:** The olive fruit fly, *Bactrocera oleae*, is a major pest of olive crops and its expansion is restricted to the geographic areas where olive trees are grown. The knowledge of the within and between populations genetic variability can help to understand the history of a species and monitoring the origin and spread of invading populations. Such information could be crucial to define appropriate strategies for eradication or control. In the present work, PCR amplification of inter-simple sequence repeats (ISSR technique) was applied to the analysis of the genetic variability of four Iberian populations of *B. oleae*. Flies from four different geographical areas, representing the Iberian distribution range of the species, were collected by harvesting infested fruit and allowing the larvae to pupate in the laboratory. Four random primers (817, 820, 820, 847, UBC primer set no. 9) were used to assess their genetic variation. The results show considerable levels of genetic polymorphism in the analysed samples, ranging from 78.5% to 82.1%. Regarding the distribution of this variability, most of the genetic variation was found within populations (92.6%). Likewise, a substantial level of gene flow (5.36) was deduced. Thus, ISSR are highly sensitive markers for variability detection in *B. oleae* and could help to answer fundamental questions related to the population structure and dynamics of the fly and, hence, to improve management control.

## Syrphid community in organic olive groves: can morphospecies be used as surrogates for species?

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**Abstract:** Syrphids are known as biological control agents of hemipteran pests and, in the olive grove, several species have been identified as predators of *Euphyllura olivina* (Costa). The objectives of this work were: (1) to study the syrphid community in organic olive groves and (2) to test the potential for the use of syrphid morphospecies as a surrogate for species. The field work was conducted in two organic olive groves located near Mirandela (Northeast of Portugal). The sampling period occurred in two seasons, from the middle of August to the end of October 2009 and from the beginning of April to the end of July 2010. Syrphids adults were separated in morphospecies by a parataxonomist in accordance with their morphological differences. Then, the species were identified by a taxonomist. The number of individuals collected was 64 in 2009 and 27 in 2010, for a total of 91. The morphological identification showed the presence of 12 morphospecies that corresponded to six species: *Episyrphus balteatus* (De Geer) the most abundant with 51.7% of relative abundance, followed by *Sphaerophoria scripta* (L.) with 23.1% and *Eupeodes corolla* (Fabricius) with 18.6%. The proportion of correctly assigned morphospecies to taxonomic species was 3.3%. However, during the identification, a 93.4% splitting and a 3.3% lumping error occurred. This study showed that species identification was relatively difficult when based only on parataxonomic traits, requiring a careful examination of characteristics such as the size of the eyes and their hair, the form of the antennae, the humeral plate and the squama, as well as the femur color and the size of the bands on the abdomen.

**Key words:** Syrphidae, morphospecies, *Episyrphus balteatus*, parataxonomy

### Introduction

In agro-ecosystems, syrphids (Diptera: Syrphidae) are pollinators, important biological control agents against pests and have been used as indicators of agricultural pollution, habitat disturbance and habitat quality (Sommaggio, 1999; Burgio & Sommaggio, 2007). Syrphid larvae are known to attack a wide range of pests, in particular, hemipterans. In the olive grove, the syrphid species *Xanthandrus comtus* (Harris) was described by López-Villalta (1999) as a predator of *Euphyllura olivina* (Costa) nymphs and by Sacchetti (1990) as a predator of larvae of the phylophagous generation of the olive moth, *Prays oleae* (Bernard) (Lepidoptera: Praydidae). Moreover, *Episyrphus balteatus* (DeGeer), *Meliscaeva auricollis* (Meigen), *Scaeva mecogramma* (Bigot), *Epistrophe lineola* Zetterstedt and *Chrysotoxum* sp. were also indicated as predators of *E. olivina* (Pereira *et al.*, 2007). Syrphids are attracted by different species of flowers that provide nectar and pollen to adult stages (Gomes, 1978; Gilbert, 1994; MacLeod, 1999, Dinkel & Lunau, 2001; Katzourakis *et al.*, 2001). Therefore,

the increase of their populations can be successful by creating ecological infrastructures that will provide essential resources to them (Altieri, 1999).

As with other arthropod groups, the identification of Syrphidae species that occur in agro-ecosystems is being affected by the taxonomic skill crisis, with many groups suffering from a taxonomic impediment in terms of identification. In this context, Syrphidae is a very diverse family comprising of approximately 6000 species described worldwide (Sommaggio, 1999; Katzourakis *et al.*, 2001) and the problem of species identification could be reduced by using a parataxonomic approach where morphospecies can be readily separated by morphological differences that are obvious to researchers without extensive taxonomic training. This approach will allow for the fast estimation of richness or biodiversity and can be a cost-effective technique for studies where time and money are scarce or in regions where taxonomic information is limited (Oliver & Beattie, 1996; Derraik *et al.*, 2002; Krell, 2004; Derraik *et al.*, 2010). The aim of this work was to research the reliability of the use of syrphid morphospecies as surrogates of species since, based on our knowledge, this was the first study related with syrphids. Thus, the specific objectives of the present work were: (1) to study the species richness of syrphids in the olive grove; (2) to compare the efficiency of two different approaches: parataxonomic identification through the establishment of morphospecies and taxonomic identification, through the identification of species by a specialist (Alexandre Gomes).

## Material and methods

The study was conducted in two olives groves in organic production located in Cedães (41°29'20.76''N, 7°07'36.02''W) and Valbom dos Figos (41°32'51.07''N, 7°08'41.34''W), near Mirandela (northeast Portugal).

The sampling period occurred in two different seasons, the first in the beginning of August to the end of October 2009 in Cedães using 1.5l bottle traps half-filled with 3% ammonium di-hydrogen phosphate and the second from the beginning of April to the end of July 2010 in Valbom-dos-Figos, using two different methods, bottle traps half-filled with ethanol (70%), water (29%) and detergent (1%) and a hand-net.

Both in 2009 and 2010, bottle traps were hung on a branch of the tree canopy. The bottles were surveyed once a week and all the arthropods trapped were transferred to a clean bottle and carried to the laboratory. The collected syrphids were separated and preserved in Eppendorf tubes with ethanol (70%). The identification process took place in two phases: (1) parataxonomic identification based on morphological characters such as the pattern of the spots of the abdomen, the color and size of the legs and the length of the specimen and (2) taxonomic identification that was done by Alexandre Gomes, a taxonomist of syrphids, and was based on dichotomous keys produced by Séguy (1961) and Gilbert (1986). The comparison between morphospecies and species allowed us to assess the degree of "splitting" (when a taxonomic species was separated into several morphospecies) and "lumping" (when two or more taxonomic species were gathered in one morphospecies) that occurred due to the parataxonomic identification. When one taxonomic species corresponded to one morphospecies the correct separation was 1:1.

## Results and discussion

The total number of individuals collected was 64 in 2009 and 27 in 2010, for a total of 91 syrphids belonging to 12 morphospecies that corresponded to six taxonomic species (Table 1). *E. balteatus* was the most abundant species with 51.7% of relative abundance, followed by *Sphaerophoria scripta* (L.) with 23.1%, *Eupeodes corolla* (Fabricius) with 18.6%, *Syrphus vitripennis* Meigen with 3.3%, *Melanostoma mellinum* (L.) with 2.2% and *Xanthandrus comtus* (Harris) with 1.1%.

Table 1. Number of morphospecies and species of Syrphidae and quality of identified morphospecies.

Morphospecies	Number of individuals	Species	Nr. species: Nr. morphospecies	Result
1	2	<i>Episyrphus balteatus</i> (De Geer)	1:4	Splitting
2	33	<i>Episyrphus balteatus</i> (De Geer)		
3	1	<i>Eupeodes corollae</i> (Fabricius)	1:3	Splitting
4	14	<i>Eupeodes corollae</i> (Fabricius)		
5	11	<i>Episyrphus balteatus</i> (De Geer)		
6	3	<i>Syrphus vitripennis</i> Meigen	1:1	Correct
7	1	<i>Episyrphus balteatus</i> (De Geer)		
8	3	<i>Sphaerophoria scripta</i> (L.)	1:3	Splitting
9	17	<i>Sphaerophoria scripta</i> (L.)		
10	1	<i>Sphaerophoria scripta</i> (L.)		
11	2	<i>Eupeodes corollae</i> (Fabricius)		
12	3	<i>Xanthandrus comtus</i> (Harris) (1) <i>Melanostoma mellinum</i> (L.) (2)	2:1	Lumping

The proportion of correctly assigned morphospecies to taxonomic species was 3.3%. However, during the identification, a 93.4% of splitting and a 3.3% of lumping error occurred.

The main splitting errors occurred when four different morphospecies were identified as being only one species, i.e. *E. balteatus*, three morphospecies belonged to *E. corollae* and three others belonged to *S. scripta*. Lumping occurred only when three specimens belonging to morphospecies 12 were identified by the taxonomist as being two different species, *X. comtus* and *M. mellinum*.

In this study, the separation of syrphid individuals by morphospecies resulted in an overestimation of species richness due to the degree of intraspecific variations. Thus, the parataxonomic separation of syrphids was based on several morphological traits such as the color patterns of the abdomen, the size of the individuals or the structure of the wing (venation and wing areas). However, taxonomic identification revealed that these characters were very different among individuals of the same species, resulting in a high rate of splitting. Moreover, other intraspecific variations such as sexual dimorphism also contributed to the overestimation of species.

A more accurate approach could be achieved by basing the taxonomic identification on specific structures and characters, such as the size of the eyes and their hair, the form of the antennae, the humeral plate and the squama, as well as the femur color and the size and pattern of the bands on the abdomen (Gilbert, 1986). *E. balteatus*, for example, typically has two small black bands on every segment of the abdomen whereas *S. scripta* has a continuous yellow stripe on both sides of the thorax and *E. corollae* has three pairs of yellow commas on its abdomen reaching the outside edge but, even so, they are variable flies. The dissection of genitalia could be necessary if the morphological identification was not enough to identify the species.

This type of approach using parataxonomy to distinguish morphospecies was previously applied to other groups of arthropods and different accuracy rates were obtained. The best results were obtained for the separation of Lepidoptera morphospecies that reached an accuracy rate of 91%, also with a slight overestimation of taxonomic species. This result could be attributed to the great differences between adults of several families and species (Derraik *et al.*, 2002). Considering coleopteran morphospecies, species were correctly identified in 63% of the cases (Derraik *et al.*, 2002). For some families of Coleoptera, such as the family Coccinellidae, the relationship between morphospecies and taxonomic species was 62% (Cotes *et al.*, 2009). In the case of spiders, the frequency of correct separation of species in taxonomic morphospecies was 50% (Derraik *et al.*, 2002). The lowest value was obtained for Hymenoptera with 44% of the morphospecies correctly assigned to species (Derraik *et al.*, 2010).

Although the identification of syrphid morphospecies usually takes less time and cost, these results suggest that their use can produce a substantial error due to the intraspecific morphological variability that exists in this family. To overcome some of the identification errors, the prior training of the non-specialist is of primary importance, and would most probably improve the accuracy of the morphospecies separation. Therefore, using characteristics of the family, such as the size of the eyes and their hair, the form of the antennae, the humeral plate and the squama, as well as the femur color and the size of the bands on the abdomen will increase the reliability of syrphid identification.

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