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3 [First record of the invasive pest \*Drosophila suzukii\* in Ukraine indicates multiple sources of invasion](#)

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21 Abstract

22 *Drosophila suzukii* is an invasive polyphagous fruit pest, which has recently emerged as a global threat to agriculture in  
23 the Americas and in Europe. [Due to the rapid spread, great economic losses and its pest behavior, \*D. suzukii\* represents](#)  
24 [a powerful model for invasion biology and pest management studies.](#) However, despite the current research efforts in  
25 Europe, the invasion routes and current distribution, as well as level of genetic diversity in natural populations of  
26 *D. suzukii* still remains unknown. Here, we present the first report of the occurrence of the *D. suzukii* in Ukraine.  
27 [Moreover, the sequence of a fragment of the cytochrome oxidase I gene was compared with genetic data on this species](#)  
28 [collected from populations in Europe, USA and Asia available in public databases.](#) Our data points to an early stage of  
29 invasion of *D. suzukii* in Ukraine and reveals a comparatively high genetic diversity in the Ukrainian population of this  
30 pest species suggesting a complex invasion scenario from multiple sources. Monitoring the level of genetic variation  
31 across space and time as well as understanding the invasion routes of this major invasive insect pest is essential for  
32 developing successful pest management strategies.

33 Key words: cherry fruit fly; invasive species; [Spotted Wing Drosophila](#); [population genetics](#)

34 Key Message

- 35• [Drosophila suzukii is an invasive agricultural pest that is expanding rapidly worldwide since the late 2000s.](#)
- 36• [Our data represent the genetic pattern at the start of an invasion by \*D. suzukii\* in Ukraine.](#)
- 37• [COI sequence data reveals high genetic diversity in the Ukrainian \*D. suzukii\* population.](#)
- 38• [Our study indicates the potential for multiple sources of \*D. suzukii\* invasion into Europe, along with possible recurrent](#)
- 39 [introductions of this major invasive insect pest.](#)

## 40 Introduction

41 Invasive species represent significant threats to biodiversity, with impacts on non-native habitats potentially having  
42 deleterious ecosystem effects (Crooks 2002; Gurevitch and Padilla 2004) and strong economic burden (Pimentel et al.  
43 2005). *Drosophila suzukii* (Matsumura, 1931), also known as the spotted wing drosophila (SWD), is an invasive,  
44 destructive crop pest, which represents a good example of one of the most severe ongoing biological invasions of the  
45 Western Hemisphere (Asplen et al. 2015).

46 Most drosophilids attack overripe, rotten or damaged fruits, and generally are not considered as pests. Differently,  
47 *D. suzukii* is one of the very few drosophilids that can oviposit in healthy, ripe fruits thus can cause considerable  
48 damage to crops (Mitsui et al. 2006; Lee et al. 2011) resulting one of the most damaging pests of cultivated soft-skinned  
49 tree fruits and berry crops in the temperate regions (Saguez et al. 2013). Economic losses due to *D. suzukii* can be  
50 enormous; for example, within the USA the estimated annual economic losses attributed to crop damage by *D. suzukii*  
51 are in the order of \$700 million (Bolda et al. 2010; Walsh et al. 2011; Wiman et al. 2016). The fast-paced global spread  
52 of *D. suzukii*, accompanied with a severe economic burden, highlights the importance of tracing current distribution and  
53 invasion routes of this novel invasive insect pest.

54 SWD is native to Asia (Kanzawa 1939; Calabria et al. 2012) but, by 2008, *D. suzukii* invasion was simultaneously  
55 reported in both in the USA (California) (Hauser et al. 2009) and in western Europe (Spain) (Calabria et al. 2012).  
56 Since 2008, *D. suzukii* has expanded its non-native range quite rapidly becoming a key pest species throughout the  
57 Americas (Hauser 2011; Deprá et al. 2014; Wang et al. 2016) and in Europe (Cini et al. 2012; Asplen et al. 2015; Arnó  
58 et al. 2016). Despite the invasions has been reported at the same time, genetic data indicate that the invasion to North  
59 America and Europe by *D. suzukii* were independent demographic events (Adrion et al. 2014). Interestingly, levels of  
60 genetic diversity in the North American populations of *D. suzukii* are quite high, but are relatively low in Europe (and  
61 also the island of Hawaii) (Adrion et al. 2014). Low genetic diversity of *D. suzukii* in Europe compared with  
62 populations in the US is hypothesized to be a consequence of a strong genetic bottleneck, yet the idea is based on data  
63 from only one European population from Spain (Adrion et al. 2014). More recent genotype data, based on a large  
64 (n=28) panel of microsatellite loci, showed similar levels of polymorphism and strong genetic differentiation between a  
65 population from France and a population from Hawaii, consistent with the pattern described above (Fraimout et al.  
66 2015). Thus, despite the wealth of genomic data (Chiu et al. 2013; Ramasamy et al. 2016) and present research efforts  
67 in many other fields of *D. suzukii* biology (Hamby et al. 2016), it is still inconclusive whether European *D. suzukii*  
68 populations are characterized by low genetic diversity. Understanding the level of genetic variation in natural  
69 populations of *D. suzukii* has a particular importance from the perspective of developing successful global pest  
70 management strategies (Haye et al. 2016), as genetic diversity often positively correlates with adaptation (Reed and  
71 Frankham 2003; Barrett and Schluter 2008). Moreover, genetic diversity should inform the size and number of  
72 independent and/or recurrent introductions that could be occurring, which would inform appropriate management  
73 actions.

74 Although, *D. suzukii* is the only species of the *Drosophila* group in Europe that is characterized by (1) a large,  
75 pointed and serrated ovipositor in the females, and (2) by a dark spot near the wing tips of adult males, these characters  
76 do not allow unambiguous morphological identification during biodiversity surveys by non-specialists. For example,  
77 the conspicuous male wing spots require up to 2 days to fully develop, and large serrated ovipositor is similar to that of  
78 females of closely-related species such as *Drosophila subpulchrella* (Hauser 2011). Finally, the immature stages cannot  
79 be identified by their morphology. Thus, there is a need to support surveys of *Drosophila* species with genetic data to  
80 provide unambiguous confirmation of the identity of any suspected *D. suzukii* (Murphy et al. 2016). One of the most  
81 widely used methods of identifying of *D. suzukii* is through DNA barcoding; sequence analysis of a fragment of the  
82 mitochondrial *cytochrome oxidase I (COI)* gene (Hebert et al. 2003; Hauser 2011).

83 Here, we report (1) the Drosophilidae species composition of seven localities distributed across Ukraine, with  
84 individuals collected during two years of sampling, (2) the first record of *D. suzukii* from Ukraine, but only in the most  
85 southern locality that was sampled (Yalta, Crimea) and (3) *COI* gene sequence data that, by comparison with sequenced  
86 data available from public databases, indicate high genetic diversity of *D. suzukii* in Ukraine that points to a complex  
87 invasion scenario, potentially from multiple sources. Our study sets the baseline of presence/absence data of this species  
88 for invasion of Ukraine, which may contribute to further monitoring studies of this pest species and to understand its  
89 dispersal behavior. Our study highlights the importance of performing biodiversity surveys and tracking genetic  
90 diversity in natural populations of this major invasive insect pest.

## 91 Materials and Methods

### 92 *Sample collection*

93 Specimens of *D. suzukii* were found during routine biodiversity surveys for *Drosophila* species that were undertaken in  
94 the summer-autumn seasons of 2014 and 2015. The seven sampling sites throughout Ukraine were: Yalta, Odesa,  
95 Uman, Kharkiv, Piryatin, Kyiv and Chornobyl (Fig. 1). According to Köppen classification most of Ukraine is  
96 characterized by a humid continental climate, except for the South of Crimea (*i.e.* the Yalta sample location), which is  
97 subtropical (Köppen W 1931; Kottek et al. 2006).

98 Fig. 1 Geographical distribution of *D. suzukii* sampling localities. (a) World map with sampling localities of *COI*  
99 analyzed specimens derived from GenBank (Online Resource HGB1), denoted with a circles. A star symbol represents  
100 location with specimens sequenced in the present study. Color of the symbols correspond to the sampling locations in  
101 the haplotype network (Fig.2). (b) Seven locations across Ukraine surveyed for *Drosophila* species. Sampling locations  
102 are denoted by open and closed circles, closed circle (Yalta, Crimea) indicates the location where specimens of *D.*  
103 *suzukii* were collected

104 In each locality, flies were netted over four traps that had been baited with 6-7 smashed fermented apples and about 300  
105 ml of commercially available wheat beer (Serga et al. 2015). The baited traps made of thick colorful, flexible plastic  
106 film, were kept at each location for 72 hours. A brief description of the sampled localities and their GPS coordinates can  
107 be found in Serga et al. (2015), with the only difference being the sample site coordinates in Yalta, where sampling  
108 during 2014-2015 was performed in Yalta city center in a private garden that had mixed vegetation, fig tree  
109 (*Ficus carica*) and raspberry (*Rubus idaeus*) nearby (GPS coordinates: 44°30'03.0"N 34°10'00.0"E). Captured flies  
110 were identified on the basis of their external morphology under a 7x-45x stereomicroscope (Konus Crystal, Italy).  
111 Morphological identification of *D. suzukii* was carried out according to Hauser (2011) and putative specimens preserved  
112 at -20°C in 96% ethanol for molecular analyses.

### 113 Molecular methods

114 Genomic DNA was extracted from nine individuals of *D. suzukii* (sampled during summer 2015) using a standard high  
115 salt method, modified from Aljanabi and Martinez (1997). A 693 bp fragment of the *COI* mtDNA gene was amplified  
116 using the universal primers LCO1490 and HCO2198 (Folmer et al. 1994). Polymerase chain reactions (PCR) were  
117 performed in 10 µL reactions containing 2 µL of DNA, 5 µL of 2X DreamTaq Green PCR Master Mix (Thermo  
118 Scientific) and 0.5 µM of both forward and reverse primers. Amplification conditions were: 95°C for 3 min, then 35  
119 cycles of: 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, followed by 72°C for 7 min. For sequencing, the PCR  
120 products were purified with Exonuclease I and FastAP Thermosensitive Alkaline Phosphatase (Thermo Scientific),  
121 cycle sequenced in both directions using BigDye v.3.1 chemistry (Applied Biosystems), and visualized with an  
122 ABI3730 (Applied Biosystems). Sequences were edited manually with BioEdit v.7.2.5 (Hall 1999) and aligned with  
123 MEGA6 (Tamura et al. 2013) and then translated to confirm the absence of stop codons. Representative sequences of  
124 *D. suzukii* were deposited in GenBank (KX268719-KX268727).

125 To infer the genetic relationships of these Ukrainian *D. suzukii* with other native (e.g. Asia) and invasive (e.g. Europe  
126 and the USA) populations of *D. suzukii*, *COI* gene sequences for *D. suzukii* were downloaded from GenBank. We only  
127 used those *COI* sequences that (1) corresponded to the same fragment that we sequenced and (2) whose GenBank  
128 record contained detailed information about the sampling location. We obtained 74 sequences that represented *D.*  
129 *suzukii* samples from Europe (Portugal, Spain, Italy and Serbia), USA (California, Oregon, Washington and Arkansas),  
130 China and Japan (for further details see Online Resource HGB1). A haplotype network was constructed with TCS  
131 v.1.21 (Clement et al. 2000) and edited using tcsBU (TCS Beautifier) (Múrias Dos Santos et al. 2016).

### 132 Results

133 *Drosophila* individuals were collected from seven regions throughout Ukraine, between 2014 and 2015, and identified  
134 as belonging to 14 different *Drosophila* species (see Online Resource SD2). The *Drosophilidae* species fauna of  
135 Ukraine is a quite typical for the Palearctic region (Bächli and Rocha-Pite 1982). *D. suzukii* was collected during  
136 August on both sampling years from Yalta (Crimea) (Table 1), which represents the first record of *D. suzukii* in  
137 Ukraine.

138 Table 1 Sample locations with GPS coordinates, sampling dates, total number of individuals collected (in parentheses)  
139 from the Ukraine during 2014 and 2015

140 Genomic DNA extraction was obtained from nine wild individuals from Yalta collected in 2015. After sequencing  
141 using *COI* universal primers, we identify 4 different haplotypes. Our study of the genetic diversity of *D. suzukii*  
142 individuals from Ukraine was based on a haplotype network that incorporated an additional 74 *D. suzukii* *COI*  
143 sequences available from GenBank (see accession numbers in Online Resource HGB1), with 606 bp of the *COI* gene  
144 used in the final alignment (Fig. 2). The haplotype network uncovered substantial genetic diversity, most of which was  
145 present in samples from *D. suzukii*'s putative native range in Japan. Higher amount of *COI* haplotypes was found in the  
146 samples from Ukraine compared to other European countries, similar in level of diversity to the samples from the USA.

147 The most frequent haplotype in Europe was also found in individuals from Ukraine as well as in samples from Japan.  
148 The second notable feature of our data is that Ukrainian *D. suzukii* COI haplotypes clustered with both invaded by  
149 *D. suzukii* areas, the samples from Europe and with the samples from the USA (both areas invaded by *D. suzukii*). In  
150 addition, it is worth mentioning that there are one haplotype shared between the USA and Chinese *D. suzukii*  
151 individuals only (Fig. 2). One private haplotype was found in samples from Italy. Most interestingly, the other  
152 Ukrainian haplotypes were found in samples from the USA, China and Japan. Similarly, one haplotype from Serbia was  
153 found also in samples from the USA and China (Fig. 2), but not including other European locations.

154 Fig. 2 Haplotype network of COI gene fragment (606 bp) for *D. suzukii* sampled from Ukraine, Europe, the USA, China  
155 and Japan (see GenBank accession numbers and number of samples in Online Resource HGB1). Colors indicate sample  
156 locations. Small white circles represent undetected intermediate haplotypes and each line corresponds to a mutational  
157 step. The area of the circles represent the amount of identical COI gene sequences used in the alignment

## 158 Discussion

159 *Drosophila suzukii* is native to the mountainous temperate regions of East Asia (Kanzawa 1934; Kanzawa 1939; Asplen  
160 et al. 2015) and has likely dispersed to Western countries via trade of fruit by sea (Hauser 2011; Calabria et al. 2012).  
161 Invasion by *D. suzukii* presents a major agricultural problem, and identifying the routes of invasion, particularly the  
162 areas that represent its source, is an important issue from the pest management perspective. Here, we report the invasion  
163 of *D. suzukii* in Ukraine (Yalta, Crimea), with the population having high levels of genetic diversity in contrast to other  
164 populations of *D. suzukii* in Europe.

165 There are apparently two separate invasion routes into Europe and into the USA (Adrion et al. 2014), however the  
166 source (s) of *D. suzukii* for both regions remains unknown. In the USA, the invasion of *D. suzukii* could have occurred  
167 by a large number of founding individuals and/or from multiple sources, as could be inferred from high genetic  
168 diversity and 1 haplotype sharing with both China and Japan. Unfortunately, only 4 COI sequences from *D. suzukii*  
169 from China could be included in our analysis, as most of the *D. suzukii* COI sequence data from China had an  
170 ambiguous origin. In contrast, European *D. suzukii* was, up to now, mostly represented by one predominant haplotype,  
171 with just another haplotype unique to Italy; this pattern is consistent with a substantial bottleneck during the foundation  
172 of European *D. suzukii* populations (Adrion et al. 2014). Interestingly, the new individuals collected from Yalta  
173 (Ukraine) revealed no genetic signature of a sharp bottleneck and shared COI haplotypes with populations in both the  
174 USA and Europe, suggesting multiple sources of invasion into the Ukraine by *D. suzukii*. Alternatively, it is possible  
175 that *D. suzukii* had been established in Yalta for a significant period of time and was introduced one or few times with  
176 many individuals to maintain a genetically diverse pool. While it is hard to determine the route and timing of the  
177 invasion by *D. suzukii* into Crimea, the latter hypothesis seems highly unlikely since all our sampling localities had  
178 been surveyed for *Drosophila* species annually since 2005 (Kozerevska et al. 2008; Radionov et al. 2011; Serga et al.  
179 2014; Serga et al. 2015). The relevance of this survey effort points to the arrival of *D. suzukii* in southern Ukraine close  
180 to the summer of 2014. Thus, it seems more likely that *D. suzukii* was recently introduced to Crimea on multiple  
181 occasions from distinct sources, such as from the two introduced areas (i.e. the USA and Europe) or alternatively, from  
182 the two ancestral areas (i.e. China and Japan).

183 A combination of geographical profiling analyses and data on the trade of fresh fruit made by Cini et al. (2014) suggests  
184 that the most likely arrival location of *D. suzukii* in Europe would be the region surrounding Marseilles, southern  
185 France, from where it would have subsequently spread across Europe. This idea is supported by the first records of  
186 *D. suzukii* in California and in Spain, both occurring close to important sea ports (Rota-Stabelli et al. 2013; Cini et al.  
187 2014), as well as examples of dispersal of the *Drosophila* species that follow commercial fruit trafficking routes (Capy  
188 and Gibert 2004; Lachaise and Silvain 2004). Indeed, our report of *D. suzukii* from Yalta, touristic center which has a  
189 port and is located on the coast of the Black Sea, is concordant with the idea that trade of fruit by sea routes and  
190 travelling industry, presents an important dispersal method by *D. suzukii*. Interestingly, that port in Yalta annually host  
191 hundreds of passenger cruise liners and cargo trade ships from all over the world. Unfortunately, lack of molecular  
192 analysis data for specimens found on multiple occasions in other European countries (Asplen et al. 2015), as well as  
193 often poor description of already submitted to GenBank files might decrease resolution of *D. suzukii* population  
194 genetics studies. Which proves how important is to report the information correctly, since with wide sampling where  
195 the information of the sampling locations is properly stated the future studies can be more complete and precise.

196 The invasion of *D. suzukii* in Ukraine we report in the presents great concern, taking into account the enormous  
197 economic losses that this species has caused in USA (Wiman et al. 2016), and to some extent in Europe (Asplen et al.  
198 2015). Recent invasions of *D. suzukii* in some of Eastern European countries (e.g. Hungary, Czech Republic, Slovakia,  
199 Bulgaria and Serbia) have shown a rapid population expansion after invasion by apparently quite few individuals  
200 (Asplen et al. 2015). For example, since *D. suzukii* was first reported in Serbia in 2014, it rapidly dominated the  
201 drosophilid assemblages across the country (Tosevski et al. 2014). All individuals of *D. suzukii* sampled across Serbia  
202 shared the same haplotype (Tosevski et al. 2014), which was identical to the COI haplotypes identified from Spain,  
203 Portugal, Italy and Japan. However, an additional individual collected from southern Serbia in November of 2014 (that

204 was sequenced after the first report of *D. suzukii* from Serbia, GenBank accession number: KX273434) (Tosevski et al.  
205 2014) shares its *COI* haplotype with *D. suzukii* from the USA and Chinese populations. This again indicates the  
206 potential for multiple sources of *D. suzukii* invasion into Europe, along with potential recurrent introductions of this  
207 highly invasive pest species. Thus, in contrast with other European *D. suzukii* populations as previously argued (Adrion  
208 et al. 2014), *D. suzukii* in Ukraine and Serbia contain comparatively high levels of genetic diversity that potentially  
209 offers a new source of genetic variation in Europe. Establishment of an invasive species often benefits from standing  
210 genetic variation that allows rapid adaptation to new selection pressures (Frankham 2005; Markert et al. 2010; Bouzat  
211 2010). Potentially, high genetic diversity in *D. suzukii* populations in Europe can facilitate this species range expansion  
212 and increase fruit yield losses caused by this invasive insect pest, possible also even lessening the impact of biological  
213 control agents and pest management activities.

214 *Drosophila suzukii* populations dynamics mostly depends on temperature, humidity (Tochen et al. 2014, 2016b; Hamby  
215 et al. 2016), and the availability of essential food resources (Tochen et al. 2016a). The dry Mediterranean climate,  
216 which is similar to that in Yalta, seems not to be preferred by *D. suzukii* (Hauser et al. 2009; Calabria et al. 2012).  
217 However, the apparent absence of *D. suzukii* from continental part of Ukraine may be temporary as these areas have  
218 more suitable conditions (i.e. temperate climate, milder summer temperatures and higher humidity), which significantly  
219 increase fecundity and longevity in *D. suzukii* populations (Tochen et al. 2016b). Therefore, the invasion of this pest  
220 species into more temperate regions of Ukraine and possible recurrent introductions to other parts of Europe pose a  
221 potential threat to agriculture, especially berry crop producers. Currently, it is hard to determine, whether the high level  
222 of genetic diversity in *D. suzukii* populations in Yalta is unique, or whether it is a common pattern across Europe.  
223 Further research applying wide range of multilocus genetic markers and more samples from native or invaded  
224 *D. suzukii* areas is needed to provide more resolution. Future studies can make use of the mitochondrial genes, as well  
225 as microsatellites markers, which have been shown to be a powerful tool to understand invasion routes and genetic  
226 diversity in *Drosophila* natural populations (Pascual et al. 2007; Bahder et al. 2015). Results presented herein contribute  
227 to identification of *D. suzukii* global invasion routes and might help to establish this species as a good model object for  
228 research on invasion biology and pest management.

229 Compliance with Ethical Standards:

230 Conflict of Interest: The authors declare that they have no conflict of interest.

231 Ethical approval: All applicable international, national, and institutional guidelines for the care and use of animals in  
232 research were followed.

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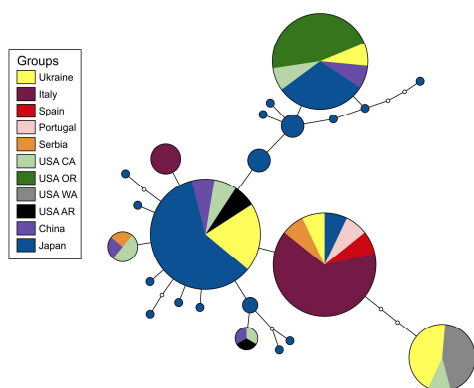
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346  
 347 Fig. 1 Geographical distribution of *D. suzukii* sampling localities. (a) World map with sampling localities of *COI*  
 348 analyzed specimens derived from GenBank (Online Resource HGB1), denoted with a circles. A star symbol represents  
 349 location with specimens sequenced in the present study. Color of the symbols correspond to the sampling locations in  
 350 the haplotype network (Fig.2). (b) Seven locations across Ukraine surveyed for *Drosophila* species. Sampling locations  
 351 are denoted by open and closed circles, closed circle (Yalta, Crimea) indicates the location where specimens of *D.*  
 352 *suzukii* were collected



353  
 354 Fig. 2 Haplotype network of *COI* gene fragment (606 bp) for *D. suzukii* sampled from Ukraine, Europe, the USA, China  
 355 and Japan (see GenBank accession numbers and number of samples in Online Resource HGB1). Colors indicate sample



356 locations. Small white circles represent undetected intermediate haplotypes and each line corresponds to a mutational  
 357 step. The area of the circles represent the amount of identical *COI* gene sequences used in the alignment

Location with GPS coordinates	Yalta		Odesa		Umanø		Kharkiv		Piryatin		Kyiv		Chornobyl		
	44°30'03.0"N 34°10'00.0"E		46°29'13.91"N 30°43'51.59"E		48°45'45.26"N 30°14'38.97"E		49°59'24.30"N 36°13'50.44"E		50°19'35.40"N 32°29'35.62"E		50°21'09.06"N 30°28'57.70"E		51°16'13.73"N 30°13'19.63"E		
Collection date	27 Aug 2014	24 Jun 2015	27 Aug 2015	22 Jul 2014	26 Jul 2015	1 Oct 2014	26 Sept 2015	28 Jul 2014	20 Aug 2015	20 Aug 2014	25 Aug 2015	24 Jul 2014	10 Aug 2015	13 Sept 2014	26 Aug 2015
<i>D. sukukii</i>	11	10	1	-	-	-	-	-	-	-	-	-	-	-	-

358 Table 1 Sample locations with GPS coordinates, sampling dates, total number of *D. sukukii* individuals collected from  
 359 the Ukraine during 2014 and 2015