The African fig fly *Zaprionus indianus*: a new invasive pest in France?

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Abstract

We report here the first observation of *Zaprionus indianus* Gupta in mainland France. Five specimens of both sexes were trapped in a liquid vinegar bait in Cap d'Antibes (French Riviera) and their taxonomic identification was then performed by morphological and molecular analysis. *Z. indianus* is recognized as a fig pest in the invaded American countries, and it could pose economic problems in this new location since figs are also grown in European Mediterranean areas. This Drosophilid species is on the EPPO pest quarantine list. Its spread and possible permanent establishment in South France should thus be monitored in the next years to assess possible agricultural damages.

Key words: Zaprionus indianus, Drosophilidae, African fig fly, striped vinegar fly, invasive pest, France.

Introduction

Terrestrial arthropods are a large part of invasive species (Pimentel et al., 2001; Kenis et al., 2009; Vila et al., 2010; Kenis and Branco, 2010; Vaes-Petignat and Nentwig, 2014; Schindler et al., 2015), and biological invasions are important threats for biodiversity, human health and economy. Although Drosophilidae (vinegar flies) are mainly known as laboratory models, some species, as the invasive Asian fly Drosophila suzukii Matsumura (Walsh et al., 2011; Calabria et al., 2012; Asplen et al., 2015), are agronomic pests. Within the EU project DROPSA ("Strategies to develop effective, innovative and practical approaches to protect major European fruit crops from pests and pathogens"), we set-up a field monitoring in the Alpes-Maritimes (South of France) to evaluate the impact of D. suzukii on local vinegar fly species. Among the drosophilid species trapped on one site (Cap d'Antibes), we surprisingly caught five specimens (3 females and 2 males) that belonged to the exotic species Zaprionus indianus Gupta (Diptera Drosophilidae), also known as the African fig fly in South America and the striped vinegar fly in the USA. Z. indianus originates from the Afrotropical region (Yassin et al., 2008a; Yassin and David, 2010; Bouiges et al., 2013), and it has become established as an important pest of commercial fruits in South and Central America (Vilela, 1999; Tidon et al., 2003; van der Linde et al., 2006). This fly is also invasive in North America where it has been found in different fruits (van der Linde et al., 2006; Joshi et al., 2014). There have been very few reports of Z. indianus presence in Europe (see discussion) and this is the first record of this drosophilid species in the French mainland. Checking its local establishment and tracking its potential geographic expansion will require more field trapping as well as the extension of the survey on wild and cultivated fruits.

Materials and methods

The trapping was performed from the 13th to the 20th January 2016, on a site located in the Cap d'Antibes (43°33'53.8"N 7°07'31.3"E, 40 m a.s.l.), during a winter trapping of Drosophilidae species in the Alpes-Maritimes department (France). This site is moderately urbanized, with many gardens of exotic plants and an arboretum in the vicinity (Villa Thuret). It is characterized by a mild winter, with 4 °C (night) to 10.9 °C (day) average temperatures during the trapping period. The Biobest Droso-traps® (red trap) used have entry holes reduced by a net $(0.5 \text{ mm} \times 0.5 \text{ mm mesh})$, and they are filled with a mixture of 2/3 apple cider vinegar, 1/3 red wine vinegar (ACV). Traps were placed randomly on trees (1.5 m from the ground) and recovered eight days later. The captured insects were filtered from the ACV using a fine-mesh and stored in 100% ethyl alcohol for further identification. All specimens were morphologically identified to genus and species levels.

For molecular analysis, DNA was extracted from the whole fly (two separate individuals) using the prepGEM Insect kit (Zygem), with 3 h of 75 °C incubation time. The mitochondrial Cytochrome Oxidase I (COI) gene was amplified by PCR (Taq PCR Master Mix kit, QIAGEN), in a 25 µl final reaction volume, using the "universal" DNA primers LCO 1490 (forward) and HCO 2198 (reverse) (Folmer et al., 1994) and the following conditions: 15 min denaturation at 95 °C, 40 cycles of 30 s at 95 °C, 1 min 30 s at 50 °C, and 1 min at 72 °C, with a final 30 min extension step at 60 °C. The purity of the amplicon was checked on gel (1.5% Agar) and the PCR samples were sent for sequencing (Genewiz, France). The comparison of nucleotide sequences with sequences available in the NCBI database (GenBank) was performed using Blastn (Altschul et al., 1990) with standard settings.

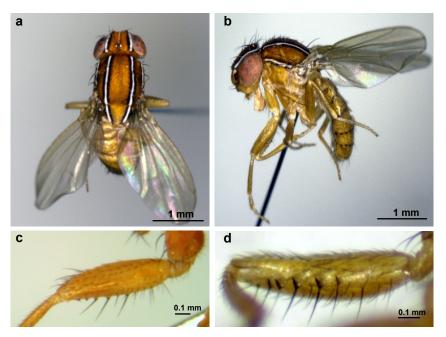


Figure 1. Pictures of a male *Z. indianus* specimen: **a)** dorsal view showing the two silvery-white stripes running from the head bristle to the scutellum and **b)** lateral view of the same specimen; **c-d)** enlarged picture of the forefemur with the very long dark bristles/spines arising from a black spot characteristic of the species. (In colour at www.bulletinofinsectology.org)

Phylogenetic analysis of sequence data was done with the Mega6 software (Tamura *et al.*, 2013). Sequences were aligned manually and phylogenetic trees were built using the neighbor joining (NJ) method (Saitou and Nei, 1987), with Bootstrap values based on 1000 replications. Nucleotide distances in NJ trees were estimated by the Kimura's two-parameters method (Kimura, 1980).

Results

The five specimens (3 females and 2 males) were first identified as belonging to the exotic genus Zaprionus Coquillett, based on the presence of characteristic longitudinal white stripes on the frons and the mesonotum (figure 1a-b) as well as of dark spines at the base on the forefemur (figure 1c-d) (Tsacas and Chassagnard, 1990; Yassin and David, 2010; van der Linde, 2010; see also discussion). The subsequent thorough morphological examination of the individuals with the classification key of Yassin and David (2010) identified them as belonging to the same species, Z. indianus. This belonging was further confirmed by results of the partial sequencing of the COI gene from two individuals. One amplicon was indeed obtained for each individual, from which about 630 bp were accurately sequenced. The two nucleotide sequences were 100% identical, with no stop codon in the predicted partial protein. The best NCBI match (100% identity) was obtained with the sequence gb|KC994629.1| that corresponds to Z. indianus. The nucleotide sequences were then used to build a phylogenetic tree confirming the identification (figure 2).

Discussion

The Zaprionus genus is phylogenetically very close to the genus Drosophila and it includes 59 species: 48 species belong to the Afrotropical subgenus Zaprionus sensu stricto, the other 11 ones being part of the Oriental-Australasian subgenus Anaprionus Okada (Yassin and David, 2010; Yassin et al., 2010).

As all the species included in the Zaprionus subgenus, Z. indianus is native to tropical Africa. However, thanks to an Eastwards migration, it invaded India in the middle of the 20th century and was described from this country, hence its name (Yassin et al., 2008a; 2008b; Commar et al., 2012). About 20 years ago, another independent migration across the Atlantic Ocean led the colonization of America (Yassin et al., 2008b; David et al., 2006). The American colonization is well documented, Z. indianus was first recorded in Brazil (Vilela, 1999) and rapidly spread to South and Central America (van der Linde et al., 2006; Castrezana, 2007; Lavagnino et al., 2008). It is now widely distributed in the USA and even further North in Canada (Joshi et al., 2014; Markow et al., 2014). In Europe, the presence of Z. indianus is poorly documented: old records from Austria (1985), Italy (1988) and Malta (1985) can be found in TaxoDros Database (http://www.taxodros.uzh.ch/), and there is one individual description from Spain (Carles-Tolrá, 2009). Yet, none of these reports provided molecular identification. As said previously, Z. indianus, as all other species of the genus, is a tropical species which is not expected to colonize cold temperate places due to its low tolerance to cold (Araripe et al., 2004). This is in agreement with the extant distribution in middle east, India and South and Central America. However, the

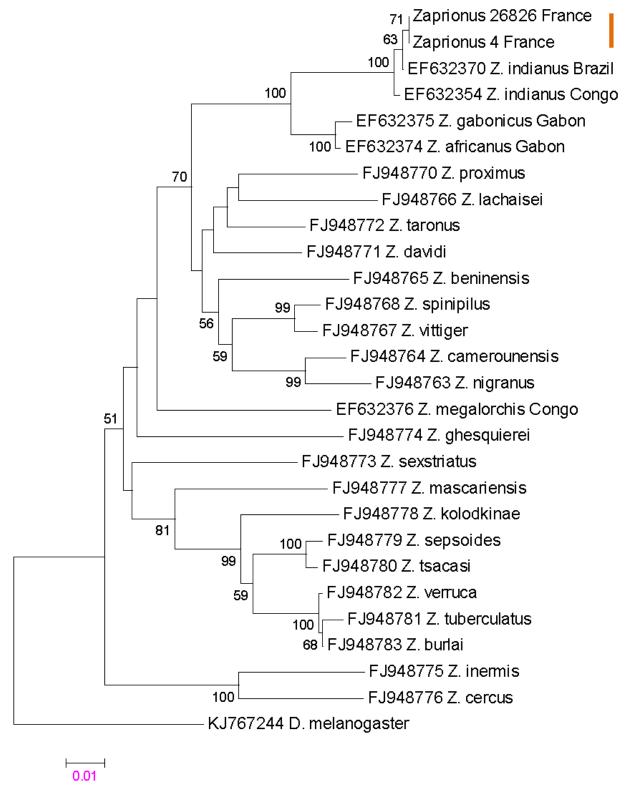


Figure 2: Molecular clustering of the *Z. indianus* French individuals. The neighbor joining (NJ) tree is based on partial COI sequences (French individuals, see the orange line). *Drosophila melanogaster* Meigen was used as an outgroup. Only bootstrap values >50 are shown.

numerous and recurrent observations in cold places, notably in South America, USA and Canada, are puzzling. Generally, these observations were done during the warm season, and they might correspond to an accidental introduction followed by a complete extinction of the propagule due to lower temperatures. In this respect, our collection of Z. indianus during winter in Cap d'Antibes is unexpected and very surprising. It is unlikely that such flies could produce a permanent population because of the low temperature at this time of the year. We must however consider that, in the perspective of a global warming, the climatic conditions in Southern France will become progressively more favourable to the establishment of a permanent population. The capacity of Z. indianus to adapt to different climates is also demonstrated by the phenotypic comparison of African, Indian and American populations. Clinal variations corresponding to an increase of body size were observed in Africa and India, i.e. in countries harbouring long established populations, while no latitudinal variations were found in recent American populations (David et al., 2006). So, a further genetic capacity to adapt to still cooler conditions might be expected (Karan et al., 1999; da Mata et al., 2010).

With respect to its ecology, *Z. indianus* is a generalist, polyphagous and often domestic species, which can breed in many wild and cultivated fruits (Lachaise and Tsacas, 1983; Yassin and David, 2010). Females usually oviposit in decaying or damaged fruits where eggs will develop. Thus, *Z. indianus* may likely develop on a large number of wild fruits in temperate countries making its eradication difficult once established. Male and female can survive up to three months under laboratory conditions

(25°C), and a female produces about 60 offspring (Setta and Carareto, 2005). The generation time is about 20 days so that several generations may occur per year in temperate regions. Although Z. indianus is not considered as an agronomic pest in its native area and in USA, it is not the case in other invaded areas. In Brazil, the fly infests healthy and undamaged fig fruits, causing up to 40% of economic losses on figs crops (Vilela, 1999; Stein et al., 2003; Commar et al., 2012; Joshi et al., 2014). This ability to attack figs forged its name "African fig fly" in South America although oranges and peaches were also reported to be attacked, likely because of particular cultural practices (over-ripening of fruits on trees). The establishment and spread of Z. indianus could thus have significant economic consequences in Europe where about 60,000 tons of figs are grew per year, France contributing to about 4,000 tons (http://faostat3.fao.org; http://agreste.agriculture.gouv.fr/IMG/pdf/R9315C01.pdf). This fly is already considered as harmful in several countries and it was thus included in the EPPO pest quarantine list (https://gd.eppo.int/reporting/article-3252).

It is worth noting that the presence of Zaprionus tuberculatus Malloch, a close species to Z. indianus, was also recently reported in Turkey (Patlar et al., 2012) and Trentino (Italy) (Raspi et al., 2014), suggesting that different members of the Zaprionus genus are currently expanding in Europe. The potential agronomic impact of Z. tuberculatus is still unknown. Yet, Zaprionus species possibly found in sympatry in Europe, including Zaprionus ghesquierei Collart, reported in Cyprus only (Chassagnard and Kraaijeveld, 1991), can easily be distinguished morphologically using the following key:

1	- Forefemur without a row of spines
	- Forefemur with a row of spines wearing long bristles (figure 2c, d)
2 (1)	- Forefemur with a protruding tubercule bearing a bristle; frons with a median white stripe
	- Forefemur without a protruding tubercule; frons without a median white stripe

The expansion of these invasive drosophilid species has likely been facilitated by their biology (polyphagy, high population increase rate and ability to infest fruits previously injured by other species), the global fruit trade (Commar *et al.*, 2012), and possibly the global warming. As already noticed for *D. suzukii*, it will be important to document the impact of these new species on native communities of Drosophila and their associated natural enemies.

For the assessment of both agronomic risks and ecological impacts, we thus recommend a more precise monitoring of *Zaprionus* species in the French Riviera with special emphasis on figs, wild persimmons and prickly pears.

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