



First Record of *Zaprionus tuberculatus* Malloch, 1932 (Diptera: Drosophilidae) in Minas Gerais, Brazil

Marina Magalhães Moreira^{1*} , Luísa de Paula Bouzada Dias¹,
Letícia Carlesso de Paula Sena¹, José Lino Neto^{1,2}, Hermes Fonseca de Medeiros³,
Karla Yotoko^{1,2} 

¹ Universidade Federal de Viçosa, Programa de Pós-graduação em Entomologia, Viçosa, Minas Gerais, Brasil.

² Universidade Federal de Viçosa, Departamento de Biologia Geral, Viçosa, Minas Gerais, Brasil.

³ Universidade Federal do Pará, Campus de Altamira, Faculdade de Biologia, Altamira, Pará, Brasil.

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ABSTRACT

The Afrotropical *Zaprionus* Coquillett, 1902 (Diptera: Drosophilidae) has gained visibility since the 1990s due to the geographic expansion of *Zaprionus indianus* Gupta, 1970. More recently, a second species, *Zaprionus tuberculatus* Malloch, 1932, invaded regions outside its original African regions, causing economic concerns, particularly in Europe. In 2021, *Z. tuberculatus* was captured for the first time in the Americas, specifically in urban parks, and preserved fragments of the Brazilian Cerrado, causing concerns about the competition with native drosophilids. Here we report the occurrence of *Z. tuberculatus* 900 km from its first record in urban and rural areas of Viçosa, MG, but not in forest fragments. Considering the great capacity for dispersion and the potential of *Z. tuberculatus* to compete with native drosophilids species, as well as the potential harm to fruit production when co-occurring with *Drosophila sukukii* (Matsumura, 1931), further studies would be necessary to monitor this invasion and create mechanisms to control it.

In the late 1990s, *Zaprionus indianus* Gupta, 1970 (Diptera: Drosophilidae), a species with high reproductive potential and recognized as a fruit production pest (Pfeiffer et al., 2019), invaded various cultivation areas outside of Africa (Vilela, 1999; Yassin et al., 2008), shedding light to the genus *Zaprionus* Coquillett, 1902, which had previously been limited to tropical Africa.

Another species of the genus *Zaprionus* that has recently drawn attention from researchers and fruit producers is the Afrotropical *Zaprionus tuberculatus* Malloch, 1932 (Kuyulu et al., 2019; Kamel et al., 2020). According to the Invasive Species Compendium, it is an invasive species with the potential to alter the composition of native communities (CABI, 2023). Moreover, this species has high dispersal and adaptation capacity in different habitats and has already been sampled outside its original African regions, causing concerns for fruit production, particularly in Europe (Patlar et al., 2012; Raspi et al., 2014; Leivadaras et al., 2017). In 2021, *Z. tuberculatus* was reported for the first time in the Americas, in urban parks and preserved fragments of the Brazilian Cerrado (Cavalcanti et al., 2022).

In 2022, after observing *Zaprionus* adults perching on *Morus nigra* L. (blackberry) fruits in a resident's backyard in an urban area, we set traps in urban, rural, and fragmented forest areas to capture adults of the genus (see Table 1). We initially searched for specimens of *Zaprionus*, which we diagnosed by white stripes on the head and thorax. As the association of *Z. indianus* and *Drosophila sukukii* (Matsumura, 1931) can potentially damage fruit production (Pfeiffer et al., 2019), we also searched for males of this *Drosophila*, which are easily recognizable by a dark spot on the wings. As soon as we started sorting the traps, we found evidence that we were dealing with more than one species of *Zaprionus*.

The external characters were used to differentiate the two species of the genus *Zaprionus* (see Fig. 1). While *Z. tuberculatus* group species have a tubercle on the first pair of legs (profemoral tubercle) and a mid-frontal stripe, *Z. indianus* lacks these features and has black dots on the insertion of abdominal bristles (Yassin and David, 2010). To differentiate among species of the *tuberculatus* subgroup, we set isofemale lineages to check on the egg projections, the shape of the aedeagus, the length of the testicles, and the surface of the spermatheca of their progeny (*results not shown*) (Yassin and David, 2010). We eventually concluded that we were dealing with only two species: *Z. indianus* and *Z. tuberculatus*.

*Corresponding author.

E-mail: moreira.marina95@gmail.com (M.M. Moreira).

Table 1
Sampling points (GPS coordinates), with their respective description, sampling dates, main plants around the traps, and the number of *Zaprionus indianus* and *Zaprionus tuberculatus* captured in traps baited with banana mixed with *Saccharomyces cerevisiae* yeast (Medeiros and Klaczko, 1999). At each collection point, three traps were placed, which were kept there for 48 hours.

GPS coordinate	Description	Sampling date	Plant	<i>Z. ind</i>	<i>Z. tub</i>
20°45'48"S; 42°51'53"W	UFV preserved area	2016/03	NA	0	0
		2019/03	NA	0	0
		2022/04	NA	0	0
		2022/11	NA	0	0
		2023/02	<i>Ficus carica</i> L.	0	0
20°45'28"S; 42°53'26"W	Urban backyard	2022/11	<i>Morus nigra</i> L.	5	19
20°51'32"S; 42°52'33"W	Rural Area	2022/12	<i>Morus nigra</i> L. and <i>Solanum lycopersicum</i> L.	5	20
20°45'58"S; 42°52'55"W	Urban backyard	2023/02	<i>Malpighia emarginata</i> DC.	12	75



Figure 1 External anatomical details of *Zaprionus tuberculatus* and *Zaprionus indianus* observed using the Zeiss SteREO Discovery.V20 stereomicroscope. Males and females exhibit the same diagnostic characteristics. A. Abdomen of *Z. indianus* (female) showing black spots (BS) at the base of the setae. B. Abdomen of *Z. tuberculatus* (male) without black spots. C. Lateral view of *Z. tuberculatus* highlighting the spur borne or a salient tubercle (T) on the medioventral margin of the forefemur. D. Dorsal view of *Z. tuberculatus* displaying the medium-white stripe at the frons (FS). Scale bars = 0.2 mm.

We also added evidence to the determination of species by sequencing a COI fragment obtained with universal primers that we deposited in GenBank under the ids: OQ869608 and OQ869609.

The number of adults of *Z. indianus* and *Z. tuberculatus* captured in each sampling area and the fruit trees around traps are in Table 1. Among all the sampling points collected over eight different days, we

captured *D. suzukii* (five males) in association with *Zaprionus* species at just one location: the backyard sample taken in November 2022. Unlike Cavalcanti et al. (2022), who found *Z. tuberculatus* in urban and preserved areas, we found no *Zaprionus* specimens in the Atlantic Forest fragment. It indicates that *Z. tuberculatus* may compete with Cerrado native species but has yet to be able to invade Atlantic Forest

fragments, at least in our region during our sampling efforts. It is worth mentioning that previous studies have reported the presence of *Z. indianus* in fragments spanning from the northern to the southern limits of the Atlantic Forest in the states of Pernambuco (Coutinho-Silva et al., 2017) and Rio Grande do Sul (Hochmüller et al., 2010), respectively. This suggests that the areas included in our study should be monitored over time to detect possible invasions by these two species.

It is remarkable that our discovery of *Z. tuberculatus* 900 km from its initial record in Brazil, in a distinct biome, suggests that it has already achieved a wide distribution throughout the country and potentially even across the American continent. Furthermore, our samples revealed that the number of *Z. tuberculatus* specimens is significantly higher than that of *Z. indianus* in all the locations where the species were found. It is also concerning that we found both species of *Zaprionus* along with *D. sukukii* at one of the collection points. In fact, Pfeiffer et al. (2019) demonstrated that when *Z. indianus* co-occurs with *D. sukukii*, the former is able to lay eggs in unripe fruits, suggesting that the co-occurrence of *Z. tuberculatus* with *D. sukukii* has a high potential to impact fruit production (Chireceanu et al., 2015). Consequently, given the potential of *Z. tuberculatus* to compete with native drosophilid species and pose a risk to fruit production, further studies are warranted to monitor its spread and develop mechanisms for its control.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

MMM and KY conceived the study and contributed to the study's design. KY obtained the financial support, supervised the work, and provided guidance throughout the research. MMM, LPBD, and LCPS conducted the fieldwork and collected valuable data. HFM played a critical role in confirming the identification of *Z. tuberculatus*. JLN, MMM, LPBD, and LCPS, under the supervision of JLN, contributed to obtaining the photographs used in the study. MMM initiated the manuscript writing process. KY, LPBD, and LCPS participated in revising subsequent drafts of the manuscript. All authors actively reviewed and approved the final manuscript for publication, and all authors unanimously agreed on all aspects of the work.

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