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Parasitoids of Drosophilidae with potential for parasitism on *Drosophila suzukii* in Brazil.

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Abstract

The spotted wing drosophila is an invading polyphagous species, which causes damage on small stone fruits. In Brazil, the species was detected in 2013. This study registers the occurrence of parasitoids *Leptopilina boulardi* (Barbotin, Carton & Kelner-Pillault) (Hymenoptera: Figitidae) and *Trichopria anastrephae* Lima (Hymenoptera: Diapriidae) on blackberry and strawberry fruits attacked by *Drosophila suzukii*.

Introduction

The spotted wing drosophila, *suzukii* (Matsumura, 1931) (Diptera, Drosophilidae), is a species endemic to Asia, first registered as an invading species in Hawaii in 1980 (Kaneshiro, 1983). It has rapidly spread worldwide in the last few years, arising as one of the main pests of small stone fruits (Asplen *et al.*, 2015). In 2008, it was recorded simultaneously in California, in the United States (Bolda *et al.*, 2010), and several other localities in Europe (Calabria *et al.*, 2012). For South America, records include Brazil (Deprá *et al.*, 2014; Schlesener *et al.*, 2014; Geisler *et al.*, 2015), Uruguay (González *et al.*, 2015), and Chile (Medina-Muñoz *et al.*, 2015).

This species presents a short life cycle and high biotic potential (Emiljanowicz *et al.*, 2014; Tochen *et al.*, 2014), which may lead to a rapid increase in population when environmental conditions are favorable (Wiman *et al.*, 2014) possibly resulting in considerable economic losses to commercial crops (Beers *et al.*, 2011; Walsh *et al.*, 2011). Currently, the main control method applied is chemical (Cancino *et al.*, 2015), wherein several insecticide classes – such as pyrethroids, spinosyns and organophosphates – have been proven effective over *D. suzukii* (Bruck *et al.*, 2011). Nevertheless, the fly's rapid life cycle, which allows for the occurrence of many generations during a single production cycle, and its occurrence in the fruit's ripening phase, require frequent applications endangering human health and the environment via residue in the fruits, insecticide resistance in insects, as well as the negative effects to pollinators and biological control agents (Cini *et al.*, 2012).

Therefore, alternative strategies for the control of *D. suzukii* are demanded. Biological control, particularly by use of parasitoids, may help suppress regional *D. suzukii* population even in crop adjacent habitats (Wang *et al.*, 2016). A great diversity of parasitoids associated with the *drosophila* genus has been recorded (Fleury *et al.*, 2009). Larvae parasitoids are the most recurring ones, especially those from the

Asobara (Braconidae), *Leptopilina*, and *Ganaspis* (Figitidae) genera and pupal parasitoids from the *Trichopria* (Diapriidae) and *Pachycrepoideus* (Pteromalidae) genera (Rohlf and Hoffmeister, 2004; Wertheim *et al.*, 2006; Mitsui and Kimura, 2010). Regarding *D. suzukii*, numerous field and lab studies performed in Europe and North America have reported that there is an association between the insect and several species belonging to the aforementioned genera, although few have been proven effective in regulating population growth (Mitsui *et al.*, 2007; Mitsui and Kimura, 2010; Miller *et al.*, 2015; Nomano *et al.*, 2015). In its native area, the efficiency of indigenous parasitoids has been evaluated, aiming at exploring a classical biological control strategy, by introducing and establishing natural enemies from the fly's original area in the invaded areas (Guerrieri *et al.*, 2016). Another approach to the biological control of *D. suzukii* would be an increase on control agents already present in the recently invaded areas (Cini *et al.*, 2012).

This study aimed to detect the presence of potential biological control agents in four small-fruit producing areas, in the southern region of Rio Grande do Sul, Brazil. By means of Köppen climate classification, the region has a Cfa climate type, characterized by its humid temperate climate (Kottek, 2006), wherein the temperate climate small-fruit crops stand out, such as strawberry, blueberry, blackberry, and raspberry. Of those, strawberry crops are the most representative fruits (Fachinello *et al.*, 2011).

Materials and Methods

The occurrence of potential *D. suzukii* parasitoids has been verified in four small-fruit producing areas during the 2015/2016 crop, places where the presence of the invading species had already been detected via capture traps for adults and insects emerged from previously sampled fruits. The subject areas are located in Pelotas City, Rio Grande do Sul, Brazil, specifically Rincão da Caneleira (31°32'36"S), Cristal (31°35'19"S), Cascatinha (31°38'23"S), and Cerrito Alegre (31°35'12"S). On a weekly basis, blackberry (*Rubus* spp.) fruits were collected from the first three locations and strawberry (*Fragaria x ananassa*) fruits from the last location, respectively.

Fruits were weighed and accommodated individually in plastic containers with a fine layer of vermiculite and a screened orifice at the lid. Fruits were kept in an acclimatized room ($24 \pm 2^\circ\text{C}$, $70 \pm 10\%$ relative humidity and 12h photo phase) until the emergence of flies and/or parasitoids. Emerging insects were aspirated from the containers and placed in Eppendorf tubes containing 70% alcohol for later identification. *D. suzukii* specimens were identified by taxonomical characteristics according to Vlach (2013). Parasitoid identification was performed as established by Costa Lima (1940), Norlander (1980), and Guimarães *et al.* (2003), and voucher species were deposited at the "Oscar Monte" Entomophagous Insect Collection (Biological Institute, Campinas, SP, Brazil; curator: Valmir A. Costa). Parasitoids were transferred to glass tubes (20 mm \times 80 mm) containing *D. suzukii* larvae and pupae originated from laboratory breeding, as to confirm parasitoidism and initiate the breeding of such specimens on said host for later studies (Figure 1). Parasitoidism was allowed for 72 hours and, afterwards, specimens were transferred to Eppendorf tubes containing 70% alcohol following identification based on previous species identification.



Figure 1. *Trichopria anastrephae* females over *D. suzukii* pupae.

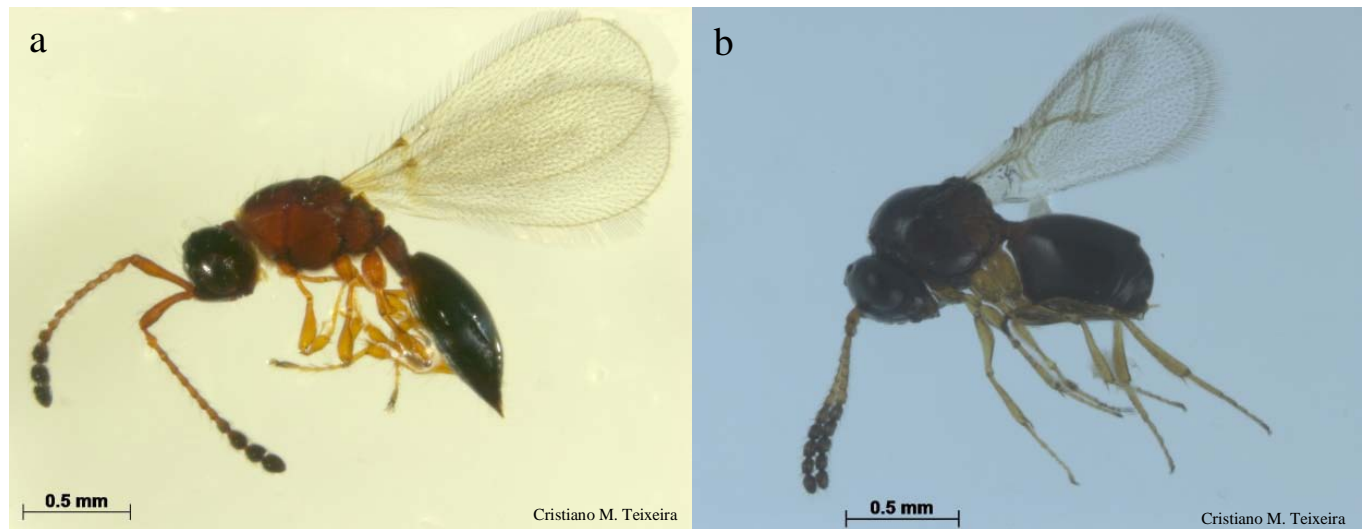


Figure 2. *Trichopria anastrephae* (a) and *Leptopilina boulardi* (b) females.

Results and Discussion

During the study period, 1,582 fruits were collected, out of which 638 were strawberries and 944 blackberries. The recovered parasitoids were *Leptopilina boulardi* and *Trichopria anastrephae* (Figure 2). In addition to *D. suzukii*, other drosophilid species emerged from the infested fruits, suggesting a secondary attack after the initial damage caused by the species. *L. boulardi* occurred in a small proportion in relation to the *T. anastrephae* (Table 1).

Table 1. *D. suzukii*, *L. boulardi* and *T. anastrephae* specimens emerged from blackberry and strawberry fruits collected in four locations in countryside Pelotas, RS, Brazil, during the 2015/2016 crop.

Location	Infested fruits	<i>D. suzukii</i>		<i>L. boulardi</i>		<i>T. anastrephae</i>	
		♂	♀	♂	♀	♂	♀
<i>Rubus sp.</i>							
Rincão da Caneleira	132	685	730	18	29	55	68
Cascatinha	92	332	378	3	1	6	3
Cristal	479	3,142	3,363	9	12	64	51
<i>Fragaria x ananassa</i>							
Cerrito Alegre	169	735	754	7	4	19	23
Total	872	4,894	5,225	37	46	144	145

This fact can be related to the cellular answer presented by *Drosophila* larvae against hymenopterous parasitoids, unchaining an encapsulation process on their eggs, unlike pupae parasitoids, which are less affected (Kacsoh and Schlenke, 2012). In *D. suzukii* this immune response is more potent than in other drosophilids, as can be verified by the higher rate of parasitoid egg encapsulation (Poyet *et al.*, 2013).

Leptopilina boulardi is a Drosophilidae larval parasitoid, particularly to species from the *Drosophila* genus (Allemand *et al.*, 2002), which lay their eggs individually in second instar larvae (Krzemien, 2008). In the event of eclosion by the hymenopterous larvae, they consume the fly's internal tissue, and adult parasitoids

emerge from the dipterous pupae (Kacsoh and Schlenke, 2012). Such parasitoids are originally from Africa, but they are currently distributed in tropical and warm temperate climate regions (Seyahooei *et al.*, 2011), like Europe, Asia, Africa, the American continent, and Caribbean islands (Allemand *et al.*, 2002). Brazil has recorded them in several states, such as São Paulo (Nordlander, 1980), Minas Gerais and Rio de Janeiro (Guimarães *et al.*, 2003).

The *T. anastrephae* species was described by Lima (1940), who obtained the specimens from *Anastrepha serpentina* (Wiedemann) and *Anastrepha* spp. (Diptera: Tephritidae) puparia. Species from the *Trichopria* genus are pupae parasitoids, whose females lay their eggs in the *Drosophila*'s hemocoel and their larvae consume the pupae's internal tissues, from where the adult emerges (Kacsoh and Schlenke, 2012). *Trichopria anastrephae* is distributed throughout Minas Gerais (Silva, 2003), Goiás (Marchiori and Penteado-Dias, 2001), Santa Catarina (Garcia and Corseuil, 2004), and Rio Grande do Sul (Cruz *et al.*, 2011) states. It has also been recorded in Argentina (Turica and Mallo, 1961) and in Venezuela (Boscán and Godoy, 1996).

The spotted wing drosophila is already present in the agroecosystems of the southern Rio Grande do Sul region, and there have been reports of losses to several crops. Therefore, this drosophilid presents a risk to local fruit production, since the region presents climate characteristics and host plants to allow for its establishment. The results found demonstrate the importance of *L. boulandi* and *T. anastrephae*, species, parasitoids that occur naturally in those areas, and which can help reduce *D. suzukii* population.

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New chromosomic paracentric inversions in *Drosophila cardinoides* (Diptera, Drosophilidae) at Santa Catarina Island, South of Brazil.

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Introduction

Included in the *Cardini* group, which is characterized by drosophila with polymorphisms of abdominal pigmentation and inhabits neotropical region, *Drosophila cardinoides* is a representative species in southern Brazil that can be collected many times in the island of Santa Catarina, mainly on the border of the forest. This species is characterized by a dark abdominal pigmentation, wings and abdomen with bright appearance, but has the body morphology very similar to *D. procardinoides*, forming a monophyletic group. Thus, they are not taxonomically decisive features. Cytogenetically, the chromosomal inversions of *D. cardinoides* are more fixed, following the pattern of the *Cardini* group. According to previous studies, it was expected that the number of inversions of *D. cardinoides* was not so wide, since it is less polymorphic compared to other species, such as *Drosophila polymorpha*, for example. Even with significant advances on the chromosomal map of *D. cardinoides*, there are many gaps and investigations to be made in order to contribute to evolutionary and phylogenetic studies of this species group.

Material and Methods

In the southern part of the island of Florianópolis, in Caieira da Barra do Sul (Figure 1) there is a conserved Atlantic Forest area remaining at the Serra do Tabuleiro State Park, where *Drosophila* were collected in the years of 2015 and 2016 during summer and spring. For this, an entomological network was used over baits with bananas and yeast. From these collections, eleven isolineages of *Drosophila cardinoides* were established, maintained in culture media at a constant temperature of 17°C. To obtain the polythenic chromosomes, cytological slides were prepared with third stage larvae using the Ashburner technique (1967) with small modifications and for the chromosomal analysis, the Rohde and Valente (1996) and Cordeiro *et al.* (2014) methods.



Figure 1. Point shows the collecting point at Florianópolis Island (S 27°48'; O 48°56').