Watermelon-infesting Tephritidae fruit fly guild and parasitism by *Psyttalia phaeostigma* (Hymenoptera: Braconidae)

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**Abstract**

An ecological guild of Tephritidae fruit flies exploits cucurbit vegetable fruits, tremendously reducing their production worldwide. Knowledge of the composition of the guild of infesting flies in the field and information on their natural enemy species, might improve pest management strategies. Our aim was therefore to identify Tephritidae species infesting the watermelon *Citrullus lanatus* (Thunb.) Matsum. & Nakai in the Republic of Benin. Morphological and molecular identification of parasitoid species present in the field collections was also done. Infested watermelons were sampled in one of the main watermelon-production areas in the country. Adult tephritid flies emerging from watermelons were identified as *Dacus bivittatus* (Bigot), *D. ciliatus* Loew, *D. punctatifrons* Karsch, *D. vertebratus* Bezzi, *Zeugodacus cucurbitae* (Coquillet), and *Ceratitis cosyra* (Walker). In this study period, *D. vertebratus* was consistently the most abundant species emerging from watermelon. *Dacus ciliatus* was the second most common species followed by *Z. cucurbitae*. The number of emerging fruit flies per kilogram of watermelon varied with collection date and month, and was most variable for *D. vertebratus*. Parasitism in the fruit flies was 1.6 ± 6.4% and occurred through one wasp species that was identified as *Psyttalia phaeostigma* Wilkinson (Hymenoptera: Braconidae). This solitary parasitoid is closely related to other members of the *P. concolor* species complex, some of which are used in biological control. Problems associated with identifying *Psyttalia* species and possibility of using this wasp as a biological control agent against tephritid flies were discussed.

**Keywords** Cucurbitaceae · *Citrullus lanatus* · Morphological and molecular identification · Parasitoid · Biological control agent · Diptera

**Introduction**

The composition of insect pest species infesting agricultural crops is a result of the interaction of the pest with the host, the environment, other pests, their natural enemies, and their host spectra (Wyckhuys et al. 2012; Parisey et al. 2016; Charlery de la Masselière et al. 2017). A good knowledge of the composition of pest communities and of factors governing their occurrence is crucial for developing sustainable plant protection management strategies (Wang et al. 2005). Watermelon, *Citrullus lanatus* (Thunb.) Matsum. & Nakai (Cucurbitaceae) is, like many other cucurbitaceous fruits, infested by true fruit flies of the Tephritidae family. Tephritid fruit flies are ranked as the most notorious pests of economic importance on cucurbit horticultural crops (Dhillon et al. 2005; Sapkota 2010; FAO 2012; Ekesi et al. 2016). Watermelon, has some of the highest fruit fly infestation rates observed among cultivated Cucurbitaceae grown in Africa (Gnanvossou et al. 2008; Badii et al. 2015; Kambura et al. 2018; Mwatawala et al. 2010). The fruit fly guild and the dominant species infesting cucurbit fruit species in Sub-Saharan Africa vary, yet the most common species include *Dacus bivittatus* (Bigot), *D. punctatifrons*, *D. vertebratus* Bezzi, *D. frontalis* (Becker), *D. lounsburyii* (Coquillet), *D. ciliatus* Loew and *Zeugodacus cucurbitae* (Coquillet) (White and Elson-Harris 1992; Gnanvossou et al. 2008;...
Mwatawala et al. 2010; Kambura et al. 2018). Records on watermelon infestation from West Africa are scarce. Gnanvossou et al. (2008) reported D. vertebratus as the dominant species in Benin, representing 80% of the tephritid guild, while in Ghana, Bactrocera dorsalis, Z. cucurbitae, and D. bivittatus were reported as the dominant fruit fly species infesting watermelon (Badii et al. 2015). The species D. bivittatus, D. ciliatus, D. punctaftrons, D. vertebratus, D. frontalii, and D. lounsberryi are indigenous to Africa (De Meyer and Ekési 2016), while Z. cucurbitae may have arrived from Asia at the beginning of the 1900. However, the most recent African invasions originated from the expansion of local populations (Virgilio et al. 2010). The introduction of Z. cucurbitae is suspected to influence the African guild of watermelon-infesting tephritid species, since it is considered an invasive species that could out-compete native species (Vayssière et al. 2002; Mwatawala et al. 2010). Watermelon production is increasingly important in West Africa (Fatondji et al. 2008). Economic losses caused by tephritid fruit flies drive growers into an extreme use of pesticides despite their ecological harm (Essumang et al. 2013; Mahmud et al. 2015; Achigan-Dako 2016). It is therefore important to investigate more sustainable alternative control approaches against these pests.

The composition of the Tephritid fruit fly guild might be influenced by temperature, rainfall, host species availability and interspecific competition (Ye and Liu 2007; Kaplan and Denno 2007; De Meyer et al. 2015). Specific species-related precipitation- and temperature requirements are known to contribute to high populations of B. dorsalis Hendel (Diptera: Tephritidae) during high precipitation periods, while high Ceratitis cosyra (Walker) (Diptera: Tephritidae) populations occur during low rainfall periods (Vayssière et al. 2009). Parasitism by native parasitoid species might also affect fruit fly populations (Ovurski et al. 2005; Copeland et al. 2006). Owing to the specificity of certain parasitoids from the Opiinae subfamily of the Braconidae family, they have regularly been used as biological control agents in tephritid fruit fly management (Wharton and Gilstrap 1983; Bautista et al. 2004; Copeland et al. 2006; Harris et al. 2010). The known braconid species parasitizing Dacus and Zeugodacus species on cucurbits are from the genera Psyttalia, Diachasmimorpha, Opius, and Fopius, sub family Opiinae (Wharton and Gilstrap 1983; Copeland et al. 2004; Bautista et al. 2004; Rugman-Jones et al. 2009; Harris et al. 2010). Opiinae species known or suspected as parasitoids of D. ciliatus include Fopius caudatus (Szépligeti), Diachasmimorpha carinata (Szépligeti), Psyttalia perproximus (Silvestri), Diachasmimorpha longicaudata Ashmead, Psyttalia concolor (Szépligeti), Psyttalia fletcheri (Silvestri), Psyttalia phaeostigma Wilkinson, Diachasmimorpha brevislyi Paoli, and Opius incisii Silvestri (Silvestri 1913; Thompson 1943; Narayanan and Chawla 1962; Wharton and Gilstrap 1983).

Although many of these species have been found parasitizing the Dacus species, P. fletcheri is the only species reported to have been mass-produced and released in cucurbits production fields, apart from the early releases reported in the first decades of the 1900th (Silvestri 1913). Our objective in the present study was to investigate the current guild of tephritid fruit flies infesting watermelon in one of the main watermelon-production areas in the Republic of Benin; and to evaluate the diversity of any associated parasitoids. These results would contribute information towards developing appropriate pest management strategies of fruit flies in watermelon.

Materials and methods

Study area

The study was conducted in watermelon fields in the Atlantic Department, in the southern part of the Republic of Benin, from 2016 to 2017. The choice of field sites was done based on a previous survey (Achigan-Dako 2016). The farmer-managed fields used for this study were located in the village of Agbanzin-Kpota (06.35515°N; 002.2266°E, Altitude 9 m), in the neighbourhood of the town of Ouidah in Southern Benin. The study fields covered a total area of 2.5 ha, split in small plots of 0.1–0.25 ha, about 200 m apart. Conventional production schemes were mainly done, with insecticides (principal with products containing mixtures of Lambda-cyhalothrin and acetamiprid) applied every 2–3 days. The watermelon variety used by all farmers in our study fields was Kaolack (Technisem, France). The area is characterized by a bimodal rainfall pattern, with a long rainy season from April to July, and a short one around October to November. From December until March, the precipitation is nearly zero and there is almost no watermelon production in the area. The main production period in the study area falls typically between June and September, and a minor production occurs in April to May.

Fruit collection

Samples of supposedly infested watermelon fruits were collected directly from the fields weekly, from onset of the fruiting until harvest was completed in the plots. The two harvesting seasons fell in July to October in 2016, and April to August in 2017. Signs of oviposition holes (necrosis around the puncture mark), deformation, decomposition, and/or visible presence of larvae were the criteria used to separate infested fruits from un-infested ones during collection. Fruit samples (at least 500 g) were weighed and the number of fruits per sample was adjusted with randomly selected fruits from the same plots. The average sample weight was 753 ± 47 g.
The samples were thereafter arranged in plastic containers on a metal mesh placed on sand for incubation. The plastic containers were covered with a fine fabric (mesh size: 104 × 94, 300 μm aperture), held by an elastic band and labelled. Sand was changed in the containers when it became too damp. Pupae were collected every three days for at least three weeks, counted and placed directly in a Petri dish covered with mesh (mesh size: 26 × 24, 1220 μm aperture) for fly and parasitoid emergence. The emerging adults were gently removed and kept for rearing and identification. In 2016, 143 fruit samples were collected against 84 samples in 2017, with a total weight of 83 kg and 101 kg respectively.

**Morphological identification of tephritid flies and braconid parasitoids**

A sub-sample of fruit flies emerging from the pupae was placed in a refrigerator at 6 ± 2 °C for 30 s to inactivate them, and then identified under an inverse reflected light stereomicroscope (Olympus XZS10) according to Virgilio et al. (2014). Similarly, a sub-sample of the parasitoid was separated for morphological identification using available identification keys, descriptions, and pictures (Wharton and Gilstrap 1983; Rugman-Jones et al. 2009). Lengths of ovipositor, ovipositor sheath, and hind tibia were measured on 30 female parasitoids for the identification.

**Molecular identification of parasitoids**

Female individuals of the parasitoid wasp were preserved in 70% alcohol at −20 °C for molecular identification. DNA was extracted, following the LIVAK protocol (1984), from a total of 10 specimens and sent to INQABA Biotech (Pretoria, South Africa), for subsequent PCR amplifications and identification. A 722pb target region of the Cytochrome Oxidase I (COI) gene was amplified using the barcoding primers LCO-1490 (5′ GGTCAACAAATCATAAAGATATTGG 3′) and HCO-2198 (5′ TAAACTTCAGGGTGACCAAAAAATCA 3′) (Folmer et al. 1994). The PCR products were gel extracted, purified and sequenced in the forward and reverse direction, using the PCR primers, on the ABI PRISM™ 3500xl Genetic Analyse (INQABA Biotech).

Sequences were edited in BioEdit (vers. 7.2 (Hall 1999)) manually inspected against sequence nucleotide calls for nucleotide scoring. The forward sequences were then aligned with the reverse complements of the reverse sequences and joined to verify the sequences in both directions and to manually edit potentially erroneous nucleotide calls. The termini of the concatenated sequences were then trimmed at the primer sequence to reveal the clean sequence, which was then translated into protein sequences to further assess sequencing errors, since mtCOI is an expressed sequence. The resulting sequences were then compared with the nucleotide sequence collection in NCBI using nucleotide BLAST to determine any similarities with previously reported sequences and assess species identification. Nucleotide similarity of 98% was used as a basis of barcode species identification (Hebert et al. 2003). Nucleic acid sequences from sequenced species were deposited in GenBank and voucher specimens were deposited in the reference insect collection at IITA-Benin.

**Statistical analyses**

The number of emerging tephritid fruit fly species and braconid wasps were counted per sample, omitting the few samples from which no fruit fly pupae were recovered. To analyse whether the emergence count of fly species differed and by how much, a generalized linear mixed model with log link function was ran. A mixed model was also used to account for the variability due to the collection period expressed in month, treated as random effect in the model. Since the samples used to count the fruit fly emergence had different weight, a log-transformed fruit sample weight was used as offset in the model. Due to convergence problem encountered, brms package (Bürkner 2018) which provides an interface to fit Bayesian generalized linear multivariate multilevel models was used. Thus 2000 iterations on 4 chains was parameterized with default prior specification. The convergence of algorithm was accessed through the potential scale reduction factor (Rhat) on split chains where at convergence, its value is equal to 1. All statistical analyses were performed with the R software (version 3.6.0, R Development Core Team 2019).

**Results**

**Morphological identification of tephritid flies**

Based on morphological characteristics, according to Virgilio et al. (2014), a total of six Tephritidae species were identified. Fruit flies with an abdomen with fused tergites were identified as belonging to the genus *Dacus* (16,708 individuals), while flies with separate tergites were identified as belonging to the genus *Zeugodacus* (957 individuals). Hence, four *Dacus* species and one *Zeugodacus* species were identified among the fruit fly specimen. Morphologically, *D. vertebratus* and *D. frontalis* are relatively similar, with orange-brown thorax, a transverse suture with a triangular yellow mark, and no stripe on the scutum. The two species were distinguished through the differences in their wing and femur coloration. The wing of *D. vertebratus* has a costal band with an apical spot rounded at the end of the vein R₄₊₅ and has an anal streak, while *D. frontalis* does not have a distinguished spot, and the costal band reaches further than the end of the vein R₄₊₅. The fore, mid and hind femurs of *D. vertebratus* are dark in the apical half, which also differentiates it from *D. frontalis* which was
only dark on the apical mid femur. We identified *Dacus vertebratus* in our collections, yet not *D. frontalis*. *Dacus ciliatus* was identified based on its predominantly orange-brown scutum without yellow or orange stripes, with two small dark spots on the lower scutum. Its narrow costal band with a small apical point extending midway between the R4+5 and M veins, the third tergite containing two small black spots on each side, are other key characteristics. *Dacus punctatifrons* was identified based on its orange-brown colour tending towards black with lateral and median stripes on the scutum. The wings contained a costal band up to half of the R4+5 vein with an anal streak, and its fore, mid and hind femora are yellow or orange at the basis and dark apically. Dark flies with lateral and median yellow stripes on scutum, very deep apically enlarged costal band, and fore and mid femurs that are dark on 2/3 of the apical end were identified as *D. bivittatus*. They were differentiated from *D. lounsburyi* through the coloration of the femur, which on the latter species is more yellowish. The only non-*Dacus* species (apart from the few *C. cosyra* species identified) was *Z. cucurbitae*, which has lateral and median stripes on the scutum, and typical wing coloration with a pre-apical cross-band at the dm-cu vein and a large apical point surrounding half of the R4+5 cell with an anal streak present.

### Tephritid species guild and infestation

The samples were heavily infested and we collected a total of 25,129 fruit fly pupae from which 17,665 adults emerged. Of the samples collected, 87% yielded fruit flies and 18% yielded parasitoids. The average emergence of fruit flies in 2016 and 2017 was 92.1 ± 12.1/kg and 125.6 ± 17.5/kg, respectively. The six identified fruit fly species included four *Dacus* species (*D. vertebratus* [n = 13,153], *D. ciliatus* [n = 3441], *D. punctatifrons* [n = 101], and *D. bivittatus* [n = 5]), one *Zeugodacus* species (*Z. cucurbitae* [n = 957]), and one *Ceratitis* species (*C. cosyra* [n = 8]). Overall, *D. vertebratus* was the dominant species and represented approximately 65% of all emerging flies (Table 1). The variation of emergence was nevertheless highest for *D. vertebratus*, followed by *D. ciliatus* (Table 2). The variation was greatest (43%) between *D. vertebratus* and *D. bivittatus* while the difference between *D. ciliatus* and *Z. cucurbitae* was only 5%. The monthly variation showed that *D. ciliatus* were present in high number during August and September and that *Z. cucurbitae* were present mainly in July and August (Fig. 1).

### Parasitism

A total of 233 parasitoid specimens emerged from the pupae from the field-collected watermelon in Agbanzin-Kpota (Agbanzin-Kpota, 06.35515°N, 002.2266°E, 9 m; 16.viii.2016, 24.viii.2016, 31.viii.2016, 08.ix.2016, 15.ix.2016, 25.iv.2017, 09.vi.2017, 14.vi.2017, 21.vi.2017, 29.vi.2017, 04.vii.2017, 12.vii.2017, 19.vii.2017, 21.vii.2017, 28.vii.2017, and 04.viii.2017, coll. B. F. R. Layodé). The level of parasitism (calculated as number of emerging parasitoids/emerging fruit flies per sample) was low with an average of 1.6 ± 6.4% from all the samples.

### Morphological and molecular identification of parasitoid

The external morphology of 30 parasitoid females was examined and appeared consistent with the characters used to identify *Psyttalia* spp. adults (Wharton 2007). The wings had numerous veins and cells, a presence of the RS + M vein and absence of the 2 m-cu vein (Fig. 2a), as for Braconidae species. The mandibles had a normal appearance, narrowing apically and overlapping when closed (Fig. 2b); and a sternaulus was present as a single sculptured groove (Fig. 2c), as for species in the subfamilies Opiinae and Braconinae. The mesonotum had an unsculptured notauli and a median stripe, the latter being a new feature for the species (Fig. 2d). The forewings had a large 2nd submarginal cell and a m-cu vein extending to the first submarginal cell and a second 4-sided submarginal cell (Fig. 2a). The parasitoids had hind wings with a relatively longer sub-basal cell, extending relatively longer than about half the distance to the origin of m-cu (the m-cu being absent, Fig. 2a). Finally, the parasitoid had a short clypeus and a large labrum exposed, which are descriptions used to distinguish the genus *Psyttalia* from other Opiinae genera e.g. *Opis* (Fig. 2b). The ovipositor was noticeably long (Fig. 2e), with ovipositor sheaths at least 2 times longer than hind tibia (Wilkinson 1927). Mean length of ovipositor were 3.74 ± 0.097 mm, ovipositor sheath 2.80 ± 0.08 mm, tibia 1.13 ± 0.031 mm and ratio between ovipositor sheath and hind tibia equalled 2.49 ± 0.051.

The nine sequences mtCO1 amplified were identified as *Psyttalia phaeostigma* (Hymenoptera: Braconidae). The 509–645 bp fragments had 99–100% homology (e = 0E00; Gaps =0/606) to the only available sequence previously deposited in GenBank from one individual collected in Kenya.

*Int J Trop Insect Sci (2020) 40:157–166*
(Rugman-Jones et al. 2009). Our samples are archived in GenBank under accessions n° MK105570 - MK105576 and MK143392 - MK143393. The sequences obtained were compared with all available mitochondrial COI sequences in GenBank through phylogenetic analysis by maximum likelihood, and our specimens were clustering in the same supported clade along with the Kenyan specimen identified as *P. phaeostigma*, also showing relationship with other members of the *Psyttalia concolor* species complex (Fig. 3).

**Discussion**

This study presents an assessment of watermelon colonization by tephritid fruit flies and identification of parasitoids in the system which will be the basis for further investigation of management methods for the most devastating fruit fly species in West Africa. Tephritid fruit fly guild and population density differed among the collection months, yet *D. vertebratus* was the dominant species overall, followed by *D. ciliatus* and *Z. cucurbitae*. Previous report on tephritid-infestation of watermelon from Republic of Benin, showed likewise, a dominance of *D. vertebratus*, followed by *D. ciliatus*, and a very low infestation of *D. punctatifrons*, *Z. cucurbitae*, and *D. bivittatus* (Gnanvossou et al. 2008). From these two studies, we hypothesis that infestation by both *D. ciliatus* and *Z. cucurbitae* are becoming more important. In studies conducted in Ghana in 2011–2013, *B. dorsalis*, *Z. cucurbitae* and *D. bivittatus* were observed emerging from watermelon (Badii et al. 2015), which is greatly different from our findings. Another records on watermelon infestation from West Africa reported infestation of only *Z. cucurbitae* (Vayssières et al. 2007). In Tanzania watermelon is infested mainly by *D. ciliatus* (70%), followed by *Z. cucurbitae* (20%), and

**Table 2** Difference in Tephritidae fruit fly species emergence (fixed effect) and variation due to date and month (Random effect)

| Parameter | Estimated fruit fly emergence [mean ± SD] | 95% credible interval\(^a\) | Rhat\(^b\) |
|-----------|-----------------------------------------|--------------------------|----------|
| Fixed effect | | | |
| Intercept \((D. bivittatus)\) | 2 ± 2 | 0 5 | 1.01 |
| *D. ciliatus* | 18 ± 2 | 7 55 | 1.01 |
| *D. punctatifrons* | 4 ± 2 | 1 12 | 1.00 |
| *D. vertebratus* | 54 ± 2 | 22 167 | 1.01 |
| *Z. cucurbitae* | 15 ± 2 | 6 48 | 1.01 |
| Random effect | | | |
| Intercept | Month | 2 ± 1 | 1 4 | 1.00 |

Values are transformed to the original scale from the logarithm model, using exponential function

\(^a\) Two-sided 95% credible intervals

\(^b\) Potential scale reduction factor (Rhat) for convergence. Rhat = 1 indicate that parameters converge well in the model

**Fig. 1** Monthly emergence of Tephritidae fruit flies per kilo watermelon
**D. bivittatus** (5%), while **D. vertebratus** and **D. punctatifrons** are scarce (Mwatawala et al. 2010). In Kenya the watermelon-infesting species are mainly **Z. cucurbitae** (50%) and **D. vertebratus** (50%) (Kambura et al. 2018). The guild and the dominating fruit fly species are hence greatly variable on the continent and within regions, yet the dominating three species are **D. ciliatus**, **D. vertebratus** and **Z. cucurbitae**. Due to the documented dominance of the exotic **Z. cucurbitae** over the indigenous **Dacus** species such as **D. ciliatus** in Africa (Vayssières et al. 2002), we had expected a greater presence of this invasive species over the native ones. Because of its larger body size, higher fecundity, higher fertility, longer life span, and shorter development time, in comparison to native species such as **D. ciliatus** (Vayssières et al. 2002), **Z. cucurbitae** was expected to increase in prevalence after its introduction in 2004 (Vayssières et al. 2007). Somewhat surprisingly, we did observe that the native **D. ciliatus** emerged in as high proportion as 70–90% during the initial production period in 2016, and that the native **D. vertebratus**, together with **D. ciliatus**, were the dominating species in the remaining months. Possibly, an interspecific competition occurs between **Z. cucurbitae** and **D. ciliatus**, hence, the increase of the former coincided with a reduction of the latter, yet the competitive superiority among species is yet to be disclosed, since conflicting results have been obtained. Studies conducted in Pakistan showed that **D. ciliatus** overcame the population of **Z. cucurbitae** by suppressing its larval development in interspecific competition (Quereshi et al. 1987), while in Reunion Islands, **Z. cucurbitae** outcompeted **D. ciliatus** (Vayssières et al. 2008). It is often stated that **D. ciliatus**
is the major competitor of *Z. cucurbitae* in Africa (Vayssières et al. 2008; Mwatawala et al. 2010); yet, here we observed that, *D. vertebratus* also competed with *Z. cucurbitae* in at least one watermelon. The reason for the inverted presence of *D. ciliatus* and *Z. cucurbitae* can also be related to the specific species-related precipitation and temperature requirements. It is therefore possible that the difference in rainfall pattern between the two years might have affected their abundance. In 2016 the precipitation during May and June was high, causing inundation of the area, whereas the first rainy season in 2017 was in contrast, moderate. Due to the inundation, the production started late in 2016, while in 2017 it was almost continuous from April to August (when our collections ended).

In our study, the field was treated with insecticides, primarily to manage the fruit fly infestation. The prospect of having fruits from a production without insecticides is very low as the tiny fruits (<2 cm) are often attacked (Vayssières et al. 2000, own experience). We did not quantify the percentage of reduction of infested fruits due to these applications, yet we have experience of producing without insecticides and not being able to harvest hardly any fruits. Our results allow us to report that tephritid fruit flies are causing heavy damage to watermelon production, even under conventional pesticide application regimes, as infested fruits were always observed in spite of the high insecticide applications rate of every 2nd or 3rd day, with a fruit fly emergence rate of approximately 100 individuals per kg fruit collected. Insecticide use in watermelon production in neighbouring Nigeria, to manage fruit flies, causes insecticide-residue levels in watermelon peel, pulp, seed, leaf and stem that exceeds acceptable daily intake value levels, and the maximum residue limits set for vegetables and soil (Mahmud et al. 2015). In a previous study, even non-target watermelon-neighbouring crops contained elevated
levels of insecticide residues (Essumang et al. 2013), reflecting the extreme pest pressure on the crop in West Africa. The compatibility between insecticides and natural enemies is vital for biological control, hence the current fruit fly management method is a problem for the use of parasitoids as biological control agents (Harbi et al. 2017). Non-specific tephritid insecticides, incompatible with natural enemies, risk destroying any arthropod, including biological control agents (Roubos et al. 2014; Biondi et al. 2012). It was therefore surprising that parasitism was encountered in the heavily-insecticide-treated fields. It might then, be possible to combine a chemical control with biological control if planning the applications in relation to fructification period, natural enemy sensitivity, and insect activity in time and space (Hassan and Van de Veire 2004). Any Biological Control management with the identified species will have to be combined with a reduction in insecticide, and probably also fungicide use.

The parasitoid individuals were identified to be one species i.e. the solitary Braconidae species *P. phaeostigma*. Since the watermelons were removed from the field with either egg or larval staged fruit flies (not pupae), parasitization by *P. phaeostigma* could only have taken place in these stages. The parasitoid *P. concolor* (Szépligeti) species complex, whose members are difficult to distinguish morphologically (Silvestri 1913; Wharton and Gilstrap 1983). This group includes (among others) *P. concolor* sensu stricto, *P. perproxima* (Silvestri), *P. humilis* (Silvestri), *P. lounsburyi* and *P. ponerofaga* (Rugman-Jones et al. 2009). These species are closely related and are not biologically isolated, crossing resulting in viable offspring (Billah et al. 2008b). To differentiate the species, molecular methods (Rugman-Jones et al. 2009), morphometric methods (Wharton and Gilstrap 1983; Billah et al. 2008a), and their specific fruit fly host and/or fruit host preferences, have been used (Billah et al. 2008b). Molecular comparison with the single *P. phaeostigma* specimen from Kenya and the closely related species, together with the morphological features observed, confirmed this identity. However, the identity of various *Psyttalia* species is still uncertain and it is possible that the genus contains more than the current approximately 80 species reported (Wharton 2007, 2009; Wu et al. 2016), that cryptic species are present and some of the species are the same (Billah et al. 2008a, b; Wharton 2009).

The identified wasp might be an interesting biological control agent. Some *Psyttalia* species especially *P. concolor*, are used (Yokoyama et al. 2008; Miranda et al. 2008) or tested (Sime et al. 2007) in biological control programs against various tephritid fruit pests. The continuing interest in *Psyttalia* is directed primarily against such pests as *Z. cucurbitae, Bactrocera oleae* (Rossi), and *Ceratitis capitata* (Wiedemann) (Mohamed et al. 2003, 2006; Daane et al. 2008). Biological control releases of Opiniinae species such as *P. fletcheri* and *Fopius arisanus* (Sonan) have been conducted for the management of *Z. cucurbitae* in Reunion Islands and Hawaii (Marquier et al. 2014). Although *P. phaeostigma* is not yet formally identified as a biological control agent, it has been associated with tephritid fruit flies as a potential natural enemy of Cucurbitaceae pests (Wharton 2007, 2009; Billah et al. 2008a; Wu et al. 2016) and could be a candidate for controlling fruit flies of the genera *Dacus* and *Zeugodacus*.

In any attempt to initiate mass-rearing and introduction of *P. phaeostigma*, it will be important to further investigate its ability to survive within the guild of tephritid fruit flies present in watermelon. *Psyttalia phaeostigma* preference for, and survival performance, in the main (or key) watermelon-infesting fruit flies will reveal the capacity to parasitize the specific species and sustain its population. Studies of its preference for host developmental stage and its ecological requirements are also pertinent. It might also be interesting to investigate whether the parasitism by *P. phaeostigma* (or other natural enemies) contributes to the fruit fly guild variability among regions. Such information will provide useful indications of whether biological control management of watermelon-infestation of *D. vertebratus, D. ciliatus*, and *Z. cucurbitae* shall be considered using *P. phaeostigma*.

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Compliance with ethical standards

Conflict of interest We declare that these research works have no conflict of interest.

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