***Oecanthus nigricornis* (Orthoptera: Gryllidae) as the first known host of *Stylogaster neglecta* (Diptera: Conopidae)**

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Abstract

The conopid fly *Stylogaster neglecta* Williston (Diptera: Conopidae) is a parasitoid with no known host. We report this species as the first recorded dipteran parasitoid of *Oecanthus nigricornis* Walker (Orthoptera: Gryllidae) (blackhorned tree crickets). We reared field-collected *O. nigricornis* juveniles over several months in 2017 and found that larval *S. neglecta* emerged from them during late July into August. We estimated the incubation period for *S. neglecta* larvae to be around 30 days based on the length of time it took for them to emerge from the host and pupate (subsequently all hosts died). We documented several cases of multiple parasitism. In 2018, we dissected *O. nigricornis* sampled from four sites across southern Ontario, Canada and upstate New York, United States of America and found that the percentage of juvenile *O. nigricornis* parasitised ranged from 2–39%. Further sampling will be necessary to determine whether this variation represents consistent population differences or between year variation in parasitism.

Introduction

Conopidae (Diptera) larvae are obligate endoparasitoids (Gibson and Skevington 2013; Gibson *et al*. 2013). *Stylogaster* Macquart (Diptera: Conopidae) is the sister group to other conopids (Gibson and Skevington 2013; Marshall 2012). *Stylogaster* species target nymphs and adult Gryllidae (Orthoptera) and Blattodea, whereas other conopid genera use adult aculeate Hymenoptera (Freeman 1966; Kotrba 1997; Woodley and Judd 1998; Schmid-Hempel 2001; Rasmussen and Cameron 2004; Marshall 2012; Gibson *et al*. 2013). *Stylogaster* eggs have been found on other species, such as other Diptera, but the lack of egg development indicates that these may be accidental ovipositions (Couri *et al*. 2019; Couri and Barros 2010; Stuke 2012).

Stylogaster species have been studied mainly in Africa and the Neotropics and are associated with army ants (Hymenoptera: Formicidae), using hosts that have been flushed out by the ants (Lopes 1937; Rettenmyer 1961; Burt *et al*. 2015). The flies are thought to either stab their ovipositors into the abdomen of the host and insert the egg under a sclerite, or shoot the egg from the ovipositor via a flipping motion of the abdomen (Kotrba 1997; Woodley and Judd 1998). While most studies have focussed on tropical species, *Stylogaster neglecta, S. biannulata* (Say), and *S. beresfordi* Burt are found in the Nearctic region (including Canada), typically in forest understoreys where adults feed on yellow/white flowers (Burt *et al*. 2015). Only *S. biannulata* has a recorded host, *Gryllus rubens* Scudder (Orthoptera: Gryllidae) (Woodley and Judd 1998). While incubation times were not examined, Woodley and Judd (1998) found that in the few cases were adult flies emerged, it was 19–25 days after the puparia were formed. Little is known of the life history of *Stylogaster*. *Sicus* Latreille, and *Physocephala* Schiner (Diptera: Conopidae), which parasitise *Bombus* Latreille (Hymenoptera: Apidae) in Europe, North America, and Japan, oviposit during periods of host reproduction (Schmid-Hempel 2001) from

July until mid-September (Macfarlane *et al*. 1995). They kill their hosts within 10–12 days of oviposition. Hosts are induced to burrow into the ground and the fly pupates inside the dead host (Schmid-Hempel 2001).

In old fields at the University of Toronto at Mississauga in August 1996, one of us (W.B.) first found *Stylogaster neglecta* larvae (identified by Jeff Skevington, Canadian National Collection of Arthropods, Arachnids, and Nematodes, Ottawa, Ontario, Canada) emerging from juvenile *Oecanthus nigricornis* Walker (Orthoptera: Gryllidae) (blackhorned tree crickets), pupating and then eclosing. The larvae emerged and pupated between late August and mid September, with adults eclosing from late November to early January (L.F.B., unpublished data). *Oecanthus nigricornis* are found across eastern North America, hatching around mid June and reaching maturity in August. Eggs are laid in August and September and hatch the following June (Fulton 1915; Vickery and Kevan 1985). *Oecanthus* are parasitised by several hymenopteran egg parasitoids (Smith 1930a, 1930b; Udine and Pinckney 1940) and some helminths (Denner 1968; Sonin 1990). However, there are currently no known dipteran parasitoids of the genus, despite other Orthoptera (including gryllids) commonly being parasitised by Sarcophagidae (Diptera) and Tachinidae (Diptera). This includes the well-known genus *Ormia* Robineau-Desvoidy, which is attracted to songs of male gryllid hosts (Rees 1973; Cade 1975; Lehmann 2003). Our observations are the first case to our knowledge of *Oecanthus* juveniles or adults as hosts for a dipteran parasitoid and the first report of a host for *S. neglecta.*

We collected further *O. nigricornis* samples at University of Toronto at Mississauga (Ontario, Canada) in the summer of 2017 and 2018 and identified emerging adults as *S. neglecta* using Burt *et al*. (2015). In 2017, *O. nigricornis* were collected from natural populations at discrete time intervals to determine when in the season conopid parasitism occurs. In 2018, *O. nigricornis* were sampled from several sites to determine population level variation in parasitism.

Methods

Natural history of *Stylogaster neglecta*

In 2017 we sampled *O. nigricornis* approximately every two weeks from 28 June until 13 September (Fig. 1, Table 1–2) from old fields at the University of Toronto, Mississauga Campus (43.55°N, 79.66°W) (total = 131 *O. nigricornis*). The start date was chosen as the first day in which were successfully able to sample *O. nigricornis* from the site. The end date was chosen as the first sample in which we no longer found any *S. neglecta* larva emerging from the sampled *O. nigricornis*. We reared *O. nigricornis* individually in open-ended plastic cylinders, 8 cm in diameter and 7.5 cm long, with metal mesh covering the ends, at 25 ºC and 70% humidity. *Oecanthus nigricornis* were fed pollen and dry dog food and containers were sprayed with water every second day. We recorded the date on which *O. nigricornis* were caught, when they died, and whether a parasitoid emerged. *S. neglecta* pupae found in the containers were removed and placed in a petri dish on filter paper and sprayed with water daily. Chi-squared tests were used to determine if there were significant differences in the proportion of *O. nigricornis* parasitised on different sampling dates. Confidence intervals were calculated using the Clopper-Pearson method. Cases of multiple conopid parasitism of a single host were also recorded.

To examine whether *S. neglecta* was actively laying eggs the entire summer or during a shorter period of time, an analysis of variance was performed to determine if the length of time between when we collected the host and parasitoid emergence differed between collection dates. If *S. neglecta* oviposits on hosts throughout the summer, then larvae in the sampled *O. nigricornis* should be at different stages of development and there will be no correlation between date of sampling and length of time from host capture until parasitoid emergence. If however, *S. neglecta* females oviposited roughly concurrently, then we expect *O. nigricornis* sampled on the same date would have parasitoids that emerge after similar lengths of time, and that the length of time between when the host *O. nigricornis* was sampled and the parasitoid emerging will be longer earlier in the summer and shorten as the season progresses. Specimens were deposited at the Royal Ontario Museum (Toronto, Ontario, Canada).

Site differences in parasitism

In late July 2018, we collected *O. nigricornis* in southern Ontario (Canada) and New York State (United States of America): from the Koffler Scientific Reserve (King City Ontario, 44.03°N, 79.53°W), *rare* Charitable Research Reserve (referred to as *rare*) (Cambridge Ontario, 43.38°N, 80.39°W), our University of Toronto at Mississauga site previously sampled in 2017, and the Edmund Niles Huyck Preserve and Biological Research Station (referred to as Huyck) (Rensselaerville New York, 42.10°N, 74.10°W). Late July was chosen based on the results from the 2017 study, which indicated that this was the peak of *S. neglecta* oviposition activity. For each site, two to four old fields within a 1-km radius of each other were sampled by sweep netting. *Oecanthus nigricornis* caught ranged from third to fifth instar. *Oecanthus nigricornis* were frozen and then preserved in 70% ethanol before being dissected and their abdomens examined using a Wild M5A dissecting microscope (Wetzlar, Hesse, Germany). They were scored as parasitised if *S. neglecta* eggs or larvae were found (Fig. 2). A chi-squared test was performed to determine if there were significant differences in the proportion of infected *O. nigricornis* between sites. Confidence intervals were calculated using the Clopper-Pearson method.

Results

Natural history of *Stylogaster neglecta*

Twenty-four (20%) of the 120 University of Toronto at Mississauga *O. nigricornis* were parasitised by *S. neglecta*. There was a significant difference in the proportion of parasitised *O. nigricornis* collected on different dates ($\chi^2 = 37.014$, df = 2, $P < 0.01$) with the highest proportion of parasitised individuals found in the 14 August sample (Fig. 3). Parasitoid larvae emerged from the near the anus of *O. nigricornis* and pupated outside the host. Host *O. nigricornis* remained alive for hours to days after emergence and several observations of *O. nigricornis* after parasitoid emergence suggested that they had lost the use of their metathoracic legs. There were several cases of multiple parasitism: four *O. nigricornis* had two *S. neglecta* larvae, and one hosted three. Parasitoids that shared a host appeared to be smaller than parasitoids that did not (personal observation). Of the pupae, only one eclosed (in December 2017). Mean time from a *O. nigricornis* being collected to parasitoid emergence was 16.6 days (standard deviation = 9.04; range 3–31 days). No *O. nigricornis* collected before 17 July or after 29 August were parasitised. Consistent with this narrow oviposition period, mean time from host collection to parasitoid emergence was significantly shorter at later sampling dates ($F = 32.36$, $P < 0.001$) (Fig. 4).

Site differences in parasitism

Of 674 *O. nigricornis* from all four sites, 149 or 22% (CI = 0.246, 0.324) were parasitised. There were large differences in parasitism between sites (Fig. 5): at Huyck 2% (*n* = 104, CI = 0.0024, 0.069), at Koffler 11% (*n* = 178, CI = 0.079, 0.189), at *rare* 26% (*n* = 193, CI = 0.272, 0.434), and at University of Toronto at Mississauga 39% (*n* =199, CI = 0.539, 0.717) (χ^2 = 70.23, P < 0.001). *Oecanthus nigricornis* hosts were mostly third to fifth instars, and of all ***O.*** *nigricornis* parasitised, only one had reached adulthood.

Discussion

We report *S. neglecta* as the first dipteran parasitoid of *O. nigricornis* as the first known host for this parasitoid. Like *S. biannulata*, *S. neglecta* uses a gryllid cricket host in which the larvae develop internally, pupates outside the host, and occasionally exhibits superparasitism (Woodley and Judd 1998). Like the *Bombus* specialists *Physocephal*a and *Sicus*, *S. neglecta* have a narrow time window of a few weeks in which hosts are parasitised (Macfarlane *et al*. 1995; Schmid-Hempel 2001; Otterstatter 2004). Unlike the *Bombus*-hunting conopids, *S. neglecta* pupates outside of the host. *Stylogaster neglecta* also appear to have longer incubation times than the 10–12 days reported for the *Bombus* hunters (Schmid-Hempel 2001). Further work should examine the nature of overwintering strategies in *S. neglecta* and whether it resembles that of other conopids.

The time from host capture until parasitoid emergence decreased over the season with longer intervals in July and shorter ones in August. We did not find any parasitised ***O.***

nigricornis in June or September. The shorter intervals in the late summer are because the larvae had already been developing for some time before we collected their hosts. *Stylogaster neglecta* appears to parasitise hosts from late July to early August and is not active throughout the rest of the summer. This is consistent with unpublished adult collection records of this species by Burt and others (unpublished data), which examined records of adult *S. neglecta* in collections across North America. Of the 128 *S. neglecta* caught in southern Ontario, eight were caught in June, 114 in July, five in August, and one in September. Such a narrow time of parasitoid adult activity is expected in hosts with discrete generation times (Godfray 1994). Parasitoids that emerge too early may suffer from greater mortality waiting until hosts emerge, while those emerging late may have difficulty either in finding unparasitised hosts, or in subduing larger-sized hosts.

Assuming that all *S. neglecta* larvae have a similar incubation times, and the longer lengths of time from host capture until parasitoid emergence are roughly equivalent to the incubation time, then it would appear that *S. neglecta* has an incubation period of about 30 days in its host. This estimate is higher than estimates for other conopid genera, such as 10–12 days found in *Physocephala* and *Sicus* (Schmid-Hempel 2001). However, as noted earlier, the mean time from our collecting the host *O. nigricornis* until parasitoid emergence will be shorter than actual mean incubation time given that eggs had been laid before collection. The actual incubation time is likely to be longer than we recorded but less than 44 days, as there were no indications of parasitism two weeks before the earliest sample.

We found significant differences in the proportion of *O. nigricornis* that were parasitised across four different sites in southern Ontario and upstate New York. The proportion of parasitised individuals ranged from 39% in University of Toronto at Mississauga to 2% in the Huyck Preserve. While there were significant differences between sites in terms of parasitism rate, our sampling only took place over a single season so we cannot confirm if these are long term site differences. There appears to be an inter-year difference in parasitism at University of Toronto at Mississauga, with the parasitism rate in late July 2018 being higher than the parasitism rates found in July 2017. However, the parasitism rate in August 2017 was higher than the single 2018 sample. It appears that the rate of parasitism at University of Toronto at Mississauga peaked slightly earlier in 2018 than in 2017. Parasitoid-host relationships often show cyclical population dynamics across multiple years (Hassell and May 1973; Godfray 1994; Godfray *et al*. 1994) which may explain the between-site differences. Long term sampling will be necessary to determine if these differences are consistent from year to year.

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Figure 1. *Stylogaster neglecta* female raised from a pupa after emerging from *Oecanthus nigricornis*. Collected as a pupa on 28 August 1996 by Luc Bussière and emerged as an adult 9 January 1997.

Figure 2. Juvenile fourth instar *Oecanthus nigricornis*, parasitised by *Stylogaster neglecta*. Caught at *rare* Charitable Research Reserve.

Figure 3. Proportion of *Oecanthus nigricornis* collected in the summer of 2017 that were parasitised at the University of Toronto at Mississauga, with exact binomial confidence intervals and sample sizes.

Figure 4. Time in days between collection of parasitised *Oecanthus nigricornis* (University of Toronto at Mississauga 2017) and when larval *Stylogaster neglecta* emerged for all *O. nigricornis*.

Figure 5. Time in days between collection of parasitised *Oecanthus nigricornis* (University of Toronto at Mississauga 2017) and when larval *Stylogaster neglecta* emerged, averaged for each collection date, with standard deviation.

Figure 6. Proportion of *Oecanthus nigricornis* parasitised from samples collected across four sites in July 2018. Exact binomial confidence intervals and sample sizes are given. Huyck, Edmund Niles Huyck Preserve and Biological Research Station; KSR, Koffler Scientific Reserve; UTM, University of Toronto at Mississauga; rare, *rare* Charitable Research Reserve.

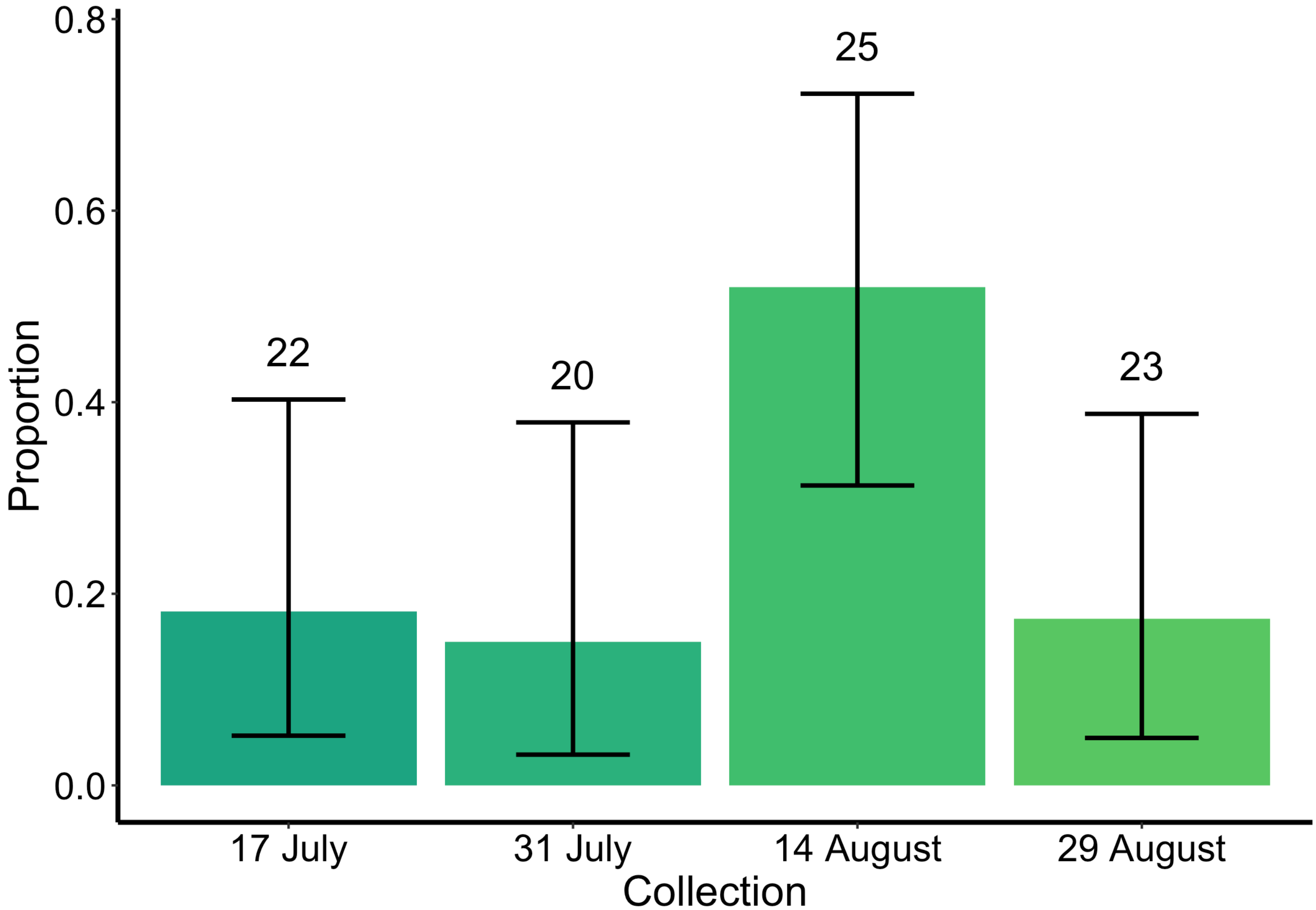
Table 1. Sex, location, voucher identification number, and collector of samples used in analysis and referenced.

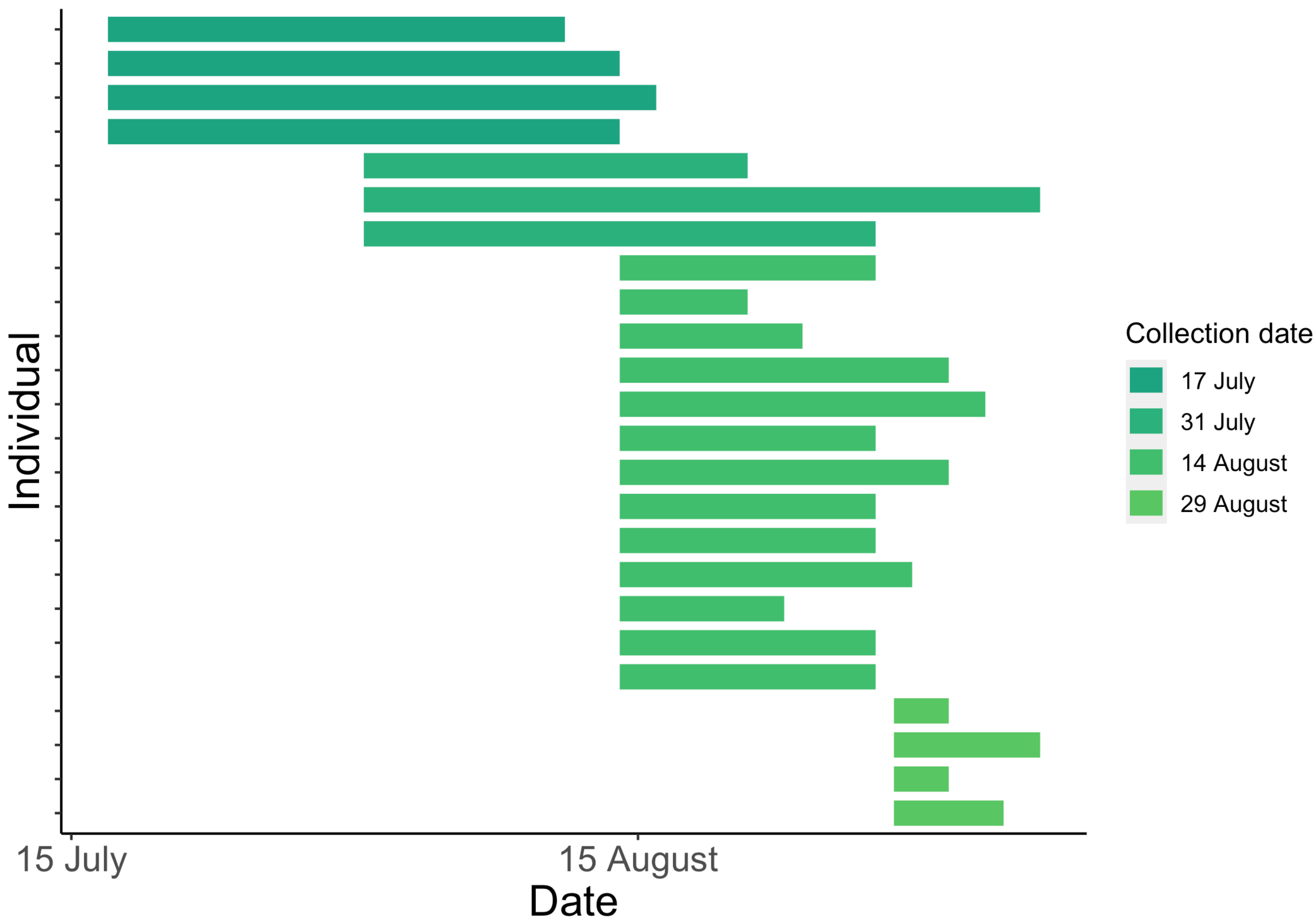
Voucher ID	Species	Location	Date	Collector	Sex
ROME179756	Oecanthus nigricornis	University of Toronto at Mississauga	31 July 2017	Erik Etzler	Male
ROME179757	Oecanthus nigricornis	University of Toronto at Mississauga	28 June 2017	Erik Etzler	Female
ROME179758	Oecanthus nigricornis	University of Toronto at Mississauga	31 July 2017	Erik Etzler	Female
ROME179759	Oecanthus nigricornis	University of Toronto at Mississauga	17 July 2017	Erik Etzler	Male
ROME179768	Stylogaster neglecta	University of Toronto at Mississauga	11 August 2017	Erik Etzler	N/A
ROME179769	Stylogaster neglecta	University of Toronto at Mississauga	December 2017	Erik Etzler	N/A
ROME179770	Stylogaster neglecta	University of Toronto at Mississauga	January 1997	Luc Bussière	Male

ROME179771	Stylogaster neglecta	University of Toronto at Mississauga	28 November 1996	Luc Bussière	Female

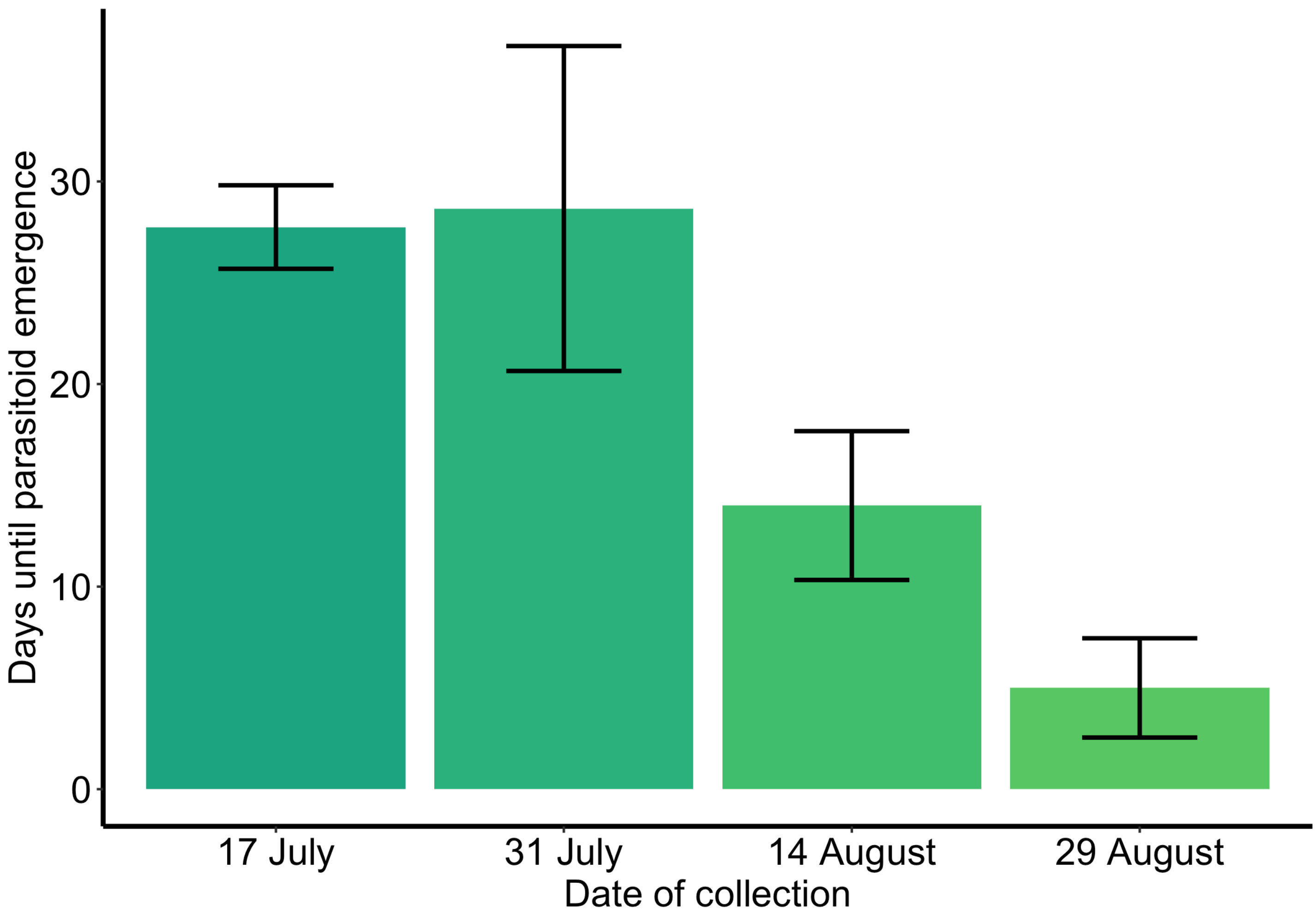
Table 2. Dates of collections done over the summer of 2017, with number of samples collected and number of parasitised individuals in each collection.

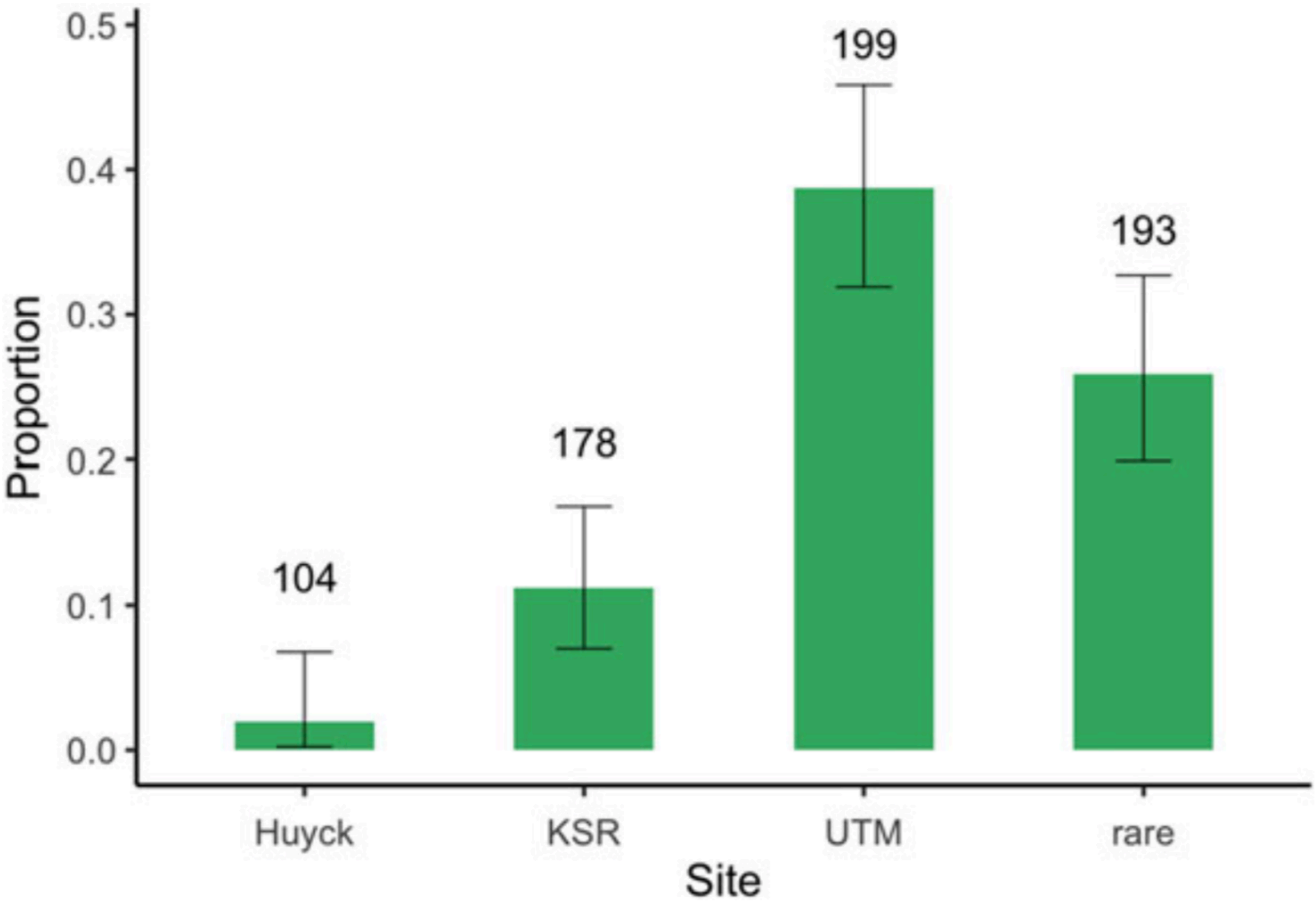
Collection	Date	Number of samples	Number parasitised
1	26 June	21	0
2	17 July	22	4
3	31 July	20	3
4	14 August	25	13
5	29 August	23	4
6	13 September	20	0


0.8
0.6
0.4
0.2
0.0
Proportion
22
20
25
23
17 July
31 July
14 August
29 August
Collection



Individual
15 July
15 August
Date
Collection date
17 July
31 July
14 August
29 August


Days until parasitoid emergence
30
20
10
0
17 July
31 July
14 August
29 August
Date of collection



0.5
0.4
0.3
0.2
0.1
0.0
Proportion
199
193
178
104
Huyck
KSR
UTM
rare
Site