Parasitology

cambridge.org/par

Research Article

Cite this article: Balzer ZS, Davis AR (2020). Life history traits and interactions of *Stylops advarians* (Strepsiptera) with its bee host, *Andrena milwaukeensis*. *Parasitology* **147**, 410–417. https://doi.org/10.1017/S0031182020000037

Received: 12 October 2019
Revised: 17 December 2019
Accepted: 18 December 2019
First published online: 22 January 2020

Key words:
Abundance; *Andrena*; Hymenoptera; intensity; life history; manipulation; prevalence; Strepsiptera; *Stylops*

Author for correspondence:
Arthur R. Davis, E-mail: art.davis@usask.ca

Life history traits and interactions of *Stylops advarians* (Strepsiptera) with its bee host, *Andrena milwaukeensis*

Zach S. Balzer and Arthur R. Davis

Department of Biology, University of Saskatchewan, 112 Science Place, Saskatoon, Saskatchewan S7N 5E2, Canada

Abstract

Specimens of *Stylops advarians* were sampled by collecting foraging bees of *Andrena milwaukeensis* along the South Saskatchewan River within Saskatoon, Saskatchewan. As the foraging season progressed from early May till late June over three consecutive years (2016–2018), most stylopized bees possessed endoparasitic adult (neotenic) females of *S. advarians* protruding from the bee gaster's dorsum. In contrast, very few adult bees stylopized by male puparia, and no free-living males, were encountered. Over the sampling period, prevalence remained around 22% each year; mean intensity was 1.2 (range of 1–3 female parasites per bee); and parasite abundance was 0.27. Also newly reported for *Stylops* is the occurrence of one bee bearing four *Stylops* (two neotenic females and two males with puparia), plus another bee with a male puparium extruded from its gaster's sternites. Around 2 May each year, a high proportion of the earliest captured female bees were stylopized. However, non-stylopized female bees typically were not encountered until about 10 days later, suggesting the parasites manipulate female bee hosts to emerge earlier, in close synchrony to male bee emergence. First-instar larvae of *S. advarians* appeared from 22–25 May, indicating that adults of *S. advarians* matured and mated at similar times each season.

Introduction

Strepsiptera is an endoparasitic order of Insecta that has a unique and complex life cycle caused by the extreme sexual dimorphism of the adult males and females. Adult males are winged and free-living, and actively seek females to mate. However, adult females are permanently endoparasitic (except those in Mengenillidae), and only their cephalothorax extrudes from the host's gaster (abdomen) to allow for copulation and release of mobile first-instar larvae (Kinzelbach, 1971; Kathirithamby, 1989, 2009).

There are eight extant families of Strepsiptera, the three largest being Halictophagidae (115 species), Stylopidae (163) and Xenidae (117) (Kathirithamby, 2018). However, many species of the large genus *Stylops* may have been misidentified. Thus, based on molecular identification, the genus likely has around 67 valid species (Straka *et al.*, 2015), instead of 117 (Kathirithamby, 2018). *Stylops* stylopizes important bee pollinators of *Andrena*, causing the inhibition of ovary development (Pèrez, 1886; Smith and Hamm, 1914; Kathirithamby, 1989, 2009). Within Canada, Peck (1991) reported four species of *Stylops*, namely *S. advarians*, *S. childreni*, *S. leechi* and *S. vicinae*. Without disclosing their identity, Straka (2019) recently reported 15 species of Stylopidae.

Despite these established host–parasite relationships, there are surprisingly little data on standard life-history characteristics (prevalence, intensity, abundance) of strepsipteran parasites infecting their host populations, and much of the data cover a single year of investigation alone. Therefore, greater attention to annual studies at a common location to discover fluctuations in life-history traits over consecutive years is required. Strepsipterans that stylopize true bugs have been examined in this regard (Waloff, 1981; Melber, 1989; Melber and Pohl, 1997; Roy and Hazra, 2016), whereas fewer studies have focused on Stylopidae and Xenidae (see Pierce, 1909 and references therein), which infect bees and wasps, respectively. Pierce (1909) found that the intensity of stylopized bees was 1–3 parasites per host, but abundance data that include non-stylopized bees were not provided. In addition, for five *Andrena* species collected from several studies and locations, prevalence varied widely (35–90%) among species (Pierce, 1909). Furthermore, all *Stylops* were extruded dorsally, from between the host bee's 4th and 5th gastral segments, in all *Andrena* species examined (Pierce, 1909).

A single species, *Stylops crawfordi*, has been examined in different years, but at different locations. In Dallas, Texas, USA, Pierce (1909) recorded that 35% of *Andrena crawfordi* were parasitized by *S. crawfordi*. Several decades later, Jones and Jones (1981) found the prevalence of *S. crawfordi* infecting *A. crawfordi* at College Station, Texas to exceed 25%, before declining to 10% a few days later. Whereas male puparia were readily found by Pierce (1909), Jones and Jones (1981) were unable to collect males, which may have lowered the prevalence.

The general difficulty of finding males of Stylopidae could be due to their timing of emergence, as observed in Xenidae. Hrabar *et al.* (2014) observed the synchronous maturation of females and males of *Xenos peckii*; males emerge from their puparia concurrently when females protrude from their hosts' gasters, thereby increasing the likelihood of males finding female strepsipterans. Mating is also aided by a behavioural change observed in female *Andrena* infected by *Stylops* (Linsley and MacSwain, 1957; Straka *et al.*, 2011). Male *Andrena* normally emerge earlier than females (Westrich, 1989, as cited by Straka *et al.*, 2011), but stylopized females were observed emerging simultaneously with both non-stylopized and stylopized males (Brandenburg, 1953; Linsley and MacSwain, 1957; Straka *et al.*, 2011). The advanced emergence of stylopized female bees ensures that all host bees would be outside of their nest, increasing the probability that when a male *Stylops* emerges, a mature female will be available to mate. Males of *Stylops pacifica* have been observed mating with a female while her host bee was foraging (Linsley and MacSwain, 1957; Daly *et al.*, 1998), whereas others were only found mating while bees were near their nest site (Ulrich, 1933). The minute size and short life-span of these males also contribute to the difficulty in collecting them (Kathirithamby, 2009).

Over three consecutive seasons (2016–2018), we have undertaken an investigation of *Stylops advarians* interacting with its host, *Andrena milwaukeensis*, at a regular site within the western Canadian prairies. Phoresy and structure of the first-instar larvae (Balzer and Davis, 2019*a*) and adult male (Balzer and Davis, 2019*b*) of *S. advarians* have been described. The objectives of this article, however, are to provide standard life-history traits (prevalence, intensity and abundance) detailing the ecology of *S. advarians* within this population of *A. milwaukeensis*, and to investigate the seasonal dates of the parasite's activity, including initial emergence of the stylopized hosts and the emergence of the host-seeking first-instar *Stylops* larvae from the adult female parasites. Accordingly, this work also advances our general understanding of the potential impact of Strepsiptera on bees, which are particularly beneficial as pollinators.

Materials and methods

Study site

Specimens of *S. advarians* Pierce were collected by capturing their foraging host, bees of *A. milwaukeensis* Graenicher, from 2016 to 2018. In early May each spring, bees of *A. milwaukeensis* were found foraging on *Shepherdia* flowers at the northeast region of Cosmopolitan Park, a natural setting within Saskatoon, SK, near the South Saskatchewan River (52°07′43.8″N, 106°38′50.2″W). As the season progressed, bees of *A. milwaukeensis* were taken 1.1 km southwest (52°07′18.0″N, 106°39′25.2″W) several days later (Fig. S1). Moreover, two of four bees collected on 17 April 2019 for histological investigation near the newly constructed Collaborative Sciences Research Building (CSRB) at the University of Saskatchewan (52°07′56.4″N, 106°37′57.6″W) were found to be unusual in terms of parasite intensity and the host body's location of the extrusion of the parasite in the host body, and are included herein.

Specimen collection and analysis for parasitism

Adult bees of *A. milwaukeensis* were collected by sweep-netting and placed in vials. Bees of this species are discernible in the field due to their reddish-orange tergal hairs, and afterwards their identity was confirmed by their long clypeus and rounded pygidial plate (LaBerge, 1980). Bees of *A. milwaukeensis* were examined for stylopization (the presence of strepsipteran parasites extruded from the gaster) using an Olympus SZ-ST dissecting microscope. Non-stylopized bees, and most of the stylopized bees, were euthanized using vapours of ethyl acetate; however, stylopized bees not immediately euthanized were used to collect first-instar larvae that emerged from the extruded female parasites in late May (Balzer and Davis, 2019*a*).

Results

Seasonal occurrence of S. advarians

From 2016 to 2018, neotenic females of *S. advarians* were encountered in foraging bees of *A. milwaukeensis* as early as 2 May around the northeastern area of the study site. Bees of this species were no longer found at this location after 8 May, but instead were collected further southwest 6–10 days later, where they were found visiting flowers of *Cotoneaster* and *Syringa*. Thus, bees of *A. milwaukeensis*, both stylopized and non-stylopized, were continually collected as the season progressed throughout May and most of June. Host bees of *A. milwaukeensis* do not die upon the emergence of males of *S. advarians*; indeed, bees with empty puparia were occasionally collected several weeks after the emergence of the male from the puparium. After 22 June, bees of *A. milwaukeensis* ceased to forage.

In 2016 and 2017, only female bees were collected. Upon micro-inspection of these hosts, only neotenic females of *S. advarians* were found with the exception of one female bee collected on 14 May 2018, which had two intact puparia (males) (Balzer and Davis, 2019*b*). In 2018, male bees also were caught until they ceased to be present from 18 May onward. Male bees were also stylopized.

Of 455 foraging bees of *A. milwaukeensis* captured from 2016 to 2018, the stylopized bees from our study site held between 1 and 3 parasites each (Tables S1–S3). However, our summarized data (Figs 1–3) do not differentiate between bees that had one, two or three female *Stylops*; instead, these figures simply illustrate the presence or absence of *Stylops*. The frequency of stylopized bees collected per day did not show a clear pattern over the 3-year sampling period (Figs 1–3), except for early in the season. Stylopized female bees were regularly caught around 2 May, with multiple days early each spring where all, or a relatively high proportion of collected bees, were stylopized (Figs 2 and 3). Indeed, non-stylopized bees were only occasionally seen from 2–4 May (Figs 2 and 3). In 2016, however, bee sampling was not undertaken in the northeast region of the study site, so the presence of stylopized bees in that area during early May is uncertain (Fig. 1).

The number of stylopized bees collected before 8 May in 2017 and 2018 was significantly higher than the number of stylopized bees collected afterward (2017: $\chi^2_{1\,\mathrm{df}} = 22.216$; $N = 151$ bees, $P < 0.01$; 2018: $\chi^2_{1\,\mathrm{df}} = 10.66$; $N = 199$, $P < 0.01$).

No seasonal pattern for the occurrence of multiple *S. advarians* per collected host was apparent. Instead, such hosts were encountered randomly. For example, in 2017, the four bees with two *S. advarians* in their gaster were taken on 5, 15 and 29 May and 12 June (Table S2). Corresponding collection dates for eight similar hosts in 2018 were 2, 4, 22 and 28 May and 4, 6, 12 and 18 June (Table S3).

Stylops advarians is viviparous, and from year to year, the host-seeking first-instar larvae were observed emerging from the brood canal opening of several of the endoparasitic, neotenic females at similar dates. First-instars were initially observed on 25 May 2016, on 24 May 2017 and on 22 May 2018. Larvae exhibited asynchronous development inside their mother's body (Kathirithamby, 2009). Thus, many more larvae emerged at dates thereafter, as well as were found within the same adult female's body, following these initially recorded emergences each year.

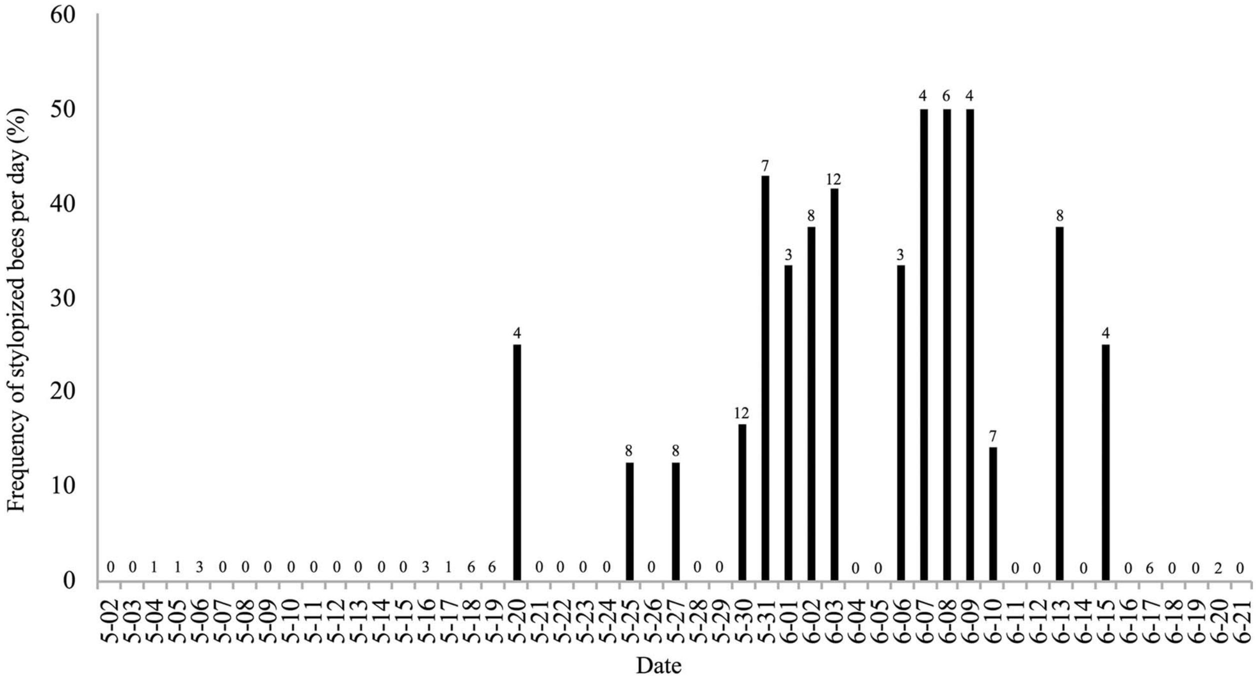

Fig. 1. Bar graph showing the frequency of stylopized bees of *Andrena milwaukeensis* collected each day in 2016. The number above each bar represents the total bees collected that day. Dates without bars are either days where collection did not occur (0), or stylopized bees were not encountered.

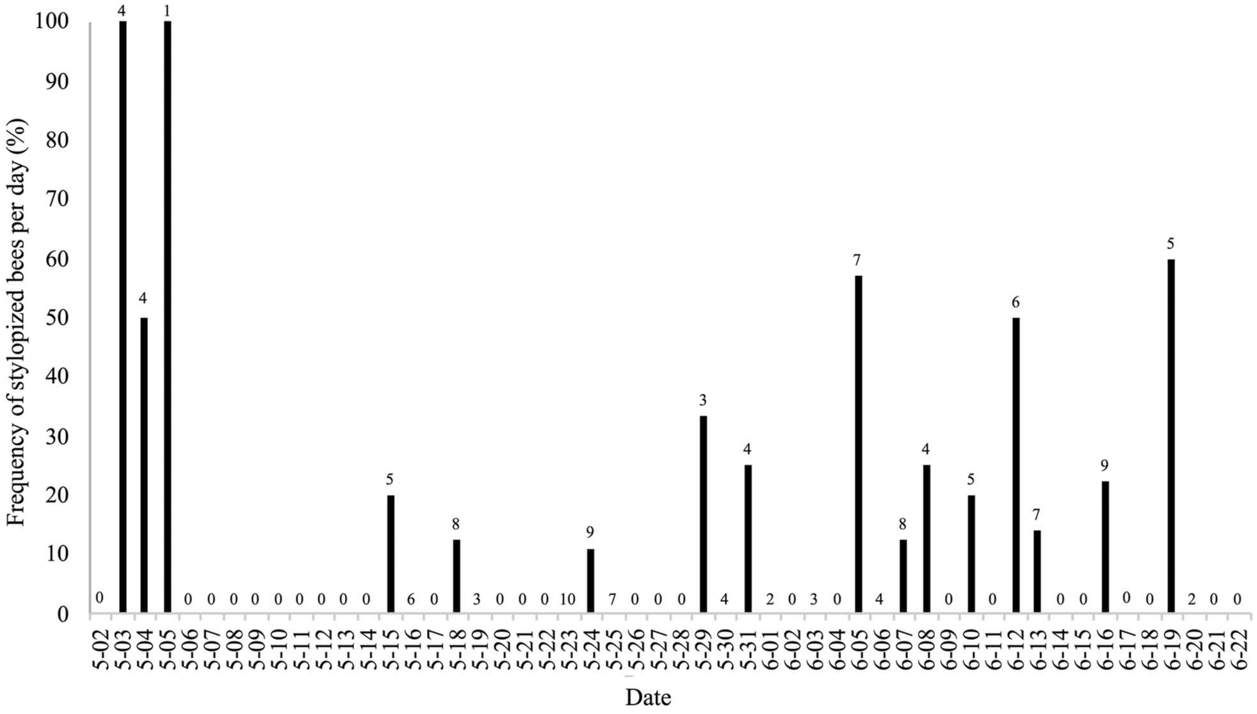

Fig. 2. Bar graph showing the frequency of stylopized bees of *Andrena milwaukeensis* collected each day in 2017. The number above each bar represents the total bees collected that day. Dates without bars are either days where collection did not occur (0), or stylopized bees were not encountered.

When mean temperature data (J. Diefenbaker International Airport, Saskatoon; 6 km from the study site) for spring–summer of 2016–2018 were plotted (Fig. S2), we could not discern correlations between temperature and the dates of advanced emergence of stylopized bees, nor dates of appearance of first-instar larvae.

Prevalence, intensity and abundance of S. advarians

Table 1 displays the annual sampling of bees of *A. milwaukeensis* collected from 2016 to 2018, as well as the parasite load of the stylopized bees. These results include only bees that held female parasites; bees with intact or empty male puparia were very rare (<0.7%).

The maximum number of *Stylops* females found per bee gaster during the collection period was three (Fig. 4), with the parasite intensity ranging from 1.17 to 1.24 and averaging 1.2 (Table 1). Of the total of 455 bees of *A. milwaukeensis* collected during May and June of 2016–2018, 356 (78%) lacked a female parasite (Fig. 4). Eighty-two bees (18%) had one female parasite (Figs 4 and 5A). Thirteen bees (3%) had two female parasites (Figs 4

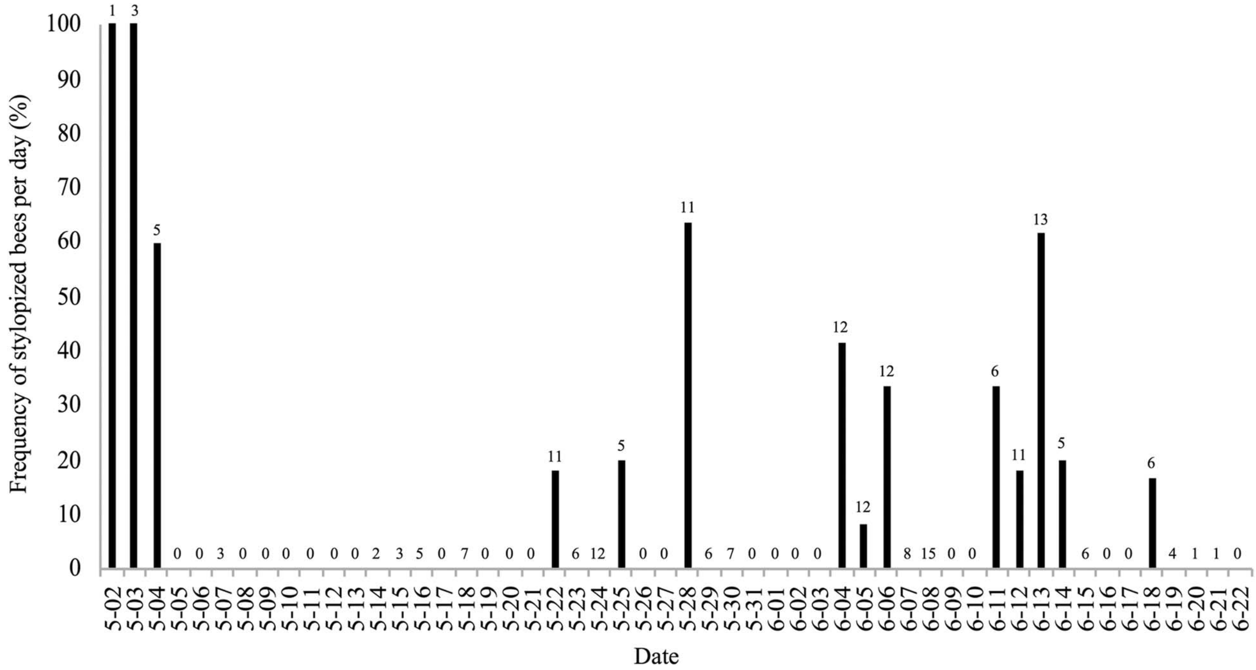

Fig. 3. Bar graph showing the frequency of stylopized bees of *Andrena milwaukeensis* collected each day in 2018. The number above each bar represents the total bees collected that day. Dates without bars are either days where collection did not occur (0), or no stylopized bees were not encountered.

Table 1. Number of bees of *Andrena milwaukeensis* sampled each year, including the number of neotenic females of *Stylops advarians* extruding per host gaster

	Number of female *Stylops* per bee								
Year	0	1	2	3	Total bees collected	Total bees stylopized	Prevalence (%)	Mean intensity	Abundance
2016	97	27	1	2	127	30	24	1.17	0.28
2017	102	23	4	1	130	28	22	1.21	0.26
2018	157	32	8	1	198	41	21	1.24	0.26

Prevalence, mean intensity and abundance for each year of the collection are also indicated.

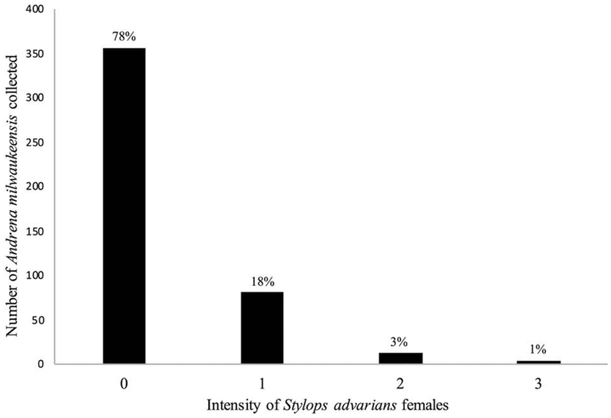

Fig. 4. A bar graph showing the intensity of infection by adult females of *Stylops advarians* within the *Andrena milwaukeensis* population of Cosmopolitan Park in Saskatoon, Saskatchewan sampled from 2016 to 2018. Percentages are shown above each bar.

and 5B), and four bees (1%) had three female parasites (Figs 4 and 5C). Thus, most bees lacked any sign of parasitism by a female *S. advarians*. Thus, the mean abundance of *S. advarians* was 0.27 (range 0.26–0.28; Table 1).

Most neotenic females of *S. advarians* protruded between the 4th and 5th tergites on the left or right side of the host bee's metasoma. A female parasite occupied the left or right side of the host's gaster, without significant bias ($\chi^2_{1\,\mathrm{df}} = 1.634$; $N = 42$ bees, $P = 0.439$). When three females were present per host, one female occupied each of the left and right sides of the host, whereas the third parasite protruded near the midline of the bee's gaster (Fig. 5C). Rarely was a bee found with a single female parasite residing at the centre of its gaster (Fig. 5D). When a bee infected with one central female parasite was found, that parasite was wider than those that resided laterally within a bee's gaster.

Unexpectedly, two abnormal specimens were acquired very early in spring (17 April 2019) at a different site (CSRB), during the intended collection of bees of *A. milwaukeensis* for histological investigations. One bee supported four *Stylops*: two puparia (one empty, one intact) and two neotenic females (Fig. 5E). The empty puparium and two females extruded between the 4th and 5th tergites, but the intact puparium extruded between the 3rd and 4th. Another host gaster held a vacated puparium on its ventral side, extruding between two sternites (Fig. 5F). Both host bees were captured while foraging on *Salix* catkins.

Discussion

The relatively advanced seasonal emergence of overwintered female bees of *A. milwaukeensis* that are stylopized corroborates previous observations (Brandenburg, 1953; Linsley and

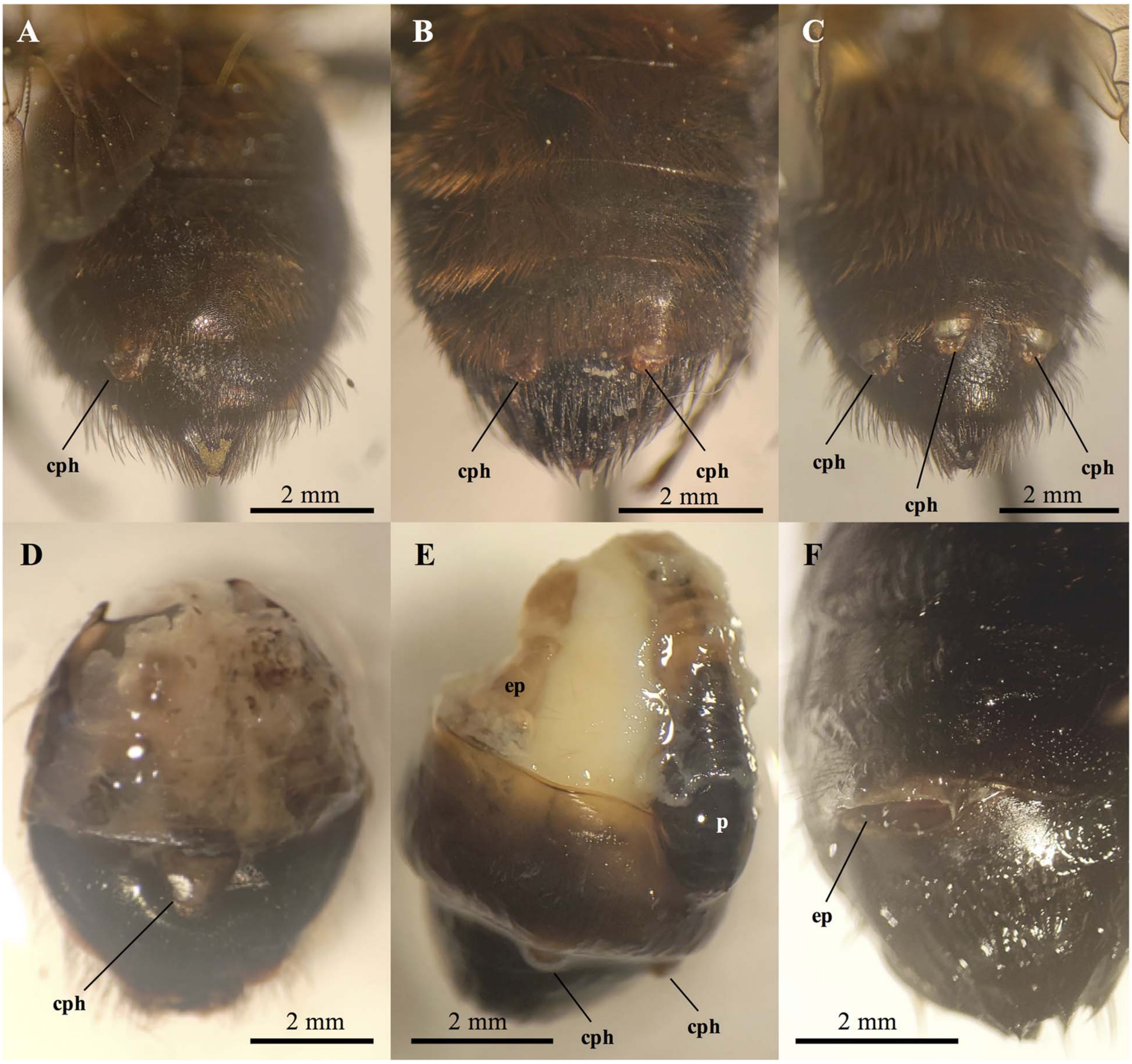

Fig. 5. Photographs of dorsal (A–E) and ventral (F) surface of gasters of *Andrena milwaukeensis* infected with *Stylops advarians*. (A) Gaster stylopized by one neotenic female. Note protruding cephalothorax of the adult female parasite. (B) Gaster stylopized by two neotenic females. (C) Gaster stylopized by three neotenic females. (D) A neotenic female at the midline of the host bee's gaster. (E) Partially dissected gaster with two neotenic females and evidence of two male puparia. (F) Gaster showing an empty *Stylops* puparium. cph, cephalothorax of adult female *Stylops*; ep, empty puparium; p, intact puparium.

MacSwain, 1957; Straka *et al*., 2011). As suggested by the latter, a *Stylops* female may physiologically manipulate its bee host – if also female – to emerge from her nest earlier than non-stylopized female bees. Consequently, stylopized female bees emerge concurrently with stylopized and non-stylopized male bees, thereby increasing the overlap of strepsipteran activity within a vicinity; as a result, the opportunity for a male of *S. advarians* to find a female should increase.

Over three consecutive years of sampling, however, free-living adult males of *S. advarians* remained elusive, not being encountered at the study site. This difficulty was likely due to factors such as their small size, the synchronization necessary between male emergence from their puparia coinciding with female maturation (Hrabar *et al*., 2014), and their short life-span (Kirkpatrick, 1937; Kathirithamby, 2009). As a result, free-living males unnoticed during their short lives go undetected for that entire season. In California, however, males of *S. pacifica* were recorded mating while the female's host bee was foraging on flowers of *Ranunculus californicus* (Linsley and MacSwain, 1957; Daly *et al*., 1998). Stylopized bees of another *Andrena* species were observed to emerge earlier than normal, but did not leave their nest site, presumably because flowers had not yet begun blooming (Ulrich, 1933). We suggest that at this study location, stylopized *A. milwaukeensis* emerge before the flowers they feed on bloom; thus, mating of *S. advarians* may take place at the bees' nest sites, rather than at open flowers. Unfortunately, nests of *A. milwaukeensis* were not found, so this supposition awaits confirmation.

Nonetheless, the annual mating period of *S. advarians* at this site likely occurs on, or shortly before, 2 May, when stylopized bees were first observed. This conclusion is based on inaugural observations of stylopized bees recorded in spring, the synchronization of male emergence with female maturation (Hrabar *et al*., 2014), the lack of intact puparia detected within bee hosts after 2 May, the detection of eggs in female *Stylops* collected in early May (unpublished results), and the emergence of first-instar larvae around 3 weeks after initial observation of the stylopized bees. Capture of a single individual bearing two intact puparia on 14 May 2018 very likely reflects that these two males died *in situ* (Balzer and Davis, 2019*b*), rather than that the mating period of

S. advarians extends over a 2-week span. After exposure of these puparia to the bright light of a microscope lamp failed to stimulate their emergence (James *et al*., 2016), the males were declared deceased, due to the short life-spans of male *Stylops* (Kathirithamby, 2009) and, for unknown reasons, having been unable to escape their puparia. The study by Jones and Jones (1981) also suggests that all males emerge and mate around the same time, as they could only detect empty puparia. Pierce (1909) found 44 bees of *A. crawfordi* hosting males of *S. crawfordi*, potentially within their puparia, though this possibility was not stated. Unfortunately, no dates of collection were given for those bees and their parasites.

Owing to the short life-span of adult males of *S. advarians*, only females were regularly collected. Because females of *S. advarians* reside within their host as neotenic adults, *A. milwaukeensis* bees were collected continuously throughout their foraging season to sample their female parasites. Stylopized bees were observed earliest on flowers of *Shepherdia*, whereas non-stylopized bees were much less commonly encountered at this time. Both stylopized and non-stylopized bees were later found further southwest, visiting *Syringa* and *Cotoneaster*. The bees' presence at different foraging areas at the study site was likely stimulated by the successive depletion of pollen and nectar sources from each taxon upon which they visited.

Inaugural issuance of first-instar larvae of *S. advarians* from their mothers occurred on similar dates each year. This comparable emergence in three consecutive springs suggests that mating of *S. advarians* occurs at similar times each year, assuming the length of development from embryo to the emergence of first-instar larvae is consistent. Linsley and MacSwain (1957) estimated that the development of first-instars of *S. pacifica*, from insemination to emergence, takes 30–40 days. Moreover, Jones *et al.* (1980) reported an incubation period of 2–3 weeks for the eggs of *S. bipunctatae*. The histological study of eggs within adult females of *S. advarians* taken around 2 May (Balzer, 2019), as well as first emergence of the viviparous first-instar larvae around 22 May, corroborates that the duration of egg incubation of *S. advarians* resembles that of *S. bipunctatae* (Jones *et al*., 1980). Like in other *Stylops* species (Ulrich, 1956; Linsley and MacSwain, 1957), the first-instar larva of *S. advarians* is deposited on flowers. By phoresy, it may be transported either internally or externally by an adult bee of *A. milwaukeensis* (Balzer and Davis, 2019*a*) to its nest, where the parasitic larva eventually can invade its host.

This study's prevalence data pertain only to stylopization by at least one female of *S. advarians*, the single bee with two puparia (males) being excluded. Thus, the true level of prevalence is undoubtedly higher than 22%. Beyond the fortuitous discovery of additional host bees with puparia (males), another line of research that would indirectly estimate the proportion of males of *S. advarians* in this population would be the determination of the sex ratio of the first-instar larvae. Assuming that both sexes have an equal probability of successfully parasitizing a host, this approach may clarify the true prevalence of *S. advarians* within this population of *A. milwaukeensis*.

The annual prevalence of stylopized bees of *A. milwaukeensis* remained consistent (21–24%) for three consecutive years of sampling. These values accord to two independent studies from different locations in Texas, USA, with an initial level of prevalence (25%) for *S. crawfordi* parasitizing *A. crawfordi* (no males), before a recorded decline to below 10% (Jones and Jones, 1981). Sampling during the foraging season of *S. crawfordi* spanned around 30 days but actually was curtailed to only 13 days of collection, due to rain (Jones and Jones, 1981). In the other study of this same bee–parasite relationship, wherein male parasites were included, the prevalence was 35% (Pierce, 1909). In a separate strepsipteran–host relationship, Hughes *et al.* (2004) found that 7% of *Polistes dominula* at their nest were stylopized, but found 25% of overwintering wasps to be stylopized. The low percentage of stylopized wasps on the nest were workers which usually abandon the nest, whereas the higher percentage was of gynes that aggregate to overwinter. The consistency in the prevalence of *S. advarians* from 3 years at the same site may reflect the vast numbers of larvae that each female parasite produces, as well as the minute probability that each first-instar successfully parasitizes a host. Since so many first-instar larvae are produced per female, annual fluctuation in the proportion of these larvae that successfully find a host evidently remains low. This presumably low success rate would cause the number of bees that become infected with *S. advarians* to remain similar each year, leading to similar prevalences.

Prevalence may be different between strepsipteran species due to several factors. The particular environment that these parasites and their hosts occupy, plus their resultant behaviour, may govern each parasite's success. For example, the behaviour of stylopized eusocial wasps of *Polistes* is different than that of the stylopized solitary bees of *Andrena*. Stylopized wasps of the former forego their social tasks and leave their colony to aggregate at the same site, facilitating mating by the parasites (Hughes *et al*., 2004). *Andrena* infected by *Stylops*, however, continue their normal behaviour of foraging and nest making (Linsley and MacSwain, 1957). Strepsipterans may also manipulate their host, as seen with *Xenos* females potentially causing their wasp host to spend more time on specific plants in order to increase the success of their larvae being deposited in that favourable place. Makino and Yamashita (1998) found that *Xenos moutoni* manipulates its *Vespa* host to visit holes and scars in trees that produce sap in order to facilitate the dispersal of first-instar larvae. More recently, Beani *et al.* (2018) found that *Polistes* wasps stylopized by a female *Xenos* spent more time at *Campsis radicans* flowers, likely for a similar reason. The prevalence of species within most other strepsipteran families is unclear and needs further study.

The intensity of parasitism throughout the collection period of 2016–2018 never exceeded three females per host. However, early in 2019, a specimen was captured with four parasites of *S. advarians* within its gaster (Fig. 5F), which we have not found documented for any *Stylops* species. Most of the 455 bees (78%) were not stylopized, and the majority of those that were had just one parasite (18%). Occasionally, bees were found with two female parasites (3%), but very rarely (1%) were three female parasites present in a host. Thus, the mean abundance of parasitism was 0.27 and remained annually consistent (range 0.26–0.28). The low mean intensity (1.2) of *S. advarians* in *A. milwaukeensis* bees is most likely due to the difficulty of a first-instar successfully finding and surviving within a permanent host. It is likely rare for a single larva of *S. advarians* on a flower to successfully travel with an adult bee to its nest and parasitize one of that phoretic host's offspring. It is therefore a successively rarer event for two, three or even four strepsipteran first-instar larvae to be carried by a single adult bee to the bee's nest, and then successfully parasitize the same immature bee in that nest. That intensity evidently rarely exceeds three strepsipterans per host also suggests that the gaster of an individual host may reach its carrying capacity. Supportive indirect evidence includes the expanded state of a centrally-located single parasite, compared to laterally-positioned parasites (see below).

Females of *S. advarians* were most often found protruding between the 4th and 5th tergites of their host's gasters, even when three females were present. The consistent location of the females within their host may signify that this position provides the female with the greatest reproductive success (Maeta *et al*., 2001). This location within the host allows the female parasite to grow to the anterior end of her host's gaster, without impacting important organs of the host in a debilitating way. A longer body may allow the strepsipteran female to produce more offspring. Female parasites were also often found in the lateral area of their host's abdomen in single- and double-parasite infections.

Occasionally, bees were found to have just one female *Stylops* that protruded from the midline of their gasters and, unlike the findings of Linsley and MacSwain (1957), we did not find undeveloped *Stylops* on either side of this single, central female. The few centrally-residing females observed were wider than those found laterally, and perhaps this larger space permits increased growth of the parasite that allows more larvae to be produced. However, if the number of eggs a single female produces is genetically based, as observed in *Drosophila* (Robertson, 1957), or is governed by nutrient acquisition (Rivero *et al.*, 2001), the lateral or central placement within the host would be irrelevant in regard to the production of larvae. That most females were found laterally, rather than centrally within their host's gaster, likely signifies how the female parasite is supported by her host's organs (unpublished results). However, there was no preference to which side of the host that a neotenic female occupied.

The consistency in the annual values of several life-history traits (prevalence, intensity and abundance), as well as their narrow ranges for 2016–2018, suggests that the host–parasite system of *S. advarians* and *A. milwaukeensis* at this location in western Canada is currently in balance. On the other hand, collections of several specimens of *A. milwaukeensis* in 2019 near the newly-constructed CSRB at the University of Saskatchewan on April 17, as they foraged on *Salix* catkins, revealed some unique features. This date is about 2 weeks earlier than bees of *A. milwaukeensis* were normally collected during 2016–2018 at the main study site. On that same date (17 April 2019), the main study site was examined and no *A. milwaukeensis* were observed. Foraging bees found near the CSRB may have nests near the building, and the soil may have warmed more quickly than the ground at the main site, perhaps related to the recent construction or presence of the building. Temperature increases are known to advance the emergence of ground-nesting bees, elsewhere (Schwartz and Karl, 1990; Schenk *et al.*, 2018). One of these bees was infected with four *Stylops* parasites. The presence of four *Stylops* in one bee of *A. milwaukeensis* is likely an extremely rare event, as this is the only specimen with four *Stylops* found in over 3 years of sampling. Another of these collected bees had an empty puparium on the ventral side of its gaster. This strategy for ventral protrusion by a male did not preclude its successful emergence from this host body's position, but it would seem detrimental for the females of *S. advarians* because of a potential impediment for a male to mate with a female residing in this ventral position on the host. Protruding from the tergites rather than the sternites is most often seen and hence likely the most successful strategy. As far as we know, this is the first recorded event of four *Stylops* parasites within a single *Andrena* bee, and the first time a *Stylops* puparium has been detected on the ventral side of the gaster of an *Andrena* bee. On the other hand, dorsal- and ventral-protruding strepsipterans are a common occurrence in wasps stylopized by xenid strepsipterans (Maeta, 1963, 1971; Kifune and Maeta, 1975; Maeta *et al.*, 2001).

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0031182020000037.

Acknowledgements. We thank Kelton Braun for his help collecting several stylopized bees. Additionally, we thank three anonymous reviewers for their constructive critiques of this submission.

Financial support. This work was supported by a University of Saskatchewan Graduate Scholarship, and an Arthur Brooks Award from the Entomological Society of Saskatchewan (ZSB), plus an NSERC Discovery Grant (ARD), for which we are grateful.

Conflict of interest. None.

Ethical standards. Not applicable.

References

Balzer ZS (2019) *Morphology of Stylops advarians (Strepsiptera) and the Effects of Parasitization on its Host, Andrena milwaukeensis (Hymenoptera)* (MSc thesis). University of Saskatchewan, Saskatoon, Canada.

Balzer ZS and Davis AR (2019a) Adaptive morphology of the host-seeking first-instar larva of *Stylops* advarians Pierce (Strepsiptera, Stylopidae), a parasite of *Andrena milwaukeensis* Graenicher (Hymenoptera, Andrenidae). *Arthropod Structure & Development* **52**, 100881.

Balzer ZS and Davis AR (2019b) Description of the adult male of *Stylops advarians* Pierce (Strepsiptera: Stylopidae). *Zootaxa* **4674**, 496–500.

Beani L, Cappa F, Manfredini F and Zaccaroni M (2018) Preference of *Polistes dominula* wasps for trumpet creepers when infected by *Xenos vesparum*: A novel example of co-evolved traits between host and parasite. *PLoS ONE* **13**, e0205201.

Brandenburg J (1953) Der Parasitismus der Gattung *Stylops* an der Sandbiene *Andrena vaga* PZ. *Zeitschrift für Parasitenkunde* **15**, 457–475.

Daly HV, Doyen JT and Purcell III AH (1998) *Introduction to Insect Biology and Diversity*, 2nd Edn. Oxford, UK: Oxford University Press.

Hrabar M, Danci A, McCann S, Schaefer PW and Gries G (2014) New findings on life history traits of *Xenos peckii* (Strepsiptera: Xenidae). *The Canadian Entomologist* **146**, 514–527.

Hughes DP, Kathirithamby J and Beani L (2004) Prevalence of the parasite Strepsiptera in adult *Polistes* wasps: field collections and literature overview. *Ethology Ecology & Evolution* **16**, 363–375.

James M, Nandamuri SP, Stahl A and Buschbeck EK (2016) The unusual eyes of *Xenos peckii* (Strepsiptera: Xenidae) have green- and UV-sensitive photoreceptors. *Journal of Experimental Biology* **219**, 3866–3874.

Jones D and Jones G (1981) Stylopization of *Andrena* spp. (Hymenoptera: Andrenidae) by *Stylops crawfordi* (Strepsiptera: Stylopidae) in Texas. *Journal of the Kansas Entomological Society* **54**, 223–227.

Jones D, Williams ML and Jones G (1980) The biology of *Stylops* spp. in Alabama, with emphasis on *S. bipunctatae*. *Annals of the Entomological Society of America* **73**, 448–451.

Kathirithamby J (1989) Review of the order Strepsiptera. *Systematic Entomology* **14**, 41–92.

Kathirithamby J (2009) Host-parasitoid associations in Strepsiptera. *Annual Review of Entomology* **54**, 227–249.

Kathirithamby J (2018) Biodiveristy of Strepsiptera. In Foottit RG and Adler PH (eds), *Insect Biodiversity: Science and Society*, Vol. **2**. New York, USA: John Wiley & Sons Ltd, pp. 673–703.

Kifune T and Maeta Y (1975) A new subgenus and new species of the genus *Xenos* (Strepsiptera, Stylopidae) from Japan. *Kontyû* **43**, 446–455.

Kinzelbach R (1971) Morphologische Befunde an Fächerflüglern und ihre phylogenetische Bedeutung (Insecta: Strepsiptera). *Zoologica* **41**, 1–256.

Kirkpatrick TW (1937) Studies on the ecology of coffee plantations in East Africa. II. The autecology of *Antestia* spp. (Pentatomidae) with a particular account of a strepsipterous parasite. *Transactions of the Royal Entomological Society of London* **86**, 247–343.

LaBerge WE (1980) A revision of the bees of the genus *Andrena* of the western hemisphere. Part X. Subgenus *Andrena*. *Transactions of the American Entomological Society* **106**, 395–525.

Linsley EG and MacSwain JW (1957) Observations on the habits of *Stylops pacifica* Bohart. *The University of California Publications in Entomology* **11**, 395–430.

Maeta Y (1963) Some biological notes on *Pseudoxenos iwatai* Esaki (I) its biology and natural history. *Kontyû* **31**, 1–15.

Maeta Y (1971) Some biological notes on *Pseudoxenos iwatai* Esaki (IV) additional notes on its biology and natural history. *Kontyû* **39**, 105–119.

Maeta Y, Gôukon K, Kitamura K and Miyanaga R (2001) Factors that determine the positions where *Pseudoxenos iwatai* Esaki (Strepsiptera: Stylopidae) extrudes from the host abdomen. *Tijdschrift voor Entomologie* **144**, 203–215.

Makino S and Yamashita Y (1998) Levels of parasitism by *Xenos moutoni* du Buysson (Strepsiptera, Stylopidae) and their seasonal changes in hornets (Hymenoptera, Vespidae, *Vespa*) caught with bait traps. *Entomological Science* **1**, 537–543.

Melber A (1989) Die Parasitierung der Heidezikade *Ulopa reticulata* (F.) (Hom., Auchenorrhyncha, Cicadellidae) durch *Halictophagus silwoodensis* Waloff (Strepsiptera, Halictophagidae) in nordwestdeutschen *Calluna*-Heiden. *Braunschweiger naturkundliche Schriften* **3**, 419–428.

Melber A and Pohl H (1997) Erster Nachweis einer Strepsipterenparasitierung bei Wanzen in Mitteleuropa (Insecta, Strepsiptera et Heteroptera). *Bonner Zoologische Beiträge* **47**, 69–76.

Peck, SB (1991) Order Strepsiptera, twisted-winged parasites. In Bousquet Y (ed.), *Checklist of Beetles of Canada and Alaska*. Ottawa, Canada: Research Branch, Agriculture Canada, pp. 366–367.

Pérez J (1886) Des effets du parasitisme des *Stylops* sur les apiaries du genre *Andrena*. *Actes de la Société linnéenne de Bordeaux* **40**, 21–63.

Pierce WD (1909) A monographic revision of the twisted winged insects comprising the order Strepsiptera Kirby. *Bulletin of the United States National Museum* **66**, 1–232.

Rivero A, Giron D and Casas J (2001) Lifetime allocation of juvenile and adult nutritional resources to egg production in a holometabolous insect. *Proceedings of the Royal Society B – Biological Sciences* **268**, 1231–1237.

Robertson FW (1957) Studies in quantitative inheritance XI. Genetic and environmental correlation between body size and egg production in *Drosophila melanogaster*. *Journal of Genetics* **55**, 428–443.

Roy S and Hazra N (2016) Seasonal variation of stylopization on white leafhopper *Cofana spectra* (Distant) (Homoptera: Cicadellidae) by *Halictophagus australensis* Perkins (Strepsiptera: Halictophagidae). *Journal of Entomology and Zoology Studies* **4**, 82–85.

Schenk M, Mitesser O, Hovestadt T and Holzschuh A (2018) Overwintering temperature and body condition shift emergence dates of spring-emerging solitary bees. *PeerJ* **6**, e4721.

Schwartz MD and Karl TR (1990) Spring phenology: nature's experiment to detect the effect of 'green-up' on surface maximum temperatures. *Monthly Weather Review* **118**, 883–890.

Smith GW and Hamm AH (1914) Studies on the experimental analysis of sex – part 11. *Quarterly Journal of Microscopial Science* **60**, 435–461.

Straka J (2019) Strepsiptera of Canada. *Zookeys* **819**, 377–382.

Straka J, Rezkova K, Batelka J and Kratochvíl L (2011) Early nest emergence of females parasitized by Strepsiptera in protandrous bees (Hymenoptera Andrenidae). *Ethology Ecology & Evolution* **23**, 97–109.

Straka J, Juzova K and Nakase Y (2015) Nomenclature and taxonomy of the genus *Stylops* (Strepsiptera): an annotated preliminary world checklist. *Acta Entomologica Musei Nationalis Pragae* **55**, 305–332.

Ulrich W (1933) Fang und Züchtung von Strepsipteren. *Abderhalden, Handbuch der biologischen Arbeitsmethoden*, Abt. 9, 7, 259–237.

Ulrich W (1956) Unsere Strepsipteren-Arbeiten. *Zoologische Beitrage* **2**, 177–255.

Waloff N (1981) The life history and descriptions of *Halictophagus silwoodensis* sp.n. (Strepsiptera) and its host *Ulopa reticulata* (Cicadellidae) in Britain. *Systematic Entomology* **6**, 103–113.