



Host-parasitoid network in extreme conditions: the case of cereal aphids in wheat crops in Saskatchewan, Canada

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With 3 figures and 1 appendix

Abstract: In agricultural areas, the climatic conditions at the northern limit of crop cultures could impact pest regulation. In this paper, we study the aphid-parasitoid-hyperparasitoid network in cereal fields around Saskatoon (SK, Canada), one of the most harsh climatic conditions for cereal crops in the world, with an extremely short growing season. We have investigated the aphid-parasitoid-hyperparasitoid species relative abundances over two consecutive years. In 2014, a cold spring year, aphids were only found late in the growing season, and their arrival probably corresponded to late Southern winds. In 2015, aphids, parasitoids and hyperparasitoids arrived in the cereal fields in July and August, whereas the cereal harvest is typically complete by the end of the month of August. Three species of primary parasitoids were found for a total parasitism rate of 7.62%, and four genera of hyperparasitoids, with a hyperparasitism rate of 20.1%. There were 87.4% apterous aphids, and the proportion of apterous and winged (alate) aphids did not differ among the 3 aphid species, or among use by parasitoid species and hyperparasitoid species. The sex ratio of parasitoids in the complex was biased towards females for most of the species. In areas with extreme weather conditions and short growing seasons, effective biocontrol through parasitism is limited, although the synchronization level of parasitoids to their aphid hosts is high. We discuss the consequences of the limited length of the growing season on the provision of ecosystem services and on the seasonal ecology of insects of agronomic interest. We also provide information about morphological criteria for identification of parasitoids from the genus *Aphidius*.

Keywords: Trophic web, harsh conditions, biological control, aphid-parasitoid-hyperparasitoid, cereal crops, identification, diapause

1 Introduction

The development and survival of ectotherms are intimately linked to ambient temperature. These organisms have evolved a diversity of morphological, physiological, and behavioral adaptations to survive within a given range of climatological conditions. Temperature governs species distribution, ecosystem function, community composition, population abundances, life history traits of individuals and synchronization of life-cycles of interacting species (Bale et al. 2002; Hance et al. 2007). At the extreme limits of ectotherm's distribution areas, food webs can be less complex (i.e. with less species richness and fewer trophic links) compared to those of more favorable areas. Hammill et al. (2015) showed that food web simplicity generally decreased the persistence of species. Therefore, in the northernmost distribution area of species occurring in the Northern Hemisphere, the multitrophic

interactions among ectotherms can be random, with two possible scenarios: annual colonization from southern areas or *in situ* overwintering (Brodeur & McNeil 1989, 1994; Irwin et al. 1988).

In agricultural areas, which represent highly-disturbed and less resilient ecosystems, pest regulation at the periphery of crop cultivation could be different than in more concentrated crop growing regions. Indeed, a positive relationship between biodiversity and ecosystem functioning has been observed in many studies. For example, generalist predators often benefit from high prey diversity which is itself favored by high plant diversity (Symondson et al. 2002; Miyashita et al. 2012). Several studies have shown that more diverse communities are functionally less susceptible to environmental stresses (Mulder et al. 2011; Steudel et al. 2012). Host-parasitoid systems are particularly suited to investigate the links between biodiversity and biological control

ecosystem services. In cereal crops in temperate areas, food webs of aphid host and their parasitoids and hyperparasitoids are well known. However, this information is presently lacking at the northern extreme of crop areas in some overlooked agricultural areas, where the food webs should be simpler than in areas with more favorable climatic conditions.

In this paper, we study the aphid-parasitoid-hyperparasitoid network in cereal fields around Saskatoon, Saskatchewan (SK), Canada, which has severe climatic conditions for cereal crop production, including an extremely short growing season (130–140 days). Before obtaining a temporal series of data in the northernmost limits of cereal crop production, it is first necessary to establish baseline information on the seasonal ecology of insects, the composition of food webs, the sex ratio of the parasitoids which influences their dynamics, as well as the proportion of diapausing individuals (Andrade et al. 2016; Tougeron et al. 2017). For sex ratio, in parasitoids, it can be strongly biased towards females in favorable environments (Godfray 1994), as it is for example commonly observed in cereal aphid-parasitoid systems under favorable conditions (Andrade et al. 2015). Several studies have focused on the influence of climate (Stireman et al. 2005) or land cover patterns (Bennett & Gratton 2012) on species richness, abundance and diversity of host-parasitoid communities. The fitness of parasitoids is linked to the spatial and temporal availability of the host species they exploit (Henry et al. 2008). Therefore, one can expect the specific composition of the host community and the relative abundance of the different species to be major factors explaining the relative abundances of parasitoid species. The proportion of alate and apterous aphids could provide information about their migrations.

To improve our understanding of the food web in Saskatchewan Canada and the potential of biocontrol from parasitoids, we compared our results with previous studies of aphid food webs in USA (with more favorable climatic conditions) and with Europe (the area of origin of the aphids and many of their parasitoids). Indeed, the pest status of aphids in cereals differs across the world: they are major pests in most invaded regions (e.g. North America), but often minor pests in their native regions (e.g. Europe). This status could account for the lower number of natural enemies and the simpler food webs observed in non-European countries and thus explain the higher impact of those pests in cereal systems outside of Europe. Three aphid species largely present in cereals in North America probably came from Europe; the bird cherry-oat aphid, *Rhopalosiphum padi* (Linnaeus), which has been present in North America at least since 1900 (Blackman & Eastop 2007), the greenbug aphid, *Schizaphis graminum* (Rondani) for which the first North American report was on wheat and barley in Virginia around 1882 (Webster & Phillips 1912) and the English grain aphid, *Sitobion avenae* (Fabricius), a native of Europe, which was probably accidentally introduced everywhere in the world along with its cereal crop hosts (Asia, North and South

America) (Brewer & Elliott, 2004). In most non-European countries, biological control programs against *S. avenae* were organized and several species of parasitoids were introduced, and sometimes native parasitoid species adapted themselves to these new aphid hosts (Brewer & Elliott, 2004). Therefore, the host-parasitoid communities could be very similar in all the parts of the world where cereals are cultivated (for example Andrade et al., 2016 for Europe, Zuniga, 1990 for South America, Horrocks et al. 2010 for New Zealand) but in Saskatchewan, the climatic conditions may be too harsh for the survival of many of the parasitoids that were introduced to the USA. The native *Aphidius avenaphis* (syn. *Praon avenaphis* Fitch) is a generalist North American aphid parasitoid (Marsh 1979; Pike et al. 1997) and was reported to occur in the US and Canada as early as in the 19th century (Fitch 1861, Muesebeck & Walkley 1951 in Kos et al. 2011). *Aphidius avenaphis* prefers the English grain aphid when this resource becomes the most abundant aphid in cereal crops, as does *A. uzbekistanicus* (Kos et al. 2011).

We investigated the species relative abundances in an aphid-parasitoid system in cereal fields in Saskatchewan, during the two consecutive years of 2014 and 2015. Relative abundances of parasitoid species and their aphid hosts in cereal fields were determined during the very short summer period. We aimed at investigating; (1) the species of aphids, parasitoids and hyperparasitoids present, their phenology, sex ratio and their links within the trophic web; (2) whether parasitoids and hyperparasitoids exploited different host species at distinct levels (i.e. resource specialization or resources equally exploited).

We predicted that: (1) this host-parasitoid system shows a high inter-annual variability due to the paucity of species and the harsh conditions; (2) two complementary scenarios could occur in regards to the origins of the aphids and the parasitoids: they may arrive on the south winds, and/or part of the population could overwinter onsite near fields; (3) the sex ratio is not female biased for parasitoids and hyperparasitoids, since conditions are not favorable; (4) diapause is observed as early as August among parasitoids in response to early harsh climatic conditions encountered in Saskatchewan. (5) this system harbors a limited number of parasitoid and hyperparasitoid species compared to the same aphid-parasitoid systems in the USA or in Europe; (6) this system harbors proportionally more parasitoid and hyperparasitoid generalist species than the same system in the USA or in Europe due to the small amount of resource available and the potential spatio-temporal variability in hosts' abundances.

2 Material and methods

2.1 Field data

To establish the food webs, sampling was conducted in wheat fields near Saskatoon and in a radius up to 200 km north east of Saskatoon. Five fields where cereal aphids

appeared were monitored over time each year in 2014 and six in 2015. In 2014, the monitored fields were near Alvena (52.50°N, 106.02°W), the University of Saskatchewan's Kernen Research farm (52.16°N, 106.55°W), the Agriculture and Agri-Food's (AAFC) Melfort Research farm (52.82°N, 104.60°W), AAFC Saskatoon Research Farm (52.13°N, 106.63°W), and a grower's field near Yellow Creek (52.754°N, 105.220°W). In 2015, the sampling sites were the same or adjacent fields to those of 2014, except with the addition of a field at the Saskatchewan Conservation Centre near Prince Albert (53.07°N, 105.83°W). All fields were conventionally rather than organically managed but in this research area, the wheat was not sprayed with insecticides because it is not economically viable for farmers or research farm managers to treat their wheat crops unless a threshold of 12–15 aphids per head is reached. The wheat fields used were varieties that also contained the *Sml* wheat midge-resistance gene so that wheat fields were not treated with insecticide in July for wheat midge. Sweep sampling was used to determine when aphids appeared in the fields, especially during periods of low-levels of aphids which could not be detected by visual sampling. For sweep sampling, we took 20 sweeps of 180 degree with a 38 cm diameter sweep net in five transects per field per date, at distances into the field of 5m, 10m, 25m, 50m and 100m from the field edge, with all transects parallel to the field edge and the adjacent roadside ditch for a total of one hundred sweeps per field. When aphid presence was noted in sweep samples, visual observations were also performed. For visual observations, living aphids were collected directly from the wheat plants and brought to the laboratory to allow for aphid mummy formation (i.e., dead aphids containing a developing parasitoid pupa). Aphid mummies of parasitoids or hyperparasitoids also were collected directly from the plants and brought to the laboratory until their emergence. Cereal aphids and their primary parasitoids were then identified to the species level based on morphology, and enumerated, and hyperparasitoids were identified at the genus level (Pike et al. 1997). As 2014 and 2015 were very different years in regards to weather and the target insect's presence and abundances, the sampling protocols were slightly different for each year, but both years included a sweep sampling and visual sampling.

For 2014, fields were visited at two-week intervals and sweep net sampling was done between late June (prior to wheat heading) and the beginning of September. The dates of visual sampling were August 28, September 2, 5, 9 and 12. Because very few aphids were found in this year, and these aphids were limited to late-senescing, green wheat plants growing in low, wet areas of a few, unharvested fields, it was impossible to evaluate the aphid density per field. In each field, all the visually encountered aphids were collected and kept in the laboratory at 20°C, RH 35%, DL 16:8 h, to observe the possible formation of mummies (which did not occur). Therefore, the available data consisted of the total number of aphids per field, and their identification to the species level.

For 2015, fields were visited at two week intervals and sweep net sampling was done between late June and early September. Mummy collection began at the time of first occurrence of aphid mummies on August 11, 2015. Before this date, no mummies were found via sweep netting or visual counts but a low population of cereal aphids were present in wheat fields as detected by sweep netting. Visual sampling was done by visually inspecting tillers chosen randomly from the field edge to the field center with a sampling effort of one hour per field. In the period of maximum aphid abundance, meaning August 25–September 2, a measure of aphid abundance and parasitism rate was performed. For aphid abundance, 50 stems per field chosen randomly from the field edge to the field center were thoroughly examined for aphid presence in the field. The living aphids observed were counted and their species were identified based on morphological traits (Blackman & Eastop 2005). The number of mummies was counted and the parasitism rate calculated as the number of mummies divided by the number of mummies + aphids. Another count of relative abundance of the different species of aphids was performed by removing 50 tillers containing aphids per field. Each plant was carefully examined in the lab and each aphid was identified and counted. Mummies were collected randomly from the field until a minimum of 250 mummies were collected per field. Each mummy was placed in an individual gelatine capsule and kept in the lab at 20°C, RH 35%, DL16:8 h until parasitoid emergence. The species of the emerged parasitoids and the species of the aphids from which those parasitoids emerged were identified based on morphology using various binomial keys (Pike et al. 1997, Kavallieratos et al. 2005, 2013, Kavad & Korat 2014, Tomanović et al. 2003, 2012). The proportion of winged aphids was evaluated on both living aphids and on aphid mummies (wings remain visible after mummification). The sex ratio was evaluated for each species of primary parasitoid. Each unhatched mummy was dissected around one month after the peak of emergence to observe the occurrence of mortality of parasitoids in aphid mummies and the percentage of diapausing individuals (gold-yellow prepupae; Brodeur & McNeil 1989; Tougeron et al. 2017).

2.2 Meteorological data

To understand the differences of food webs between the 2 years of sampling, meteorological data for the wheat growing season (May–September) was downloaded from the Environment and Climate Change Canada (ECCC) Saskatoon weather station (Saskatoon, SK, Canada, 52.17°N; 106.72°W, elevation 504m) for the 2014 and 2015 seasons (Historical Data, Environment and Climate Change Canada 2014, 2015). For each sampling season, the mean, lowest and highest daily temperatures were analyzed, as well as the precipitation amount (mm). Meteorological data were then used to explain fluctuations in aphid and parasitoid abundance at a fine temporal scale, based on aphid and parasitoid developmental temperature thresholds in the field (Sampaio et al. 2007).

2.3 Statistical analyses

Quantitative food web graphical representations (Memmott et al. 1994) were made based on the identified aphid mummies and their respective emerging parasitoids for 2015, in order to illustrate the degree to which relative abundances of aphids and parasitoids were linked.

We used the “bipartite” R package (Dormann et al. 2009) to analyze the 2015 quantitative food web using different metrics. The “connectance” describes the proportion of realized links in a food web among all possible links. Co-occurrence of parasitoids on hosts describes the similarity in host-preferences. The “generality” index describes the mean number of prey species per predator. Finally, the “H2” index measures the degree of specialization of species (from 0: generalist to 1: specialist) in a network.

Generalized Linear Mixed Models (GLMM) with a binomial error distribution were fitted to the data to assess differences in the proportion of winged and apterous aphids between each aphid species and between each parasitoid species, by considering the sampling field as a random effect.

Chi² tests were performed to compare the sex-ratios of parasitoids and hyperparasitoids with the null hypothesis of a 1 to 1 sex ratio. Cox models were performed to compare development time between primary parasitoids and hyperparasitoids, and other Cox models were performed to compare development time among primary species and among hyperparasitoid species.

Meteorological data: Before inter-annual comparison of temperatures was performed, the seasonal aspect had to be removed from the dataset, as it is trivial that winter is colder than summer. To do so, mean minimal temperature from 2014 and 2015 were subtracted for each date in order to obtain stationary data (i.e., mean temperature difference between 2014 and 2015, at each date). The autocorrelation function was calculated and we found that up to six consecutive days had their mean temperature correlated. The temperatures were compared between 2014 and 2015 and confidence intervals (95%) were calculated by accounting for the autocorrelation factor. Differences in mean wind speed and snow cover between 2014 and 2015 were assessed using Generalized Least Squares models (GLS, with autoregressive process of order 1) as no strong seasonal component was to be accounted for (snow only occurs in winter and wind is not likely to be stronger in any season than in another).

3 Results

3.1 Aphids, parasitoids and hyperparasitoids

Year 2014

In the year 2014, sweep net sampling only detected aphids in wheat fields at the end of August. Only two species of aphids were found, with a total of 432 *R. padi* and 285 *S. avenae* collected in the 5 fields. *Rhopalosiphum padi* were regu-

larly distributed among the 5 different fields, whereas for *S. avenae*, 273 of the total 285 were found in the same Alvena field, with one or two individuals in each of the other four fields. No mummies formed from any of these aphids when they were collected and held under laboratory conditions, and no mummies were collected in the fields. All data analyses were performed using R software (R Development Core Team 2015). Means and standard errors (SE) are provided.

Year 2015

Species occurrence and trophic web

Aphids: In 2015, sweep netting detected low levels of cereal aphids in mid-July. *Sitobion avenae* individuals were captured in fields with a mean of 17.4 ± 5.9 SE per 100 sweeps in the wheat fields in the week of July 13–17 2015. Visual surveys however, failed to detect these low levels of cereal aphids at this time. In the second week of August (10–14 2015), we observed 777 ± 421 SE aphids per 100 sweeps with great between field differences in numbers. The next week (Aug 17–21 2015), aphid populations presented a mean of 502 ± 306.5 SE, followed by a mean of 67.3 ± 54.3 SE when the wheat crop began to senesce (Aug 26–Sept 2 2015). In the visual surveys, three aphid species were found, with unequal abundances. On the 50 tillers per field that were randomly sampled the week of August 10–14, a total of 485 aphids were found, from which 397 were *S. avenae* (81.8%), 74 were *R. padi* (15.2%) and 14 were *S. graminum* (2.8%). Aphid densities were calculated as the mean number of aphids per plant (50 tillers) in five different fields surveyed. *Sitobion avenae* had a density of 1.58 ± 0.38 , *R. padi* of 0.29 ± 0.11 and *S. graminum* of 0.06 ± 0.03 per plant. A total of 40 mummies were found on these 50 plants per field, for a parasitism rate of $40/525 = 7.62\%$. This parasitism rate did not take into account any parasitoid larvae that might have been present inside the aphids. In this possibility, we do not know if these parasitoid larvae will overcome the defensive system of the aphids and mummify.

Another count of relative abundance was performed where 50 wheat tillers with aphids per field were brought to the lab and the numbers of each species of aphid were counted. On these 50 plants with aphids per field, 2034 aphids total were counted; 90.1% were *S. avenae*, 9.0% were *R. padi* and 0.8% were *S. graminum*. Fifty-two tillers out of the total 250 had at least two different species of aphids.

Parasitoids: The first mummies were observed on the 11th of August 2015, concurrent with the cereal aphid population peak in the fields. In total, 1179 mummies were taken from the five fields and their emergences followed in the lab. There were 98 dead parasitoids inside the mummies (8.2%), 304 empty mummies (25.5%), and 30 mummies (2.5%) containing a larva/prepupa in diapause (among the dead mummies, 55 contained a dried larva that may have died during diapause due to dry storage conditions in the capsules, so

the diapause level might be underestimated). The wing status of the mummies was noted and 90% of the mummies were apterous and 10% were alate.

From the 747 mummies from which wasps emerged, there were 597 primary parasitoids and 150 hyperparasitoids; a hyperparasitism rate of 20.1%. Three species of primary parasitoids were found, *Aphidius avenaphis* (Fitch) (549 individuals, 92.0%), *Aphidius ervi* (Haliday) (47 individuals, 7.8%), *Diaeretiella rapae* (M'Intosh) (only one individual, 0.2%, ignored in Fig. 1), and four genera of hyperparasitoids, *Asaphes suspensus* (Nees) (Hymenoptera: Pteromalidae) (102 individuals, 68%), *Dendrocerus bicolor* (Kieffer) (syn. *Dendrocerus aphidum* (Rondani)) (Hymenoptera: Megaspilidae) (40 individuals, 26.6%), *Alloxysta* sp. (Hymenoptera: Figitidae) (4 individuals, 2.7%) and *Coruna* sp. (Hymenoptera: Pteromalidae) (4 individuals, 2.7%). The quantitative food web composed by these species is shown in Figure 1. Connectance was 0.77, co-occurrence of parasitoids on one given host was 1.9 out of 3 maximum and co-occurrence of hosts parasitized by the same parasitoid species was 3.7 out of 6 maximum, showing that all hosts and parasitoid species were strongly linked in the food web. The mean number of parasitoid species attacking a particular aphid species was 2.43 and the mean number of hosts per parasitoid species was 1.85. There was no specialization as the parasitoids and hyperparasitoids used the available aphid species as a function of their relative abundance ($H_2' = 0.01$). Apterous aphids comprised 87.4% of the collection, and the proportion of apterous and alate aphids did not differ between the three aphid species (GLMM, $df=2$, $\chi^2=3.2$,

$p=0.209$), nor in use between the primary or hyperparasitoid species (GLMM, $df=5$, $\chi^2=8.1$, $p=0.152$). The sex ratio was biased towards females for the primary parasitoids *A. avenaphis* and *A. ervi* (62% and 65%, respectively, $\chi^2=30.9$, $df=1$, $p<0.001$ and $\chi^2=4.1$, $df=1$, $p<0.05$ respectively), and for the most abundant hyperparasitoid species, *A. suspensus* (87%, $\chi^2=40.5$, $df=1$, $p<0.001$). This ratio was close to 50% for *D. bicolor*. The sex ratio was not measured for *Alloxysta* sp. and *Coruna* sp. as there were only a few individuals.

The developmental duration of the hyperparasitoids was longer than that of the primary parasitoids when kept in the laboratory at 20°C (Fig. 2), with hyperparasitoids emerging later than primary parasitoids, (Cox model, $\chi^2=871$, $df=1$, $p<0.001$, mean development time of 4.6 ± 0.15 days and 29.3 ± 1.9 days for primary and secondary parasitoids respectively, all species pooled together). In primary parasitoids, *A. avenaphis* took longer to emerge from the mummies than *A. ervi* (4.6 ± 0.2 days and 3.5 ± 0.3 days, respectively, Cox model, $\chi^2=11.1$, $df=1$, $p<0.001$). In secondary parasitoids, there were no differences in mean development time among species (29.5 ± 9.8 , 30.7 ± 2.3 , 43.7 ± 13.8 and 24.1 ± 3.7 days, for *Alloxysta* sp., *Asaphes suspensus*, *Coruna* sp. and *Dendrocerus* sp., respectively, Cox model, $\chi^2=3.4$, $df=3$, $p=0.34$).

3.2 Meteorological data

We observed no differences in the snow cover (GLS, $F=0.04$, $df=1$, $p=0.85$) or wind speed (GLS, $F=0.88$, $df=1$, $p=0.36$) between the two years. Year 2014 was colder than 2015

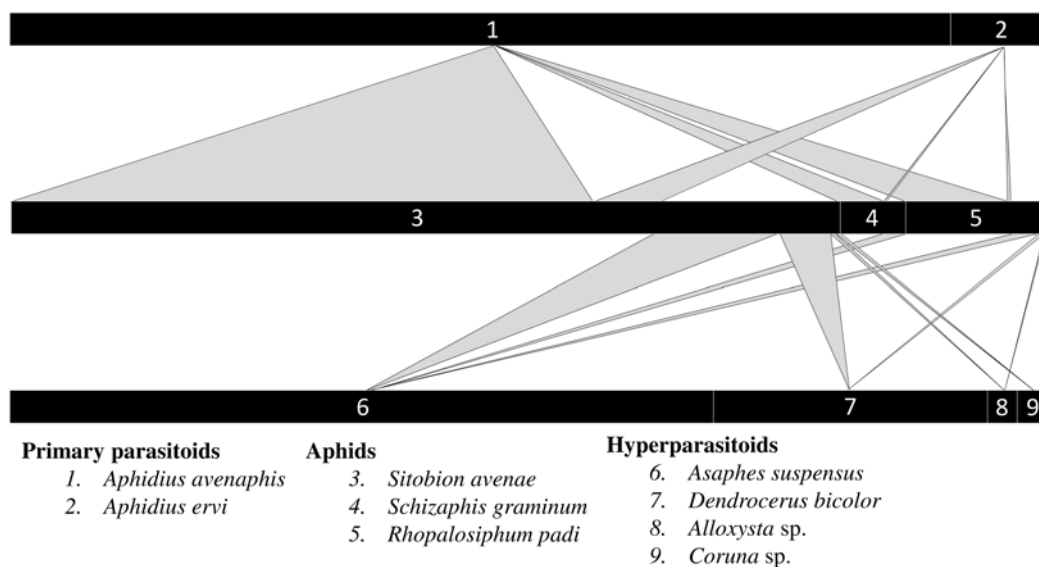


Fig. 1. Trophic web of cereal aphids, their parasitoids and hyperparasitoids collected from wheat fields in northeastern Saskatchewan, Canada, in the summer of 2015. Upper bars represent primary parasitoid relative abundance, middle bars represent aphid relative abundance and lower bars represent hyperparasitoid relative abundance for each species. The thickness of the arrows between two bars is proportional to the relative number of trophic interactions between species.

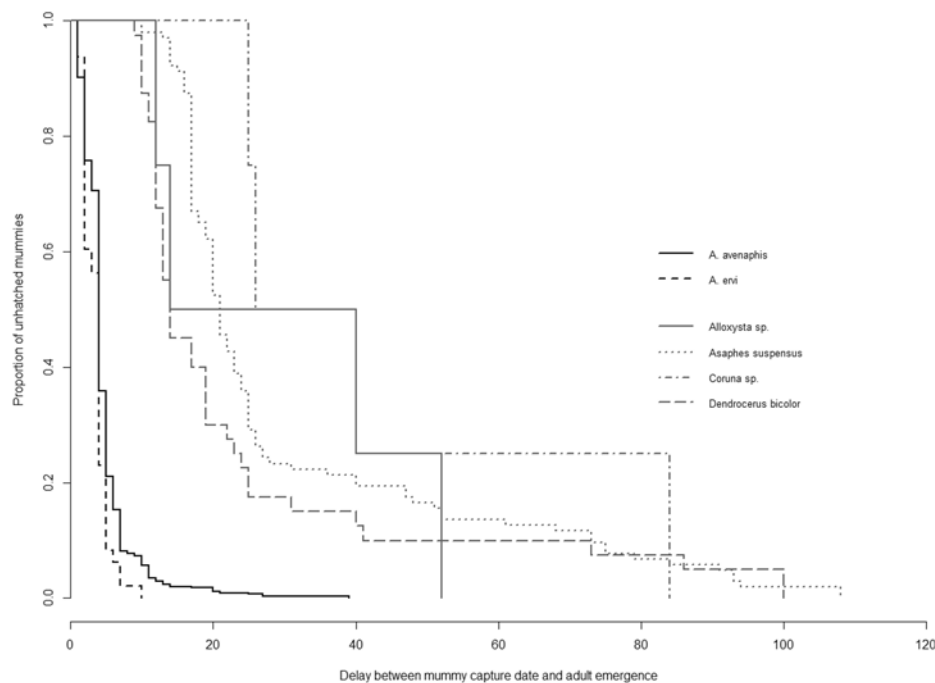


Fig. 2. Time between mummy collection and emergence date at 20°C in the laboratory for primary and hyperparasitoid species of cereal aphids from Saskatchewan, Canada, in 2015.

(mean difference in minimal temperatures -3.3°C , IC95% $[-4.3; -2.3]$) (Fig. 3). Precipitation was higher at the beginning of the season (May–June, GLS, $F=8.6$, $df=1$, $p<0.001$) and similar during the summer (July–August, GLS, $F=1.1$, $df=1$, $p=0.2$) in 2014 compared to 2015 (Fig. 3). Spring 2014 was rainy through May and early June but for the months of July and August 2014, little precipitation occurred until a thunderstorm at the end of July and one in the third week of August, 2014 (Fig. 3). The arrival of aphids to Saskatchewan wheat fields in 2015 however, coincided with three major rainstorms that tracked South to North across the Canadian prairies at the end of July and the beginning of August and resulted in weekly averages of 8.4 mm of rain at the end of July and 5.5 mm of rain in the beginning of August (Fig. 3). The mean minimum temperature during the growing season (May–August) was $8.71 \pm 0.48^{\circ}\text{C}$ in 2014 and $10.35 \pm 0.47^{\circ}\text{C}$ in 2015. The mean maximum temperature was $21.32 \pm 0.52^{\circ}\text{C}$ in 2014 and $23.61 \pm 0.50^{\circ}\text{C}$ in 2015.

4 Discussion

The trophic web linked to cereal aphids in Saskatchewan was very variable between the two sampling years; one year without any parasitoids and one year with two upper trophic levels (parasitoids and hyperparasitoids), which confirms our first prediction of high inter-annual variability in the trophic

web. These diverse results indicate that the populations are probably not well established in Saskatchewan and are likely limited by the weather preceding or during the growing season, as none of the upper trophic levels was observed during the colder year of 2014 whereas a more complete trophic web was present during the warmer year of 2015.

Concerning the second prediction, we observed that the overall proportion of alate aphids was much lower than the proportion of apterous aphids. Primary parasitoids used both alate and apterous aphids, at the same rate as their relative proportions, meaning that they encounter both types of aphids and that they did not prefer one aphid type over the other. Therefore, we cannot decipher between the two possible scenarios: 1. Migration of insects from the south and 2. Onsite overwintering populations. For the first scenario, if southerly winds carrying aphids arrive early enough in the spring, they could establish populations in Saskatchewan fields and be parasitized. This is supported by the meteorological data of 2015: In 2015, the probable arrival of aphids to Saskatchewan was during the week of 15-July 2015 and precipitation data from ECCC confirms that three rain events occurred that week, including one on 15-July 2015. Low pressure events, such as occur with rainstorms, bring migrating insects such as aphids down out of the low level jet stream (Dickison et al. 1983; Hopkinson & Soroka 2010; MacRae et al. 2011) so wind trajectories coupled with rainstorms in 2015 were likely the driving force for aphid migration into

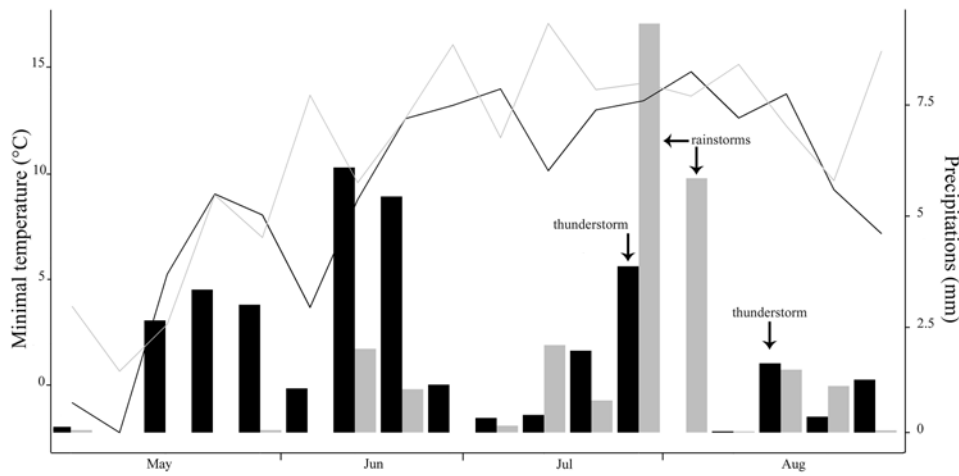


Fig. 3. Combined graph of minimal temperatures (lines) and precipitation (bars) for the growing seasons of 2014 (black) and 2015 (grey), from Saskatoon, Saskatchewan, Canada, data averaged weekly.

Saskatchewan fields. An opposite situation occurred in 2014, with few south winds occurring except one with an origin in California, arrived in the sampling area around Saskatoon on the 15-Aug 2014 (Owen Olfert, Ross Weiss, and Serge Trudel 2014, Prairie Pest Monitoring Blog with ECCC wind trajectory data) when most wheat crops had begun to senesce.

Aphids may have been parasitized before they migrated into the wheat fields in 2015. As evidenced by sampling aphid mummies, the first generation of parasitoids was present within four days of the peak aphid population in fields which informs the scenario that parasitoids could arrive during the immature instar inside the migrant aphids (documented for UK by Vorley & Wratten 1987). These south to north migrations can be in successive waves, with some of them rarely reaching the northern areas, or arriving late in the season, more in the summer than in the spring (Spencer 1922). In the state of Washington where 13 species of primary parasitoids are found, Pike et al. (1997) showed that five primary parasitoids occur as soon as April, whereas other species arrive later, with *A. avenaphis* observed from June to November, *Ephedrus* sp. from August to October and *A. matricariae*, detected only during the summer growing season. Migrations of aphids from southern to northern USA were well documented in several crops, for example flights of *Myzus persicae* Sulzer into potato fields (Zhu et al. 2006), Messina (1993) for *Diuraphis noxia* Durjumov (Homoptera: Aphididae) in cereals in Utah or Irwin et al. (1988) for *R. maidis* and *S. graminum* in cereal fields in Illinois. However, the lag in mummification time compared to the beginning of aphid infestation can also be explained by parasitoids that have overwintered in Saskatchewan, either in cereal aphid mummies or alternate aphid hosts (Brodeur & McNeil 1994, Shands et al. 1965). Sweep sampling in 2014 indicated that hyperparasitoids were present in cereal fields even when the

parasitoids were not, so the hyperparasitoids likely also have a resident population in Saskatchewan. Our data of 2015 supports the presence of a resident population of at least one species of primary parasitoids. Indeed, the first aphids mummified in 2015 were a mix of both alate and apterous and if the primary parasitoids arrived only within already parasitized aphids then we would expect the first mummies to be all alate aphids. Thus, another explanation (second scenario of overwintering populations), that does not exclude the possible occurrence of the first one, is that parasitoids are overwintering in Saskatchewan on alternate aphid hosts, or in cereal aphid mummies in micro-habitats (Brodeur & McNeil, 1989). These overwintering parasitoids would only emerge from their winter diapause to seek aphid hosts when sufficient heat units accrue during June–July, thus we only observe mummies in cereal aphids a few weeks after. The dominance of *A. avenaphis* (>90%), a North American native and the paucity of other primary parasitoids in the complex though suggests that this species could be resident and overwinters either in Saskatchewan or in the northern States of the USA. In regards to alternate hosts, *A. avenaphis* and *A. ervi* were recorded emerging in small numbers from pea aphid, *Acyrtosiphon pisum*, mummies on faba bean, *Vicia faba*, in Saskatchewan (Wist: unpublished). Alternate aphid hosts from aphids on surrounding flowering plants and trees could provide a reservoir for *A. avenaphis* if its host range is quite general but alternate aphid hosts were not tracked in this study. With the most-abundant primary parasitoid being a native North American species, and few other primary-parasitoids found that do occur in more southerly-parts of North America, coupled with the presence of diapausing *A. avenaphis* prepupae, it is likely that *A. avenaphis* does overwinter in Saskatchewan. Parasitized aphids can have their behavior modified by parasitoid larvae and leave host plants

to find protected overwintering sites (Brodeur & McNeil 1989) so our estimation of mummies entering diapause may be under-represented if *A. avenaphis* infested aphids leave wheat plants prior to mummification. The second scenario, of a resident population of *A. avenaphis*, based upon our mummification data, is thus most likely.

The sex ratio of most of the parasitoids and hyperparasitoids was female biased as is typical of these insects in more favorable climatic areas (Andrade et al. 2016, Tougeron et al. 2016), which is opposite to our third prediction of a non female-biased population. This is probably due to the fact that the harsh climatic conditions do not result in a biased sex ratio, at least for the dominant native parasitoid species *A. avenaphis* and the most abundant hyperparasitoid species, although it was shown that other types of stressful conditions result in male-biased sex ratio (van Baaren et al. 1999).

Concerning the fourth prediction (diapausing parasitoids could be observed in the first parasitoid generation), the growing season in Saskatchewan is short (typically 90 days in the north to 130–140 days around the center, and 140–150 in the south); the first parasitoids were observed in the middle of August, and wheat is harvested by the end of August or the beginning of September so another generation of parasitoids on this cereal aphid population is unlikely. Diapause of the parasitoids could occur as soon as the first generation of parasitoids is produced, meaning that for some species only one generation occurs during the wheat growing period, before the population enter winter diapause. These first generation diapausing parasitoids are probably lost from the population/community after the harvest and thus would not participate in overwintering. The duration of development of our hyperparasitoids was longer than that of the primary parasitoids. Because aphids and primary parasitoids arrived late in the growing season, there is little probability of a second hyperparasitoid generation in northern fields before the cereals are harvested and the temperature decreases in the fall. Some of the hyperparasitoids were in diapause, as were a portion of the primary parasitoids. In Quebec, Canada, diapause begins as early as August under natural conditions (Brodeur & McNeil 1994; Tougeron et al. 2018). In Saskatchewan, the first generation should enter into diapause or it will not survive. As hyperparasitoids are highly generalist, aphid mummies on other host plants might suffice as hosts for a spring generation of hyperparasitoids but this hypothesis requires further testing.

For our fifth prediction, the trophic web was dominated by two major aphid species in cereals, *S. avenae* and *R. padi* as is common on other continents (Andrade et al., 2016 for Europe, Zuniga, 1990 for South America, Horrocks et al. 2010 for New Zealand where there is *R. padi* but not *S. avenae*). In the colder year of 2014, *R. padi* was the dominant aphid, whereas in the warmer 2015 it was *S. avenae*. In Western Europe, *R. padi* is more cold resistant than *S. avenae* (Alford et al. 2014) and may have better overwintering success in Saskatchewan in areas close to its primary

hosts, bird cherry, *Prunus padus* or choke cherry, *P. virginiana*. There was only one native primary parasitoid species found in our sample in Saskatchewan, *A. avenaphis*, a generalist whose original host range is unknown, and one other *Aphidius* species, *A. ervi*, which was introduced into North America in the mid-1960s (Halfhill et al. 1972). At least four genera of hyperparasitoids were found, which are probably generalist native species and were also found previously in Saskatchewan on cereal aphids (Doane et al. 1991 in Olfert et al. 2002). The trophic web presented only a few species which follows our fifth prediction that the system in North America harbors a limited number of species compared to the same system in Europe and further south in North America. The total number of aphid species (three) is comparable or smaller to this observed on other continents (three to five), whereas the number of primary parasitoid species (three with only one occurrence of *D. rapae*) is strongly reduced. For example, there can be up to 13 primary parasitoid species in Washington state, USA (Pike et al. (1997), 8 primary parasitoids of cereal aphids in the West Central Great Plains USA (Noma et al. 2005), 7 species in western Europe (Andrade et al. 2016), and 11 in South America (Zuniga 1990). Moreover, for primary parasitoids, *A. avenaphis* represented more than 90% of the parasitoids sampled in Saskatchewan. No mummies or adults of any *Aphelinus* species were found in Saskatchewan and this contrasts with the two species found in the West Central Great Plains of the USA (Noma et al. 2005) and the three species of *Aphelinus* in Washington State, USA (Pike et al. 1997). The number of genera of hyperparasitoids is similar to that observed elsewhere in the world, but little is known about hyperparasitoid abundance and diversity in other aphid systems. For example, Noma et al. 2005, recorded a much lower hyperparasitism rate in Colorado than we did in Saskatchewan but did not speciate their hyperparasitoids.

The parasitoids and hyperparasitoids that we found are all generalists that exploited all the aphid hosts species available, which agrees with our last prediction that there would be proportionally more generalist species in the northernmost-growing region of North America than in the same system elsewhere. There is 100% generalist parasitoid species in Saskatchewan whereas there are some specialist species in all other cereal aphid systems.

Kaneryd et al. (2012) showed that highly specialized systems are particularly vulnerable to variable environments. In host-parasitoid communities, the fluctuations in the quality and quantity of host resources are expected to exert a strong influence on the resource exploitation strategy of parasitoids (Andrade et al. 2013; Andrade et al. 2015). A high variance in the availability and the quality of host resources would be favorable for generalist parasitoids. A prevalence of specialist behaviour is expected in a stable environment (Peers et al. 2012). Our trophic web was not equilibrated, with one highly dominant species (*A. avenaphis*) and other, less frequently collected, species. Hammil et al. (2015) showed that

the poorer in number of species an ecosystem is, the less persistent in time it is. Kamjunke et al. (2004) showed that in extreme conditions, the food webs presented only a few species, with no functional redundancy, as many species are excluded due to the stressful conditions compared to more favorable habitats. In such systems, the effects of the higher trophic level is enhanced.

Finally, if we consider the possible impact of these parasitoid species as biological control agents against the cereal aphids, it likely would be quite limited, as the parasitoids, even with concurrent arrival with the cereal aphids, had the opportunity of only one and maybe two generations, and their numbers are reduced by hyperparasitoids. In this system, the parasitism rate was less than 10%, considerably lower than what is observed in other areas of the world and below the minimum of 22–24% that begins to lead to a decrease of aphid populations (Plečáš et al. 2014). The impact of predators on cereal aphid populations might be higher than that of parasitoids. Cereal aphid predators were noted in sweep samples in 2015 (Wist: unpublished) as two species of green lacewing larvae (Neuroptera: Chrysopidae), lady beetle adults and larvae (Coleoptera: Coccinellidae), mostly *Coccinella septempunctata*, minute pirate bugs (Hemiptera: Anthocoriidae), and damsel bugs (Hemiptera: Nabidae) which is identical in species composition to the complex found in 2012 and 2013 in wheat grown in Saskatchewan (Wist et al. 2013). Pérez-Rodríguez et al. (2015) showed the importance of a good synchronization between the different trophic levels, and particularly between the spring migrations of aphids and their parasitoids in England must occur to ensure efficient biocontrol. However, in areas with extreme weather conditions and short growing seasons, the limited length of the growing season does not presently allow effective biocontrol through parasitism, although the synchronization level of parasitoids to their aphid hosts is high. With global climate change, the shortening of the winters in Western Canada could induce an earlier arrival of aphids in the spring and a faster increase of their population with consequent crop damage. However, their parasitoids will also arrive sooner and this earlier arrival could increase both the richness of the food web and the efficiency of the parasitoid biological control. As cereal crops are a major aspect of the Canadian prairie landscape (Dosdall et al. 2011), it is important to continue to study the evolution of these food webs in future years. Indeed, the high level of synchronization between aphids and parasitoids observed in this study is the key for effective biological control strategies, where efficiency can be increased by conservation biological control methods. The latter can enhance the diversity (abundance and richness) of natural enemies, which increases the potential outcomes from a biological control program. The large amounts of semi-natural habitats around the fields in such a harsh and cold area represent a good opportunity to develop these methods, which have already shown their efficiency in

winter in areas with mild winter conditions (Damien et al. 2017).

These two year results of natural survey of the system of aphids-parasitoids and hyperparasitoids of cereals at the northern limit of cereal cultivation represent a first set of data which show that this system is particularly relevant to answer two fundamental ecological questions: Firstly, the importance of food web complexity in resilience and stability of an ecosystem, by comparing the stability of this system for several years to the same system in southern areas. Second, this system could also be interesting in a study comparing spatially the biological control service on a gradient of food web complexity, to measure the link between food web complexity and biological control service.

Compliance with ethical standards

Funding: This research was funded by the FP7-PEOPLE-2013-IRSES program (project APHIWEB, grant no. 611810 to Nicolas Desneux (coordinator) and Joan van Baaren and Kevin Tougeron (participants)).

Conflict of Interest: Authors declare that they have no conflict of interest

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Acknowledgments: The authors thank Agriculture and Agri-Food Canada for offering vehicles for field data collection and growth cabinet space to rear the aphids and parasitoids. The authors thank Prof. Jean-Sébastien Pierre for help in the analysis of meteorological data.

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- male and female, and compared with the available data in the literature. For the *Aphidius* genus, one criteria of identification – among others (Tomanović et al., 2003) – is the patterning of the anterolateral area of metasomal tergite 1. We observed some individuals with forms that had never been described. Image sequences of aphid species, mummies and anterolateral area of metasomal tergite 1 and heads and antennae of *Aphidius* wasps were captured with a Nikon Digital Sight DS-L3 Digital Camera System mounted to a Nikon SMZ25 dissecting microscope and aligned and z-stacked with the Nikon Elements BR package software (Nikon Canada).

Results

Identification of the mummies and of the parasitoids of the genus *Aphidius*

Mummies of *S. avenae* had dark and long cornicles, and long antennae which reached the cornicles (Fig. S1a). Mummies of *S. graminum* had medium length, pale cornicles and medium length antennae (Fig. S1b). Mummies of *R. padi* had the shortest cornicles and the shortest antennae (Fig. S1c).

In several identification keys (INRA-Encyclop’aphid, Pike et al., 1997, Kavallieratos et al., 2005, 2013, Kavad and Korat 2014, Tomanović et al., 2003, 2012), two morphological characters are important for the identification of the different species of the genus *Aphidius* – the patterning of the anterolateral area of metasomal tergite 1 and the number of antennomeres (Fig. S2a). Individual *A. avenaphis* typically have an anterolateral area of metasomal tergite 1 with thin striations (costulate), as do *A. rhopalosiphii*, *A. matricariae* and *A. uzbekistanicus* (Fig. S2b). Most of the individuals of *A. avenaphis* we sampled had the typical patterning of their anterolateral area of metasomal tergite 1 of this group of species; however a few individuals had a anterolateral area of metasomal tergite 1 with thicker striations (Fig. S2c), but less thick than the pattern of striations of *A. avenae* or *A. colemani* which presented with fewer thick striations (Fig. S2d). The *A. ervi* specimens all had coarse anterolateral area of metasomal tergite 1 typical of *A. ervi* (Fig. S2e).

Table S1 summarizes the numbers of antennomeres found on the individuals of *A. avenaphis* and *A. ervi* that we sampled in 2015 compared to data in the literature. For *A. avenaphis*, we observed 129 females (78%) with 16 antennomeres and four (2%) with 15 antennomeres and 33 (20%) with 17 antennomeres. For the males, 59 individuals (49%) had 19 antennomeres (Fig. S2a), 6 (5%) presented 18 antennomeres, 41 (42%) had 20 antennomeres and 5 (4%) had 21 antennomeres. For *A. ervi*, the number of antennomeres varied from 17 (one individual, 6%), to 18 (nine individuals, 56%) and 19 (six individuals, 38%) for the females and from 19 (three individuals, 27%), to 20 antennomeres (four individuals, 36%) and to 21 antennomeres (four individuals, 36%) for males.

Appendix

The morphological criteria of identification of the parasitoids from the genus *Aphidius* are not completely consistent in the literature, especially concerning the number of antennal antennomeres. The number of antennomeres (flagellomeres + the scape and pedicel) was counted for each

Manuscript received: 10 December 2018

Revisions requested: 27 June 2019

Modified version received: 22 July 2019

Accepted: 25 July 2019



Fig. S1. Representative mummies of **a.** *S. avenae*, **b.** *S. graminum*, and **c.** *R. padi*.

Table S1. Number of antennomeres on antennae according to different sources from the literature for both male and female individuals of the main Aphidiinae species. Counting of antennomeres included the scape and the pedicel for (a), and is not specified for the other references. References: (a) Our observations, (b) Pike et al., 1997, (c) Kavallieratos et al., 2005, 2013, (d) Hullé et al. 2006, (e) Kavad & Korat 2014, (f) Tomanović et al., 2012.

Species	Males	Females
<i>Aphidius avenae</i> Haliday	(18–21) ^a	(16–17) ^f (17–18) ^{db}
<i>Aphidius avenaphis</i> (Fitch)	(19–21) ^a	(15–16–17) ^a (16–17) ^b
<i>Aphidius ervi</i> Haliday	(19–21) ^a	(17–19) ^a (17–18) ^d (18–20) ^f
<i>Aphidius colemani</i> Viereck	(16–17) ^a	(13–14) ^d (15–16) ^{bdf}
<i>Aphidius matricariae</i> Haliday	(16–17) ^a	(14–15) ^{abcdf}
<i>Aphidius rhopalosiphii</i> De Stephani Perez	(18–19) ^a	(16–17) ^{df} (17–18) ^b
<i>Aphidius uzbekistaniscus</i> Luzhetskii	(18–19) ^a	(16–17) ^{db}
<i>Ephedrus plagiator</i> (Nees)	(11) ^{ad}	(11) ^{acd}
<i>Diaeretiella rapae</i> (M'Intosh)	(16–18) ^{ac}	(13–15) ^{adef}
<i>Praon volucre</i> (Haliday)	(18–21) ^a	(16–18) ^{dc}

Discussion

In this study, we determined the primary parasitoids by two morphological criteria that are recognized as important for the identification of the different species of the genus *Aphidius*, the number of antennomeres and the patterning of anterolateral area of metasomal tergite 1 (Hullé et al. 2006, Pike et al., 1997, Kavallieratos et al., 2005, 2013, Kavad & Korat, 2014,

Tomanović et al., 2003, 2012). We evaluated the variation level in the number of antennomeres, which is quite high, both in *A. avenaphis* and *A. ervi*, with around 20% of the individuals harboring a higher or a lower number of antennomeres than average. Some individuals presented asymmetric antennae with a different number of antennomeres on the two antennae (around 3% of the individuals). For *A. avenaphis* we found some males (5%) with 18 antennomeres, and

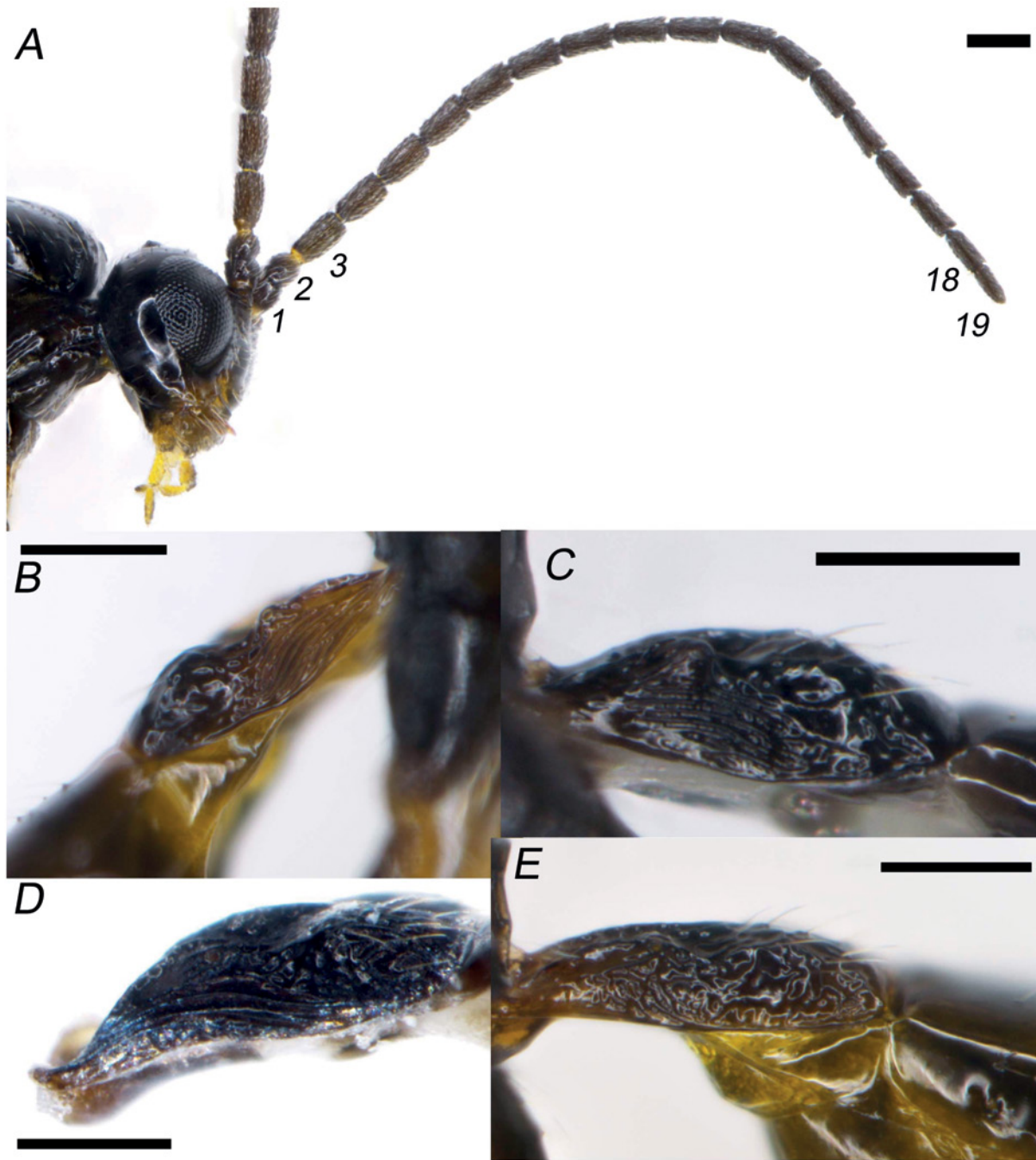


Fig. S2. a. Head and antennae of an *Aphidius avenaphis* male showing the antennomere numbering pattern, b. Anterolateral area of metasomal tergite 1 of a typical *A. avenaphis*, c. of individuals with thicker striations, d. of a typical *A. avenae*, and e. of a typical *A. ervi*. All individuals were sampled around Saskatoon, Saskatchewan except *A. avenae* which came from Brittany, France. Scale bars = 100μm.

this lower number has not been mentioned in other studies. It should be noted that the number of antennomeres varied between the different studies (list of references in the Table S1), showing either local variations, or a sample with too few individuals taken to record all the possible variability. For example, in our study we sampled few *A. ervi*. This

species represented less than 10% of our sampling and we did not observed all the range of antennomeres mentioned in the literature, whereas with our large sample of *A. avenaphis*, we found a larger variation than was mentioned in the literature. However, we cannot exclude some local differences, as a species like *A. ervi* has worldwide distribution. This

variability is probably common within species and further taxonomic studies should bring consensus. Concerning the second criterion, the patterning of the anterolateral area of metasomal tergite 1, individuals of *A. avenaphis* typically have a anterolateral area of metasomal tergite 1 with thin striations (costulate), as do *A. rhopalosiphii*, *A. matricariae* and *A. uzbekistanicus*, and in our sampling we found this typical pattern in most of the individuals. However, a few individuals had an anterolateral area of metasomal tergite 1 with thicker striations, but thinner than the pattern of striations of *A. avenae* or *A. colemani*, which have fewer but thicker striations. It is difficult to make hypotheses about this variation. As no *A. avenae* or *A. colemani* were sampled, the hypothesis of a possible hybridization is difficult to consider, although these species occur in the state of Washington to the southwest of our sampled area. In conclusion, the morphological criteria of parasitoid species identification available in the literature are not always conclusive, with the observation of some intermediate forms.

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