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BIOLOGICAL CONTROL

Taxonomic Status and Population Oscillations of *Aphidius colemani* Species Group (Hymenoptera: Braconidae) in Southern Brazil

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Keywords

Cereal aphid, parasitoids, taxonomy, cryptic taxa

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Abstract

Aphidius colemani (Viereck) was reported in Brazil before the Biological Control Program of Wheat Aphids (BCPWA) when Mediterranean genotypes were introduced from France and Israel. This species was re-described as a complex called *A. colemani* group composed of three species. Consequently, uncertainty remains about which parasitoid of the group is occurring in southern Brazil. This study has two main objectives: (i) re-examine the species status of *A. colemani* group collected during the introduction of parasitoids and from a 10-year (2009–2018) monitoring program in wheat fields in northern Rio Grande do Sul (RS), Brazil; (ii) describe the variation in the population density of parasitoids and its association with meteorological factors during this period. We examined 116 specimens from the Embrapa Wheat entomological collection, and those collected in Moericke traps in Coxilha, RS. All the parasitoids of the *A. colemani* group from the BCPWA period were identified as *Aphidius platensis* (Brèthes). In traps, 6541 cereal aphid parasitoids were collected, of which 61.9% (n = 4047) were from *A. colemani* group and all those were identified as *A. platensis*. Temperature was the factor that effected population density with the highest number of parasitoids recorded in the winter months. Sex ratio changed between years varying from 0.50 to 0.97. The parasitoid *A. platensis* was the only species in the *A. colemani* group sampled during 10 years of monitoring.

Introduction

The Biological Control Program of Wheat Aphids (BCPWA) in Brazil, coordinated by Embrapa Wheat (with the support of FAO and the University of California) during the 1970s and 1980s, was very successful. During this program, 1,442,544 parasitoids were released and 17.7% of these parasitoids were *Aphidius colemani* (Viereck) (Hymenoptera: Braconidae), introduced from France and Israel (Salvadori & Salles 2002). This species was the most frequently recaptured in mummies of *Schizaphis graminum* (Rondani), during the release period (Zúñiga-Salinas 1982).

Starý *et al* (2007), analyzing records of *A. colemani*, in Brazil, registered that this species was found before the BCPWA, and they verified that this species can parasitize 28 species of aphids and explore different environments such as crops, weeds, vegetables, and ornamental plants. In addition, the dominant species parasitizing *Rhopalosiphum padi* (Linnaeus) on cereal crops has been *A. colemani* (Zúñiga-Salinas 1982, Ronquim *et al* 2004).

The taxonomy of *A. colemani* has been controversial with synonymized species, revised, and re-described. *Aphidius platensis* (Brèthes) was recognized as a synonym of *A. colemani* (Starý 1975, Starý *et al* 2007). However, recent studies based on molecular and morphometric analysis brought back the taxonomic issues and *A. platensis* was re-described into a group of three species, called *A. colemani* group, including *A. colemani*, *Aphidius transcaspicus* Telenga, and *A. platensis* (Tomanović *et al* 2014). *Aphidius colemani* has a Mediterranean distribution, while *A. transcaspicus* has a Mediterranean and Central Asia distribution (Tomanović *et al* 2014), and *A. platensis* has been found in Brazil (Souza *et al* 2019), Chile, and Iran (Tomanović *et al* 2014). Tomanović *et al* (2014) suggested that the records of *A. colemani* in South America should be re-examined, due to the uncertainty about exactly which species are established in that region.

The correct identification of a parasitoid introduced in Brazil as *A. colemani* decades ago to control aphids in wheat is an important issue. Which species of *A. colemani* group are occurring in northern Rio Grande do Sul (RS) is still not confirmed. Although Embrapa has a 10-year (2009–2018) monitoring program in wheat fields by Moericke traps in northern Rio Grande do Sul, confirmation of the species group had not been performed yet.

Despite the importance of parasitoids in the biological control of wheat aphids, studies to understand how the environment influences the population of *A. colemani* group in the Neotropical Region used a short period of time, no longer than 2 years (Bartoszeck 1976, Pimenta & Smith 1976, Gravena 1979, Lazzari 1985, Mendes *et al* 2000, Pinto *et al* 2000, Ronquim *et al* 2004, Alves *et al* 2005). The pest population and its natural enemies must be monitored in long time series to comprehend the influence of biotic and abiotic effects on its outbreaks (Moraes 1987, Leslie *et al* 2009, Sampaio *et al* 2017). Abiotic factors affect predators and parasitoids directly or indirectly by influencing the phytophagous or host plant in the so-called bottom-up effect (Harrington *et al* 1999, Hoover & Newman 2004, Gillespie *et al* 2012, Dyer *et al* 2013). Temperature affects the fitness of aphids and their parasitoids (Le Lann *et al* 2011, Souza *et al* 2017), and depending on the rainfall intensity, it can increase or reduce the survival of aphids according to their intensity (Thackray *et al* 2004, Sampaio *et al* 2017). Thus, it is important to understand how these factors determine the occurrence of *A. colemani* parasitoids in wheat crop systems after the biological control program was established.

The *A. colemani* group had an important role in the biological control of wheat aphids during the BCPWA in Rio Grande do Sul (Zúñiga-Salinas 1982). New taxonomic information about this group (Tomanović *et al* 2014) was recently published, and according to these standards, it is not known which species of the *A. colemani* group was recovered by Zúñiga-Salinas (1982). Similarly, the *A. colemani* species currently occurring in Brazil or the effect of climatic factors on them is unknown. The objectives of this study were as follows: (i) to re-examine the species status of *A. colemani* group in northern RS; (ii) to evaluate the meteorological influence on parasitoid populations in a 10-year survey.

Material and Methods

To verify the taxonomic status of *A. colemani* group in northern Rio Grande do Sul (RS) collected during the period that aphid parasitoids were introduced to Brazil, the parasitoids identified as *A. colemani* deposited in the Embrapa Wheat entomology collection (Passo Fundo, RS, Brazil) were re-examined using taxonomic information from Tomanović *et al* (2014). The specimens examined included 70 females and 46 males emerged from mummies of Aphidini tribe aphids (Hemiptera: Aphidinae) collected in 1979 and 1980, by Zúñiga-Salinas at Fazenda Limeira, Carazinho, RS.

A parasitoid monitoring program was implemented in northern RS to observe the *A. colemani* group occurrence and population fluctuation. The sampling was conducted in a 5500-m^2 area in a wheat crop region (Coxilha, RS, 710 m altitude, 28°11′42.8″S and 52°19′30.6″W). The sampling period was from January 2009 to December 2018, totalizing 10 years and 522 weeks. Data on rainfall, air temperature, and relative humidity were obtained from Passo Fundo meteorological station (28°15′S, 52°24′W, 684 m), located 10 km away from the experimental area. The field was cultivated in no-till farming system.

Aphidius colemani group was monitored with Moericke traps (yellow tray, 45 cm long × 30 cm wide × 4.5 cm tall) filled with solution (2 L) prepared with water, 40% formalin (0.3%), and detergent (0.2%). Each tray had three side holes (5 mm diameter), near the border, protected by a fine screen to prevent leakage and solid content loss during the rains. Four traps were distributed on the borders of crop rotation trials. The traps were leveled approximately 20 cm from the ground, with bricks. The crop rotation area was cultivated with cereal crops (oat, wheat, and triticale), radish, and fallow during the winter season and in the summer with soybeans, corn, and *Brachiaria* sp. Every 7 days, the solid content of the trays was separated from the solution through the sieve and collected. The biological material was conserved in a glass bottle with 70% alcohol. In the laboratory, the aphids and parasitoids were separated, identified, and counted under a stereoscopic microscope.

Parasitoid species were identified based in Tomanović *et al* (2014) key for adult female *A. colemani* species group. Scanning electron microscopy was performed with Vega 3–TESCAN Equipment at the Multiuse Center of the University of Passo Fundo, RS, Brazil. Photos were analyzed of the anterolateral tergites of some specimens in good condition collected by Zúñiga-Salinas (1979 and 1980) and during the parasitoid monitoring program.

The *A. colemani* group population variation throughout the sampling period was described by calculating the mean parasitoids per month and per year. The maximum, mean, and minimum daily air temperature, accumulated rainfall, and relative humidity during the week preceding each sampling were compared with the parasitoid density per trap in the subsequent week. A zero-inflated generalized linear mixed model (GLMM) was applied to fit the mean air temperature and accumulated rainfall effects on the parasitoid populations (P_{ij}) considering each year. The other meteorological variables were dropped from the model to avoid collinearity. Due to the high variance in the mean numbers of captured parasitoids, it was considered a negative binomial distribution, described as:

$$E(P_{ij}) = \mu_{ij} \text{ and } Var(P_{ij}) = \mu_{ij} + \frac{\mu_{ij}^2}{\vartheta}$$

where $E(P_{ij})$ is the mathematical expectation of P_{ij}; $Var(P_{ij})$ is the variance of P_{ij}; ϑ is the dispersion parameter. The sampling years and weeks were considered random effects in the model. The mean number of parasitoids, the temperature *i*, and rainfall *j*, were described as:

$$\mu_{ij} = e^{\alpha + Temp_i + Prec_j + Temp*Prec_{ij} + a_1 + a_2}$$

where α was the intercept; a_1 was the random effect of the year; a_2 was the random effect of the weeks within the year. Inferences of deviance analysis for the fixed factors were based on chi-square statistic, because the dispersion parameter was known. The GLMM estimated equation was plotted with respective 95% confidence interval.

Sex ratio (*SR*) was determined by the following equation:

$$SR = \frac{Number\ of\ females}{Number\ of\ females + Number\ of\ males}$$

The χ^2 test of heterogeneity was applied to compare the SR between years. Tests were performed with packages: Agricolae "Statistical procedures for agricultural research" (version 1.2-8, 2017), Stats (version 3.5.2, 2018), and glmmADMB package "Generalized linear mixed models" (version 0.8.3.3, 2016); all analyses were accomplished on R Studio (version 1.1.383), R (version 3.4.3).

Results

Species status of ***Aphidius colemani*** *group*

During 10 years of monitoring, 6541 cereal aphid parasitoids were collected and 61.9% (*n* = 4047) were identified as *A. colemani* group specimens. These parasitoids and those collected in Carazinho during the BCPWA have the forewing length of nervure R1, varying from approximately the same length to shorter than the pterostigma (Fig 1a, Table 1), maxillary palpomeres with four segments and labial palpomeres with two segments and anterolateral area of the petiole

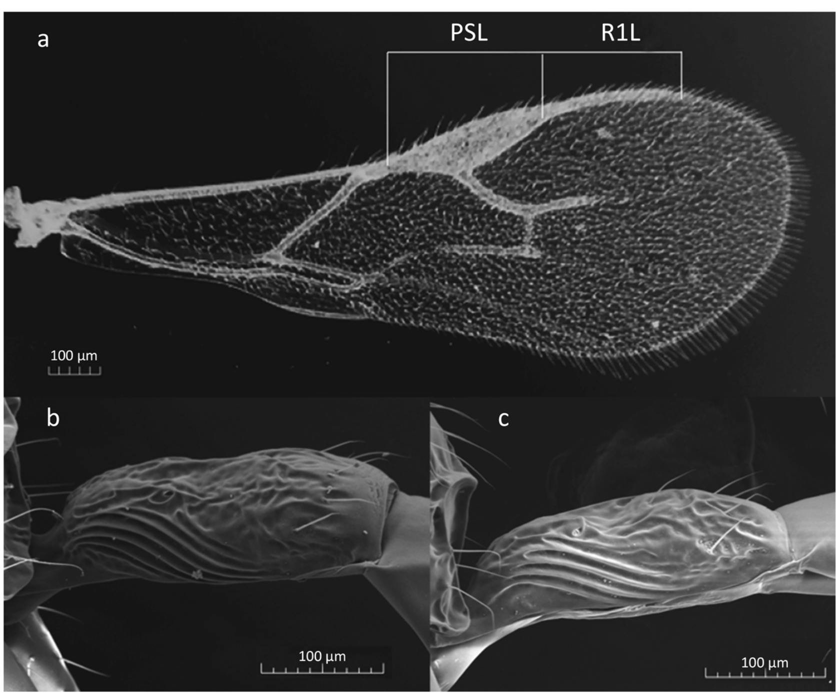

Fig 1 *Aphidius platensis*, females. **a** Forewing, **b** anterolateral area of petiole: standard of insects collected by Zúñigas Salinas (between 1979 and 1980), **c** anterolateral area of petiole: standard of insects from 10 years of monitoring 2009–2018. PSL, pterostigma length; R1L, nervure R1 length.

Table 1 Morphological characters, used on identification of *Aphidius colemani* group (Tomanović et al 2014) and of the parasitoids collected in south of Brazil.

Morphological characters	Tomanović *et al* (2014)			Carazinho, Coxilha, RS
	A. colemani	*A. platensis*	*A. transcapicus*	*A. platensis*
Anterolateral area of petiole	Bluntly costate	Sharply costate	Sharply costate	Sharply costate
Labial palps	2 palpomeres	2 palpomeres	3 palpomeres	2 palpomere
R1 Length	Approximately the same length of pterostigma	1/3 shorter than the l ength of pterostigma	Shorter than half the length of the pterostigma	Varying approximately the same length to shorter than the pterostigma

sharply costate (Fig 1b, c). Therefore, the parasitoids from Coxilha and Carazinho were identified as *A. platensis*, and this species is the most frequent cereal aphid parasitoids in Coxilha, RS, during the survey.

Meteorological factors effect

The zero-inflated GLMM adjusted for the parasitoid population sampled showed significant effect for the estimate of temperature (− 0.4476 ± 0.0516; χ^2 = 74.99***) and non-significant effect for rainfall (− 0.0043 ± 0.014615; χ^2 = 0.073) and interaction (0.0004 ± 0.00088; χ^2 = 0.245). The raise of one degree in the mean air temperature decreases by 0.4476, the exponent of the base *e* that estimates the parasitoid population (Fig 2). For the aleatory effects, the model estimated a variance of 0.000004 between the years and a variance of 7.03 between the weeks in each year, demonstrating a stronger effect on the population of the weeks during a year than through the years. *Aphidius platensis* was collected from March to November (Fig 3)

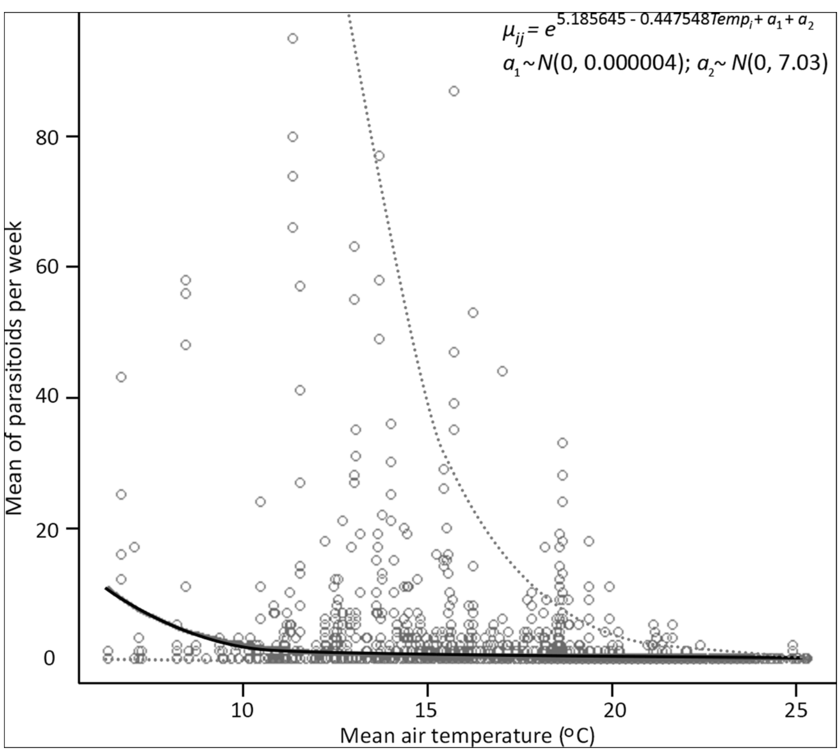

Fig 2 GLMM estimated equation with respective 95% confidence interval (traced line); each circle represents the mean number of *Aphidius platensis*/ per week and mean air temperature, samplings with Moericke traps from 2009 to 2018 in Coxilha, RS.

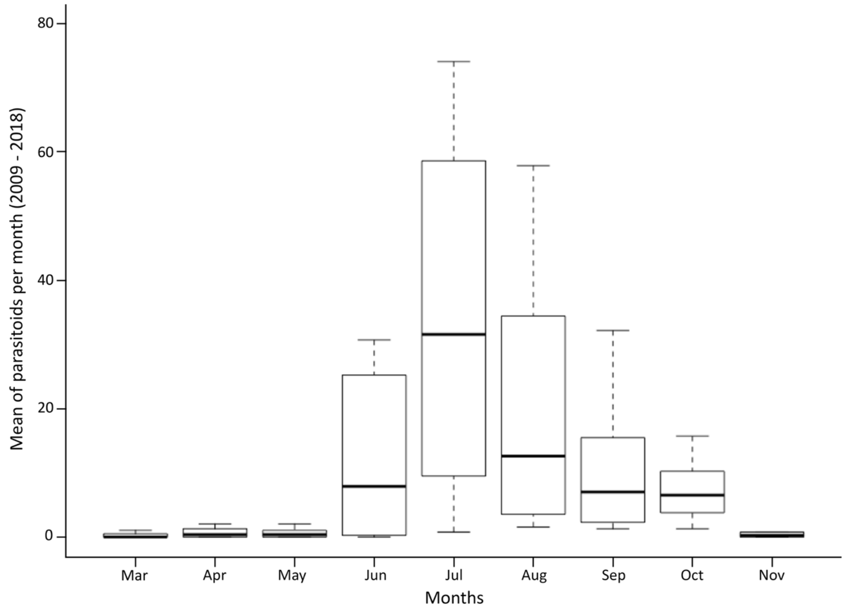

Fig 3 Boxplot of *Aphidius platensis*/per month, parasitoids sampled with Moericke traps, from 2009–2018 in Coxilha, RS. For each box plot, the central horizontal line corresponds to the sample median, minimum, and maximum observations (not including outliers) are indicated by the horizontal line at either end of the bar, while the end of the bar represents the interquartile range (between 25 and 75%).

and was not registered in the summer (December, January, and February), and it was more abundant in the winter (June, July, and August). During winter, mean minimum air temperature was 9.0°C, mean maximum was 18.9°C, and the mean temperature was 13.1°C. The mean number of parasitoids/per trap in the month of July was the highest (45.52). In 2016, the largest parasitoid populations were found and July was the month with the highest *A. platensis* population peaks (170 parasitoids/per trap) (Fig 4c). Thus, the prior month temperature (max 15.7°C, mean 9.7°C, min 5.4°C during June) may have favored *A. platensis* and host aphid populations. The mean temperature in June for the other monitoring years was high (2009–2018: max 17.8°C, mean 12.3°C, min 8.6°C) (Fig 4a, b).

The sex ratio of *A. platensis* ranged from 0.50 (2009) to 0.97 (2018) showing differences between years (χ^2 = 759.59; df = 9; P < 0.0001) (Table 2). In the total sampling period, 3332 females and 991 males were collected, with 0.73 sex ratio (Table 2).

Discussion

The genus *Aphidius* Nees is the most taxonomically problematic group of the subfamily Aphidiinae, with few reliable characteristics for species identification (Starý 1973, Pungerl 1986, Kambhampati & Mackauer 1988, Unruh *et al* 1989, Tomanović *et al* 2003). Parasitoids collected in Coxilha and Carazinho have petiole sculpture similar to what was described by Tomanović *et al* (2014) for *A. platensis.* These authors found that the anterolateral area of *A. platensis*'s petiole is sharply costate while this area in *A. colemani* is bluntly costate (Table 1). The sculpture of the anterolateral area of the petiole is a reliable criterion to identify *Aphidius* species (Eady 1969, Starý 1973, Pungerl 1986, Petrović *et al* 2018). According to Tomanović *et al* (2014), *A. transcaspicus* differs from *A. colemani* and *A. platensis* because its nervure R1 is shorter than half the length of the pterostigma. Furthermore, *A. transcaspicus* labial palps has three palpomeres (Table 1). Based on the description from Tomanović *et al* (2014), *A. colemani* has R1 approximately the same length as its pterostigma, and for *A. platensis* the length of the R1 is one third shorter than the length of its forewing stigma (Table 1). However, the ratio between the lengths of pterostigma and R1 was not reliable to determine the species collected in Brazil, overlapping those ratios described by Tomanović *et al* (2014) for *A. colemani* and *A. platensis* (Table 1, Fig 1a). The relationship between the forewing nervure R1 and pterostigma length is recognized as a criterion with wide intraspecific variation (Tomanović *et al* 2013, Villegas *et al* 2017, Petrović *et al* 2018). Thus, Rabasse *et al* (1985) analyzing *A. colemani* group specimens collected in Passo Fundo, northern RS, found similar results, in which the parasitoid's R1 nervure varied from 0.85 to 1.08 of the pterostigma length. Thus, these morphological characteristics should be observed more carefully by other researchers during the identification process.

The potential hosts in the field must be considered in the prevalence of *A. platensis*. Five cereal aphid species have been registered as *A. colemani* host in Brazil: *Metopolophium*

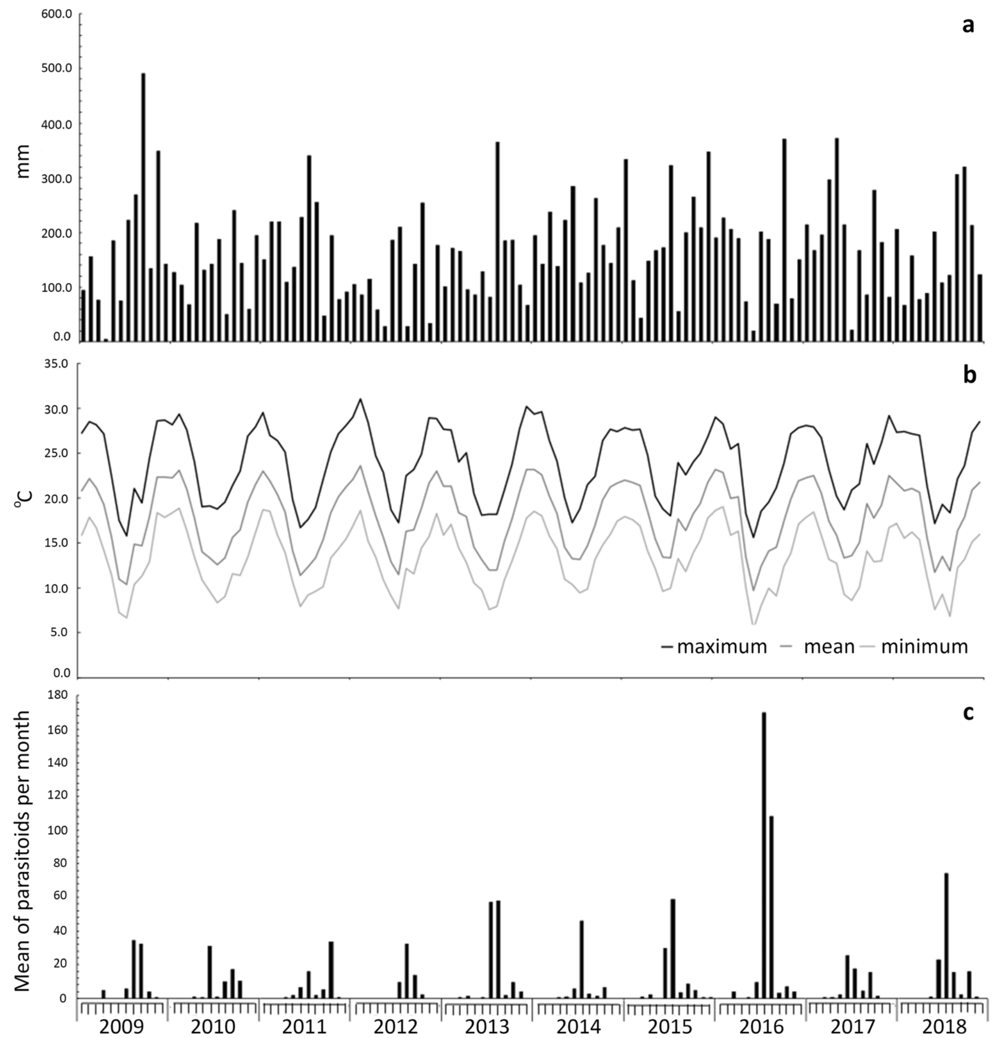

Fig 4 Data series from 2009 to 2018/per month (tick marks), Weather Station, Passo Fundo, RS (12 km away from traps), (**a**) accumulated rainfall, (**b**) air temperature fluctuation, (**c**) mean of *Aphidius platensis* in weekly samplings/per trap/per month (Coxilha, RS).

Table 2 Number of male and female, and sex ratio of *Aphidius platensis* per year, total number sampled from Moericke traps, January 2009 to December 2018 Coxilha, RS.

Year	N		Sex ratio
	♀	♂	
2009	162	162	0.50
2010	166	115	0.59
2011	185	74	0.71
2012	202	28	0.88
2013	442	85	0.84
2014	161	90	0.64
2015	237	199	0.54
2016	1,063	158	0.87
2017	203	63	0.76
2018	511	17	0.97
Total	3,332	992	0.73

dirhodum (Walker), *R. padi*, *S. graminum*, *Sitobion avenae* (Fabricius), *Rhopalosiphum maidis* (Fitch), and *Rhopalosiphum rufiabdominalis* (Sasaki) (Zúñiga-Salinas 1982, Starý *et al* 2007). In northern RS, all these species are associated with winter cereal crops (Rebonatto *et al* 2015). According to these authors, *R. padi* and *S. graminum* make up 65.3% of species during wheat crop seasons of 2008 and 2009 in Coxilha, RS. Furthermore, *R. padi* was considered an ideal host for *A. colemani* (Frank 2010). Cereal crops like barley, oats, rye, and wheat are considered beneficial to improve the fitness of *A. colemani*, when parasitizing *R. padi* (Jandricic *et al* 2014). Starý *et al* (2007) reviewed associations of parasitoids and hosts in Brazil and suggested that Aphidiine fauna is characterized by a typical complex consisting of the dominant species *A. colemani*, *Diaeretiella rapae* (M'Intosh), and *Lysiphlebus testaceipes* (Cresson). Based on the prevalence of *A. platensis* in northern RS, the species may be the same one identified as *A. colemani* by Starý.

In the Middle Plateau of RS, the parasitoid occurrence is related to mild temperature with mean air temperature less than 25°C, which is in accordance with favorable conditions for this species (Auad *et al* 1997, Sampaio *et al* 2005b). Sampaio *et al* (2005a) found *A. colemani* from May to October in Lavras, southeastern Brazil, but did not detect this parasitoid from November to April. According to these authors, the occurrence of the parasitoids coincided with the months of lower mean temperature. These results are probably due to the seasonal variations in the subtropical climate. Zero-inflated GLMM indicates that temperature affects the number of sampled parasitoids in Moericke traps. Temperature can directly affect the development and survival of parasitoids but also indirectly affecting the aphid populations (Thackray *et al* 2004, Sampaio *et al* 2007, Le Lann *et al* 2011, Sampaio *et al* 2017, Souza *et al* 2017). The adult longevity of *A. colemani* decreases with increasing temperature, ranging from 21.3 days at 16°C to 1.7 days at 28°C, as well as immature survival ranging from 83.3% at 16°C to 20.4% at 28°C (Sampaio *et al* 2007), therefore, lower temperatures during the winter could be propitious for this biological parameter and increased parasitoid population. The prevalence of *A. platensis* may be related to the current abundance of aphid species, especially *R. padi*. This species known as oat aphid may have benefited from the expansion of black oat (*Avena strigosa*, Schreb) areas that occurred with the implementation of a no-tillage system in Brazil. After harvesting the summer crops, the oats are sown offering an autumn refuge for aphids and consequently for the parasitoids.

The largest parasitoid populations found in the year 2016 occurred during phenomena La Niña (L'Heureux 2018). That year had lower average temperatures and reduced rainfall in the months prior to population peaks. The fact that a significant effect of rainfall on parasitoid populations has not been found deserves further study. In southern Brazil, rainfalls are generally well distributed throughout the year and heavy rainfalls have negative effects on aphid populations (Rebonatto *et al* 2015). However, in arid regions, rainfall may increase aphid population, which favors the parasitoids, but when exceeding certain threshold, has a negative effect on the aphid population (Thackray *et al* 2004, Leslie *et al* 2009, Sampaio *et al* 2017) and consequently their parasitoids (Sampaio *et al* 2017).

Sex ratio favored females during the assay (0.73). Elliott *et al* (1994) studying parasitism of *A. colemani* in a different host found that the female proportion changes with host species: *R. padi* (0.80), *S. graminum* (0.64), *R. maidis* (0.28). In host *Myzus persicae* Sulzer, *A. colemani* mated female produced offspring with 0.63 sex ratio (Jarošík *et al* 2003).

Questionable information remains about the introduction of *A. colemani* in Brazil during the BCPWA (1979–1980), and all specimens of *A. colemani* group sampled during the program period and deposited in the Embrapa Wheat entomology collection were identified as *A. platensis*. Throughout the 10-year monitoring program (2009–2018), only *A. platensis* among the *A. colemani* group were sampled with Moericke traps; moreover, *A. platensis* correspond to 61.87% of the cereal aphid parasitoids sampled. Species of the *A. colemani* group were recorded in Brazil prior to the BCPWA introductions (Starý *et al* 2007), however, it is impossible to know which species and how many different species of the *A. colemani* group were present previously or which species were introduced during the BCPWA. Our work evidences the prevalence of *A. platensis* in Rio Grande do Sul.

The survey developed by Embrapa Wheat for 10 years helped elucidate interactions between host, parasitoids, and meteorological conditions and can serve as a basis to develop managements programs for winter cereal aphids.

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Author Contribution Statement Douglas Lau planned, designed experimental work, and executed parasitoids monitoring program. Carlos Diego Ribeiro dos Santos, Juliana Pivato, and Marcus Vinicius Sampaio reviewed the entomology collection and parasitoids from the monitoring program. Carlos Diego Ribeiro dos Santos and Fabio Janoni Carvalho conducted data analyses. Carlos Diego Ribeiro dos Santos, Luiza Rodrigues Redaelli, Simone Mundstock Jahnke, Douglas Lau, and Marcus Vinicius Sampaio proposed and wrote the manuscript.

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