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Influence of factitious hosts on the morphometry and diversity of endosymbionts of the egg parasitoid *Telenomus remus*: insights for applied biological control

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Abstract The use of alternative hosts enables the mass creation of parasitoids in an economically viable manner, for examples, *Telenomus remus* Nixon (Hymenoptera: Platygastridae) created in *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) and *Anticarsia gemmatalis* Hübner (Lepidoptera: Erebidae). Due to the possible modifications resulting from the parasitoid-host relationship, we characterized the eggs of these hosts and studied the endosymbionts of each *T. remus* population. Scanning electron microscope images of freshly parasitized *Spodoptera frugiperda*, *A. gemmatalis*, and *C. cephalonica* eggs with *T. remus* exit orifice were taken. The morphometry of the parasitoids was based on the measurements of wing length and width, and body and tibia lengths. Polymerase chain reactions were performed to detect bacteria of the genera *Arsenophonus*, *Spiroplasma*, *Rickettsia*, *Serratia*, and *Wolbachia*. The eggs of the hosts differed in terms of morphological characteristics. *T. remus* females, when raised in *A. gemmatalis* eggs, had a longer body length than females raised in other hosts, and *T. remus* males were larger in all morphometric characters evaluated. Female parasitoids reared in *C. cephalonica* had body and tibia lengths similar to those in *S. frugiperda*; however, wing length and width were smaller than those of the other parasitoids. In the three *T. remus* populations *Serratia grimesii* were detected, and only in the population raised in *C. cephalonica* was the *Wolbachia* endosymbiont found. The results elucidated the understanding of host adaptation dynamics.

Keywords Microscopy · Alternative host · Natural enemy · Symbionts

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Introduction

The egg parasitoid *Telenomus remus* (Nixon) (Hymenoptera: Platygastridae) has a natural host *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae); however, due to high rearing costs, other hosts have been studied and show promise, for example, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) and *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Erebidae). Currently, *T. remus* is one of the most promising control agents for *S. frugiperda* (Kenis et al., 2019; Salazar-Mendonza et al., 2020). The effectiveness in controlling *S. frugiperda* is due to its higher parasitism capacity, which is twice as high as that of *T. pretiosum* (Pinto & Fernandes, 2020), and reproductive rate. In addition, its high capacity for dispersion and

adaptation to different temperatures makes this parasitoid promising for application in biological control programs (Pomari et al., 2015; Vieira et al., 2017; Queiroz et al., 2017a, b; Pomari et al., 2018).

One of the factors limiting the multiplication of *T. remus* on a large scale is the difficulty in creating the host of this parasitoid. The natural host *S. frugiperda* has larval cannibalism (Bentivenha et al., 2017). Therefore, to create *S. frugiperda* under laboratory conditions requires methodologies in which individuals in the larval phase are individualized. This production stage makes mass creation difficult, as the creation process is expensive in terms of the costs of creation material and labor. To solve this problem, an alternative is using substitute hosts that are easy to multiply and inexpensive to produce (Vieira et al., 2017).

One of the hosts with the potential for mass multiplication of *T. remus* is the rice moth, *C. cephalonica* (Stainton) (Lepidoptera: Pyralidae) (Pomari et al., 2015; 2016). Another alternative host that enables the successful development of *T. remus* is the pest lepidopteran *A. gemmatalis* (Hübner) (Lepidoptera: Erebidae), which, in the absence of the natural host *S. frugiperda*, has a parasitism rate above 80% (Bueno et al., 2014). Nonetheless, some characteristics related to both the host and parasitoid can influence the offspring of each population, causing positive or negative changes in these individuals, for example, physiological changes include changes in the size and fitness of individuals, and genetic changes influences include in the bacteria associated with these parasitoids.

The characteristics of the eggs vary from host to host, with some inherent characteristics, including shape, size, surface, and chorion, directly influencing the shape and survival of the parasitoid (Jones et al., 2015; Poncio et al., 2018). The size of the parasitoids is correlated with their performance, foraging, and efficiency in the pest control in the field (Ueno, 2015). Therefore, it is important to assess these changes using morphometrical measurements. From measuring the parasitoid morphometric characteristics, information about the parasitoid development can be obtained (Souza et al., 2018).

The association of insects with symbionts, mainly bacteria, is very common in nature. These bacteria can be inherited or acquired vertically (Werren et al., 2008). In these associations, both bacteria and insects undergo genetic, biochemical, and physiological changes that directly influence their life cycle and biology (Engel & Moran, 2013). Symbionts can play an important and useful role for insects, e.g., contributing to nutrition, providing essential amino acids, synthesizing B vitamins, and protecting against high temperatures or endoparasitoids (Oliver et al., 2014; Douglas, 2016; Feng et al., 2019). The known relationships involve the association of parasitoids with endosymbionts disrupting reproductive changes, such as the induction of cytoplasmic incompatibility or thelytokous parthenogenesis (Kageyama et al., 2012; Prabhulinga et al., 2016; Zeng et al., 2018).

Considering all these factors, in the present study, the following was undertaken: (1) characterization of *S. frugiperda*, *A. gemmatalis*, and *C. cephalonica* eggs; (2) morphometry of parasitoids from each population; and (3) identification of the bacterial community associated with *T. remus* multiplied in the eggs of the hosts, with the general objective of selecting the host that provided the best development for this parasitoid, without exerting harmful effects on the development and survival of *T. remus* as an applied biological control agent.

Materials and methods

The creations and bioassays were conducted in the laboratories of the Group for Integrated Pest Management in Agriculture, Department of Plant Protection, School of Agriculture, São Paulo State University (FCA/UNESP, Botucatu, SP, Brazil).

Insect rearing system

The insects used in the experiments were obtained from creations previously established in the laboratory, in which *S. frugiperda* and *A. gemmatalis* caterpillars were raised on an artificial diet (Greene et al., 1976; Parra, 2001) and followed similar maintenance procedures, according to the methodology described by Bueno et al. (2010). In the present study, an artificial diet was placed in plastic capsules (3 cm high × 7 cm in diameter), approximately 10 g per capsule, and the neonate caterpillars were placed in the capsule, where they remained until reaching the pupal phase. *C. cephalonica* was created according to the methodology described by Bernardi et al. (2000).

The rearing of *T. remus*, for all hosts, was undertaken as follows. For rearing maintenance, *S. frugiperda*

and *A. gemmatalis* eggs were offered within 48 h. Oviposition papers that contained the eggs of *S. frugiperda* and *A. gemmatalis* were glued with non-toxic glue to a rectangular cardboard sheet (17×12 cm). *C. cephalonica* eggs were glued with double-sided tape on a cardboard sheet (7×2 cm) and placed near a UV flow camera, 15 cm from the UV light, for 50 min. The cardboard sheet was offered to the newly emerged *T. remus* adults, and a *T. remus* population was established for each alternative host (offered S. *frugiperda* eggs to one population, *A. gemmatalis* eggs to the second population, and *C. cephalonica* eggs to the third population). Pure honey was placed inside of each rearing flask, with the help of a fine paint-brush, to feed the parasitoids,

After 48 h of parasitism, the cardboard sheet was removed and fresh eggs were offered. The parasitoid host ratio used was 1:40 (Pomari et al., 2013). This process was repeated until the fifth day of life of the parasitoids. The cardboard sheet with parasitized eggs was kept in a flat tube and, after the emergence of *T. remus* adults, more eggs were offered, continuing the rearing cycle.

Bioassays

Scanning electron microscopy (SEM) of S. frugiperda, A. gemmatalis, and C. cephalonica eggs

SEM of eggs was performed following the sample processing protocol of the Center for Electron Microscopy, Biosciences Institute of UNESP, Botucatu, SP, Brazil.

Fresh eggs of *A. gemmatalis*, *C. cephalonica*, and *S. frugiperda* (24 h old/without parasitism), parasitized by *T. remus* and with a parasitoid exit hole were collected. Each sample was fixed in 2.5% glutaraldehyde in phosphate buffer (0.1 M pH 7.3). After 24 h, the samples were washed three times for 5 min in distilled water to remove the fixative. The material was immersed in 0.5% osmium tetroxide in distilled water for approximately 30–40 min. Subsequently, the material was washed with distilled water three times for 10 min each. Dehydration was performed in an increasing series of alcohol (7.5%,15%, 30%, 50%, 70%, 90%, and 100%) two times for 10 min each.

The samples underwent critical point drying and were then mounted on stubs, metalized (Metallizer model MED 010, Balzers Union), scanned using an SEM (model SEM 515, Philips), and the eggs were measured. The terminology used in the description of the eggs was based on the study by Cônsoli et al. (1999).

Morphometry of Telenomus remus

For this bioassay, 60 *T. remus* individuals were used, in which the morphometry of 10 females and males from each population was measured. The individuals were placed in a Petri dish using an alcohol gel, and the images were captured using a camera attached to a Leica EZ4 D optical microscope. The images were stored and the morphological characters of length and width of the right anterior wing, body length, and length of the right posterior tibia were measured using the Image J 2.00 (Image J, 2019) software program.

Sampling, DNA extraction, and detection of endosymbionts via polymerase chain reaction (PCR)

A total of 50 adult *T. remus* individuals from each population were separated. The individuals were macerated and homogenized in a polypropylene tube (Eppendorf) in a solution containing 50 mL of 10% Chelex and 5 mL of KA protease. Then, the sample was transferred to an Infinigen thermocycler (model TC-96CG) and incubated for 20 min at 95 °C. After the incubation, the extracted DNA was used for the molecular detection of the endosymbionts of the genera *Arsenophonus*, *Spiroplasma*, *Rickettsia*, *Serratia*, and *Wolbachia* using specific primers (Table 1).

The PCR mix totaled 25 µL, and was composed of 12.5 µL of Taq DNA polymerase (NeoBio), 7.5 µL milliQ water, 1.0 µL of each primer, and 3.0 µL of sample DNA. The PCRs were performed under specific conditions for each endosymbiont (Table 2). The product resulting from the PCRs was visualized on a 1% agarose gel, to which a 100 bp molecular marker (Norgen) was added, and visualization was performed using a UV light transilluminator (Major Science).

For positive PCRs, DNA purification was performed using a Cellco purification kit according to the manufacturer's recommendations, followed by quantitative analysis of the sample DNA by optical density and spectrophotometry using a NanoDrop MD-1000 UV-Vis spectrophotometer. The samples were then sent to the Biotechnology Institute (IBTEC/UNESP) in Botucatu, SP, Brazil, for sequencing in an automatic DNA sequencer Sanger (Model: ABI 3500

Table 1 List of primers following endosymbionts tested for *Telenomus remus*

Endosymbionts	Target Gene	Primer Sequence 5'>3'	(pb)	Reference
Arsenophonus	*16 S rRNA*	F-CGTTTGATGAATTCATAGTCAAA AGTGTTACCCAAC R-GGTCCTCCAGTT	600	Thao and Baumann (2004)
Rickettsia	*16 S rRNA*	F-GCTCAGAACGAACGCTATC TGC R-GAAGGAAAGCATCTC	900	Gottlieb et al. (2006)
Serratia	*16 S rRNA*	F- CGCAGGCGGTTTGTTAAGTC AACCTCCA R- CTTCAAGGGCAC	268	Ribeiro (2019)
Spiroplasma	*16 S rRNA*	F-ACCGCATAACGTCGCAAGACC ACCTC R-CCTGTCTCAATGTTA	800	Montenegro et al. (2005)
Wolbachia	*16 S rRNA*	F-CGGGGGAAAAATTTATTGCT GAAAGTAAA R-AGCTGTAATACA	700	Heddi et al. (1999)

Table 2 Conditions for carrying out PCR reactions for endosymbionts: *Arsenophonus*, *Spiroplasma*, *Rickettsia*, *Serratia* and *Wolbachia*

Endosymbionts	Cycle
Arsenophonus	Initial denaturation temperature at 95ºC for 2 min, followed by 30 cycles of 95ºC at 30 s, 58ºC at 30 s, 72ºC at 1 min and final extension at 72ºC for 5 min, in a total of 1 h and 24 min.
Spiroplasma	Initial denaturation temperature at 94ºC for 5 min, followed by 30 cycles of 94ºC at 1 min, 52ºC at 1 min, 72ºC at 2 min and final extension of 72ºC for 5 min.
Rickettsia	Initial denaturation temperature at 95ºC for 2 min, followed by 30 cycles from 92ºC to 30 s, 58ºC to 30 s, 72ºC to 30 s and final extension of 72ºC for 5 min.
Serratia	Initial denaturation temperature at 95ºC for 10 min, followed by 35 cycles of 95ºC at 1 min, 62ºC at 1 min, 72ºC at 1 min and final extension of 72ºC for 1 min.
Wolbachia	Initial denaturation temperature at 95ºC for 3 min, followed by 30 cycles of 95ºC at 30 s, 55ºC at 30 s, 72ºC at 30 s and final extension of 72ºC for 5 min, totaling a cycle of 1 h and 12 min.

- Applied Biosystems) and compared with the data deposited in GenBank using the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Data analyses

The parasitoid morphometric characters were measured using Image J 2.00 software. For data analysis, the normality of residues was verified using the Shapiro-Wilk test (Shapiro & Wilk, 1965) and homogeneity of variances using the Bartlett test (Bartlett, 1937). When the assumptions were satisfied, the data were subjected to analysis of variance, and the means were compared using the Tukey test ($p < 0.05$). When the assumptions were not satisfied, a generalized linear model (GLM) belonging to the exponential family of distributions (Nelder & Wedderburn 1972) was adjusted. The quality of the adjustment was verified using a half-normal probability plot with a simulation envelope (Demétrio & Hinde 1997; Hinde & Demétrio, 1998). When there was a significant difference between treatments, the means were compared by contrast ($p < 0.05$). The analyses were performed using R, version 3.2.1 (R Core Team, 2017).

Results

SEM of *S. frugiperda, A. gemmatalis*, and *C. cephalonica* eggs

The *S. frugiperda* eggs were grouped in overlapping layers, usually covered by scales (Fig. 1a), which were approximately 0.58 mm in diameter and spherical with filaments arranged from top to bottom (Fig. 1b). The eggs contained three layers of polygonal cells, primary, secondary, and tertiary cells, forming the micropyle zone (Fig. 1c and d). Each egg had filaments throughout the structure (Fig. 1e), in which the aeropyles were arranged (Fig. 1f).

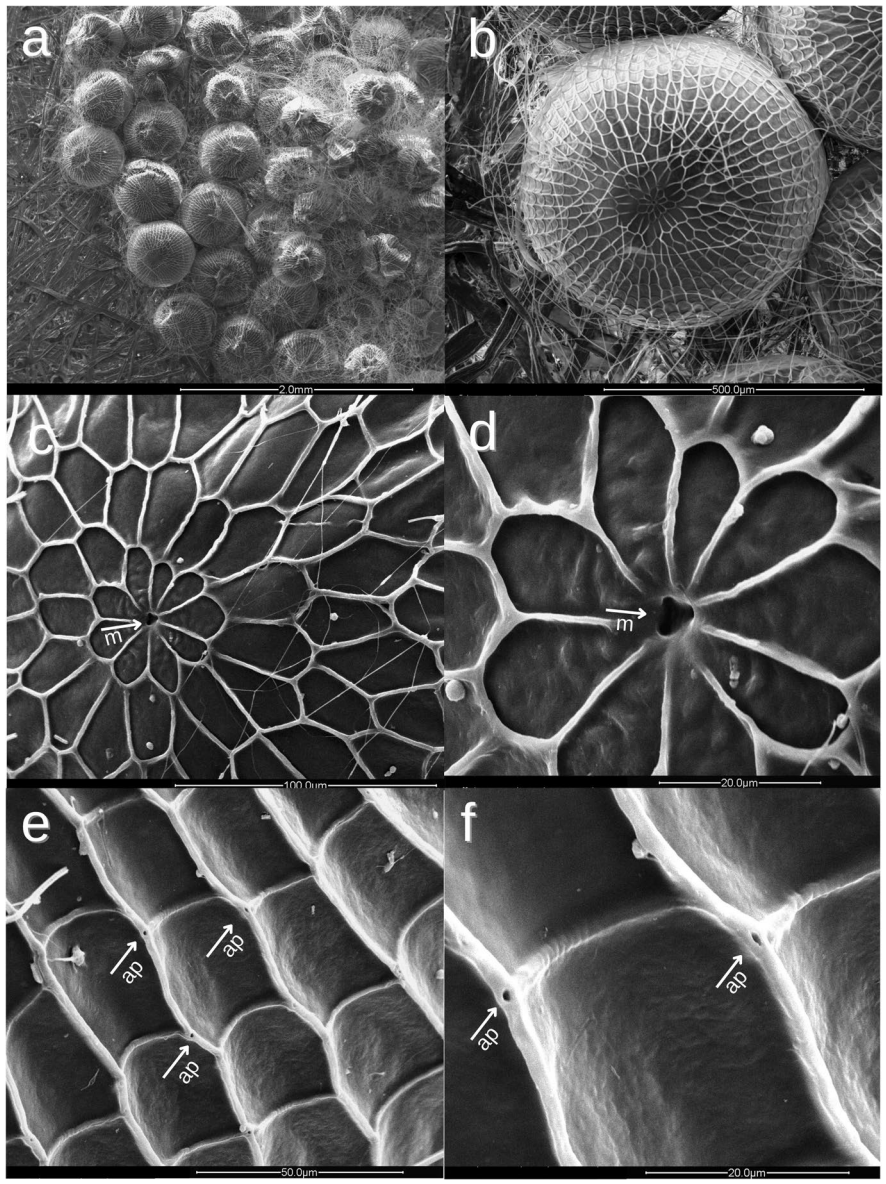

Fig. 1 Scanning electron microscopy of the surface structure of *S. frugiperda* eggs. **a** Eggs grouped in mass with the presence of scale deposited by the female; **b** Overview of the egg; **c** View of the micropyle and other upper regions of the egg; **d** Micropyle (m); **e** Grooves, filaments and aeropyles (ap); **f** Aeropyles (ap) distributed in lines in the filaments

The *A. gemmatalis* eggs were arranged individually, with a semi-spherical shape and measured approximately 0.57 mm (Fig. 2a and b). The micropyle was positioned on top of the egg (Fig. 2c), surrounded by three layers of cells (approximately six primary cells, 10 secondary cells, and nine tertiary cells). The cells progressively increased in size from the top to the bottom (Fig. 2d). These eggs had very evident lateral filaments, some reached the cells surrounding the micropyle, whereas others were shorter and connected by lateral connections, forming rectangular areas along the entire length of the egg (Fig. 2b and e). In these filaments, at the points of connections, aeropyles were located in great quantities throughout the length of the egg (Fig. 2f). Similar to *S. frugiperda* eggs, the aeropyles were arranged at the intersections between the connection bridges and filaments (Figs. 1f and 2f).

The *C. cephalonica* eggs were the smallest of all the host eggs. They had an ellipsoid shape that was 0.51 mm wide with a reticulated pattern and were arranged on the entire surface of the egg (Fig. 3b).

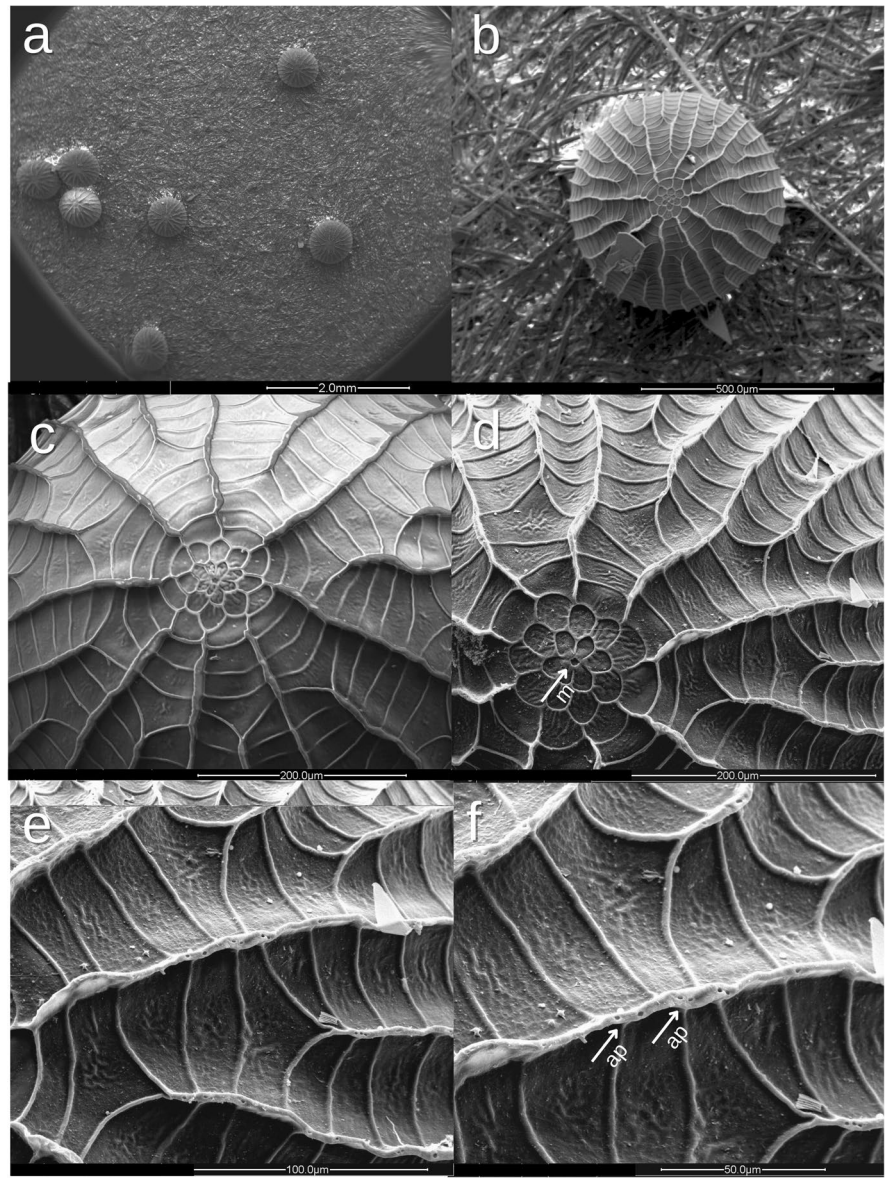

Fig. 2 Scanning electron microscopy of the surface structure of *Anticarsia gemmatalis* eggs. **a** Eggs arranged separately; **b** overview of the egg; **c** view of the micropyle and other upper regions of the egg; **d** micropyle (m); **e** side view; **f** aeropyles (ap) distributed in lines in the filaments

The eggs were individually arranged on the oviposition surfaces (Fig. 3a). The micropyle was in the anterior part of the eggs, which was at the top of the prominent projection (Fig. 3c and d). These eggs had few and small aeropyles, which were in the egg filaments (Fig. 3f).

The parasitized eggs of all hosts showed no difference after being parasitized (Fig. 4a, c and e). Regarding the exit orifice of the parasitoid from the egg, the parasitoid in all hosts made an opening in the egg from the side, with all hosts having a 0.25 mm opening area (Fig. 4b, d and f).

Morphometry of *Telenomus remus*

Female parasitoids from the *S. frugiperda* and *A. gemmatalis* eggs had equal wing length ($F = 13.07$; $df = 2.27$; $p < 0.0001$); however, the wing width was greater in female parasitoids of *A. gemmatalis* ($F = 48.90$; $df = 2.27$; $p < 0.0000$). Body length was similar in *C. cephalonica* and *S. frugiperda* populations, and greater in females from the host *A. gemmatalis* ($F = 52.46$; $df = 2.27$; $p < 0.0004$; Table 3). Parasitoids raised on *S. frugiperda* and *C. cephalonica* eggs had similar sized tibias, whereas

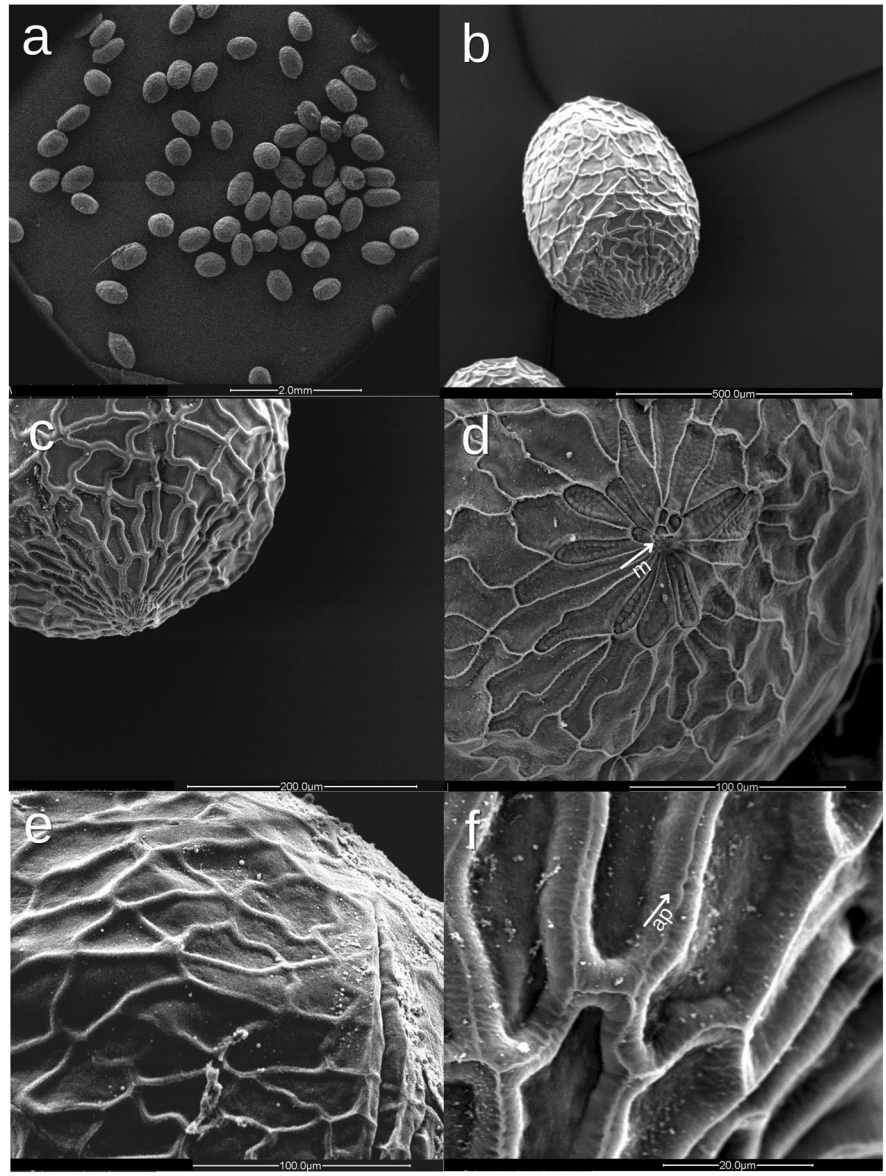

Fig. 3 Scanning electron microscopy of the surface structure of *C. cephalonica* eggs. **a** Scattered eggs; **b** overview of the egg; **c** lateral view of the micropyle; **d** micropyle (m); **e** side view; **f** aeropyles (ap)

females raised on *A. gemmatalis* eggs had larger tibias ($F=4.25$; $df=2.27$; $p<0.0247$; Table 3).

For the male parasitoids from the three populations, individuals raised on *A. gemmatalis* eggs had all the bigger morphometric characteristics. Male *T. remus* reared in *S. frugiperda* had a shorter wing length ($F=36.39$; $df=2.27$; $p<0.0000$) and width ($F=34.65$; $df=2.27$; $p<0.0000$), body length ($F=19.16$; $df=2.27$; $p<0.0000$), and tibia length ($F=26.66$; $df=2.27$; $p<0.0000$) than those reared in *A. gemmatalis* and were longer than those reared in *C. cephalonica*. In addition, male parasitoids raised in *C. cephalonica* were smaller than those of all other parasitoids in the other populations (Table 4).

Symbionts associated with *Telenomus remus*

In the three *T. remus* populations, the bacterium of genus *Serratia* was detected by PCR (Table 5) and, when sequenced, it was identified as *Serratia grimesii* (GenBank No. access: KC991303.1; 97% identity). Only the *T. remus* population reared in *C. cephalonica* tested positive for the genus *Wolbachia* (Table 5), which was confirmed by sequencing as *Wolbachia* endosymbiont

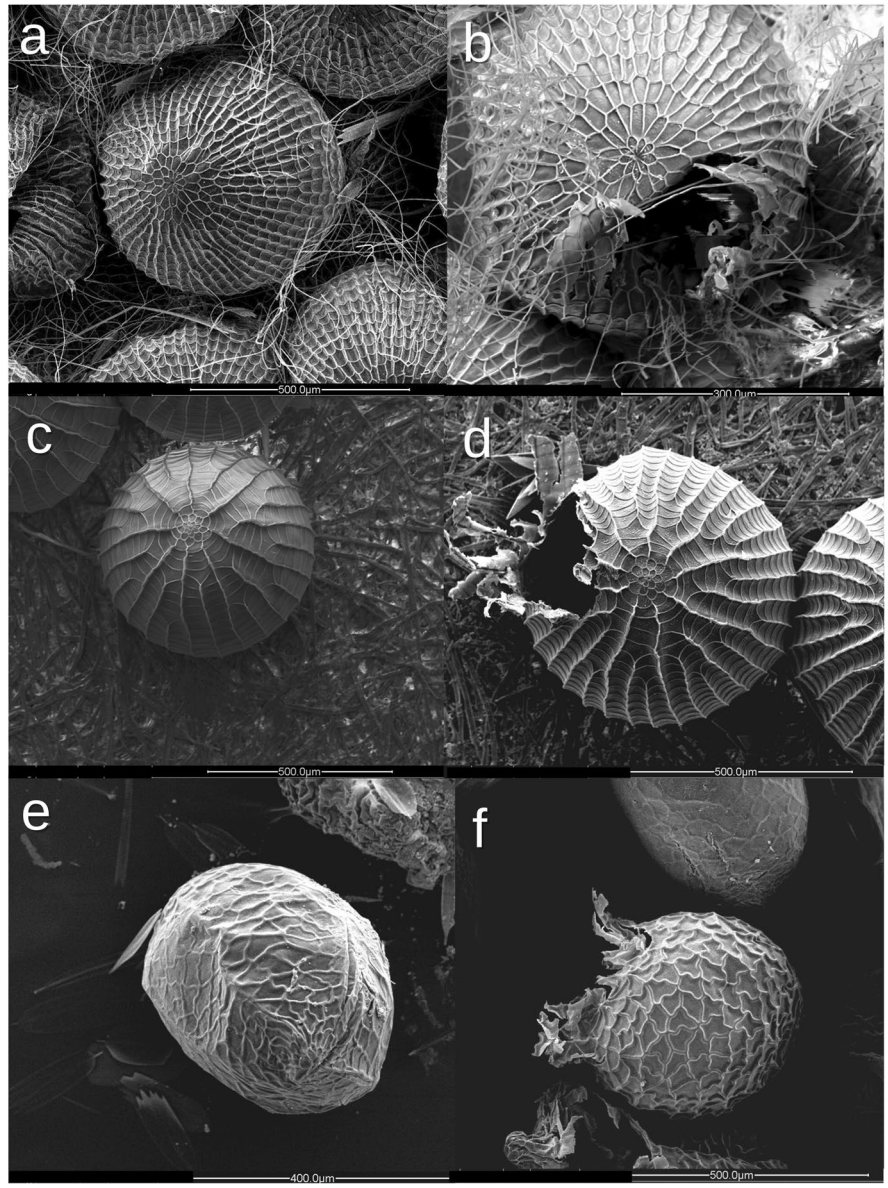

Fig. 4 **a** *S. frugiperda* parasitized eggs; **b** *S. frugiperda* eggs with *T. remus* exit hole; **c** *A. gemmatalis* parasitized eggs; **d** *A. gemmatalis* eggs with *T. remus* exit hole; **e** *C. cephalonica* parasitized eggs; **f** *C. cephalonica* eggs with *T. remus* exit hole;

(GenBank No. access: MF509296.1; 98% identity). The other symbionts tested (*Arsenophonus*, *Spiroplasma*, and *Rickettsia*) were negative in all populations.

Discussion

The morphometric characteristics of wing length and width, and body and tibia lengths indicate the ability of parasitoids to develop their hosts. The size of the parasitoid is linked to the size of the host and, subsequently, their quality (Sequeira & Mackauer, 1992; Ueno, 2015).

All three *T. remus* populations had a size compatible with that described in the literature for this species (Cave, 2000). However, *T. remus* raised on *A. gemmatalis* eggs had higher morphometric characteristics and, considering that the quality determination of parasitoids is directly related to the size of the individual (Kölliker-Ott et al., 2003), the data obtained

Table 3 Mean of wing length and width, body length and tibia (mm) of *Telenomus remus* female reared in *Anticarsia gemmatalis*, *Spodoptera frugiperda* and *Corcyra cephalonica*

Host	Measures (mm)			
	Wing length[1]	Wing width[2]	Body length[2]	Tibia length[2]
A. gemmatalis	0.60 ± 0.02a	0.20 ± 0.00a	0.72 ± 0.01a	0.15 ± 0.01a
S. frugiperda	0.60 ± 0.01a	0.19 ± 0.00b	0.62 ± 0.01b	0.14 ± 0.00b
C. cephalonica	0.53 ± 0.01b	0.16 ± 0.00c	0.59 ± 0.01b	0.13 ± 0.00b
F	13.07	48.903	52.469	4.2559
df	2. 27	2. 27	2. 27	2. 27
p value	0.0001	0.0000	0.0004	0.02474

[1] Means followed by distinct letters indicate significant differences between treatments (GLM with Gaussian distribution, followed by test of contrasts, $p < 0.05$)

[2] Means followed by distinct letters in the columns indicate significant differences between treatments (Tukey, $p < 0.05$);

Table 4 Means of wing length and width, body length and tibia (mm) of *Telenomus remus* males reared in *Anticarsia gemmatalis, Spodoptera frugiperda* and *Corcyra cephalonica*

Host	Measures (mm)			
	Wing length	Wing width	Body length	Tibia length
A. gemmatalis	0.65 ± 0.01a	0.21 ± 0.00a	0.63 ± 0.01a	0.19 ± 0.01a
S. frugiperda	0.60 ± 0.01b	0.19 ± 0.01b	0.57 ± 0.01b	0.16 ± 0.01b
C. cephalonica	0.52 ± 0.01c	0.15 ± 0.00c	0.51 ± 0.02c	0.14 ± 0.01c
F	36.39	34.65	19.16	26.66
df	2.27	2. 27	2. 27	2.27
p value	0.0000	0.0000	0.0000	0.0000

Means followed by distinct letters in the columns indicate significant differences between treatments (Tukey, $p < 0.05$)

suggest that these parasitoids may have a better performance in the field.

The size of *A. gemmatalis* parasitoids might be related to its greater egg volume (0.089 mm^3) compared to the other hosts. Larger eggs have higher nutritional content, providing better development of the parasitoid (Jones et al., 2015). This relationship is known for the parasitoid *Agrothereutes lanceolatus* (Walker) (Hymenoptera: Ichneumonidae), in which the size of the hosts had a direct influence on the size, emergence, and sex ratio of the offspring of parasites obtained (Ueno, 2015).

For *S. frugiperda* and *C. cephalonica*, the egg volume was equal (0.036 mm^3), justifying some similar characteristics related to the female parasitoids. However, the egg shape of these parasitoids differs (Cônsoli et al., 1999), suggesting that it is the size and shape of the eggs that influence the morphometry of the parasitoids.

Considering the characteristics of the eggs of the hosts with the morphometric characteristics of the parasitoids, *S. frugiperda* and *A. gemmatalis* eggs were more favorable hosts for better development of *T. remus*. However, even in populations created in *C. cephalonica* with smaller morphometric characteristics, other studies have shown that this host provides excellent development for

Table 5 Symbiont bacteria tested for populations of *Telenomus remus* raised in *Spodoptera frugiperda*, *Anticarsia gemmatalis* and *Corcyra cephalonica*

Symbiont bacteria	Host		
	S. frugiperda	*A. gemmatalis*	*C. cephalonica*
Wolbachia	-	-	+
Rickettsia	-	-	-
Arsenophonus	-	-	-
Serratia	+	+	+
Spiroplasma	-	-	-

(+) positive; (-) negative;

parasitoids, confirming its performance as similar to those created in the natural host *S. frugiperda* (Pomari et al., 2016; Queiroz et al., 2017a). Therefore, *T. remus* can complete development inside different host eggs, indicating that it has strong adaptability to different hosts.

T. remus populations had similar endosymbiont bacteria in all populations, except for the population raised in *C. cephalonica* that acquired *Wolbachia*. The main change in individuals infected with *Wolbachia* is reproductive manipulation (Prabhulinga et al., 2016; Harumoto et al., 2018). Previous studies undertaken with parasitoids from infected eggs showed that *Wolbachia* infection is a positive condition. *Trichogramma dendrolimi* that were positive for *Wolbachia* were more efficient in controlling *Ostrinia furnacalis* because the infected populations had higher population growth and parasitism rate than the uninfected populations (Dong et al., 2017). When two populations of *Trichogramma pretiosum* with different reproduction modes were studied, the thelytoky population had a higher number of females and intrinsic growth rate than a population of the same species that reproduced in arrhenotoky (Prabhulinga et al., 2016).

A previous study reported the species *Serratia grimesii* associated with the coleopteran intestinal microbiota, and other species of the genus (Hernández et al., 2015). Furthermore, the relationship between this bacterium and parasitoids remains unknown, and it is not possible to determine the performance of this species in the intestine of *T. remus*.

The presence of *Wolbachia* is yet another positive advancement in applied biological control. C. *cephalonica* is the most suitable alternative host for the mass production of *T. remus*. In the mass creation of insects for use in applied biological control programs, the multiplication of females is extremely important, as these are the control agents, and a population infected by *Wolbachia* might contribute to an increase in the number of individuals of interest for mass release (Almeida et al., 2010; Ebrahimi et al., 2019).

Wolbachia is a common bacterium in symbiotic relationships with insects; however, its frequency within the insect species is low (Sazama et al., 2019). Therefore, further studies on these interactions are crucial. The acquisition of symbionts marks the beginning of these symbiotic relationships with the parasitoid; however, the adaptation process of these bacteria to the intestine of insects characterizes the success of this relationship. Irrespective of how much bacterium has been acquired, transmission to other generations and the frequency of these organisms in the parasitoid depends on the adaptation process.

In conclusion, all hosts in the present study allowed the complete development of the parasitoids, and this is the first to include host adaptation related to the characteristics of *T. remus*. It is important to understand the dynamics of adaptation to the host given the need for an effective biological control agent to control *S. frugiperda*. Further studies involving field tests are required to determine the real aptitude of each *T. remus* population when used for the biological control of *S. frugiperda*.

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Authors' contributions All authors contributed to the study. Conceptualization: Regiane Cristina de Oliveira, and Carolane Benjamin da Silva; Methodology: Regiane Cristina de Oliveira, and Carolane Benjamin da Silva; Experiments: Carolane Benjamin da Silva, Vanessa Rafaela de Carvalho, João Pedro de Andrade Bomfim, and Nadja Nara Pereira da Silva; Formal analysis and investigation: Carolane Benjamin da Silva, Vanessa Rafaela de Carvalho, João Pedro de Andrade Bomfim, and Nadja Nara Pereira da Silva; Writing - original draft preparation: Carolane Benjamin da Silva; Writing—review and editing: Regiane Cristina de Oliveira; All authors read and approved the final manuscript.

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Declarations

Conflict of interest/Competing interests All the authors declare that they have no conflict of interest.

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