Reproductive compatibility and morphological characterization of local populations of the larval endoparasitoid *Pseudapanteles dignus* in Argentina

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Abstract In hymenopteran parasitoids, isolated geographic populations can show morphological variation and reproductive incompatibility that may lead to failures in biological control programs when they are intended to be used as biocontrol agents. Through a classical morphometric approach we confirm the specific identity of three local populations of the larval arrhenotokous endoparasitoid *Pseudapanteles dignus* (Muesebeck) (Hymenoptera: Braconidae) of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), from three main tomato producing areas of Argentina. Moreover, we examined reproductive compatibility by outcrossing the three populations. Morphometric differences among *P. dignus* biotypes were found but no evidences of mating incompatibility among them existed. This laboratory study contributes with relevant information to the original description of the species *P. dignus* and has significant implications for *T. absoluta* biological control using this parasitoid in tomato crops from different regions of Argentina.

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Introduction

The South American tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is an important lepidopteran pest of tomato (*Solanum lycopersicum* L.) crops. The species originates from the Neotropical region (Pereyra and Sánchez 2006; Silva et al. 2015) and it is reported in the Americas, Europe, Africa and Asia (Campos et al. 2017; Santana et. al 2019), mostly for open-field crops. It is expected to invade China and USA, the main worldwide tomato producer countries (Han et al. 2019; Tabuloc et al. 2019). The damage is caused by the mining habit of the larva, which perforates the leaves, stems and fruits, causing serious harvest losses that can reach up to 90%. Its control currently depends almost exclusively on the use of agrochemicals in all affected regions (Guedes et al. 2019). However, in some countries of Europe and South America, several biological control programs against *T. absoluta,* which combine the use of biocontrol agents such as parasitoids, microbials (e.g., *Bacillus thuringiensis*) and mirid predators, are commercially available (Biondi et al. 2018; Salas Gervassio et al. 2019a).

Tomato is one of the most economically important vegetable crops worldwide and Argentina is ranked among the first 25th producer countries (FAO-STAT 2019). The crop is distributed throughout different biogeographic and climatic regions in this country, with *T. absoluta* reported as a pest in all of them (Sánchez et al. 2009; Puch 2011; Garrido et al. 2017; Cáceres et al. 2019). Main tomato producing regions in Argentina are: (1) northwestern (provinces of Jujuy, Salta and Tucumán), (2) northeastern of Buenos Aires province, and (3) North Patagonia (provinces of Río Negro and Neuquén) (Argerich and Troilo 2010).

Research in this country has been conducted for many years with the aim to control the pest using the native solitary, koinobiont, larval endoparasitoid, *Pseudapanteles dignus* (Muesebeck) (Hymenoptera: Braconidae). It has a narrow host range reported for few Gelechiidae species. This wasp inflicts mortality from 30 to 64% of *T. absoluta* larvae in NE Buenos Aires province, when pesticides are avoided or rationally used (Sánchez et al. 2009; Nieves et al. 2015). Field and laboratory studies suggested that *T. absoluta* and *P. dignus* had potentially co-evolved and showed that the parasitoid has positive attributes to be a biocontrol agent for this pest (Vallina et al. 2012; Salas Gervassio et al. 2019a, b). *Pseudapanteles dignus* is a Neotropical endemic species with a known distribution in few countries of the Americas (Fernández-Triana et al. 2014). In Argentina, in addition to having been observed in NE Buenos Aires province, the species has also been reported in Northern provinces (Colomo et al. 2002; Puch 2011) and in the Patagonia region (Garrido et al. 2017).

In hymenopteran parasitoids, isolated geographic populations or biotypes of a single species can show reproductive incompatibility or certain degree of compatibility in crossing experiments (Hopper et al.1993; Quicke 1997). Different populations of a same species can be subject to different selection pressures, exerted by different biotic and abiotic conditions. This may result in both genotypic and phenotypic variations (morphological, ecological or behavioral), product of the co-adaptation of gene complexes (Luna and Hawkins 2004; Wajnberg et al. 2012). This is a crucial point for inoculative biological control programs, since parasitoids are mass-reared and released, generally at quite distant regions from which the source populations were collected, and eventually interbreed with already existing populations in releasing areas. Many causes have been pointed out to explain failures in field performance and ultimately the biocontrol success of the progeny, ranging from genetic barriers that can lead to reproductive isolation, outbreeding depression (as disruption of co-adapted genes), to the presence of *Wolbachia* or similar endosymbiontic bacteria that can alter reproduction and produce mating incompatibility in arthropods (Liu et al. 2002; Luna and Hawkins 2004; Vorsino et al. 2012; Bertin et al. 2018). Another fundamental cause of reproductive isolation is when two morphs or ecotypes differ in size and mating preferentially occurs between individuals of similar size (Crespi 1989).

The implementation of biological control using a particular species in different geographic areas requires an accurate species taxonomic identification. Classical morphometry provides tools for comparing morphological traits among biotypes and also

contributes for a more complete characterization of the species (Schwarzfeld and Sperling 2014). In this respect, Yu et al. (1992) have shown that the measurement of various elements of the wings in Hymenoptera can be an effective method to study intra- and inter-specific morphological variation.

Taking into account the known geographic distribution of *P. dignus* in Argentina, where regional populations are very likely isolated to each other, thus potentially subjected to different selection pressures, our hypothesis is that there will exist certain degree of reproductive incompatibility and morphological variation. The aim of this study was to assess the presence of morphotypes and the potential reproductive isolation among three *P. dignus* populations. First, we conducted morphological studies to confirm the species status and to analyze the anatomical variability of three biotypes of this parasitoid by measuring head, forewing, ovipositor and metatibia and further calculating different ratios. Second, we determined reproductive compatibility and performance of *P. dignus* biotypes by crossing local populations from three distant areas. Results may offer some insights into *T. absoluta* biocontrol.

Materials and methods

Study area and collection of local populations of *P. dignus*

Tuta absoluta and *P. dignus* sampling was conducted in three provinces of the main tomato cropping regions in Argentina: Salta (SA; 23° 19′ 24″ S 64° 13′ 29″ W); Buenos Aires (BA; 34° 56′ 00″ S 57° 57′ 00″ W), and Río Negro (RN; 39° 25′ 25″ S 65° 42′ 00″ W). These regions are at least 1500 km separated from each other. Foliage infested with moth larvae were randomly collected from ∼ 30 tomato crops for each province selected for this study, during 2015 to 2018. Leaves were conditioned for transportation to the laboratory at the CEPAVE (La Plata, Buenos Aires, Argentina) where they were kept isolated, according to the population (SA, BA and RN), in an environmental room (25 ± 2° C, 70 ± 5% RH, L:D 14:10) to obtain *T. absoluta* or *P. dignus* pupae. Colonies were started with ≥ 120 specimens per species and population. Each parasitoid biotype was maintained on its own host population. In the case of the endoparasitoid, we first identified the species using the keys, diagnosis and descriptions published elsewhere (Muesebeck 1938; Wharton et al. 1997; Fernández and Sharkey 2006). Then, a group of emerged female wasps was immediately fixed in 70% ethanol to perform the morphometric study meanwhile the remaining wasps were the founders to start the colonies to carry out the interbreeding trials.

Morphometric study

For taking measurements of forewings, hindlegs, ovipositor, and head, fixed females were dissected and then card- or slide-mounted using conventional techniques (Noyes 1990), photographed and measured. The images were obtained using a Nikon SMZ1270 stereomicroscope and a Micrometrics 519CU CMOS 5.0 Megapixel Camera, and measurements were taken using Micrometrics Standard Edition Premium ACCU-SCOPE Inc. software. Measures and morphological terms used followed those proposed by Azidah and Quicke (2000) meanwhile wing venation was studied according to Wharton et al. (1997) and Gauld and Bolton (1996). Forewings were extracted from their base, slide mounted, and 14 landmarks (A to N) were defined and recorded using, in most cases, intersections of the veins (Fig. 1a). From this, 13 linear measurements were defined (Table 1).

Hindlegs were extracted and slide mounted, measuring the length of the tibia from the dorsal apex of the femur to the dorsal apex of the tibia (LMT) (Fig. 1b; Table 1). Ovipositor was extracted, slide mounted and measured from anterior to posterior extremes (LOV) (Fig. 1c; Table 1). Finally, after extracting forewings, hindlegs and the ovipositor, individuals were card-mounted and the head was measured by taking dimensions of maximum length of face (MLF) from the base of toruli to the margin of clypeus and maximum face width (MWF) as the distance between eyes below the toruli (Fig. 1d; Table 1).

To minimize differences in individual estimations, the following ratios were calculated based on linear measurements (Daly 1985): A–F/D–N, A–C/C–D, A–H/B–H, A–H/H–M, G–M/H–M, H–L/I–K, I–J/I–L and I–J/J–L (forewings); MLF/MWF (head); LOV/LMT (ovipositor and metatibia). Differences in ratios between regions were tested by ANOVA whenever
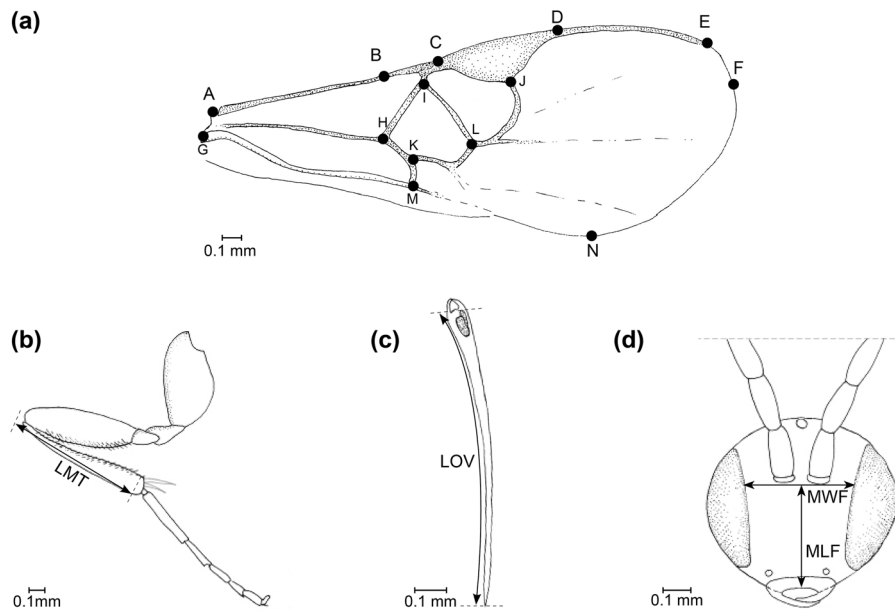
Fig. 1 Diagrams showing landmarks and measurements used in morphometric studies of *P. dignus* female body parts: **a** forewing (landmarks A to N); **b** length of the metatibia (LMT); **c** length of the ovipositor (LOV); and **d** length and width of the face (MLF and MWF, respectively)

Table 1 Linear measurements taken in different body parts for a morphometric study of *Pseudapanteles dignus* (Hymenoptera: Braconidae)

Body parts	Linear measurements (μm)	
Forewing	A–F	Maximum forewing length
	D–N	Maximum forewing width
	A–C	Length of the C + SC + R vein
	C–D	Length of stigma
	A–H	Length of M + Cu vein
	B–H	Distance in a vertical straight line from C + SC + R vein to the intersection of the M + Cu vein with M vein
	G–M	Length of 1A vein
	H–M	Distance from the intersection of the M and CU veins to the intersection of the 1A and CU veins
	I–K	Distance from the intersection of M vein with RS + M vein to CU vein
	H–L	Distance from the intersection of the M and CU veins to the intersection of the RS + M vein with m-cu recurrent vein
	I–L	Distance from the intersection of M and RS + M veins to the intersection of the RS + M vein with RS vein
	I–J	Distance from the intersection of M and RS + M veins to the beginning of the r vein in the pterostigma
	J–L	Distance from the beginning of the r vein in the pterostigma to the intersection of the RS + M vein with RS vein
Hindleg	LMT	Length of the tibia (from the dorsal apex of the femur to the dorsal apex of the tibia)
Ovipositor	LOV	Length of the ovipositor (from the anterior to the posterior extremes of the ovipositor)
Head	MLF	Maximum length of face (from the base of toruli to the margin of clypeus)
	MWF	Maximum width of face (distance between eyes below the toruli)

possible, i.e., under conditions of normality and homoscedasticity, and post-hoc analyses were performed by Tukey HSD tests. Kruskal–Wallis and post-hoc Conover–Iman tests were used in the remaining analyses. A significance level of 5% was chosen for all analyses.

A Principal Component Analysis (PCA) was accomplished to reduce the set of variables and to search for those that explain better morphological variation of different *P. dignus* biotypes. First, a correlation analysis was performed with the set of ten variables (ratios) and three highly correlated variables were eliminated. With the remaining seven variables, A–F/D–N, A–C/C–D, A–H/B–H, A–H/H–M, I–J/J–L, MLF/MWF and LOV/LMT, a PCA analysis was performed. All analyses and data processing were conducted using software R version 3.5.0 (R Core Team 2018).

P. dignus interbreeding assays

Tuta absoluta and *P. dignus* colonies of each region were established using the protocol described by Nieves et al. (2015). *T. absoluta* adults were maintained in voile cages (36 × 36 × 61 cm, Bioquip®), with honey ad libitum, and provided with tomato seedlings as substrate for oviposition. Every two days, plants bearing *T. absoluta* eggs were replaced by new ones and kept in a different cage to allow the larval development. Once larvae reached the last instar (L4), seedlings were transferred to another voile cage with a metallic grid containing sterile dry sand in the bottom to recover pupae falling which were transferred to the cage with the adults to start a new cycle.

To begin *P. dignus* colonies, newly emerged parasitoids from each population were sexed and paired in plastic capsules (8 cm diameter × 1.8 cm height), honey fed, and allowed mating. Then, each female was transferred individually into a plastic jar (750 ml) with a bouquet of tomato leaves infested by 20–30 *T. absoluta* larvae from the same region as the parasitoid as oviposition substrate, and covered with voile. Larvae were replaced every 48 h, fed with fresh tomato leaves until cocoon formation. Adults (F1) were used in the trials.

Outcrossing experiments were performed to determine reproductive compatibility between BA and SA, and between BA and RN populations. SA and RN populations could not be crossed because the field samplings were taken three years apart and colonies could not be maintained simultaneously. Four different types of inter-population crosses (treatments) were performed, with 15 replications each: (1) ♀ BA × ♂ SA, (2) ♀ BA × ♂ RN, (3) ♀ SA × ♂ BA, and (4) ♀ RN × ♂ BA. Intra-population crosses of each biotype were replicated 15 times as controls (♀ SA × ♂ SA, ♀ BA × ♂ BA, and ♀ RN × ♂ RN). Only 24 h old virgin individuals were used in each treatment. Couples were allowed to mate during 48 h, after which females were placed individually in an experimental unit (as described for the parasitoid colony), provided with 20–30 *T. absoluta* larvae infesting tomato leaves for other 48 h for oviposition. Exposed larvae were reared until the *P. dignus* adult progeny (F2) was obtained and individuals were sexed and counted. The interbreeding assays were carried out in an environmental room under the same controlled conditions as the colonies. Considering that *P. dignus* is an arrhenotokous species (Nieves et al. 2015) and that preliminary monitoring for infections of *Wolbachia* in all *P. dignus* local populations came out negative (Vallina et al. 2018), the presence of female offspring was taken as an evidence of mating among biotypes. Sex ratio was calculated as percent females, and then mean sex ratio (MSR) was estimated per each cross. Differences among sex ratio were examined with a χ^2 test and test of differences in proportions for all possible pairs was performed, with an adjustment of the p-value by the Sidak method (level of significance ≤ 5%). The p-value of each comparison was 0.00244.

Reproductive compatibility (RC) was assessed by calculating the relative value of the proportion of females in the progeny of an inter-population cross to that of the corresponding intra-population crosses of the female (Pinto et al. 1991): (MSR (A ♀ × B ♂)/ MSR (A ♀ × A ♂)) × 100) and (MSR (B ♀ × A ♂) / MSR (B ♀ × B ♂)) × 100). Values of RC equal, lower or higher than 100 indicate that the inter-population cross produced the same, lower or higher proportion of females as the intra-population crosses, respectively.

To evaluate the degree of reproductive isolation among geographical populations, we estimated two indexes proposed by Rolán-Alvarez and Caballero (2000) using the program JMATING (Carvajal-Rodríguez and Rolán-Álvarez 2006). The index of pair sexual isolation (I_{PSI}), that can range from −1 to 1, being −1 when pairs correspond to parents of

different biotypes, 0 when there exists random mating, and 1 when pairs are of the same biotype; and the asymmetry index (IA_{PSI}), which values vary from 1 (symmetry) to those significantly different from 1 (asymmetry). This last index helps detecting asymmetry in sexual isolation, i.e., whether mating occur more frequently when females from population A are paired with males from population B than the reciprocal cross (Bertin et al. 2018; Carvajal-Rodríguez and Rolán-Álvarez 2006; Rolán-Álvarez and Caballero 2000). A bootstrap analysis (10,000 iterations) was used to calculate the SD of I_{PSI} and IA_{PSI} and to test the hypothesis that the sexual isolation estimate deviates significantly from random mating ($I_{PSI} = 0$) and the asymmetry index deviates significantly from symmetrical crosses ($IA_{PSI} = 1$).

Results

Morphometric characterization

Through the morphological study of *P. dignus*, and the descriptions made by other authors, it was confirmed that the populations collected in the three regions of Argentina belong to the same species. Average of each linear measurement taken in the forewing, head, metatibia and ovipositor of *P. dignus* from three geographic populations is provided as Supplementary Table S1. Significant differences among populations were found in six out of ten ratios studied: four wing ratios A-F/D-N ($F_{2,221} = 49.13$, $p < 0.001$), A-C/C-D ($\chi^2 = 8.74$, df = 2, $p = 0.013$), H–L/I–K ($F_{2,221} = 4.55$, $p = 0.012$), I–J/J–L ($\chi^2 = 24.16$, df = 2, $p < 0.001$); the ratio between ovipositor and metatibia LOV/LMT ($F_{2,186} = 9.16$, $p < 0.001$); and the ratio between length and width face MLF/MWF ($F_{2,170} = 52.01$, $p < 0.001$) (Fig. 2).

The RN population differed in all these variables when compared with the other two populations (BA, SA), showing RN individuals with longer and narrower wings, ovipositor 1.36 × as long as metatibia and face wider than long. SA and BA populations turned out to be morphologically more similar, differing only on values for three ratios measured for wings (A–F/D–N, H–L/I–K and I–J/J–L) and the relation between metatibia and ovipositor. No differences were found regarding the length and width of the face, being longer than wide compared to those of RN.

As for the wings, those of BA turned out to be shorter and wider than those of SA, and as for the ovipositor, in population BA it is 1.33 × as long as metatibia, and 1.32 as long as metatibia in population SA. Statistical parameters of post-hoc tests are shown in Table 2.

Regarding the PCA, the first three components explained 59% of the total variability, with each component contributing 22.2%, 20.6% and 16.2%, respectively (Fig. 3). The first component sorted individuals of *P. dignus* with rather longer and narrower wings, and wider than long faces from individuals with shorter and wider wings, and faces longer than wider. The second component separated groups according to whether the ratios measured in wings A–C/C–D, A–H/B–H and A–H/H–M increase or decrease together, and the third component arranged individuals based on the ratios measured in wings A–H/B–H, I–J/J–L and that relating ovipositor and metatibia LOV/LMT.

Interbreeding

All inter-population crosses exhibited reproductive compatibility, producing female progeny in at least 50% of the replicates (i.e. $CFO_f \geq 0.5$). The mean sex ratios differed between groups ($\chi^2 = 49.736$; df = 6; $p < 0.001$). Sex ratio of ♀SA × ♂BA and ♀BA × ♂ SA crosses were the lowest (≈ 0.30), when compared with other inter- and intra-population crosses.

Differences in sex ratio values were found for the following crosses: ♀SA × ♂BA with the three intra-population matings and ♀ RN × ♂ BA (all $p < 0,0001$); ♀ BA × ♂ SA with ♀ RN × ♂ RN and ♀ RN × ♂ BA ($p < 0.0002$ and < 0.0007, respectively). Other comparisons were not significantly different (Table 3).

RC values indicated a reduced reproductive compatibility between SA and BA local populations. The greatest values of RC were registered for the crosses of BA and RN biotypes, when parents were tested in both reciprocal combinations ($> 82\%$ of sexual compatibility), and were quite similar to their intra-population crosses. There was no sexual isolation or asymmetry in the interbreeding of all biotypes since I_{PSI} was not significantly different from 0 (SA × BA 0.21 ± 0.15 and RN × BA -0.02 ± 0.15; $p > 0.05$) and IA_{PSI} no different from 1 (SA × BA 1.07 ± 0.22 and RN × BA 1 ± 0.07; $p > 0.05$), indicating that biotypes mate randomly and symmetrically (Table 3).
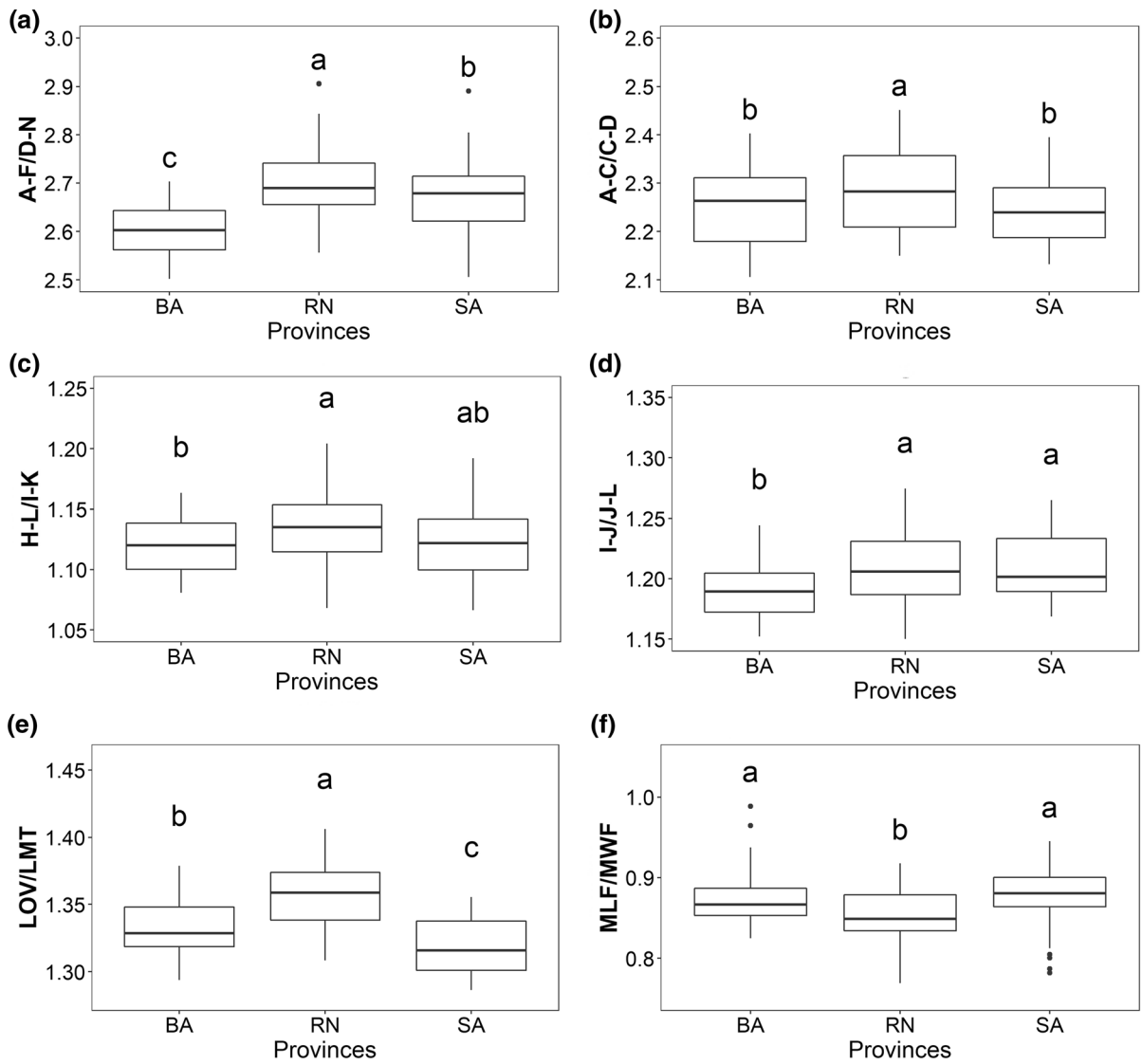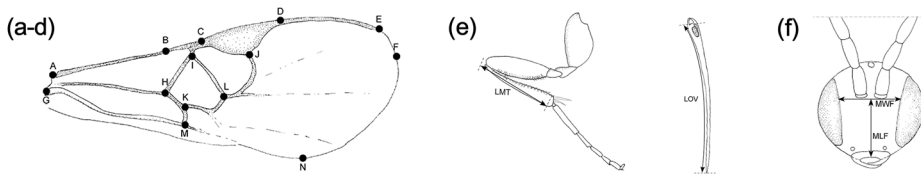
Fig. 2 Boxplot diagrams showing data distribution of the morphological ratios measured in forewing (**a–d**), ovipositor (**e**), metatibia (**e**) and head (**f**) of *P. dignus* female wasps that resulted significantly different among three Argentinian geographic populations (BA, Buenos Aires; RN, Río Negro; and SA, Salta provinces). The boxes and the horizontal line within each box indicate the quartiles and the median value of each ratio. Outliers are plotted as circles. The different lower-case letters indicate the significant differences between populations ($p < 0.05$; Tukey's HSD test)

Table 2 Results of post-hoc Tukey (T) and Conover Iman (CI) tests to compare means of morphometric ratios estimated for three *P. dignus* geographic biotypes in Argentina

Ratio	Test	Biotypes compared	p-value	Ratio	Test	Biotypes compared	p-value
A–F/D–N	T	BA-RN	< 0.001	I-J/L-J	CI	BA-RN	< 0.001
		BA-SA	< 0.001			BA-SA	< 0.001
		RN-SA	0.02			RN-SA	0.17
A–C/C–D	CI	BA-RN	0.02	MLF/MWF	T	BA-RN	0.004
		BA-SA	0.26			BA-SA	0.76
		RN-SA	0.001			RN-SA	< 0.001
H–L/I–K	T	BA-RN	0.001	LOV/LMT	T	BA-RN	< 0.001
		BA-SA	0.82			BA-SA	0.002
		RN-SA	0.06			RN-SA	< 0.001

Geographic biotypes: BA, Buenos Aires; SA, Salta; RN, Río Negro. Overall significance level: 5%. Variable legends are explained in the text
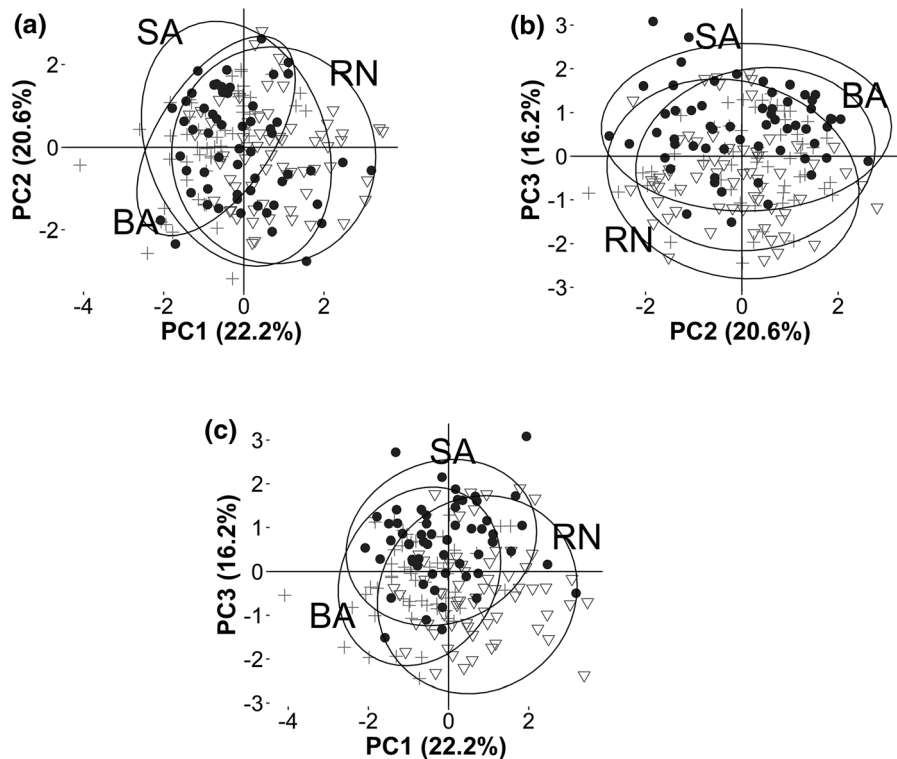
Fig. 3 PCA ordination of the three *P. dignus* biotypes (BA, Buenos Aires (+); RN, Río Negro (inverted open triangle); and SA, Salta (filled circle) provinces) according to seven morphological mean ratios measured. Confidence ellipses contain 95% of data

Discussion

The results of the present study provide novel information on the solitary endoparasitoid *P. dignus*, one of the most important natural enemies of *T.*

absoluta in Argentina. Our hypothesis that local populations from different distant horticultural areas of Argentina exhibit certain degree of reproductive incompatibility and morphological variation is not completely supported by the results of this study. In

Table 3 Mean sex ratios (MSR) measured as proportion of females from the progeny, proportion of couples that produced female offspring (CFO_f) and percentages of relative compatibility (RC) for interbreeding experiments among three *P. dignus* geographic biotypes of Argentina

Crosses	MSR ($\pm$ SE)	CFO_f ($\pm$ SE)	RC (%)
Inter-population			
♀ BA × ♂ RN	0.41 ($\pm$ 0.03)	0.79 ($\pm$ 0.11)	82
♀ RN × ♂ BA	0.51 ($\pm$ 0.03)	0.86 ($\pm$ 0.09)	100
♀ BA × ♂ SA	0.34 ($\pm$ 0.04)	0.50 ($\pm$ 0.13)	68
♀ SA × ♂ BA	0.29 ($\pm$ 0.03)	0.63 ($\pm$ 0.12)	62
Intra-population			
♀ BA × ♂ BA	0.50 ($\pm$ 0.02)	0.80 ($\pm$ 0.10)	
♀ RN × ♂ RN	0.51 ($\pm$ 0.02)	0.79 ($\pm$ 0.11)	
♀ SA × ♂ SA	0.47 ($\pm$ 0.02)	0.88 ($\pm$ 0.08)	

Geographic biotypes BA (Buenos Aires); RN (Río Negro); SA (Salta)

addition, we generated relevant information to be integrated to the original description of the species *P. dignus* made by Muesebeck (1938) and revised in part by Cardona and Oatman (1971) and Fernández-Triana et al. (2014).

We found a slight morphological variation among the three *P. dignus* biotypes from geographical areas in Argentina. Individuals from Río Negro (RN) biotype showed greater morphological differences when compared to Buenos Aires (BA) and Salta (SA) populations. Variation in allopatric populations was also recorded for other biocontrol agents, as for *Ephedrus persicae* Froggatt, a parasitoid of aphids (Zikic et al. 2009), and for *Opius bellus* (Gahan), a parasitoid of fruit flies (Marinho et al. 2014), among others. These evidences show that a rigorous morphological characterization of a species intended to be used as a biological control agent is required for its accurate taxonomic identification, as well as to recognize the range of phenotypic variation of the species, as reported by Gebiola et al. (2015) in an integrative study for species of *Necremnus* attacking *T. absoluta* in Europe. It is worth mentioning that the slight morphological differences found in *P. dignus* populations could be due, in part, to the potential effect of host quality or size, since parasitoids were reared in its original *T. absoluta* population. However this source of variation was not considered in this study.

Local populations from RN and BA provinces in spite of exhibiting morphological differences were highly compatible reproductively. Conversely, a lower reproductive compatibility existed between SA and BA biotypes, which could suggest that breeding separation is incipiently occurring between these populations. Compatibility of intraspecific crosses was shown also for other parasitoid species targeted as biocontrol agents, at very different geographical scales, as *Diadromus collaris* (Gravenhorst), a pupal parasitoid of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Liu et al. 2002) and the studies conducted on *Trichogramma galloi* Zucchi, a parasitoid of the sugarcane borer *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) (Bertin et al. 2018). Complete or partial reproductive isolation between biotypes has been shown for other several parasitoids, such as *Aphidius ervi* Haliday, a parasitoid of aphids (Takada and Tada, 2000); *Bracon hebetor* Say, a parasitoid of *Heliothis* spp. (Lepidoptera: Noctuidae) (Heimpel et al. 1997); *Cotesia flavipes* Cameron complex, parasitoids of larvae of noctuids and crambids (Joyce et al. 2010); *Cotesia plutellae* (Kurdjumov), a parasitoid of *P. xylostella* (Rincón et al. 2006), and *Spalangia cameroni* Perkins, a pupal parasitoid of muscoid flies (Birkemoe et al. 2012).

In classical and augmentative biological control programs the characterization of local populations of a wide geographically distributed natural enemy is a key issue to recognize the presence of cryptic species, to prevent failures in mass rearing of biocontrol agents through in- or outbreeding depression and to avoid undesirable outcomes in post-releasing control when reared and wild populations cross in the field (Luna and Hawkins 2004; Wajnberg et al. 2012). Two aspects of the integrative taxonomy addressed in this study (morphological and reproductive traits) evaluated in distant geographic populations of *P. dignus* showed first evidences to resolve the species status of this parasitoid in Argentina. Nevertheless, other study approaches, such as behavioral and molecular biology, ecology, among others, would be desirable to develop for a more rigorous delimitation of the species and to discern if a speciation process in *P. dignus* is ongoing (Schlick-Steiner et al. 2010).

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