



## RESEARCH ARTICLE - WASPS

## Nesting Biology, Sexual Dimorphism, and Populational Morphometric Variation in *Podium denticulatum* F. Smith, 1856 (Hymenoptera: Sphecidae)

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### Abstract

*Podium denticulatum* occurs from Mexico to southern Brazil, including northeastern Argentina. Females use pre-existing cavities to build nests, consisting of cells separated by walls of mud and resin and massively provisioned with paralyzed cockroaches. Trap-nests were disposed in three localities in the state of São Paulo, Brazil (Araras, São Carlos, Rifaina), resulting in the sampling of 201 nests from December/2003 to June/2007. The founding nests were brought to the laboratory, opened and the pupae transferred to identified vials until the emergence of the adults, when they were then weighed, sexed and stored at -20 °C. The nesting activity was highly seasonal, since almost all nests were collected in the warm and rainy period of the year. The number of constructed cells ranged from one to nine per nest. The emergence rate of adults in the 716 brood cells was 74%, with mortality homogeneously distributed by egg, larva and pupa stages. This mortality was partly due to parasitism observed in 39% of nests, predominantly by *Melittobia* sp. A 1:1 sex ratio was observed among the newly emerged adults of each area analyzed. Strong sexual dimorphism was characterized by linear measurements of wings and body mass, with females and males showing a mass between 27-116 mg and 14-70 mg, respectively. The geometric morphometry confirmed this dimorphism and revealed significant variation of wing size and shape among individuals of the analyzed populations, a result that deserves subsequent studies to point out the factors that account for this differentiation.

### Introduction

Studies on nesting biology in wasp and bee species using preexisting cavities have enhanced our understanding of behavior, life cycle, trophic niche and sex ratio issues, among others, contributing to the enlightenment of the life histories, ecology and evolution of these insects (Michener, 2007; Costa & Gonçalves, 2019). Additionally, knowing the nesting biology of solitary species reveals the ecological relevance of these species and contributes to their conservation.

The evolution of behaviors associated with different levels of parental investment (differences in nesting and provision) and sociality have been successfully investigated using species of the Sphecidae family as model organisms (Matthews, 1991; Melo, 2000).

The species of the *Podium* Fabricius genus of the Sphecidae Family are grouped into the *rufipes* (four species), *agile* (four species) and *fumigatum* (twelve species) groups. Foraging is preferably done with cockroach nymphs and adults and is one of the few known sphecids that uses resin in nest building (Bohart & Menke, 1976).

*Podium denticulatum* F. Smith, 1856, a species of the *P. (fumigatum)* group, occurs in a large area of the Neotropical region, from Mexico to southern Brazil and northeastern Argentina, whose females show the nesting behavior in pre-existing cavities (Bohart & Menke, 1976; Krombein, 1958; Genaro, 1994; Morato & Campos, 2000; Morato, 2001; Gazola, 2003). Information on the nesting biology of *P. denticulatum* has already been reported (Camillo et al., 1996; Assis & Camillo, 1997; Camillo, 2001; Gazola, 2003), as well



as aspects of its behavior (Ribeiro & Garófalo, 2010) and description of its immature stages (Buys et al., 2004).

Data available in the literature also refer to the materials used for nest building, number and size of cells per nest, number of preys in each cell and their identification, cocoon size, adult size, natural enemies and nesting times in nests of *Podium rufipes* Fabricius, *P. luctuosum* F. Smith, *P. fulvipes* Cresson and *P. angustifrons* Kohl (Rau 1937, Bohart & Menke, 1976; Camillo et al., 1996; Assis & Camillo, 1997; Camilo, 2001; Buschini & Buss, 2014), as well as immature stages of *P. fumigatum* Perty and *P. aureosericeum* Kohl (Buys et al., 2004).

In this study, aspects of the nesting biology of *P. denticulatum* were investigated, seeking to emphasize traits not yet highly explored in previous studies, such as population sex ratio, size and shape sexual dimorphism and morphometric differences between individuals from different populations. For such, analyzes with a large number of nests and individuals from three populations coming from sampling in successive years were performed using a methodological approach that has not yet been explored in this species, the geometric morphometry, to estimate its intra and interpopulation variation.

## Material and Methods

### Trap-Nests

The trap-nests consisted of bamboo canes of varying length and diameter, cut in half horizontally and sealed with masking tape; the closing of one end of the trap-nest was through the very knot of the bamboo.

### Study sites and sampling method

The study was conducted in three areas of São Paulo state with favorable nesting conditions, such as areas of typical savanna (Cerrado) or Atlantic Forest vegetation and water bodies. The geographic coordinates, altitude, pluviometric index, Köpen climate, annual medium temperature and sampling period in the three sampling areas are: i) UFSCar Campus in Araras (22°21'S, 47°23'W; 614 m; 1312 mm; Cwa; 20,3°C), from December/2003 to June/2007; ii) Rio Branco Farm at Rifaina (20°04'S, 47°25'W; 575 m; 1531,6 mm; Aw; 23 °C), from July/2004 to June/2007; iii) UFSCar Campus at São Carlos (22°01'S, 47°53'W; 856 m; 1440 mm; Cfa; 19,7°C), from November/2004 to November/2006. The São Carlos and Araras locations are 64.6 km apart and their distance from Rifaina is 220.1 and 253.1 km, respectively.

In each sampling area, packages with 8-12 bamboo canes of similar length and diameter were disposed at selected sites 100-300 meters apart each other. In Araras, Rifaina and São Carlos, 90, 60 and 24 packages were made available monthly at nine, six and three sites inside these sampling areas, respectively.

Samples were taken once a month at each site. The found nests were identified and replaced by new ones and taken to the laboratory (Hymenopteran Evolutionary Genetics Laboratory), then opened and some of their characteristics noted, such as: absence or presence of closing wall, vestibular

cell, back wall, bottom cell, presence of parasitoids and the position of each cocoon inside the nest.

After the annotations were made, the cocoons (spindle-shaped, smooth, brittle, bright brown with one end rounded and the other tapering to a dark mass, probably feces) were placed individually in vials closed with cotton and remained there for room temperature until the brood emerged, when the date, sex and body mass of each emerged individual were recorded. Then the adults were stored at -20°C. If the individual was still in the larva phase when the nest was opened, the nest was closed again and only reopened when the nest was in the pupal phase.

Some *P. denticulatum* individuals were mounted on entomological pins and sent for identification by specialists (Dr. Sérgio Túlio Pires Amarante/MZUSP and José Carlos Serrano/FFCLRP-USP) in order to be used for the identification of new individuals. Specimens were deposited at the Museu de Zoologia da Universidade de São Paulo and at the LGEH.

### Wing linear measurements

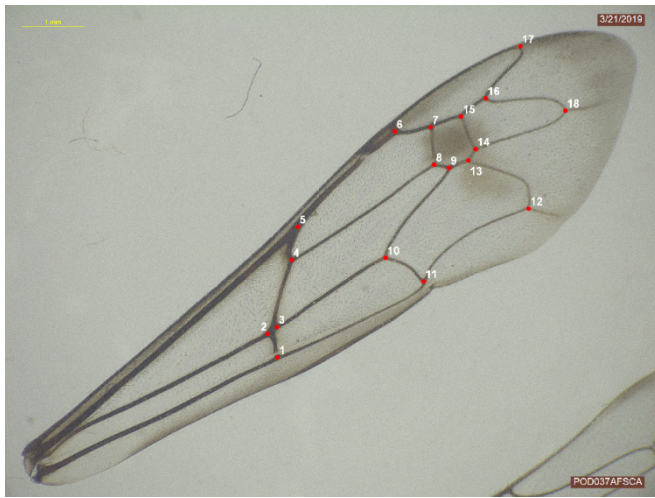
Wings of 190 adults of *P. denticulatum* (97 females and 93 males) were mounted between microscopic blades. With the aid of a camera attached to a Leica stereomicroscope, images were captured with the Win TV 2000 system. Then, measurements were taken with the LEICA LAS version 4.1 software for each of the following traits: right anterior (RAWL) and posterior (RPWL) wings length; right anterior (RAWW) and posterior (RPWW) wing width, number of hammuli of the right (Hr) and left wings (Hl). Additionally, their body mass was also taken. Each character was measured five times and the average of them was used as a measurement value to reduce errors.

### Wing geometric morphometry

Images of the right anterior wings of 181 adults (90 females and 90 males, see Table 1) were taken for geometric morphometric analysis by a Leica S6D digital camera coupled to the Leica DFC450 stereomicroscope. For the analysis of partial deformations, the images were converted to format.tps with the aid of the software tpsUtil64 version 1.79 (Rohlf, 2009). Eighteen homologous landmarks (Fig 1) were manually plotted twice independently at the junctions of the wing venation using the tpsDig2 software, version 2.16 (Rohlf, 2010) (Figure 1). This number of landmarks is similar to that of others studies in insects (Benítez et al., 2013; Perrard & Loope, 2015). In the MorphoJ software version 1.6 (Klingenberg, 2011), the images underwent Procrustes adjustment, allowing the performance of Discriminant Function Analysis (DFA), Principal Component Analysis (PCA) and Canonical Variables Analysis (CVA) from the dataset generated. An ANOVA was performed, based on centroid shape and size data, to assess the variation in size and shape in samples from different sexes and populations. All software used here is freely accessible and is available at <http://life.bio.sunysb.edu/morph> and <http://www.flywings.org.uk/MorphoJ>.

**Table 1.** Number of males and females of *Podium denticulatum* from three study sites analyzed for wing size and shape by geometric morphometry.

Study site - City (State)	Study site code	Females	Males
Araras - SP	ARR	73	65
Rifaina - SP	RFA	10	10
São Carlos - SP	SCA	7	19
<b>Total</b>		<b>90</b>	<b>91</b>



**Fig 1.** Right forewing of a female of *Podium denticulatum* with 18 landmarks plotted at the vein intersections.

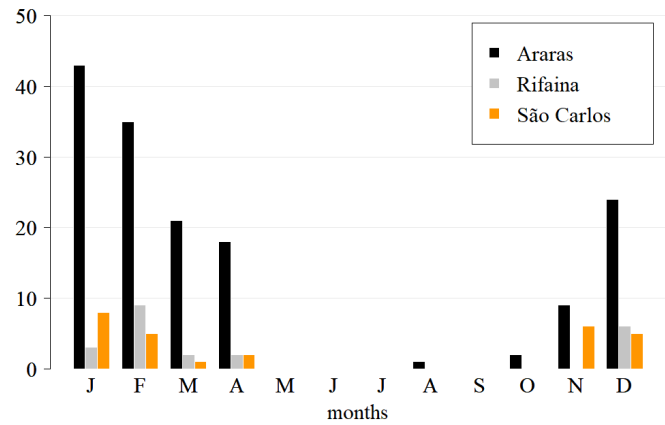
### Statistical analysis

To perform the statistical analysis, the BioEstat 5.0 software (Ayres et al., 2007) was used. Verification of a 1:1 sex ratio was performed by Chi-square test. To evaluate the differences in mass between males and females and the asymmetry between individuals who entered or not in diapause, the Kolmogorov-Smirnov test and Student's t-test ( $\alpha = 0.05$ ) were used. Wing measurements and masses were associated by Pearson linear correlation. Statistical analyzes were made in agreement with Zar (1999).

## Results

### Number of nests and seasonal abundance

In the three areas, 201 nests of *P. denticulatum* were obtained from December 2003 to June 2007. In Araras, 153 nests were sampled at seven of the nine sites, and 79% of the



**Fig 2.** Number of nests of *Podium denticulatum* sampled monthly in three locations at São Paulo state during 2004 – 2007.

nests were founded in two closer sites. In São Carlos, 26 nests were obtained in two of the three sites. In Rifaina, 22 nests were sampled at four of the six available sites, and 75% of the nests were founded in two sites only.

In this study, we observed an effect of seasonality in the nest productivity (Fig 2). Nesting activity was concentrated in the hot and rainy period (September - April), with very low activity in the cold and dry period (May - August), since only one nest with four cells was collected in August 2004 in Araras.

### Nest Architecture

*P. denticulatum* nests presented linearly constructed cells separated by mud walls, with the inner surface rough and convex and the outer surface smooth and concave. The number of cells observed was from one to nine cells per nest, with an average of  $3.7 \pm 1.8$  cells per nest (Araras:  $3.7 \pm 1.8$ ; Rifaina:  $3.6 \pm 1.5$ ; São Carlos:  $3.7 \pm 2.2$  cells per nest). The presence of closure plug and deep plug that were observed in some nests is illustrated in Fig 3. Table 2 shows the number and percentages of these and others architectural elements of nests from each sampling site. The length of the trap-nests used by *P. denticulatum* ranged from 7.5 cm to 42.3 cm, with an average of  $20.4 \pm 5.6$  cm ( $19.6 \pm 4.9$  cm,  $26.7 \pm 5.7$  cm, and  $19.3 \pm 5.5$  cm in the nests of Araras, Rifaina and São Carlos, respectively) (see Fig 4A). Otherwise, the diameter of the trap-nests used ranged from 4.1 to 17.4 mm, with an average of  $10.0 \pm 2.7$  mm ( $10.1 \pm 2.7$ mm,  $9.3 \pm 2.2$ mm and  $9.8 \pm 2.4$ mm in the nests founded in Araras, Rifaina and São Carlos, respectively) (Fig 4B).

**Table 2.** Number of *P. denticulatum* nests (Nn) from Araras (ARR), Rifaina (RFA) and São Carlos (SCA), and number (%) of nests with deep plug (DP), deep cell (DC), closure plug (CP) and vestibular cell (VC).

Study site	Nn	DP	DC	CP	VC
ARR	153 (76.1%)	43 (28.1%)	19 (12.4%)	57 (37.2%)	68 (44.4%)
RFA	22 (10.9%)	4 (18.2%)	3 (13.6%)	11 (50%)	7 (31.8%)
SCA	26 (12.9%)	-	1 (3.8%)	10 (38.5%)	6 (23.1%)
<b>Total</b>	<b>201</b>	<b>47 (23.4%)</b>	<b>23 (11.4%)</b>	<b>78 (38.8%)</b>	<b>81 (40.3%)</b>



Fig 3. *P. denticulatum* nest. Closure plug (1) and deep plug (2) are shown.

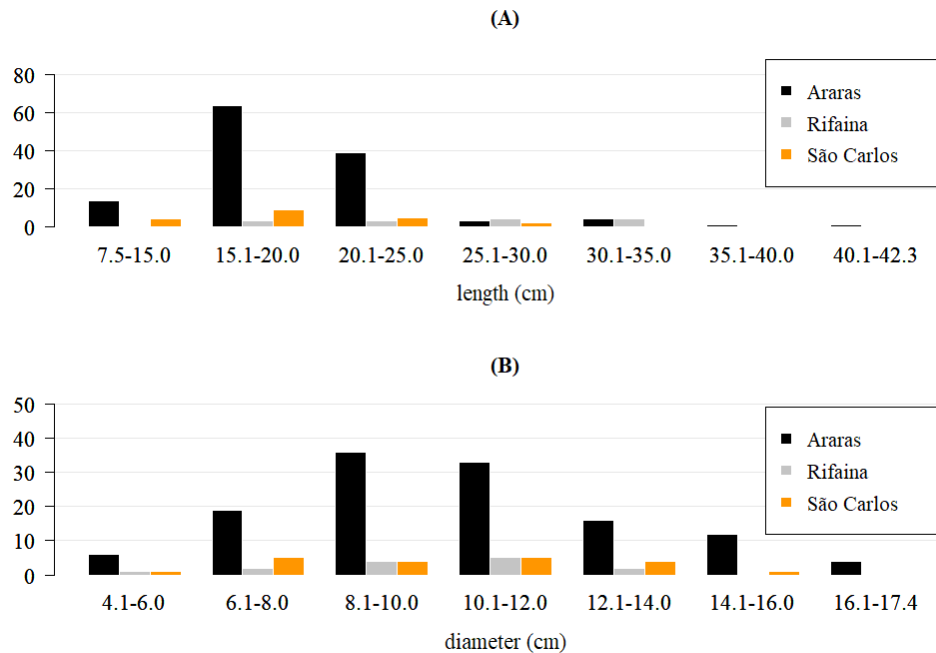


Fig 4. Length (A) and diameter (B) of the trap-nests used by the females of *P. denticulatum* in the three sampled areas.

In nine of the *P. denticulatum* trap-nest samples, a second species also built in the same cavity. (*Trypoxylon aurifrons* Shuckard, *Trypoxylon lactitarse* Saussure, *Trypoxylon rogenhoferi* Kohl, or *Centris* sp.). In five nests, the first species to nest was *P. denticulatum*, followed by nesting by *Trypoxylon* sp., whereas in the other four nests, the first species to nest was *Trypoxylon* sp. (n = 3) or *Centris* sp. (n = 1), followed by nesting by *P. denticulatum* (Fig 5).

#### Mortality

In the 201 nests sampled, 716 brood cells were observed, from which 531 adults (74%) emerged. The mortality of the offspring in the other cells was evenly distributed among the egg, larval or pupal stages (Table 3).

Parasitoids were found in 39.8% of nests (n = 80), and in 97.5% (n = 78) of these nests was found the parasitoid *Melittobia* sp. (Eulophidae). In 2.5% (n = 2) of the nests, pupae of Tachinidae (Diptera) were found in the last nest cell.

#### Interval between collection date and emergency date

The time elapsed between the date of nest collection and the emergence of individuals ranged from 1-41 days (mean =  $17 \pm 8.2$ ), with slight differences among nests of different locations (Araras:  $18.0 \pm 8.1$  days; São Carlos:  $15.1 \pm 7.7$  days; Rifaina:  $13.0 \pm 7.5$  days).

Most of the individuals who emerged between 46 and 366 days after the date of collection (mean =  $129.0 \pm 73.5$ ) entered diapause. In Araras, 21.8% of those collected in



Fig 5. Nest foundation by *Centris* sp., followed by nesting of *P. denticulatum* in a trap-nest.

January and 93.7% of them collected in April emerged from 60 to 366 days after the date of collection (mean = 151.0 ± 69.8). In São Carlos, 68.4% of individuals collected in January and all collected in April entered diapause, emerging 46 to 129 days after collection (mean = 58.0 ± 22.3 days).

In the nests where diapause was observed, all individuals of the nests entered diapause, except in a nest collected in Araras in April 2005. In this nest, four cells had been built - in the first two, the individuals emerged 7 days after collection; in the next two cells, brood entered diapause and emerged 166 days after collection. The emergence of individuals which

entered diapause occurred throughout the year, even in the cold and dry season.

*Sex ratio and nest position*

In the three studied localities, a 1:1 sex ratio was verified (Araras – 155 females and 153 males;  $\chi^2 = 0.013, P > 0.05$ ; São Carlos – 23 females and 22 males;  $\chi^2 = 0.022, P > 0.05$ ; Rifaina – 14 females and 26 males;  $\chi^2 = 3.6, P > 0.05$ ). The position of the sexes in *P. denticulatum* nests was not random, with the first cells (cells further away the nest entrance) presenting more females than males (Fig 6); so, males usually emerged before females.

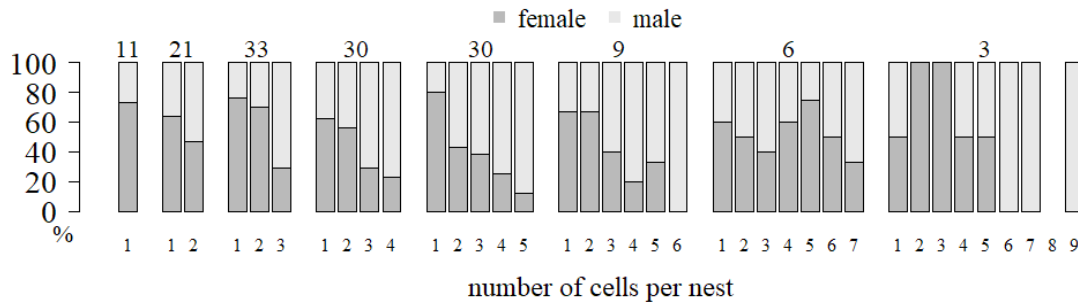
*Body mass*

A large variation in body mass of females ( $X \pm SD = 80.1 \pm 20.1$ ; amplitude: 27-116 mg; n = 157) and males ( $X \pm SD = 35.46 \pm 11.6$ ; amplitude: 14-69 mg; n = 137) was observed (Fig 7). When compared, these values were highly significant (Student's t = 19.63, P = 0.0001).

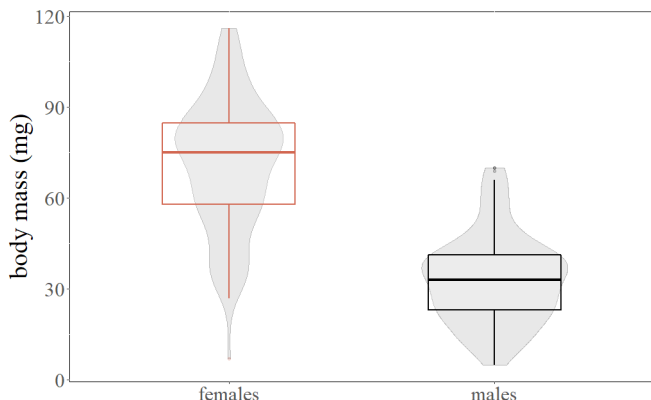
Females that entered (n = 30) and not entered in diapause (n = 129) presented body mass between 32 - 116mg ( $X \pm SD = 67.6 \pm 23.2$ ) and 32 - 115mg ( $X \pm SD = 74, 4 \pm 19.9$ ), respectively. Males that entered (n = 28) and not entered in diapause (n = 112) had body mass between 17 - 69mg ( $X \pm SD = 36.1 \pm 12.7$ ) and 9 - 70mg ( $X \pm SD = 32, 9 \pm 14.5$ ), respectively. The results observed in females and males of the two groups are not significantly different (t = 1.617, P = 0.1084; t = 1.0683, P = 0.287, respectively).

**Table 3.** Number of nests (Nn) and cells (Cn), number and percentage of emerged adults before collection (Bc) or at the laboratory (Lab). Number of died individuals in egg, larva or pupa stage of *P. denticulatum* nests sampled in three study sites (ARR: Araras-SP, RFA: Rifaina-SP, SCA: São Carlos-SP).

Sites	Nn	Cn	Emerged adults		Egg	Larva	Pupa
			Bc	Lab			
ARR	153	560	45 (8.0%)	366 (65.4%)	45 (8.0%)	51 (9.1%)	53 (9.5%)
RFA	22	75	11 (14.7%)	51 (68.0%)	5 (6.7%)	0	8 (10.7%)
SCA	26	81	5 (6.2%)	53 (65.4%)	8 (9.9%)	1 (1.2%)	14 (17.3%)
<b>Total</b>	<b>201</b>	<b>716</b>	<b>61 (8.5%)</b>	<b>470 (65.6%)</b>	<b>58 (8.1%)</b>	<b>52 (7.3%)</b>	<b>75 (10.5%)</b>



**Fig 6.** Emergence (%) of females and males in relation to the position of the cell. In some of the analyzed nests, one or more individuals died before emergency.



**Fig 7.** Violin and box-plots were used to visualize the body mass (mg) distribution of newly emerged females (orange) and males (black) of *P. denticulatum*.

*Wing linear measurements*

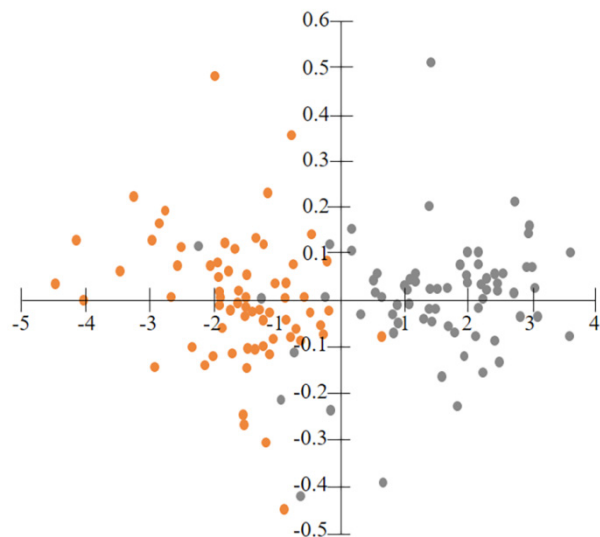
Wings of 190 individuals (97 females, 93 males) were measured for the following traits: right anterior (RAWL) and posterior (RPWL) wings length; right anterior (RAWW) and posterior (RPWW) wing width, number of hammuli of the right (Hr) and left wings (Hl), and body mass. The range of variation, mean and standard deviation, and coefficient of variation for each of these characters in males and females are shown in Table 4. It is interesting to note that the coefficient of variation for RAWL, RPWL, and RAWW were higher in males than in females.

**Table 4.** Wing linear measurements (LWM) in adult females and males of *P. denticulatum*. Range, average (X), standard deviation (SD) and coefficient of variation (CV) for seven wing features: right anterior wing length (RAWL), right posterior wing length (RPWL), right anterior wing width (RAWW), right posterior wing width (RPWW), number of hammuli from right (Hr) and left wings (HI).

LWM (mm)	Females (n = 97)			Males (n = 93)		
	range	X ± SD	CV (%)	range	X ± SD	CV(%)
RAWL	7.98-13.20	11.6 ±0.9	7.7	7.24-12.20	9.89 ±1.00	10.0
RPWL	5.9-9.9	8.8 ±0.7	7.9	5.3-9.2	7.5 ±0.7	10.0
RAWW	2.15-3.40	3.01 ±0.24	7.9	1.7-3.1	2.5 ±0.3	11.0
RPWW	1.3-2.8	2.25 ±0.27	16	1.2-2.4	1.9 ±0.3	13
Hr	21-35	28.2 ±2.7	9	17-29	23.1 ±2.5	10
HI	21-35	28.3±3.1	14	16-34	23.5±2.9	12

A discriminant analysis was performed on basis of 140 individuals (67 males, 73 females) from Araras, for which we had the values for all the variables measured. The analysis showed a clear differentiation between males and females of *P. denticulatum* (Fig 8) based on the traits analyzed, largely due to the association of body mass values to the wing linear measurements.

The asymmetry estimated by the difference between the width of the right and left hind wings was not statistically different in diapausing and not diapausing individuals (Student's  $t = 0.397, P = 0.6922$ ) (Table 5).

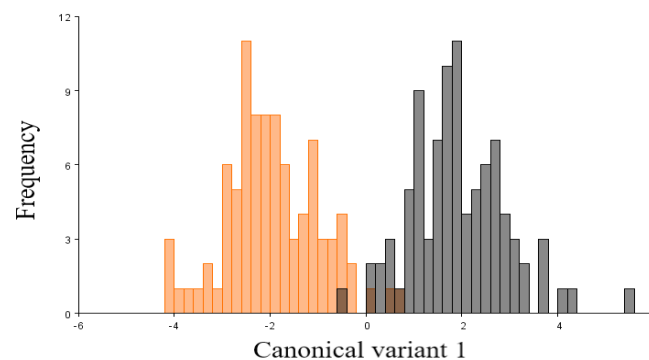


**Fig 8.** Discriminant Analysis based on four wing linear measurements (see Table 4) and body mass of males (grey) and females (orange) of *Podium denticulatum*.

Body mass (mg) was highly correlated with RAWL ( $r=0.86$ ), RPWL ( $r=0.88$ ), RAWW ( $0.87$ ), and RPWW ( $r=0.73$ ), indicating that mass is a good size estimator; it also contributed significantly to highlight the sexual dimorphism showed by the wing linear measurements (Fig 8). A significant correlation was found between number of hammuli and RPWL in males ( $r = 0, 32; P = 0.013$ ), but these two variables were much more correlated in females ( $r = 0.85; P = 0.0001$ ).

*Geometric morphometry (GM) of wing size and shape*

The males and females (n = 181) described in Table 1 were also analyzed by geometric morphometry to detect possible differences in the size and shape of the right anterior wing of males and females. The PCA generated 32 components responsible for the total variation of the data, and the first 10 components explained 86.16% of the variation (PC1 - 27.02%;



**Fig 9.** Canonical Variate Analysis (CVA) showing morphometric dimorphism between males (grey) and females (orange) of *P. denticulatum*.

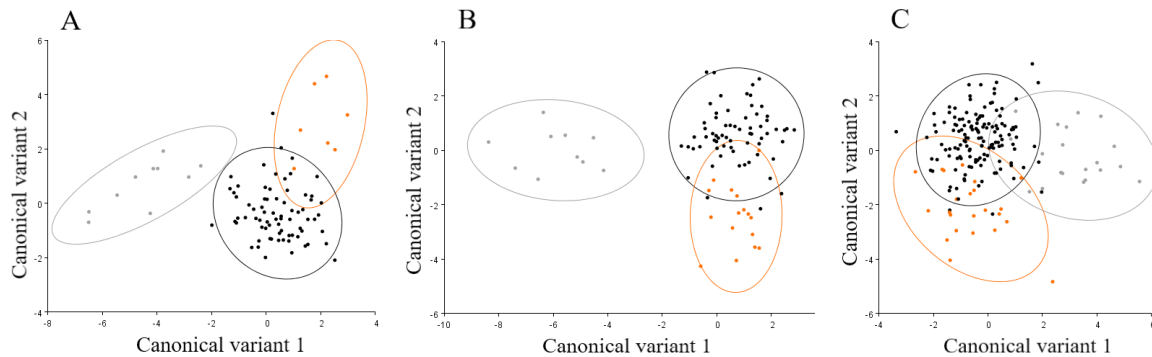
**Table 5.** Wing comparison (difference in mm between the right and left wing width) from females and males adults of *P. denticulatum* that entered (E) or not entered (N) diapause during the development.

Development	Females		Males	
	range	X ± dp	range	X ± dp
E	0.000-0.094	0.0214±0.0198	0.000-0.056	0.0228±0.0175
D	0.002-0.074	0.0215±0.0202	0.000-0.075	0.0197±0.0204

PC2 - 18.80%). The DFA showed that 95.18% of individuals were correctly assigned to their sites of origin, while the percentage in the cross-validation test was 83.51%. The accuracy in the correct attribution of the sexes was 97.78%, with an average of 93.37% in the cross-validation.

The GM analysis showed a marked dimorphism between females and males (Fig 9), and wing shape differences between males and females of the three areas were detected by CVA

(Fig 10). Differential interpopulational differentiation was detected through the Mahalanobis distances of wing size among females, males and females+males of *P. denticulatum* from the three study sites (Araras, Rifaina, and São Carlos) (Table 6). The Procrustes ANOVA values for centroid size (CS) and shape (SH) in *P. denticulatum* females of the three study sites are presented in Table 7, corroborating the morphometric variation among the three populations of this species.



**Fig 10.** Canonical Variate Analysis (CVA) for morphometric variation of the wing shape based on the joint analysis of males and females (A), only females (B) and only males (C). The samples of São Carlos, Rifaina, and Araras are indicated by the colors orange, grey and black, respectively.

**Table 6.** Mahalanobis distances of wing size between females and males of *P. denticulatum* from the three study sites (ARR: Araras-SP, RFA: Rifaina-SP, SCA: São Carlos-SP).

		ARR	RFA
<b>Females</b>	RFA	4.9545	-
	SCA	4.1055	5.6414
<b>Males</b>	RFA	6.7093	-
	SCA	2.9807	8.6982
<b>Females and males</b>	RFA	3.5097	-
	SCA	2.5613	4.2208

## Discussion

Nesting activity by *P. denticulatum* in trap-nests has been previously reported by Camillo et al. (1996), Assis and Camillo (1997) as well as Ribeiro and Garófalo (2010). Camillo (2001) observed *P. denticulatum* reusing inactive nests of *Brachymenes dyscherus* (Saussure). It is noteworthy that the trap-nests we offered in the selected areas were also used by species of wasps of the family Crabronidae (*Trypoxylon aurifrons*, *T. nitidum* F. Smith, *T. lactitarse* and *T. rogenhoferi*) and solitary bees of the genus *Centris* sp.

A seasonal nesting activity in *P. denticulatum* was found in those previous studies (Camillo et al., 1996; Ribeiro & Garófalo, 2010). This seasonal pattern has been reported in other wasps, such as *Trypoxylon aurifrons* (Santoni & Del Lama, 2007), but not in bees as *Tetrapedia curvitaris* Friese (Campos et al., 2018). Low nesting activity in the cold and dry period has also been previously reported by Camillo et al. (1996) and Camillo (2001). The greater nesting activity in Araras suggests the existence of a well-structured population in this area, although the shorter sampling time in São Carlos and Rifaina may have contributed to the lower foundation rate in these locations.

According to Ribeiro and Garófalo (2010), the nesting activities of a female of *P. denticulatum* are initiated by the search for a suitable nest building site, a determining factor for the success of nest foundation in *P. rufipes* (Krombein, 1970). Variation in nesting rates at different sites in the collection areas was found, a fact usually seen in many similar reports. Santoni and Del Lama (2007) observed variation in *Trypoxylon aurifrons* nesting rates in the same collection areas of our study, what was explained by the authors by factors as philopatric behavior, preference and/or competition for nesting sites, different periods of sampling or simply the availability of trap-nests.

**Tabela 7.** Procrustes ANOVA for wing centroid size (CS) and shape (SH) in *P. denticulatum* females of the three study sites. Sum of squares (SS) and mean squares (MS) are in dimensionless units of Procrustes distances.

	Effect	SS	MS	df	F	p
<b>CS</b>	Individual	6018364.469201	3009182.234601	2	7.10	0.0014
	Residual	36898857.494009	424124.798782	87		
<b>SH</b>	Individual	0.00248785	0.0000388726	64	3.18	<0001
	Residual	0.03399638	0.0000122113	2784		

The observed architecture of *P. denticulatum* nests, with linearly constructed cells separated by mud walls, was very similar to that found by Camillo et al. (1996), Assis and Camillo (1997) as well as Ribeiro and Garófalo (2010). Otherwise, Krombein (1967, 1970) and Gazola (2003) described that nests of *P. rufipes* are made up of a single cell that occupies almost the entire trap-nest cavity.

The average number of cells per nest was higher in our study than in previous reports (Camillo et al., 1996; Assis & Camillo, 1997; Ribeiro & Garófalo, 2010). Nests with more than 6 cells were not observed in those previous studies, which may be explained by the trap-nests with longer length we offered.

According to Camillo et al. (1996), the closure and compartmentalization processes of *P. denticulatum* nests are very similar to *P. rufipes* (Krombein, 1970) and *Penepodium goryanum* (Lepelletier) (Garcia & Adis, 1993) nests. These similarities include the use of mud and resin in partitions, the presence or absence of double walls and the insertion of pieces of leaves, lichens and wood fragments into the nest camouflage. These walls with physical insulation and sometimes camouflage functions are externally coated with resin.

The habit of building a deep plug, this “initial mud deposit” or “preliminary wall” at the beginning of nesting was also observed in *Trypoxylon* species (Santoni, 2008). Based on studies in *T. rogenhoferi*, Garcia and Adis (1995) suggest that this behavior is linked and restricted to species whose larvae use mud to make puparia. As *P. denticulatum* does not have this behavior, the frequency of construction of these walls is low.

Relative to the diameters and lengths of trap-nests used by the females, it is possible to verify their preference to trap-nests with medium diameters and lengths, a result already reported by Camillo et al. (1996), Assis and Camillo (1997) as well as Ribeiro and Garófalo (2010). However, nesting by *P. denticulatum* females in trap-nests over 25 cm in length is being described for the first time. The diameter of nesting cavities is selected by wasps as a function of species size (Krombein, 1967), prey size (Fricke, 1991; Garcia & Adis, 1995) and the effectiveness of internal wall thickness against parasitoids (Coville & Coville, 1980).

Trap-nests containing brood cells constructed by *P. denticulatum* and a female of a second species have already been described. Camillo et al. (1996) reported this type of nesting involving females of *Centris* sp., *Megachile* sp. and *Trypoxylon* sp.. However, this is the first description of nests founded by *P. denticulatum* that was also used by a wasp or bee female.

The mortality rate estimated here is very similar to that observed in nests of the species analyzed by Assis and Camillo (1996). Parasitoids such as *Melittobia* sp., *Antrax* sp., Perilampidae, Phoridae and Tachinidae were observed by Camillo et al. (1996). Assis and Camillo (1997) described the parasitoids *Melittobia* sp. and *Antrax* sp. in *P. denticulatum*

nests, while Ribeiro and Garófalo (2010) observed the parasitoids *Melittobia* sp., Tachinidae and Chrysididae in nests of this species.

Pre-pupae entered diapause were also observed by Camillo et al. (1996) also Ribeiro and Garófalo (2010). Diapause occurrence in the pre-pupal stage is a strategy used by many wasp and bee species in response to adverse weather conditions, which, in turn, also affect the availability of food resources used by these organisms, especially by decreasing their supply, a fact that can be explained by the transition from the hot and rainy period (September to April) to a cold and dry period (May to August).

The time between nest collection and adult emergence was similar to that observed by Camillo et al. (1996), except for individuals who entered diapause, whose emergence occurred over a longer period of time. It is possible that temperature variation inside the laboratory may have interfered with the brood developmental time and occurrence of diapause.

Camillo et al. (1996) proposed the occurrence of two generations per year in *P. denticulatum*, while Ribeiro and Garófalo (2010) suggested the occurrence of five/six generations per year in the species. Data from our work, such as i) nests where individuals emerged prior to collection, ii) a nest collected in the cold and dry season, and iii) individuals that emerged during the cold and dry season - indicate that more than six generations may occur, with one generation overlapping the other and generations where some individuals go through prolonged diapause.

Observing some nesting in the cold and dry period, when some nests went through the diapause phase, Camillo et al. (1996) concluded that most of the population of *P. denticulatum* survives the adverse period of the year as adults. However, the low amount of nesting observed during this period indicates that most of the population survives the adverse period of the year in pre-pupal diapause, a condition that *Penepodium goryanum* pupae use to cope with periods of adverse conditions (Garcia & Adis, 1993).

A 1:1 sex ratio was verified in our three samples studied, confirming previous data in *P. denticulatum* (Ribeiro & Garófalo, 2010) and *Podium rufipes* (Krombein, 1967). Otherwise, Camillo et al. (1996) observed in *P. denticulatum* the ratio of 1.7 males: 1 female in one generation, but they warn that there are numerous factors related to sex ratio that have not been analyzed. In most panmitic populations, equal investment in both sexes is expected if fitness return is the same with a production of a daughter or a son (Fisher, 1930). However, in many hymenopterans there are deviations of this proportion, often attributed to ecological, physiological and behavioral factors (Trivers & Hare, 1976).

Although in most nests male and female emerged, from a few nests only male or female emerged. The emergence of males only can be explained for different reasons, including: i) females are not fertilized; ii) option for the least expensive sex; iii) because they are of advanced age (Tepedino & Torchio, 1982; Frohlich & Tepedino, 1986).



The observed non-random pattern of sex emergence in trap-nests of *P. denticulatum* was already reported by Camillo et al. (1996) also Ribeiro and Garófalo (2010) and in other solitary wasp species. In *T. aurifrons*, for example, from the first cells emerged more males than females (Santoni & Del Lama, 2007), a pattern opposite to that observed here.

The size sexual dimorphism showed here in *P. denticulatum*, with females larger than males, has been previously reported (Camillo et al., 1996; Ribeiro & Garófalo, 2010) based on head width measurements, a trait especially useful for revealing sexual dimorphism in Sphecidae (Bohart & Menke, 1976). The developmental interruption promoted by diapause did not result in significant changes in wing size (width) and body mass of males and females, an unexpected result if we consider diapause as due to some disturbance in the environment experienced by individuals.

Body size is one of the key features of organisms. Ecological traits such as survival, resource acquisition rate, and reproductive capability usually are size-dependent (Takahashi & Blanckenhorn, 2015). Differences in body size between males and females, so called sexual size dimorphism (SSD), are common in many taxa (Fairbairn, 2005; Stillwell et al., 2010). In insects, females are larger than males in >70% of the taxa in all major insect orders, except Odonata (Stillwell et al., 2010). SSD has been attributed to growth rate and developmental time in arthropods (Blanckenhorn et al., 2007); however, our understanding of the causal proximate and ultimate factors of body size variation is still poor due to the low number of studies about the genetic and developmental mechanisms underlying SSD (Takahashi & Blanckenhorn, 2015).

We found higher coefficient of variation for three wing linear measurements (RAWL, RPWL, and RAWW) in males than in females. It is expected that the levels of genetic variance underlying body size traits would be larger in males if these traits are under lower intensity of sexual selection over them. Evidences of a stronger sexual selection for female than for male size have been shown in many species due to a positive correlation between female body size and fecundity (Molunby, 1997; Strohm & Linsenmair, 2000; Peruquetti & Del Lama, 2003).

The marked sexual dimorphism revealed by traditional (Fig 8) and geometric morphometry (Fig 9) demonstrates the high resolution of these methodologies to detect subtle differences in male and female morphologies, although GM can show higher resolution when compared to traditional morphometry (Quezada-Euán et al., 2015). On the other hand, when the population analysis was performed with the males and females (Fig 10A), a better differentiation between populations is reached when males and females are analyzed separately, as shown in Fig 10B and 10C, respectively. As wing morphology analysis of males and females clearly show that there are definite small-scale differences, these subtle shape differences between sexes plays a role when using wing morphology to distinguish between families (colonies),

populations or species. So, a separate analysis of each gender rather just one analysis may be critical at lower taxonomic levels; at the higher levels, differences between sexes may not greatly affect results when males and females are considered together (see Pretorius, 2005).

Based on wing shape and size analysis, the three populations of *P. denticulatum* showed morphometric variability. Mahalanobis distances (Table 6) and CVA analyses (Fig 10A, 10B and 10C) revealed that the Rifaina population is differentiated from the populations of São Carlos and Araras, an expected result when we consider that Rifaina is geographically more distant from Araras and São Carlos, and presents phytophysiognomy and other abiotic factors distinct from those verified in the two other localities. Wing size accessed by the centroid size showed significant higher variation among populations than wing shape (Table 7). This is an expected finding since size variation in insects are related to quantity and quality of the resources provided to the brood in the larval stage (Peruquetti, 2003; Campos et al., 2018). So, the higher wing size variation may reflect the differential offer of food resources at each environment, as the supply dynamics certainly vary temporally in different phytophysiognomies (Campos et al., 2018). Alternatively, this variation may be associated rather to differential responses of these populations to the features of their local environments (Grassi-Sella et al., 2018), or to local variations in landscape elements (Ribeiro et al., 2019), and specially, to higher developmental constraints to shape than to size variation. The morphometric variation found may also be at least partially the result of genetic interpopulational variation. Further studies are needed to investigate the significance of these findings, as well as to estimate the contribution of genetic and/or environmental factors to this variation, a theme little explored in bees and, in particular, wasps.

#### *Concluding remarks*

The data obtained in this work generally confirm previous data on nesting biology in *P. denticulatum*. However, new information was obtained: i) nesting by females in longer trap-nests, which may have resulted in the larger number of cells per nest observed here; ii) longer periods of diapause; iii) the first description of nests founded by a *P. denticulatum* female that was also used by a second species (bee or wasp); iv) the possible occurrence of more than six generations per year.

The sex ratio 1:1 in the three populations analyzed constitutes data that corroborates previous information obtained in populations from other areas. The demonstration of a marked sexual dimorphism by traditional and geometric morphometry (GM) confirms the effectiveness of these methodologies for detecting wing morphology variation. In addition, GM proved to be effective in demonstrating wing size and shape differences between individuals from geographically close populations, especially when such analysis is done separately from males and females from

different populations. This result deserves further studies to point out the factors that account for the observed variation, contributing to evaluate its possible functional significance, the central task of evolutionary biology.

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### Authors' Contribution

MA Del Lama - Conceptualization, methodology, supervision and writing

L Shibata - Conceptualization, methodology, investigation, formal analysis and writing

MM Santoni - Investigation, formal analysis and writing

V Oliveira e Silva - formal analysis (molecular analyses) and writing.

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