Floral Biology and Pollination in Native and Crop Plants
Revista UNISANTA Bioscience Vol. 9 nº 5 (2020) ISSN 2317-1111
Número especial:
“Floral Biology and Pollination of Native and Cultivated Plants (Intensive Course)"

REPRODUCTIVE BIOLOGY OF *Antigonon leptopus* HOOK. & ARN. (POLYGONACEAE) IN PARANÁ, BRAZIL

Nicholas A. Belliveau [1]; Gustavo H.S. Pereira[2]; Douglas Galhardo[2] & Amanda do Carmo[3]*

[1]University of Saskatchewan (USask), Graduate Programme in the Department of Biology [2]Universidade Estadual de Maringá, PPG em Zootecnia, Maringá, PR, Brasil [3]Universidade Estadual de Maringá, PPG em Biologia Comparada, Maringá, PR, Brasil

* Corresponding Author: nick.belliveau@usask.ca

Abstract

The floral morphology and pollination biology of *Antigonon leptopus* are poorly understood. This hermaphroditic plant exhibits protandry, where male fertility precedes female fertility. This study compares the reproductive traits of three populations of *A. leptopus* in Paraná, Brazil to those described in the literature. The plant’s floral visitors were recorded, and their behaviors compared. Variable floral traits (*e.g.* perianth diameter, tepal number) were quantified and general descriptions of morphology and protandry were made. The taxonomic identities of floral visitors and the duration and frequency of their visits were recorded. Variable merosity, a morphological novelty, was observed, with 4 or 5 tepals and 7 or 8 stamens per flower. The means of these variables concerning the perianth’s height and diameter were lower than the values described in the literature, according toStudent’s t-tests. Protandry was observed in three phases (chronologically: male, hermaphroditic, female), contrary to the literature’s male and female phases. PERMANOVAs showed that throughout the day, visitor assemblages differed not between flowers in different phases, but between populations. Tukey’s HSD tests demonstrated that certain bees (*e.g. Apis mellifera*, *Scaptotrigona* sp.) performed frequent, but short visits, while halictid bees made fewer, but longer visits. Overall, this study presents novel observations of floral morphology and protandry in *Antigonon leptopus*, which differ from what was previously described. Our results, along with new floral visitor data, may assist the understanding of how this exotic, pantropically introduced plant continues to persist outside its natural range.

Keywords: Floral biology, Floral visitors, Floral morphometrics, Pollination, Protandry

Resumo

A morfologia floral e a biologia da polinização de *Antigonon leptopus* são pouco compreendidas. Essa planta hermafrodita exibe protandria, quando a fertilidade masculina precede a feminina. Esse estudo compara as características reprodutivas de três populações de *A. leptopus* no Paraná, Brasil, com aquelas descritas na literatura. Os visitantes florais da planta também foram listados e seus comportamentos comparados. Características florais variáveis (por exemplo, diâmetro do perianto, número das tépalas) foram quantificadas. Foram realizadas descrições gerais dobre a morfologia e protandria. As identidades taxonômicas dos visitantes florais, a duração e frequência de suas visitas foram registradas. Observou-se merosidade variável, uma novidade morfológica, com 4 ou 5 tépalas e 7 ou 8 estames por flor. As médias dessas variáveis quanto a altura e diâmetro do perianto foram inferiores aos valores preconizados pela literatura, de acordo com os testes *t* de Student. A protandria foi observada em três fases (cronologicamente: masculina, hermafrodita, feminina), ao contrário das fases masculina e feminina da literatura. PERMANOVAs mostraram que, ao longo do dia, as assembléias de visitantes diferiram não entre flores em diferentes fases, mas entre populações. Os testes de HSD de Tukey demonstraram que certas abelhas (por exemplo, *Apis mellifera*, *Scaptotrigona* sp.) faziam visitas curtas e frequentes, enquanto as abelhas halictídeos faziam menos visitas, porém mais demoradas. Além disso, esse estudo apresenta novas observações da morfologia floral e protandria em *Antigonon leptopus*, que diferem do que foi descrito anteriormente. Nossos resultados, juntamente com novos dados de visitantes florais, podem ajudar a entender como essa planta exótica introduzida pantropicalmente continua a persistir fora de sua área natural.

Palavras-chave: Biologia floral, Morfometria floral, Polinização, Protandria, Visitantes florais

Introduction

The morphology of flowers is a key determinant in an angiosperm's life history and, in particular, its pollination syndrome (BARRETT, 2002; SAUQUET et al., 2017). Floral and pollination biology are particularly important to our understanding of the angiosperm's evolution, and their ecological interactions with visitors, pollinators, nectar robbers, and predators (COTA-SÁNCHEZ et al., 2013; SAUQUET et al., 2017). The mechanisms of reproductive biology furthermore play a crucial role in the range expansion of native and introduced plant species, rendering them uncontrollably invasive under ideal conditions (BALOGH & BARRETT, 2018).

The family Polygonaceae belongs to the order Caryophyllales and includes about 59 genera and 1384 species (PLANT LIST, 2013). Due to the extensive plasticity that allowed its diversification, this family occupies several ecosystems, from humid and floodable places to sandy deserts (SANCHEZ et al., 2009). The reproductive system of these plants allows for a fast occupation, mainly through sexual reproduction with dispersion of achenes in the water, or with tuberous roots in the ground (BURKE & DITOMMASO, 2011).

Antigonon leptopus (Hook. & Arn.) is a climbing vine in the Polygonaceae (HOOKER & ARNOTT, 1841). It occurs naturally in Mexico and Central America, but has been introduced as an ornamental plant in India, Java and Brazil(RAJU et al., 2001; BURKE & DITOMMASO, 2011). Its presence is recorded in the majority of Brazilian states, including Paraná state in the south of the country (JBRJ, 2019; TOLEDO et al, 2003). *A. leptopus* has received some attention in the literature owing to its medicinal properties (MULABAGAL et al., 2011) and industrial value (GANAIE et al., 2018). However, its reproductive biology has remained poorly studied since its discovery and description by Hooker & Arnott (1841). Limited research on these aspects of *A. leptopus*' life history has been pursued in part because of the species' documented ability to invade tropical areas outside its natural range (RAJU et al., 2001; BURKE & DITOMMASO, 2011). Some taxonomic work on the genus *Antigonon* has been done by Ewing (1982), albeit with little morphological characterization of the flowers. Notably, some literature has described *A. leptopus* as protandrous (CRUDEN & HERMANN-PARKER 1977; RAJU et al., 2001), *i.e.* having a distinct staminate phase followed by a staminate-pistillate or pistillate phase (LLOYD & WEBB, 1986). This type of breeding system helps to avoid self-fertilization, and therefore promotes the exchange of genetic material between individuals (LLOYD & WEBB, 1986).

This study has two primary objectives relating to *A. leptopus* in Paraná, Brazil: (1) characterize the floral morphology and protandrous syndrome, and (2) document floral visitor taxa and behaviours and compare the latter between the protandrous phases. We hypothesize that (1) flowers in different phases of protandry have significantly different morphology; (2) the protandrous syndrome and morphology of the flowers reflect those described in the literature; (3) flowers in different phases of protandry attract different assemblages of visitors; and (4) different floral visitors exhibit different behaviors.

Material and methods

Study area - The three evaluated populations were located on the campus of the State University of Maringá (UEM) in Maringá, Paraná, Brazil (Table 1; Fig. 1).

Table 1 - Locality information for three populations of *Antigonon leptopus* on the campus of the UEM. Altitudes are above sea level. Zones, eastings and northings are based on Universal Transverse Mercator.

Population	Altitude (m)	Zone	Easting (m)	Northing (m)	Distance from population	
					2	3
1	550	22S	404611	7411181	282 meters	628 meters
2	537	22S	404449	7410950	—	586 meters
3	506	22S	404043	7411450	—	—

Flower sampling - We sampled flowers from populations one and two only, from February 26 to 28, 2019. Population three was not sampled because it had few inflorescences, which were necessary to carry out the observations of floral visitors. From each population (one and two), we took six inflorescences which appeared to be in a relatively early stage of flowering, mostly bearing flowers as opposed to flower buds or fruits. We sampled three inflorescences per population, each at dawn and at midday, to capture any temporal variability in the flowers.

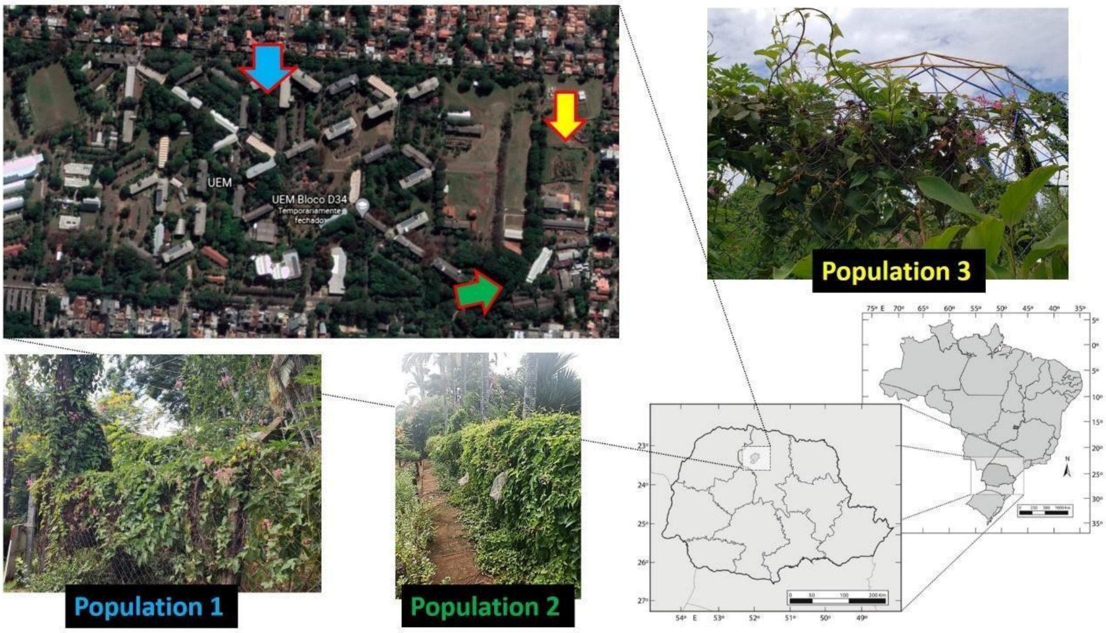

Figure 1. Locality information for three populations of *Antigonon leptopus* investigated in the campus of the State University of Maringá, Paraná Brazil. Modified in part from Google Earth Pro (2019) and Puentes et al. (2019).

Morphometric data collection - The 12 inflorescences were inspected in the laboratory, and five flowers per inflorescence (except two inflorescences, from which we selected four flowers) were selected to measure 14 flowers' attributes. We assessed seven characters visually, either by count or by ruler measurement. For the other seven traits, we imaged each flower under a Leica EZ4D Stereoscopic Microscope with the Leica Application Suite Software (version 1.8), with millimetric paper in the background. We measured the attributes from these photographs with the ImageJ software package (RASBAND, 1997- 2018). Table 2 summarizes the 14 floral attributes and their methods of quantification.

Table 2. List of floral attributes and their respective methods of measurement.

Attribute	Method	Attribute	Method
Perianth diameter	Ruler	Maximum stamen height	ImageJ
Perianth height	Ruler	Minimum stamen height	ImageJ
Number of tepals	Count	Maximum staminode height	ImageJ
Number of stamens	Count	Minimum staminode height	ImageJ
Number of filaments	Count	Androecium diameter	ImageJ
Number of staminodes	Count	Ovary diameter	ImageJ
Number of stigmata	Count	Pistil length	ImageJ

Floral visitor sampling - We observed the floral visitors from all three populations in two ways: (1) observing individual flowers for one minute to record which visitors frequent them; and (2) observing individual visitors (predominantly mobile visitors such as bees) for one minute, or until the end of their first visit, to record the duration and frequency of their visits. We observed visitors during four intervals: dawn (06:00-08:00am); late morning (08:01-11:00am); midday (11:01am-2:00pm); and afternoon (2:01pm-5:00pm).

Statistical analysis - For each statistical analysis, we considered *p*-values lower than 0.01 as significant, and all confidence intervals were run at 99% confidence. We ran the analyses using R software (R CORE TEAM, 2018), along with the library package (OKSANEN et al., 2018).

To determine whether the morphology of flowers differed between the phases of protandry, population or time of collection, we ran four permutational analyses of variance (PERMANOVAs) with 999 permutations and all 14 floral attributes as response variables. The first three tests compared flowers of two phases (staminate vs. staminate-pistillate, staminate-pistillate vs. pistillate, and staminate vs. pistillate; *N.B.* these phases are described in the results section). The fourth test compared all three phases. We included the phase of protandry, population, time of collection and the interaction between phase and population as explanatory variables. We ran the tests using the Bray-Curtis distance, square-rooted to make it metric, because this distance is appropriate for count data and for sites of the same size (BORCARD et al., 2011).

To determine whether certain floral attributes were the same as we expected from the literature (HOOKER & ARNOTT 1841; EWING, 1982), we ran one-sample Student's t-tests with null hypothesis means of five for the number of tepals, eight for the number of stamens, three for the number of stigmata, 18 mm for the perianth diameter and 19 mm for the perianth height. The latter two are the established upper bounds for their respective attributes and so we interpreted the tests' confidence intervals, rather than their *p*-values. Their respective lower bounds were four and six mm.

We ran five PERMANOVAs with 999 permutations on the presence-absence data of floral visitors, to compare the flowers of the phases of protandry and the populations insofar as the visitor assemblages they attract. We first $\log_{10}$ + two transformed the presence-absence data using the Hellinger transformation, then ordinated them with Euclidean distance. This treatment was chosen as the data were binary and reflected species abundances (BORCARD et al., 2011). One test treated all the data irrespective of the time interval when they were collected. The other four tests were specific to each of the four intervals.

With the visitor behavior data, we ran one-way analyses of variance (ANOVAs) and, when these were significant, subsequent Tukey's Honest Significant Difference (HSD) tests. Distinct insect taxa were treated as groups (explanatory variables) in these tests, and we ran two ANOVAs: one for each visit frequency and visit duration, with these as response variables.

Voucher specimens - For each population, two voucher specimens were deposited in the State University of Maringá Herbarium (HUEM) and the W.P. Fraser Herbarium (SASK), University of Saskatchewan, Canada. We also collected and preserved various floral visitors for later identification.

Results

General observations of plants

The following morphological characteristics of the plants were characterized in the field: plant growth habit as climbing vine; leaves cordate with sinuate margins, alternate or in whorls of two; stems terminating with a bi- or trifurcated tendril; inflorescence a racemose panicle; flowers bisexual with one tristylous pistil surrounded by a crown-like circular androecium of seven to eight of each stamens and staminode-like structures, alternately arranged; stamens of two different heights (three tall, four to five short); anthers versatile; semi-biserial perianth of four to five bright pink lobes (tepals), one or two of which are narrower than the rest (Fig. 2); fruit enclosed by persistent green perianth.

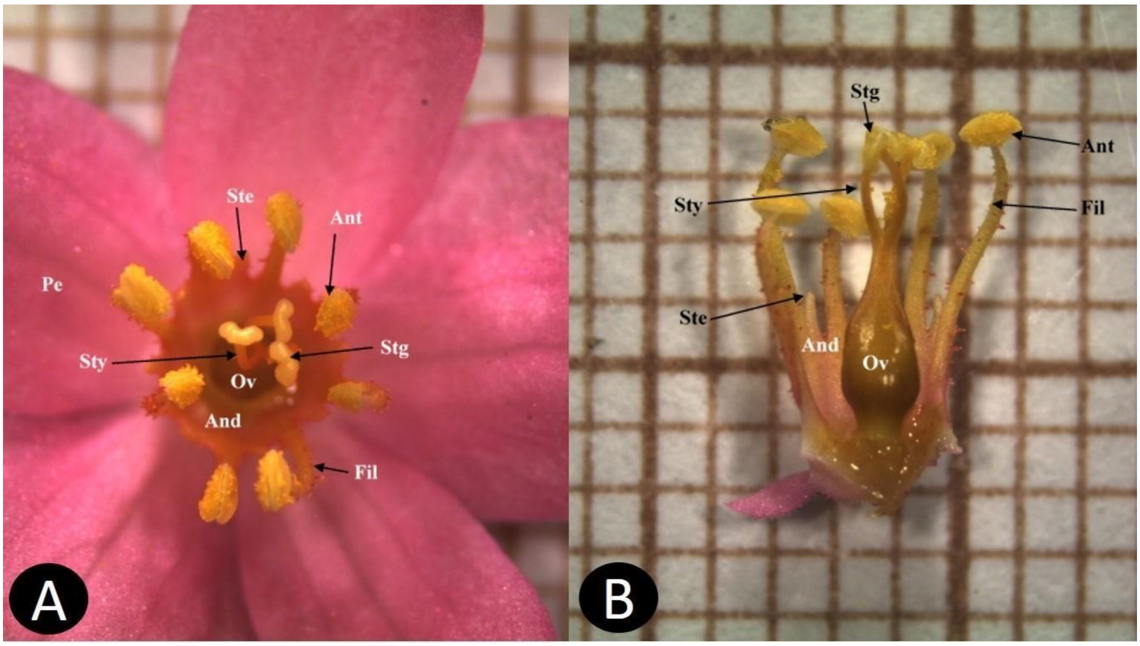

Figure 2. Floral morphology of *Antigonon leptopus.* A. Flower in frontal view. B. Dissected androecium and gynoecium in lateral view. And: androecium; Ant: anther; Fil: filament; Ov: ovary; Pe: perianth; Ste: staminode-like structure; Stg: stigma; Sty: style.

Furthermore, we observed that the plants underwent three phases of protandry, rather than the established two (staminate, then pistillate or staminate-pistillate) phases. The flowers underwent a staminate phase, then an intermediate staminate-pistillate phase and then a final pistillate phase (hereinafter the phases 1, 1.5 and 2, respectively). The flowers apparently underwent each phase sequentially within a single day.

Morphology of protandrous phases

When comparing the overall floral morphology of the three phases of protandry, all comparisons (both pairwise and overall), the flowers had significantly different sets of traits ($p \leq 0.003$). In addition, the populations from which flowers were sampled (populations one and two) had a significant effect on the flowers' morphology in all specimens ($p \leq 0.007$), but the comparison with phase 1 and 2 flowers ($p = 0.052$) (Fig. 3). There was no significant interaction between phase and population ($p \geq 0.144$). Additionally, the morphology of phase 1 and 1.5 flowers, and of all flowers irrespective of phase, was significantly different at different collection times ($p \leq 0.002$) (Table 3).

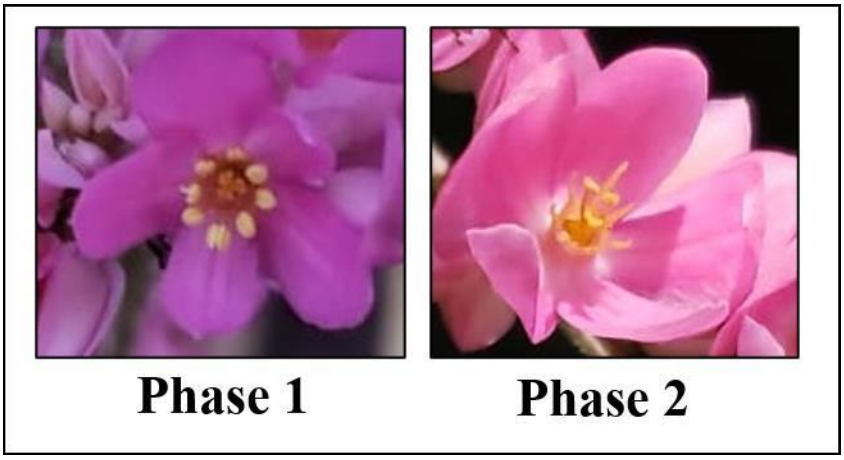

Figure 3. Photographs of *A. leptopus* flowers in Phase 1 (with anthers and pistil) and Phase 2 (with pistil but without anthers).

Table 3. *P*-values of PERMANOVAs comparing floral morphometric data across protandry phases, populations and collection times. Significant (< 0.01) *p*-values are indicated by an asterisk (*).

Compared phases	Phase	Population	Time	Phase: Population interaction
1 vs. 1.5	0.003*	0.007*	0.001*	0.144
1.5 vs. 2	0.001*	0.004*	0.195	0.667
1 vs. 2	0.001*	0.052	0.021	0.398
1 vs. 1.5 vs. 2	0.001*	0.001*	0.002*	0.286

Morphological comparisons with the literature reports

Literature searches indicated that the mean number of tepals and of stamens determined in our study was significantly lower than the numbers established in the literature ($p \leq 6.765 \times 10^{-5}$). This can be explained by the variable number of tepals (4 to 5) and stamens (7 to 8) that we observed. In contrast, the number of stigmata was uniformly three, just as described in the literature (p = 1.000) (HOOKER & ARNOTT 1841; EWING, 1982). We furthermore found, at 99% confidence, that the means of both the diameter (11.765-14.200 mm, $p = 1.084 \times 10^{-15}$) and height (9.145-10.286 mm, $p = 2.200 \times 10^{-16}$) of the perianth were within their ranges established in the literature (4-18 mm and 6-19 mm, respectively) (Table 4).

Table 4. *P*-values and confidence intervals of one-sample Student's t-tests comparing floral attributes with means established in the literature. Significant (< 0.01) *p*-values are indicated by an asterisk (*).

Trait	**Null hypothesis means**	***p*-value**	**99% confidence interval**	
			Lower bound	**Upper bound**
Number of tepals	5	9.527×10^{-6}*	4.546	4.868
Number of stamens	8	6.765×10^{-5}*	6.604	7.672
Number of stigmata	3	1.000	3.000	3.000
Perianth diameter	18 mm	1.084×10^{-15}*	11.765	14.200
Perianth height	19 mm	2.200×10^{-16}*	9.145	10.286

General observations and activities of floral visitors

Most visitors, especially bees, exhibited pollination behavior and tended to seek either nectar or pollen, or both, as floral rewards. Occasionally, bees would store pollen in their corbicula (pollen sac). A species of beetle (*Astylus variegatus* Germ.) was a frequent visitor of the flowers, mostly engaging in copulation with one another (Fig. 4A). The beetles also appeared to bite the anthers of the flowers. Leafcutter ants (cf. *Acromyrmex* Mayr sp.) were observed dismantling an inflorescence in full bloom (Fig. 4B). A jumping spider (*Frigga* C.L.Koch sp.) and a crab spider (Thomisidae) were seen predating bees (Figs 4 C-D). We suspect that the crab spider, given its color pattern is similar to some of *A. leptopus*' flowers, may have been mimetic.

Floral visitors of protandrous phases

The assemblages of floral visitors did not differ between protandrous phases (1 and 1.5 vs. 2; $p \geq 0.012$). However, the assemblages were significantly different between the populations both irrespective of sampling period and during each sampling period ($p = 0.001$), save the midday period between 11:01am and 2pm ($p = 0.017$). There were no significant interactions between the phase of protandry and population variables ($p \geq 0.062$) (Table 5).

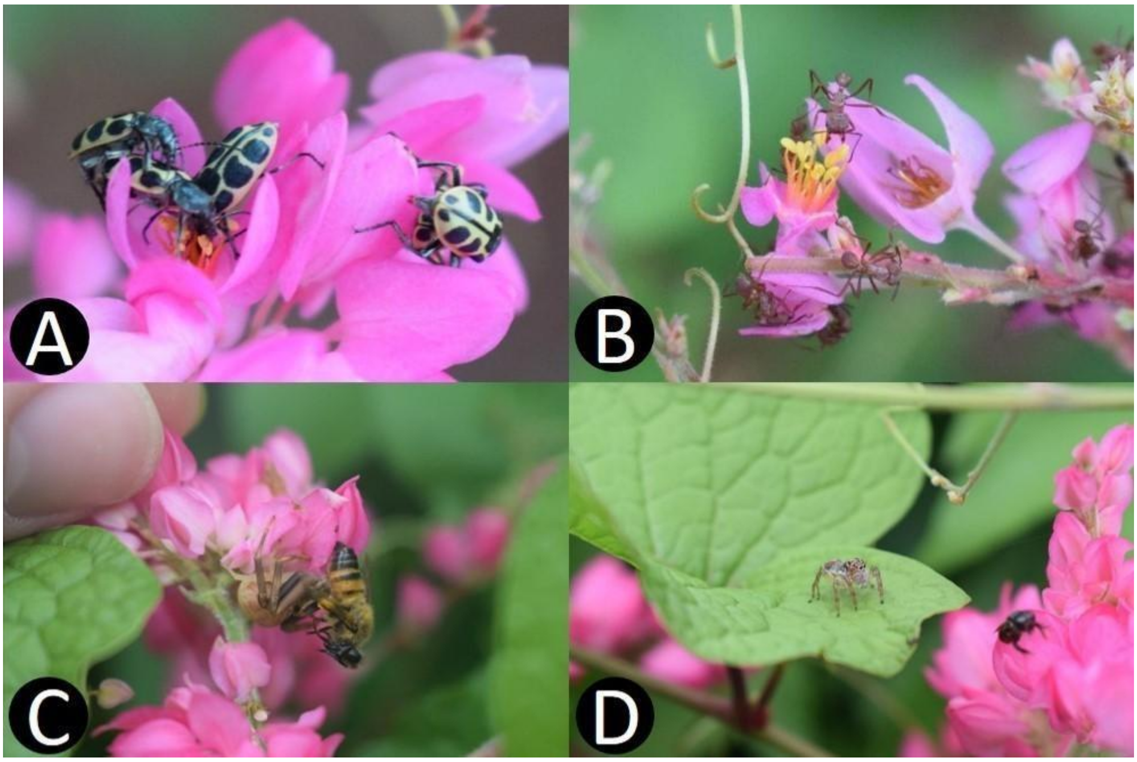

Figure 4. Floral visitors on *Antigonon leptopus.* A. *Astylus variegatus* copulating and feeding on flowers. B. cf. *Acromyrmex* sp. dismantling flowers. C. *Frigga* sp. stalking a bee (obscured in the foreground). D. Crab spider feeding on a bee and possibly mimicking the white to pink tepals of the flowers.

Table 5. Sample sizes (n) of flowers from the phases of protandry, and *p*-values for protandry phase, population and their interaction, from five PERMANOVAs comparing floral visitor assemblage data. *N.B.*: Phases 1 and 1.5 are lumped together as one group (Phase 1). Significant (< 0.01) *p*-values are indicated by an asterisk (*).

Time interval	Phase 1	Phase 2	*P* Phase	Population	Phase: Population
06:00am - 5:00pm	281	246	0.012	0.001*	0.062
06:00am - 08:00am	103	117	0.054	0.001*	0.267
08:01am -11:00am	53	67	0.100	0.001*	0.472
11:01am - 2:00pm	65	20	0.139	0.017	0.566
14:01pm -17:00pm	60	42	0.980	0.001*	0.302

Behaviours of floral visitors

The floral behaviours of five floral visitors were observed in the field. Four visitors were in the family Apidae (*Apis mellifera* Linneaus, *Scaptotrigona* Moure sp., *Plebeia* Schwarz sp. and *Tetragonisca angustula* Latreille), while members of the family

Halictidae were lumped together. Table 6 summarizes the comparisons of visit frequency and duration between taxa.

Table 6. Sample sizes (n) and *p*-values from two sets of Tukey's HSD on data used in two ANOVAs comparing the number of visits per minute across visitor taxa (above the diagonal) and the mean duration of visits across visitor taxa (below the diagonal). Significant (< 0.01) *p*-values are indicated by an asterisk (*).

Visitor	***Apis mellifera***	***Scaptotrigona***	***Plebeia***	***Tetragonisca angustula***	**Halictidae**
	n = 59	n = 98	n = 5	n = 8	n = 27
Apis mellifera		8×10^{-5}*	0.004*	$< 1 \times 10^{-7}$*	$< 1 \times 10^{-7}$*
Scaptotrigona sp.	0.999		0.278	$< 1 \times 10^{-7}$*	$< 1 \times 10^{-7}$*
Plebeia	1.000	1.000		0.102	0.319
Tetragonisca angustula	0.599	0.654	0.926		0.752
Halictidae	$< 1 \times 10^{-7}$*	$< 1 \times 10^{-7}$*	0.009*	0.034	

The differences of significant Tukey's HSD comparisons from Table 6 are further summarized in Table 7. In the case of the visit frequency comparisons, *A. mellifera* had a significantly higher number of visits in a one-minute period than the other taxa (1.957 to 7.962 visits more). *Scaptotrigona* sp. also had significantly more visits than *T. angustula* (6.005 more) and Halictidae (4.755 more). In terms of visit duration, the Halictidae spent significantly more time per visit than all other taxa, save *T. angustula* (29.423 to 30.876 seconds more).

Table 7. Differences in visit frequency (visits min^{-1}) and duration (s) between taxa which had significant Tukey HSD *p*-values, and their 99% confidence intervals.

Comparison	**Taxon 1**	**Taxon 2**	**Difference (1 minus 2)**	**99% confidence interval**	
				Lower bound	**Upper bound**
Frequency	*Apis mellifera*	*Scaptotrigona*	1.957	0.547	3.366
Frequency	*A. mellifera*	*Plebeia*	4.312	0.328	8.296
Frequency	*A. mellifera*	*Tetragonisca angustula*	7.962	4.739	11.184
Frequency	*A. mellifera*	Halictidae	6.711	4.725	8.699
Frequency	*Scaptotrigona*	*T. angustula*	6.005	2.860	9.150
Frequency	*Scaptotrigona*	Halictidae	4.755	2.896	6.614
Duration	Halictidae	*Apis mellifera*	30.876	17.035	44.717
Duration	Halictidae	*Scaptotrigona*	30.066	17.119	43.014
Duration	Halictidae	*Plebeia*	29.423	0.420	58.426

Discussion

Protandry in Antigonon leptopus

Our investigation of *A. leptopus*' protandrous syndrome differs from previous studies. Notably, we observed three distinct phases which were, in chronological order: staminate, staminate-pistillate and pistillate. This differs from Ewing (1982), who described only a staminate phase and, under ideal environmental conditions, a staminate-pistillate phase, each lasting one day.

Our findings also differ from those of Cruden & Hermann-Parker (1977) and Raju et al. (2001), who observed non-overlapping staminate and pistillate phases lasting one day each. In addition, we found that the duration of flowering lasted less than one day, as opposed to the two-day period established in the literature (RAJU et al., 2001).

We suspect that this is due to environmental factors such as visitor assemblages and climate, which are expected to be different in Brazil than in Mexico, where *A. leptopus*' is native. Alternatively, this may be caused by a developmental error described by Cruden & Hermann-Parker (1977), which causes asynchronous flowering in species with large numbers of lateral stems. Overall, the three-stage protandry observed in this study is unusual and may reflect the plant's need to reduce the entire flowering period into a shorter time frame, perhaps owing to environmental constraints as suggested by Lloyd & Webb (1986).

Novel floral attributes

In our populations, we observed several floral traits not described in the literature. Notably, the number of tepals varied from four to five, while the literature describes flowers as always having five (HOOKER & ARNOTT, 1841; EWING, 1982). We also found discrepancies for stamens, which numbered seven or eight in our flowers as opposed to the literature's eight (HOOKER & ARNOTT, 1841; EWING, 1982). Finally, we believe that the androecial structures located between each stamen, illustrated but not described by Ewing (1982), may be staminodes. This would be unusual however, because staminodes in *A. leptopus*' order (Caryophyllales) are usually derived from or associated with the perianth rather than the androecium (De CRAENE & BROCKINGTON, 2013).

Plant-insect interactions

The flowers' nectar and pollen were predominately sought after by bees. All non-bee visitors were engaged in other behaviors such as predation, copulation or leaf cutting. This is in contrast to Raju et al. (2001), who identified a wide range of insects seeking pollen and/or nectar (*e.g.* bees, butterflies, wasps, flies). This fundamental contrast in visitor assemblage composition is likely attributable to differences in climate and insect fauna between southern Brazil and India, the latter being where Raju et al. (2001) conducted their study. When comparing the primary bee visitor taxa, *Apis mellifera* visited the flowers *of A. leptopus* more frequently than any other taxon, while *Scaptotrigona* sp. visited more frequently than two other taxa. Consequently, these two taxa plus *Plebeia* sp. spent around 30 seconds less on average per visit compared to members of the Halictidae. Therefore, certain bee taxa conduct many short visits at the flowers while others spend few but longer visits. The assemblages of visitors did not differ between the phases of protandry, but in most cases they did differ between populations.

Overall, these findings suggest that *A. leptopus* has a generalist pollination syndrome which attracts a taxonomic array of visitors with varying behaviors. This generalist syndrome extends irrespective of protandrous phase, perhaps allowing for the plants to maximize their outcrossing rate. However, given the overlap of the staminate and pistillate phases of protandry, a generalist syndrome would not be evolutionarily advantageous unless the plants cannot self-fertilize (LLOYD & WEBB 1986; BARRETT, 2002). This is because when staminate and pistillate expression occurs simultaneously, the flowers have no control over the flow of pollen via pollinators (LLOYD & WEBB, 1986). During this overlap, the pollen grains from one flower can therefore be transferred by pollinators to the same flower's stigmata. Ewing (1982) found that *A. leptopus* is capable of self-fertilization and produces viable progeny via this strategy. For this reason, we considered it somewhat perplexing that the staminate and pistillate phases overlap in the plant's protandrous syndrome.

Concluding remarks

Considering the original objectives of this study, we successfully characterized the floral morphology and protandry of *A. leptopus*. We furthermore compared the assemblages of floral visitors between flowers of the protandrous phases and made general observations about visitor behaviors. Of our four hypotheses, the first was correct while the second and fourth were partially correct. We found that (1) the floral morphology of flowers in the three phases of protandry was significantly different; (2) most floral attributes (perianth height and diameter, and numbers of tepals and stamens) were lower than the numbers in previous findings, while he number of stigmata did not differ from the literature; (3) the flowers of the protandry phases did not differ in so far as their visitor assemblages; and (4) certain species of bee had substantially different visit frequencies and durations from one another. In summary, it is important to note that the data only reflect the nature of three populations of *A. leptopus* in a limited geographic area. The data therefore have a limited interpretability when assessing the reproductive biology of the species as a whole.

References

BALOGH, C.M. & BARRETT, S.C.H. The influence of floral morph ratios and low plant density on mating and fertility in a tristylous colonizing species. **Botany**, v. 96, n. 8, p. 533-545, 2018.

BARRETT, S.C.H. The evolution of plant sexual diversity. **Nature Reviews Genetics**, v. 3, n. 4, p. 274-284, 2002.

BORCARD, D.; GILLET, F. & LEGENDRE, P. **Numerical ecology with R**. New York: Springer. 306 p., 2011.

BURKE, J.M. & DITOMMASO, A. Corallita (*Antigonon leptopus*): Intentional introduction of a plant with documented invasive capability. **Invasive Plant Science and Management**, v. 4, n. 3, p. 265-273, 2011.

COTA-SÁNCHEZ, J.H.; ALMEIDA, O.J.G.; FALCONER, D.J.; CHOI, H.J. &BEVAN, L. Intriguing thigmonastic (sensitive) stamens in the Plains Prickly Pear
Opuntia polyacantha (Cactaceae). **Flora**, v. 208, n. 5-6, p. 381-389, 2013.

CRUDEN, R.W. & HERMANN-PARKER, S.M. Temporal dioecism: an alternative to dioecism. **Evolution**, v. 31, n. 4, p. 863-866, 1977.

De CRAENE, L.P. & BROCKINGTON, S.F. Origin and evolution of petals in angiosperms. **Plant Ecology and Evolution**, v. 146, n. 1, p. 5-25, 2013.

EWING, J.W. A revision of the genus *Antigonon* [master's thesis]. Bloomington (IN): Indiana University. 64 p., 1982.

GANAIE, S.U.; ABBASI, T. & ABBASI, S.A. Biomimetic synthesis of platinum nanoparticles utilizing a terrestrial weed Antigonon leptopus. **Particulate Science and Technology**, v. 36, n. 6, p. 681-688, 2018.

GOOGLE EARTH PRO. **Version 7.3.3.7786, Maringá, Paraná, Brazil, 23°24'15.77"S, 51°56'24.46"W, Eye alt 5894 feet**. Maxar Technologies 2020. Available from: https://www.google.com/earth/ [accessed 2020 Dec 4], 2018 (Jul 19).

HOOKER, W.J. & ARNOTT, G.A.W. **The botany of captain beechey's voyage**. London (UK): Henry G. Bohn. 485 p., 1841.JBRJ [Jardim Botânico de Rio de Janeiro]. **Polygonaceae in Flora do Brazil 2020 under construction**. Available from: http://reflora.jbrj.gov.br/reflora/floradobrasil/FB87242 [accessed 2019 Apr 15], c2019.

LLOYD, D.G. & WEBB, C.J. The avoidance of interference between the presentation of pollen and stigmas in angiosperms I. Dichogamy. **New Zealand Journal of Botany**, v. 24, n. 1, p. 135-162, 1986.

MULABAGAL, V.; ALEXANDER-LINDO, R.L.; DEWITT, D.L. & NAIR, M.G.Health-beneficial phenolic aldehyde in *Antigonon leptopus* tea. **Evidence-Based Complementary and Alternative Medicine**, v. 2011, 2011.

OKSANEN, J. et al. **Vegan: Community Ecology Package**. Version 2.5-3. Available from: https://CRAN.R-project.org/package=vegan, 2018.

PLANT LIST. 2013. **Version 1.1**. Available from: http://www.theplantlist.org [accessed 2020 Aug], 2013.

PUENTES, S.M.D.; LOPEZ, J.; GALHARDO, D.; OLIVEIRA, J. & de TOLEDO, V. Foraging Behaviour of *Apis mellifera* L. and *Scaptotrigona bipunctata* on *Dombeya wallichii* Flowers in Southern Brazil. **Agricultural Sciences**, v. 10, p. 1124-1134, 2019.

R CORE TEAM. **R: A language and environment for statistical computing**. Vienna (Austria): R Foundation for Statistical Computing. Available from: https://www.R-project.org/, 2018

RAJU, A.J.S.; RAJU, V.K.; VICTOR, P. & NAIDU, S.A. Floral ecology, breeding system and pollination in *Antigonon leptopus* L. (Polygonaceae). **Plant Species Biology**, v. 16, n. 2, p. 159-164, 2001.

RASBAND, W.S. **ImageJ**. Bethesda (MD): U.S. National Institutes of Health. Available from: https://imagej.nih.gov/ij/, 1997-2018

SANCHEZ, A; SCHUSTER, T.M. & KRON, K.A. A large-scale phylogeny of Polygonaceae based on molecular data. **International Journal of Plant Sciences**, v. 170, n. 8, p. 1044-1055, 2009.

SAUQUET, H. et al. The ancestral flower of angiosperms and its early diversification. **Nature Communications**, v. 8, n. 1, p. 1-10, 2017.

TOLEDO, V.A.A.; FRITZEN, A.E.D.T.; NEVES, C.A.; RUVOLO-TAKASUSUKI, M.C.C.; SOFIA, S.H. & TERADA, Y. Plants and pollinating bees in Maringá, State of Paraná, Brazil. **Brazilian Archives of Biology and Technology**, v. 46, n. 4, p. 705-710, 2003.