The specialization–generalization continuum in oil-bee pollination systems: a case study of six Brazilian species of Tigridieae (Iridaceae)

Suiane Santos Oleques, Jefferson Nunes Radaeski, Soraia Bauerman, Olivier Chauveau, Tatiana Teixeira de Souza-Chies

Biological Journal of the Linnean Society, 2020, **XX**, 1–16. With 7 figures.

The specialization–generalization continuum in oil-bee pollination systems: a case study of six Brazilian species of Tigridieae (Iridaceae)

SUIANE SANTOS OLEQUES[1*], JEFFERSON NUNES RADAESKI[2], SORAIA BAUERMAN[2], OLIVIER CHAUVEAU[1,3] and TATIANA TEIXEIRA DE SOUZA-CHIES[1,4]

[1]*Departamento de Botânica, Programa de Pós-graduação em Botânica, Universidade Federal do Rio Grande do Sul—UFRGS, Porto Alegre, Rio Grande do Sul, Brazil and Laboratório de Pesquisa em Interações Ecológicas, Universidade Federal do Pampa - UNIPAMPA, São Gabriel, Rio Grande do Sul, Brazil*
[2]*Departamento de Biologia, Universidade Luterana do Brasil – ULBRA, Laboratório de Palinologia, Canoas, Rio Grande do Sul, Brazil*
[3]*Université Paris Sud, Agro Paris Tech, —Unité Écologie, Systématique et Évolution, 91405 Orsay, France*
[4]*Departamento de Botânica, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves no. 9500, Porto Alegre, Rio Grande do Sul, 91501-970, Brazil*

Received 4 October 2019; revised 19 November 2019; accepted for publication 19 November 2019

Several South American species of Iridaceae, especially those of Tigridieae, produce floral oils as rewards to oil-bee pollinators. The present study aimed to contribute to a deeper understanding of the reproductive biology, pollination ecology and level of specialization of the interactions of species encompassed in Tigridieae. Data on breeding and pollination systems were acquired from six species native to Southern Brazil. The visitation frequency and pollen load of pollen- and oil-collecting bees were also investigated. The results strongly suggest that the studied species are distributed along a specialization–generalization continuum. Three oil-producing taxa, *Cypella herbertii*, *Cypella pusilla* and *Cypella amplimaculata*, were pollinated effectively by oil-bees, whereas in the other two studied species, *Kelissa brasiliensis* and *Herbertia pulchella*, the oil-bees appeared to function as oil thieves, owing to failure to contact the plant reproductive parts during oil-foraging behaviour. New insights into aspects of the specialization–generalization continuum of pollination systems, differences in pollinator behaviour during oil and pollen foraging, and reproductive outputs of the studied species are provided. Taken together, our results provide a significant contribution towards a better understanding of reproductive biology and plant–pollinator interactions between Iridaceae and oil-collecting bees.

ADDITIONAL KEYWORDS: elaiophores – Iridoideae – oil-collecting bees – oil-producing flowers – pollination system – specialization.

INTRODUCTION

The production of floral oils as a reward to pollinators has been reported for 11 angiosperm families and was more often lost than acquired during the evolution of angiosperms (Renner & Schaefer, 2010; Martins *et al.*, 2015). Among these plant families, Iridaceae is considered one of the most species rich in the Western Hemisphere. It encompasses ~2030 species among 65–75 genera worldwide (Goldblatt *et al.*, 2008), and shifts in pollination system are frequently associated with diversification in this florally diverse group. Floral oils are the third type of reward collected by pollinators in Iridaceae, besides pollen and nectar. The 1190 species from South African Iridaceae exhibit a wide range of pollination systems; however, epithelial elaiophores and pollination by oil-collecting bees have been recorded for only a single species, *Tritoniopsis parviflora* (Jacq.)

*Corresponding author: E-mail: suiane.bio@gmail.com

G.J. Lewis, in Crocoideae (Manning & Goldblatt 2002). In contrast, floral oil secretion and pollination by oil-collecting bees occur within numerous South American species of Iridoideae. Brazil harbours 204 species of Iridaceae, all of the subfamily Iridoideae. These species are divided into three tribes, Sisyrinchieae, Tigridieae and Trimezieae, with the first two being the most species-rich Iridaceae in South Brazil (Souza-Chies *et al.*, 2012; Eggers *et al.*, 2015). A recent phylogenetic study showed that the distribution of oil-producing trichomes among species of Iridoideae evolved repeatedly in association with the pollination system, which suggests that these glandular structures might have played a significant role in the diversification of the subfamily in the Western Hemisphere (Chauveau *et al.*, 2012).

Tigridieae currently includes 172 species distributed among 15–20 genera, which are divided into two main clades that are formally named Clade A and Clade B (Chauveau *et al.*, 2012). Most members of Clade A occur in South Brazil, where they are distributed among the following predominant genera: *Cypella* Herb. (20 spp.), *Calydorea* Herb. (12 spp.) and *Herbertia* Sweet (seven spp.) (Eggers *et al.*, 2015).

Species of Tigridieae are separated from each other by different combinations of floral attributes, the diversity of which is presumably driven by distinct functional groups of pollinators (Goldblatt & Manning, 2006); however, this has never been tested empirically, and the reproductive biology and pollination ecology of most of these species are still poorly known. A particularly intriguing aspect of floral diversity in Tigridieae is the variation in the nature and presentation of floral rewards. Chauveau *et al.* (2012) documented two types of pollinator resources for species of Clade A in Southern Brazil: flowers offering only pollen (*Calydorea*) and flowers offering pollen and oil (*Cypella*, *Herbertia*, *Kelissa* Ravenna and *Onira* Ravenna), with various arrangements of oil-producing trichomes occurring among these genera.

Lability of traits related to reproductive organs during evolution is often considered a major driver for lineage diversification and is attributed to adaptation to different pollinators (Anderson *et al.*, 2002; Foxe *et al.*, 2009; Barret, 2013). Two essential trends in plant reproduction have occurred during the evolution of angiosperm breeding systems: the transition from selfing to outcrossing, and the evolution of animal pollination (Fenster & Martén-Rodríguez, 2007; Barrett, 2010; Armbruster, 2014). Different pollinators are expected to act as agents of divergent selection pressures on floral traits. Thus, knowledge of the foraging behaviour, the morphological matching between plant and pollinators and the plant fitness are crucial to understand the role of shifts in pollination systems in evolutionary transitions related to floral rewards.

In addition to the evidence that plant–pollinator interactions can strongly influence evolutionary processes, these interactions also provide essential ecosystem services by enabling the reproduction of flowering plants (Thébault & Fontaine, 2010; Wolowski *et al.*, 2016). Moreover, studies of pollination biology are crucial to estimate population dynamics and the vulnerability of species, especially in highly threatened and poorly studied environments, such as the grasslands of South Brazil, where Iridaceae is considered to be one of the most species-rich families (Overbeck *et al.*, 2007).

The aim of the present study was to investigate the reproductive biology and pollination ecology of seven taxa belonging to Clade A of Tigridieae, which represent the different arrangements of pollination rewards observed in this clade. More specifically, our aims were as follows: (1) to identify pollinators and characterize their foraging behaviour; (2) to characterize differences in breeding system to assess self-compatibility and the extent of reliance on pollinators for reproduction; and (3) to use visitation frequencies and pollen loads of flower visitors to determine pollination systems and the associated degree of specialization for the seven studied taxa.

Given that the species studied present two combinations of resources, oil + pollen and only pollen, our hypothesis about pollination system was that *Calydorea alba* Roitman & J.A.Castillo would be pollinated exclusively by pollen-collecting bees, whereas the *Cypella* spp., *Herbertia pulchella* Sweet and *Kelissa brasiliensis* (Baker) Ravenna would be pollinated mainly by oil-collecting bees, with pollen-collecting bees acting, to different extents, as less important pollinators. Unlike *Cypella* spp. and *K. brasiliensis*, *H. pulchella* offers oil in both inner and outer tepals; therefore, we hypothesized that *H. pulchella* would be visited more by oil-bees and, as a consequence, more dependent on oil-bee pollination. Concerning the breeding system, our hypothesis was that taxa with more specialized pollination systems, i.e. more dependent on oil-bee pollination, would present higher indexes of self-compatibility. This is based on other selective forces, because an increase of pollen limitation in the absence of specialized pollinators, then high outcrossing rates could be considered, contradicting the notion that specialization reflects only selective pressures to increase outcrossing rates (Fenster & Martén-Rodríguez, 2007).

MATERIAL AND METHODS

Plant species and study area

The seven taxa studied for pollination and breeding system were *Cypella herbertii* subsp. *herbertii* (Lindl.)

Herb, *Cypella herbertii* subsp. *brevicristata* Ravenna, *Cypella amplimaculata* Chauveau & L. Eggers, *Cypella pusilla* (Link & Otto) Benth. & Hook.f.ex B.D. Jacks, *K. brasiliensis*, *H. pulchella* and *Calydorea alba*. The species *Cypella pusilla* and *K. brasiliensis*, both endemic to the State of Rio Grande do Sul (Brazil), are considered Critically Endangered and Vulnerable, respectively, according to the Red List of threatened species of the State of Rio Grande do Sul (SEMA, 2014). Vouchers of all plant populations studied were deposited in institutional herbaria, and information is provided in the Supporting Information (Appendix S1).

These taxa were chosen in order to sample the different combinations of pollination rewards and the different locations of trichomatic elaiophores observed among taxa within Clade A of Tigridieae. Specifically, the following combinations of floral reward and location of secretory structures were considered: elaiophores located on inner tepals (taxa of *Cypella* and *K. brasiliensis*); floral oil secreted from both inner and outer tepals (*H. pulchella*); and pollen as the only reward available (*Calydorea alba*) (Chauveau *et al.*, 2012). The studied taxa bloom mainly from spring to early summer, except for *Cypella pusilla*, which presents a second flowering period in March and April. Most species usually have synflorescences that expose one open flower at a time, with the exception of *Cypella herbertii* and *Cypella amplimaculata*, which could eventually expose more than one open flower at the same time.

Field observations were conducted in the state of Rio Grande do Sul, Brazil, and mainly in the Serra do Sudeste region in the South Brazilian Campos (Pampa biome, grasslands) (Schlindwein, 1998; Overbeck *et al.*, 2007). This region has an average elevation of 568 m (Supporting Information, Appendix S2), was formed mostly during the Precambrian and is characterized by a mosaic of native forest and rocky grasslands (Rambo, 1956). The area is of high conservation interest owing to the beauty of the original landscape and the endemicity of much of its flora (MMA, 2007; Overbeck *et al.*, 2007). Furthermore, the region is distinguished by a high representation of oil-producing species of Iridaceae (Schlindwein, 1998; Chauveau *et al.*, 2012; Eggers *et al.*, 2015).

Floral visitors and breeding system

The study was conducted in 2016 and 2017 from September to April, in order to encompass the entire flowering season of the different plant species, and further observations were achieved in April 2018. Floral visitors were monitored on sunny days, from the time of anthesis to closure, during periods of 15–30 min throughout the entire flowering season (approximately 3 months). Plant–insect interactions were studied methodically in two geographically distinct populations per plant taxon (Supporting Information, Appendices S1 and S2). Multiple randomly chosen flowers were surveyed in each population, and the total number of visits, in addition to the type and behaviour of visitor, were consistently recorded. Each taxon was observed for ≥ 20 h (21.0 ± 3.40 h per plant taxon, mean ± SD) during the flowering season, with the exception of *Calydorea alba*, which was observed for only 15 h, considering that the flower opening time for this species was considerably shorter (from 07.00 to 10.30 h). Floral visitors were divided into two functional groups: pollen-collecting bees and oil-collecting bees. Visitation frequency was calculated as the number of visits per minute of observation. All bee species collected were identified by a specialist using taxonomic keys, and specimen vouchers were deposited at the Museu de Zoologia (Universidade de São Paulo, Brazil).

The breeding system was determined by controlled pollination experiments, including hand cross-pollination (hand pollination with pollen from another plant); hand self-pollination (hand pollination with pollen from the same individual, usually the same flower because the majority of taxa presented one flower per individual); spontaneous self-pollination (flowers were maintained bagged, without further treatment); emasculation (anthers were removed, and flowers were bagged to verify fruit formation), which was conducted only for self-compatible taxa; and natural pollination (flowers were not bagged and were exposed to pollinators) (Dafni, 2005). We calculated the index of self-incompatibility (ISI) and ranked it into three states: (1) self-incompatible, ISI ≥ 0.8; (2) partly self-incompatible, 0.2 < ISI < 0.8; and (3) self-compatible, ISI ≤ 0.2 (Raduski *et al.*, 2012). To calculate the ISI, we used the following formula, as proposed by Lloyd (1965):

$$\mathrm{ISI} = 1 - \frac{\text{fruit set from hand self-pollination}}{\text{fuit set from hand cross-pollination}}$$

Pollen load

Initially, a library of pollen grains collected from each studied taxa of Iridaceae was prepared to identify pollen grains at the genus level, after which the number and ratio of pollen grains carried by each visitor was determined. The average pollen load for each functional group of bees was determined from a sample of 20 individuals per functional group of insect visitors.

Bees were invariably collected during foraging, and their pollen load was removed and maintained in test

tubes with 4 mL of 70% alcohol. Pretreatments and analyses of pollen samples were performed according to Erdtman (1952). After chemical treatment, slides were deposited in the Pollen Library of the Laboratório de Palinologia [Universidade Luterana do Brasil (ULBRA), Brazil]. The proportions of pollen types were classified in classes of occurrence for each functional group according to Louveaux *et al.* (1978): abundant (> 45% of total grains), common (15–45%), uncommon (3– 14%) and rare pollen (< 3%). The contribution of each functional group to pollination systems was assessed using an approach that considered both the frequency of visits and the pollen load of the bee (Krakos & Fabricant, 2014). Pollen transfer was calculated for each *Cypella* spp., *H. pulchella* and *K. brasiliensis* by applying the formula $\sum(VR_x \times PL_x)$, where VR is the total visitation frequency by any given functional group (x) and PL is the average pollen load carried by that group. The main pollination system was defined for each plant genus according to the pollinator functional group that reached > 75% of the total pollen transfer (Fenster *et al.*, 2004; Krakos & Fabricant, 2014).

Statistical analyses

Bees were systematically assigned to one of the two functional groups defined above. The frequencies of legitimate visits (*f*; visitors that came into contact with anthers and stigmas) were estimated for each of the two functional groups and compared using general linear mixed-effects models (GLMMs) with a Poisson distribution, with plant species, bee functional group and the interaction between them as fixed effects. To take into account the variation in observation times, the duration of observation was included as a $\log_{10}$-transformed offset. Differences in visitation by oil- and pollen-collecting bees among and between plant taxa were assessed with Tukey's post-hoc test using the function glht() from *multcomp* package in R (R Core Team, 2015).

To analyse binary data related to fruit set obtained from the different pollination treatments, data were fitted to GLMMs, considering pollination treatments as fixed effects and individual plants as random effects. The fit of logistic regression models was assessed using maximum likelihood analysis (Akaike information criterion). Finally, a deviance analysis was performed to test whether model deviance was greater than expected by chance and whether each model as a whole was better than the null model (Bolker *et al.*, 2008).

We performed a Wilcoxon rank-sum test to assess the difference between the numbers of pollen grains from each plant genus carried by pollen- and oil-collecting bees (Rhodes *et al.*, 2017).

RESULTS

Floral visitors

We recorded 975 flower visits during a total of 142 h of observations and identified 16 species of pollen-collecting bees and four species of oil-collecting bees (Table 1). Pollinator diversity (Fig. 1) was highest for *H. pulchella* (14 species: 13 pollen-bees and one oil-bee), followed by *K. brasiliensis* (seven species: six pollen-bees and one oil-bee), *Cypella amplimaculata* (two pollen-bees and two oil-bees), *Cypella herbertii* subsp. *herbertii* (one pollen-bee and three oil-bees), *Cypella herbertii* subsp. *brevicristata* (two pollen-bees and two oil-bees), *Cypella pusilla* (one pollen-bee and two oil-bees) and *Calydorea alba* (three species, all pollen-bees).

All oil-producing taxa were pollinated by oil-collecting bees belonging to the tribe Tapinotaspidini. *Arhysoceble picta* was observed on flowers of all six oil-producing taxa studied, and *Chalepogenus muelleri* was recorded on three species with oil flowers: *Cypella herbertii* subsp. *herbertii*, *Cypella herbertii* subsp. *brevicristata* and *H. pulchella*. *Caenonomada brunerii* and *Chalepogenus goeldianus* were found exclusively on *Cypella herbertii* subsp. *brevicristata* and *Cypella pusilla*, respectively (Fig. 1).

Arhysoceble picta and *Chalepogenus muelleri* did not contact fertile parts during their oil-foraging activities in *H. pulchella*, nor did *Arhysoceble picta* when it visited flowers of *K. brasiliensis*. Legitimate visits in these plant taxa were observed only when oil-collecting bees collected both oil and pollen. Pollen-collecting behaviour by oil-collecting bees was observed only in combination with oil-collecting behaviour (Figs 2, 3). Oil-collecting bees visited *H. pulchella* and *K. brasiliensis* exclusively to collect oil or to collect both oil and pollen, but never to collect pollen only (Table 1).

Oil-collecting bees first landed on the inner tepals of *Cypella* flowers, heading towards the distal part of the inner tepals, where the trichomes are localized. The bees contacted the reproductive organs while gathering the oil resource; consequently, pollen was attached on their anterior head and sometimes removed by bees and deposited in the scopes. Pollen-collecting bees (Halictidae) landed directly on reproductive organs of *Cypella* and collected pollen actively from the anthers. We recorded two different behaviours of oil-collecting bees on *H. pulchella* and *K. brasiliensis*: first, they landed on the outer or inner tepals of the species, respectively, gathering only the oil resource without making contact with the reproductive organs. However, during some visits, immediately after oil foraging they landed on the reproductive structure to collect pollen actively from the anthers, behaving exactly like the pollen-collecting bees we observed (Fig. 2).

Table 1. Visitation frequency for each bee species and floral reward collected for the seven taxa of Iridaceae studied

Taxa/species (authors)		Floral reward	*Cypella herbertii* subsp. *herbertii*	*Cypella herbertii* subsp. *brevicristata*	*Cypella amplimaculata*	*Cypella pusilla*	*Kelissa brasiliensis*	*Herbertia pulchella*	*Calydorea alba*
Apinae*/ Tapinotaspidini*	*Arhysoceble picta* (Friese, 1899)	Oil	**0.242 ± 0.054**	**0.800 ± 0.198**	**0.133 ± 0.036**	0.200 ± 0.021	0.052 ± 0.012*	**0.293 ± 0.081***	–
	Arhysoceble picta	Oil + pollen	**0.443 ± 0.078**	**0.260 ± 0.091**	**0.225 ± 0.104**	–	0.050 ± 0.016	**0.119 ± 0.044**	–
	Chalepogenus muelleri (Friese, 1899)	Oil	0.088 ± 0.029	**0.133 ± 0.021**	–	–	–	0.100 ± 0.000	–
	Chalepogenus muelleri	Oil + pollen	–	–	–	–	–	–	–
	Chalepogenus goeldianus (Friese, 1899)	Oil	–	–	–	**0.149 ± 0.016**	–	–	–
	Caenonomada bruneri (Ashmead, 1899)	Oil	**0.333 ± 0.000**	–	–	–	–	–	–
Apinae	*Apis mellifera* (Linnaeus, 1758)	Pollen	–	–	–	–	**0.233 ± 0.100**	**0.529 ± 0.196**	**0.316 ± 0.023**
	Trigona spinipes (Fabricius, 1793)	Pollen	–	–	–	–	–	**0.102 ± 0.026**	–
	Mourella caerulea (Friese, 1900)	Pollen	–	–	–	–	–	**0.178 ± 0.070**	–
Halictinae	*Augochlorella urania* (Smith, 1853)	Pollen	**0.162 ± 0.067**	**0.244 ± 0.058**	–	–	–	–	–
	Augochlorella iopoecila (Moure, 1950)	Pollen	–	–	–	–	**0.133 ± 0.000**	0.033 ± 0.000	–
	Augochlorella ephyra (Schrottky, 1910)	Pollen	–	–	0.067 ± 0.000	–	0.017 ± 0.000	–	–
	Augochlora amphitrite (Schrottky, 1909)	Pollen	–	**0.117 ± 0.083**	–	–	–	–	**0.258 ± 0.240**
	Augochloropsis sp. 1	Pollen	–	–	**0.100 ± 0.000**	–	–	0.067 ± 0.000	–
	Dialictus sp. 1	Pollen	–	–	**0.133 ± 0.000**	–	–	**0.133 ± 0.000**	–
	Dialictus sp. 2	Pollen	–	–	–	–	–	0.067 ± 0.000	–
	Dialictus sp. 3	Pollen	–	–	–	**0.107 ± 0.031**	–	–	**0.583 ± 0.083**
	Pseudagapostemon aff. *pruinosus*	Pollen	–	–	–	–	**0.142 ± 0.034**	0.067 ± 0.016	–
Colletinae	*Bellopria* sp. 1	Pollen	–	–	–	–	0.079 ± 0.020	0.053 ± 0.008	–
Andreninae	*Anthrenoides micans* (Urban, 1995)	Pollen	–	–	–	–	0.033 ± 0.000	0.044 ± 0.011	–
	Rhophitulus sp. 1	Pollen	–	–	–	–	–	0.091 ± 0.024	–
	Rophitulus guaraniticus (Schlindwein & Moure, 1998)	Pollen	–	–	–	–	–	**0.125 ± 0.075**	–

Data are means ± SE. Visitation rates > 0.1 are in bold.
*Bee did not contact the reproductive structures during foraging behaviour.

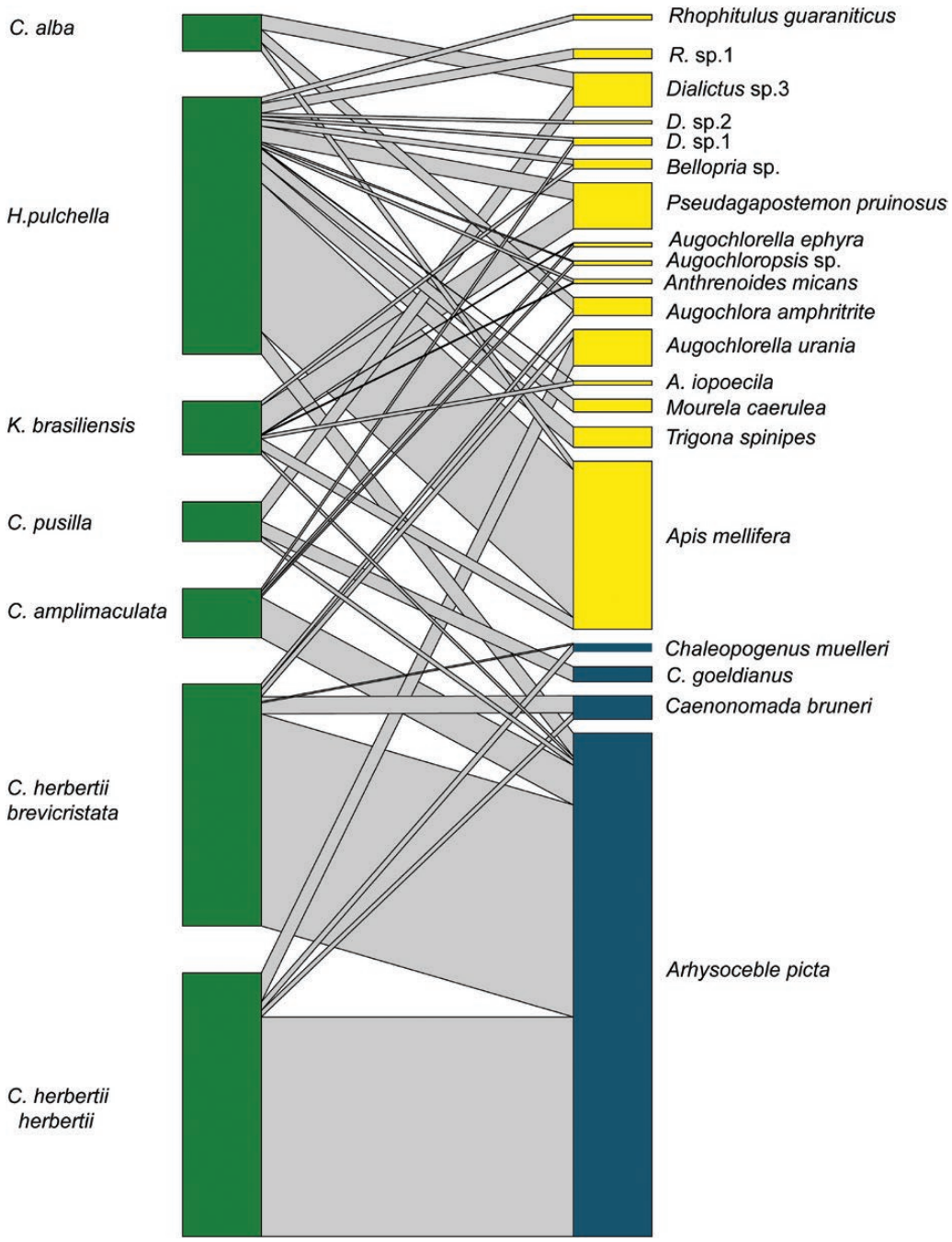

Figure 1. Network representation of plant–pollinator interactions observed. Plant species are indicated on the left by green bars and pollinators on the right by blue bars for oil-collecting bees and yellow bars for pollen-collecting bees. Grey lines represent species interactions, and the line thickness indicates the relative frequency of each interaction.

The frequency of visitation differed significantly, not only between bee functional groups for a given plant species, but also among plant species of a given functional group ($\chi^2 = 402.11$, d.f. = 7, $P < 0.001$). The frequency of flower visitation was significantly different between oil-collecting bees and pollen-collecting bees for *Cypella herbertii* subsp. *herbertii* ($Z = -15.53$, $P < 0.01$; Fig. 4), *Cypella herbertii* subsp. *brevicristata* ($Z = -10.80$, $P < 0.01$), *Cypella amplimaculata* ($Z = -8.35$, $P < 0.01$) and *H. pulchella* ($Z = 3.67$, $P < 0.01$). Among plant species, the visitation frequency of oil-collecting bees was higher for *Cypella herbertii* subsp. *brevicristata* when compared with *Cypella herbertii* subsp. *herbertii* ($Z = -9.04$, $P < 0.01$),

Figure 2. Illustration of distinct behaviour of *Arhysoceble picta*. A, inefficient oil-collecting foraging in *Herbertia pulchella* (left) and pollen-collecting foraging (right). Note that only during pollen-collecting activity does the bee contact the reproductive organs of plant species. B, efficient oil-collecting foraging in *Cypella herbertii*.

Cypella amplimaculata ($Z = -7.35$, $P < 0.01$), *Cypella pusilla* ($Z = -14.34$, $P < 0.01$), *K. brasiliensis* ($Z = -9.62$, $P < 0.01$) and *H. pulchella* ($Z = -12.19$, $P < 0.01$).

The proportion of flowers that set fruit was dependent on pollination treatment for four taxa (*Cypella herbertii* subsp. *herbertii*, $\chi^2 = 7.36$, d.f. = 2, $P = 0.02$; *Cypella pusilla*, $\chi^2 = 37.94$, d.f. = 4, $P < 0.01$; *K. brasiliensis*, $\chi^2 = 13.73$, d.f. = 1, $P < 0.01$; *H. pulchella*, $\chi^2 = 4.61$, d.f. = 1, $P = 0.031$). In contrast, pollination treatment did not significantly impact fruit set in *Cypella herbertii* subsp. *brevicristata* ($\chi^2 = 3.43$, d.f. = 2, $P = 0.179$), *Cypella amplimaculata* ($\chi^2 = 4.91$, d.f. = 2, $P = 0.08$) and *Calydorea alba* ($\chi^2 = 3.63$, d.f. = 1, $P = 0.05$; Table 2).

Fruit set from natural pollination (Fig. 5) differed significantly among plant taxa ($\chi^2 = 40.801$, d.f. = 6, $P = 0.003$). The highest value of fruit set from natural pollination was observed for *Cypella amplimaculata* (61.3%), which differed significantly from that found for *Cypella herbertii* subsp. *herbertii* (50.9%), *Cypella herbertii* subsp. *brevicristata* (30%), *Cypella pusilla* (17.4% in April and 29% in November), *H. pulchella* (40%) and *Calydorea alba* (20%).

The results of controlled pollination experiments showed that all taxa of *Cypella* studied were partly self-incompatible ($0.20 < ISI < 0.80$). However, all plant species sampled were pollinator dependent, and no evidence of spontaneous self-pollination was

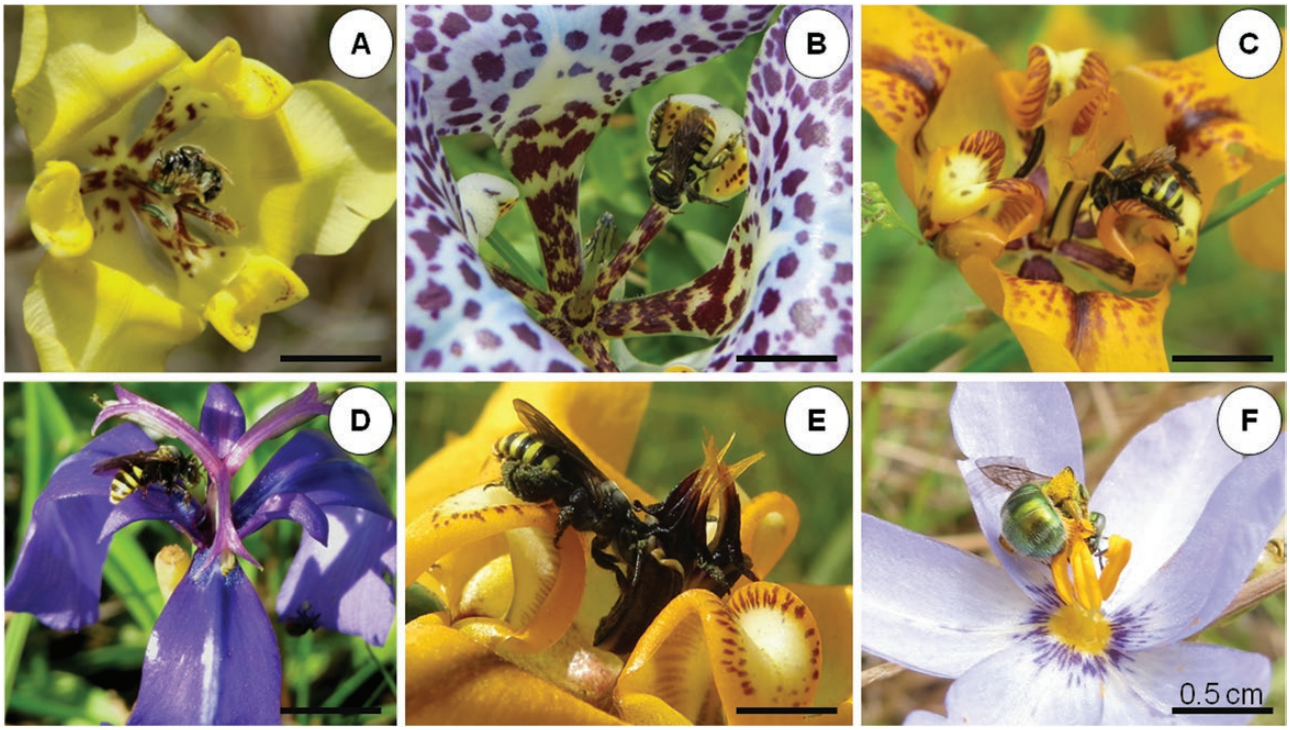

Figure 3. Plant–bee interactions observed. A, *Dialictus* sp. collecting pollen on *Cypella pusilla*. B–E, *Arhysoceble picta* collecting oil on *Kelissa brasiliensis*, *Cypella amplimaculata*, *Herbertia pulchella* and *Cypella herbertii*, respectively. F, *Augochlora amphritite* collecting pollen on *Calydorea alba*. Note that in pictures B and D, the oil-collecting bee *Arhysoceble picta* does not contact the reproductive organs of *K. brasiliensis* and *H. pulchella*.

found for any species included in our study (Table 3). Fruit set from hand self-pollination was significantly different among species ($\chi^2 = 12.529$, d.f. = 3, $P = 0.005$; Table 3; Supporting Information, Appendix S3). The highest values of fruit set from hand self-pollination were observed for *Cypella herbertii* subsp. *herbertii* (65.2%) and *Cypella amplimaculata* (42.4%), whereas *K. brasiliensis*, *H. pulchella* and *Calydorea alba* were strongly self-incompatible (ISI > 0.80).

Pollen load

A total of 83 pollen types were identified in pollen loads of visitors to Iridaceae; 29 pollen types were identified from oil-collecting bees and 54 from pollen-collecting bees (Supporting Information, Appendices S4, S5 and S6). Among oil-collecting bees, 53.57% of the pollen load was from *Cypella* (dominant pollen type), 19.16% from *Sisyrinchium* (accessory pollen type), 14.07% from *Kelissa* (isolated important), 4.85% from *Herbertia* (isolated occasional) and 8.37% from other plant families. Pollen from *Herbertia* (49.18%) was predominant in the pollen load recovered from pollen-bees, followed by *Kelissa* (21%), *Cypella* (5.7%), *Calydorea* (2.6%) and *Sisyrinchium* (1.1%); however, 24.1% of the pollen load was from other plant families (Fig. 6). There was no significant difference between the number of pollen grains carried by oil- and pollen-collecting bees of *Cypella* ($W = 38$, $P = 0.201$), *Kelissa* ($W = 10$, $P = 0.90$) and *Sisyrinchium* ($W = 37$, $P = 0.164$). However, the number of *Herbertia* pollen grains carried by pollen-collecting bees was significantly higher ($W = 46$, $P = 0.004$).

According to *F*-score analysis, *Cypella* spp. revealed a specialized pollination system based on oil-bees, which contributed 86.48% of the pollen transfer, whereas pollen-collecting bees were responsible for only 13.52%. For *K. brasiliensis*, 73.33% of the pollen transfer was from pollen-collecting bees and 26.66% from oil-collecting bees (Fig. 6), with an *F*-score value indicating contributions to total pollen transfer from both functional groups. Pollen transfer for *H. pulchella* was dependent on pollen-collecting bees for 95.49% and oil-collecting bees for 4.51%, indicating a greater contribution by generalist pollen-collecting bees (Fig. 7).

DISCUSSION

In the present study, the breeding system, visitation frequency, pollinator behaviour and pollen load were

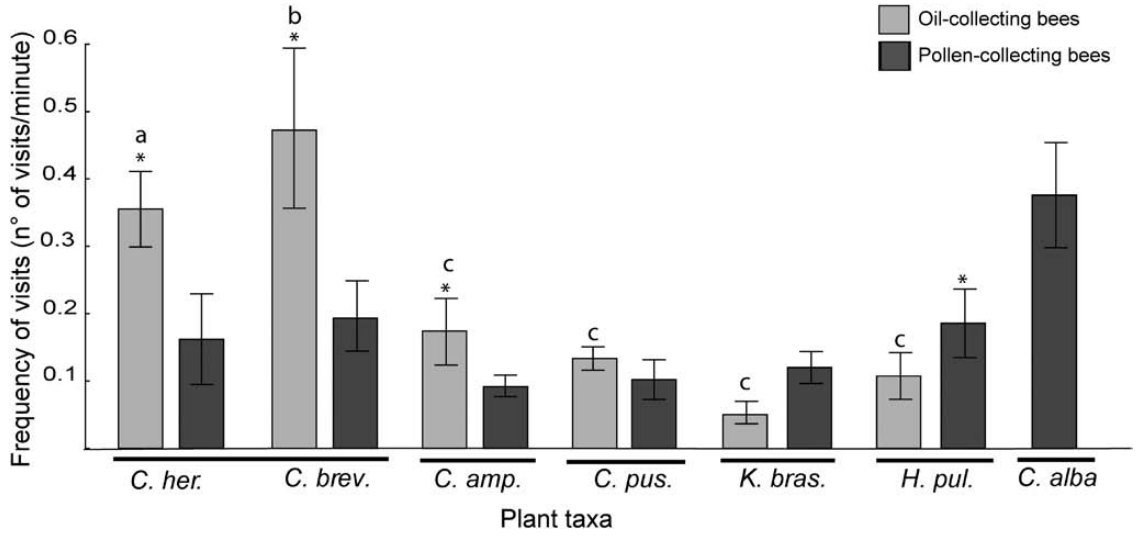

Figure 4. Visitation frequency (mean ± SE) of oil- and pollen-collecting functional groups of bees for each taxa studied. Light and dark grey bars represent oil- and pollen-collecting bees, respectively. Species names are abbreviated as follows: *C. amp.*, *Cypella amplimaculata*; *C. brev.*, *Cypella herbertii* subsp. *brevicristata*; *C. her.*, *Cypella herbertii* subsp. *herbertii*; *H. pul.*, *Herbertia puchella*; *K. bras.*, *Kelissa brasiliensis*. *Significant differences in visitation frequency between functional groups on *C. herbertii* subsp. *herbertii* ($W = 138$, $P = 0.034$). Letters indicate significant differences in visitation frequency of oil-collecting bees between *Cypella herbertii* subsp. *herbertii* and *H. pulchella* ($\chi^2 = 20.6$, d.f. = 5, $P < 0.01$). Significant differences among cross-pollination and natural pollination of two flowering periods in *Cypella pusilla*.

used to improve knowledge about plant–pollinator interactions among taxa of Tigridieae in South Brazil. New insights into aspects of the pollination systems in this tribe are provided and suggest a continuum of the importance of oil-collecting bees as pollinators of the studied taxa.

Oil is considered a specialized type of reward, because other animals are not attracted by this reward and it is collected by only a few groups of bees in Mellitinae and Apinae (Armbruster, 2017). In contrast to the expected strong functional-group specialization between oil-producing plants and oil-collecting bees, the findings of the present study suggest a more flexible pollination system and a continuum of specialization. Contrary to our expectations, this continuum emerges from highly specialized taxa to generalized taxa as follows: *Cypella* spp., mainly pollinated by three species of oil-collecting bees; *K. brasiliensis*, with a bimodal pollination system having contributions from both oil- and pollen-collecting bees; *H. pulchella*, mainly pollinated by multiple species of pollen-collecting bees although it secretes oil; and *Calydorea alba*, which is pollinated exclusively by pollen-collecting bees.

Floral visitors

This study provides the first record of specialized oil-collecting bees as pollinators of *Cypella pusilla* and *K. brasiliensis*. Previous studies have reported low frequencies or a complete absence of specialized oil-collecting bees visiting Iridaceae (Schlindwein, 1998; Devoto & Medan, 2004; Pinheiro *et al.*, 2008; Oleques *et al.*, 2016). Schlindwein (1998) recorded visits by oil-collecting bees to *Cypella herbertii* (*Chalepogenus muelleri* and *Caenonomada brunerii*) and *H. pulchella* (*Chalepogenus* sp.) in the Serra do Sudeste region. In addition to the oil-collecting bees of the tribe Tapinotaspidini, the majority of bee species recorded in the present study were small solitary bees of the subfamilies Halictinae, Colletinae and Adreninae. Three species of social bees were recorded: *Apis mellifera* and the stingless bees *Trigona spinipes* and *Mourella caerulea*.

Our observations showed that all visits from oil-collecting bees were legitimate in *Cypella* spp., with contact between the body of bees and the surface of anthers and stigmas. In contrast, oil-collecting behaviour in *H. pulchella* and *K. brasiliensis* did not result in pollen deposition on the body of the bees or contact with reproductive structures in the flowers. Nevertheless, we did not observe pollen-collecting behaviour in the absence of oil-collecting behaviour, which indicates that oil is an important reward for attracting oil-collecting bees in flowers of *K. brasiliensis* and *H. pulchella* (Schaffler *et al.*, 2015). These results have important significance, because distinct behaviour of functional groups of pollinators can apply

Table 2. Pollination breeding systems of seven plant taxa: Akaike information criterion (AIC), deviance (G2), degrees of freedom (d.f.) and deviance significance of general linear mixed-effects model analysis

Plant taxon	Model (μ)	AIC	Deviance (G2)	d.f.	Deviance significance
Cypella herbertii subsp. *herbertii*	Result ~ Treatment (2)	130.52	7.365	2	0.025*
	Result ~ Individuals (2)	143.14			
	Result ~ Treatment + Individuals (3)	142.14			
Cypella herbertii subsp. *brevicristata*	Result ~ Treatment (2)	189.39	3.433	2	0.179
	Result ~ Individuals (2)	276.00			
	Result ~ Treatment + Individuals (3)	276.00			
Cypella amplimaculata	Result ~ Treatment (2)	109.80			
	Result ~ Individuals (2)	216.26	4.917	2	0.085
	Result ~ Treatment + Individuals (3)	214.00			
Cypella pusilla	Result ~ Treatment (2)	164.52			
	Result ~ Individuals (2)	218.50	37.94	4	< 0.001*
	Result ~ Treatment + Individuals (3)	266.38			
Kelissa brasiliensis	Result ~ Treatment (2)	90.24			
	Result ~ Individuals (2)	232.77	13.703	1	< 0.001*
	Result ~ Treatment + Individuals (3)	232.77			
Herbertia pulchella	Result ~ Treatment (2)	137.65			
	Result ~ Individuals (2)	191.15	4.618	1	0.031*
	Result ~ Treatment + Individuals (3)	191.15			
Calydorea alba	Result ~ Treatment (2)	77.070			
	Result ~ Individuals (2)	170.77	3.630	1	0.056
	Result ~ Treatment + Individuals (3)	172.00			

Numbers in parentheses indicate the number of estimated parameters.
*Significant results considering $P < 0.05$.

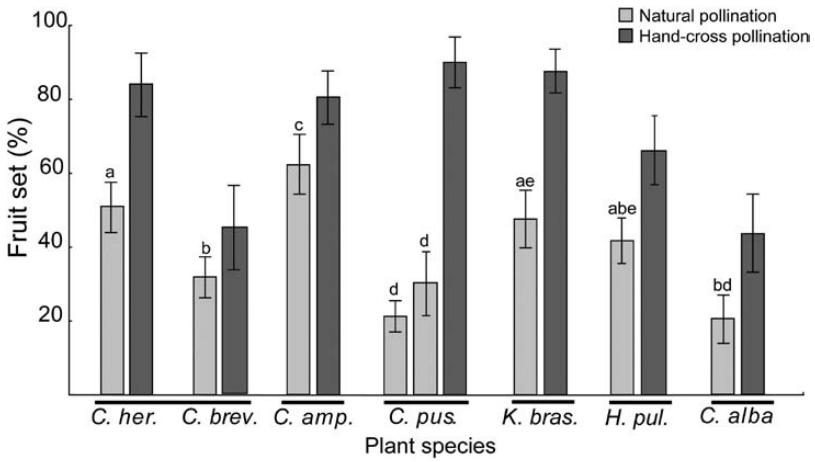

Figure 5. Fruit set (mean ± SE) of natural pollination (light grey) and hand cross-pollination (dark grey) tests. Different letters indicate differences in fruit set from natural pollination among species ($P < 0.05$). The two light grey bars for *Cypella pusilla* represent the fruit set in April and November, respectively.

distinct selective pressures on plant fitness (Ne'eman *et al.*, 2010; King *et al.* 2013; Van der Niet *et al.* 2014). In the long term, such differences in the contributions of oil-collecting and non-oil-collecting bees could result in changes in floral traits and the evolution of reward-secreting structures (Ferreiro *et al.*, 2019).

Table 3. Fruit production in seven taxa of Tigrideae after different controlled pollination treatments, and associated breeding system characteristics

Plant species	Percentage fruit set (*N*) per treatment					
	Natural pollination		Hand cross-pollination	Hand self-pollination	ISI*	Spontaneous self-pollination
Cypella herbertii subsp. *herbertii*	50.9 (55)		84.2 (19)	65.2 (23)	0.23, pSI	0.0 (13)
Cypella herbertii subsp. *brevicristata*	30.0 (100)		50.0 (20)	19.7 (20)	0.61, pSI	0.0 (28)
Cypella amplimaculata	61.3 (31)		80.9 (22)	42.4 (25)	0.48, pSI	0.0 (25)
Cypella pusilla†	April 17.4 (63)	November 29.0 (31)	90.0 (20)	27.7 (36)	0.69, pSI	0.0 (26)
Kelissa brasiliensis	47.6 (42)		87.5 (32)	0.0 (30)	1.0, SI	–
Herbertia pulchella	40.0 (70)		63.3 (30)	0.0 (28)	1.0, SI	–
Calydorea alba	20.5 (39)		43.4 (23)	0.0 (25)	1.0, SI	–

A dash indicates that an experiment was not conducted because of the absence of self-pollination.
*Index of self-incompatibility (pSI, partly self-incompatible; SI, self-incompatible).
†Natural pollination measured from both flowering periods.

Illegitimate visits can be the result of a morphological mismatch between the plant and the insect visitor (Stout, 2007; Ne'eman *et al.*, 2010; King *et al.*, 2013; Ruchisansakun *et al.*, 2016; Palacios *et al.*, 2019). Thus, an insect attracted to a flower by a specific reward, such as oil, might still fail to be a pollinator if it does not contact the anthers and/or does not contact the stigmas (Armbruster, 2017). Illegitimate visits of oil-collecting bees to oil flowers have been documented, especially in Malpighiaceae and Plantaginaceae (Sigrist & Sazima, 2004; Martins & Alves-dos-Santos, 2013). In addition, our study showed that Tapinotaspidini bees should not always be considered as pollinators during oil-collecting foraging in Iridaceae.

Breeding system

Specialized pollination systems could be greatly affected by lack of service by a pollinator, resulting in a decrease in their reproductive output, leading to a transition towards generalized pollination or, more commonly, a transition towards self-pollination (Moeller, 2005). Our results showed that *Cypella* spp. are partly self-incompatible and pollinator dependent, whereas *K. brasiliensis*, *H. pulchella* and *Calydorea alba* are totally self-incompatible. Self-incompatibility has been documented for *H. pulchella* and other species within *Herbertia* (Stiehl-Alves et al., 2017), whereas self-compatibility has been documented for *Cypella herbertii* (Devoto & Medan, 2004). Our study also provides the first evidence of the breeding system for *K. brasiliensis* and *Calydorea alba* and of self-compatibility for *Cypella pusilla*.

Although self-compatibility may have negative impacts on fitness as a result of inbreeding depression, it is especially important when conditions for outcrossing are unfavourable owing to an absence of mates or effective pollinators (Waser, 2006). Self-incompatibility could increase the risks of pollen limitation in specialized pollination systems and therefore intensify the risk of local extinction (Knight *et al.*, 2005). Nevertheless, the self-compatibility observed for *Cypella* spp. could be related to the high degree of specialization detected for their pollination system (Fenster & Martén-Rodríguez, 2007). In contrast, species with bimodal or more generalist pollination systems, such as *K. brasiliensis*, *H. pulchella* and *Calydorea alba*, are self-incompatible and could have lower risks of pollination limitation when compared with specialized species (Wolowski *et al.*, 2014).

Furthermore, the diversity and importance of non-oil-collecting bees as pollinators of *H. pulchella* was documented and suggests that further studies on the efficiency of pollination should be conducted in order to detect the true contribution of oil- and non-oil-collecting bees to plant reproductive output along the specialization–generalization continuum.

Pollen load

Our results for pollen load of the two functional groups of bees showed that oil-collecting bees carry mostly pollen types from Iridaceae (91.63%); however, the proportion of pollen load coming from this family was also especially significant for pollen-collecting bees (75.9%), whereas only 24.1% came from other plant

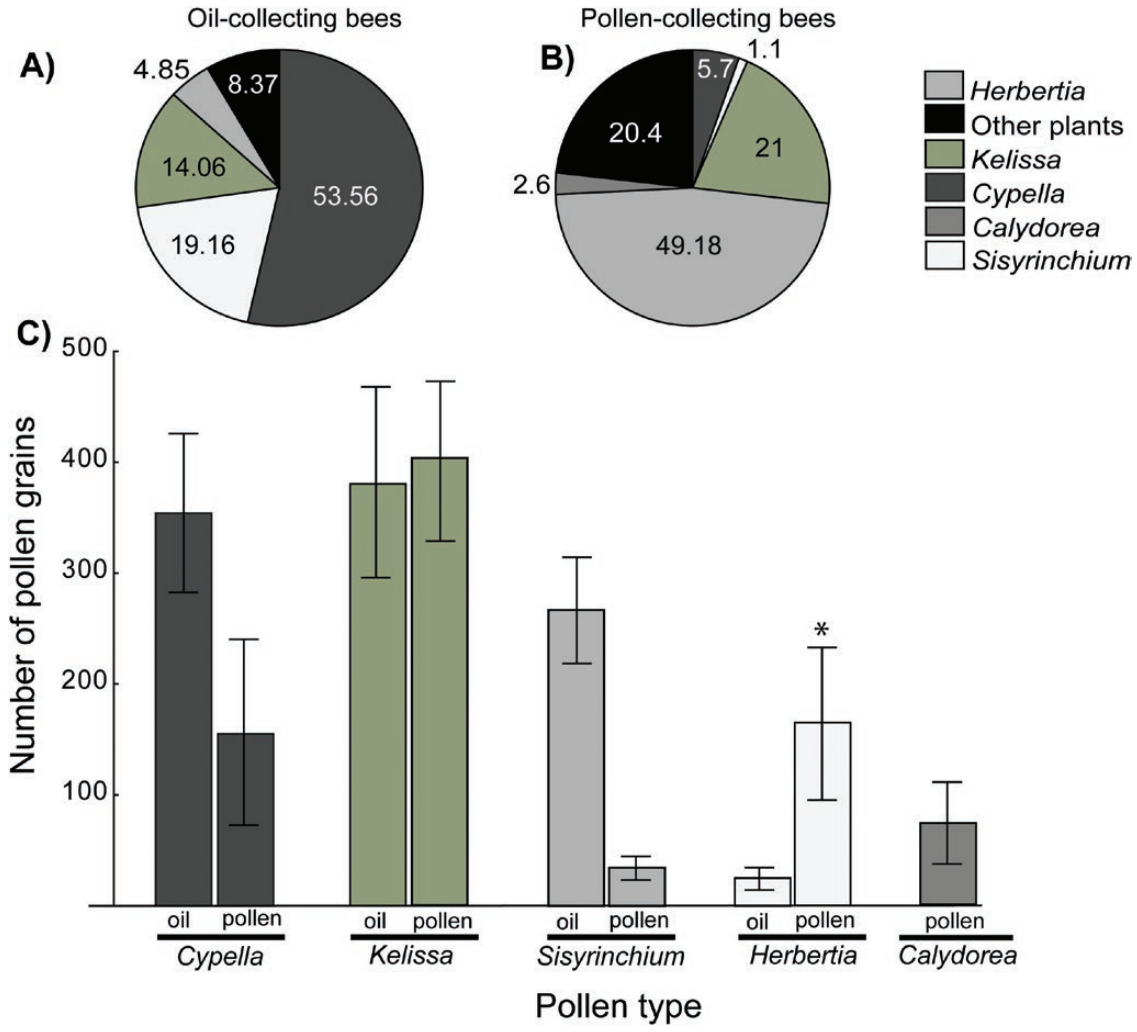

Figure 6. Results from pollen load analysis. A, percentages of pollen grains found in the oil-collecting functional group from each Iridaceae pollen type and other plant families. B, percentages of pollen grains found in the pollen-collecting functional group from Iridaceae and other plant families. C, number of pollen grains (mean ± SE) carried by oil- and pollen-collecting bees per plant genus.

families, such as Asteraceae, Solanaceae, Myrtaceae and Fabaceae. The strong attraction that oil-collecting bees have for oil-producing plant taxa could be explained by the presence of certain chemicals in the oil, mainly diacetin (Schaeffler *et al.*, 2015); however, further studies are needed to elucidate the chemical composition of the oil of Iridaceae and whether oil-collecting bees of the subfamily Apinae exhibit a similar positive response to diacetin.

Pollination systems permanently balancing on the specialization–generalization continuum and novel rewards, produced by plants to be collected by distinct functional groups of pollinators, are especially interesting because they can promote diversification through specialized plant–pollinator interactions (Armbruster & Baldwin, 1998; Waser *et al.*, 2006; Igic *et al.*, 2008; Armbruster, 2017). Our results showed that *Cypella* spp. were mainly visited by oil-collecting bees, and the pollen load results corroborated the contribution of specialized bees to the pollen transfer of the genus. Pollen transfer of *K. brasiliensis* depends on both functional groups, which visit their flowers in equal frequency, indicating a bimodal pollination system (Monty, *et al.* 2006; Shuttleworth & Johnson, 2008). The high pollen load of *K. brasiliensis* carried by oil-collecting bees results from intentional pollen collecting. Although both bee functional groups contributed complementarily to pollen transfer for *K. brasiliensis*, this bimodal pollination system includes a high level of pollen transfer by oil-collecting bees, which might have been an important factor in the evolutionary history of *K. brasiliensis* by promoting

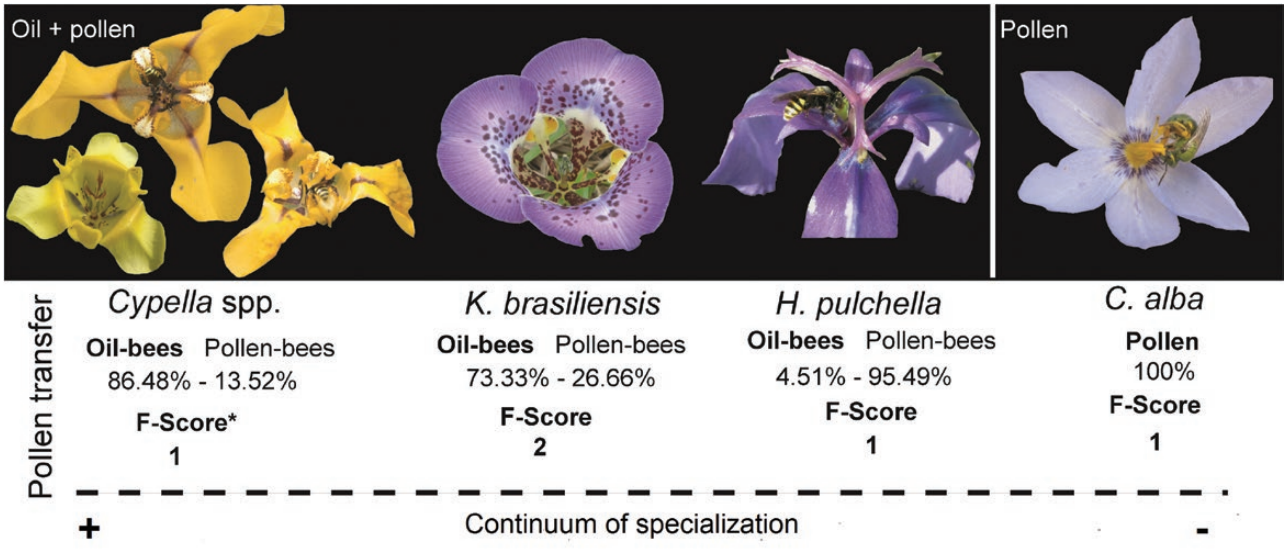

Figure 7. Diagram illustrating pollen transfer and the *F*-score for each genus along with the specialization–generalization continuum. Pollen flow (as a percentage) indicates the combination between visitation frequency data and pollen load from the bees' bodies. The *F*-score from each taxon represents the number of functional groups that contribute to > 75% of the pollen flow. Oil + pollen and Pollen indicate the type of reward available.

further floral trait displacement. Bimodal pollination systems are common, and pollination shifts are assumed to be one of the driving forces for speciation by promoting reproductive isolation in distinct plant populations (Goldblatt & Manning, 2006; Anderson *et al.*, 2009, 2016; Johnson, 2010; Ferreiro *et al.*, 2019).

Contrary to our expectation, pollen-collecting bees had a higher visitation frequency for *H. pulchella* than oil-collecting bees, assuring the pollen transfer in this species, which indicates a less specialized pollination system (Freitas & Sazima, 2003). Specialized pollination systems are most likely to develop when efficient pollinators are present (Stebbins, 1970), whereas a reduction in specialization is favoured when pollinator availability or behaviour is unpredictable (Waser *et al.*, 1996). Competition is another factor that could play an important role in the evolution of plant–pollinator interactions (Muchhala & Potts, 2007). Some of the species studied, especially *K. brasiliensis*, *Cypella amplimaculata* and *H. pulchella*, are sympatric, and all the species studies, except *Cypella pusilla*, have populations co-occuring with other oil-producing species, such as *Sisyrinchium* spp. and *Herbertia lahue*. Given that pollination is critical to plant reproduction, competition for pollinators might be expected to put strong selective pressures on coexisting species (Muchhala & Potts, 2007). When species share the same guilds of pollinators, visitation patterns can lead to competition, and competition can be reduced by specializing in different pollinators (Armbruster *et al.*, 1994). The continuum of specialization–generalization evidenced by our study could be related to distinct strategies by plants to minimize the negative effects of competition by specialized oil-bees.

Conclusions

Taken together, our results contribute to a better understanding of specialized interactions between oil-collecting bees and oil-flowers of Iridaceae. Our findings reveal a continuum in the contribution of oil-bees to the pollination system of the studied taxa. Oil-collecting bees were observed foraging in all oil-secreting plant species sampled for the present study. However, they acted as thieves in *K. brasiliensis* and *H. pulchella*, visiting flowers to collect oil without contacting the reproductive structures. Both observations of foraging behaviour and analyses of pollen load revealed that oil-bees can switch to illegitimate visitors during floral oil collection, depending on the genus studied.

ACKNOWLEDGEMENTS

The authors are grateful to Drs Kelli Ramos, Mardiore Pinheiro and Antonio Aguiar for the taxonomic identification of bee species, to Rubem Samuel de Avila Jr for support with the fieldwork, to Gabriel Martins for Figure 1A, Roberta Radaeski for Supporting Information, Appendix S2, Aline Anjos for support with pollen analysis, and Pollination Research Laboratory members (University of KwaZulu-Natal, South Africa), especially Ruth Cozien, for the criticism and support to improve the statistical analysis and discussion of

the manuscript. We also thank Mariana Tadey and one anonymous reviewer for their helpful comments. S.S.O. is thankful to the Programa de Pós-Graduação em Botânica (Universidade Federal do Rio Grande do Sul) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support and institutional scholarship (finance code 001) and grant (US$1000) from Neotropical Grassland Conservancy. O.C. received a postdoctoral fellowship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). T.T.S.-C. acknowledges CNPq for the fellowship received (grant number 304506/2016–8).

REFERENCES

Anderson B, **Alexandersson R**, **Johnson SD. 2009.** Evolution and coexistence of pollination ecotypes in an African *Gladiolus* (Iridaceae). *Evolution* **64:** 960–972.

Anderson B, **Pauw A**, **Cole WW**, **Barrett SC. 2016.** Pollination, mating and reproductive fitness in a plant population with bimodal floral-tube length. *Journal of Evolutionary Biology* **29:** 1631–1642.

Anderson GJ, **Johnson SD**, **Neal PR**, **Bernardello G. 2002.** Reproductive biology and plant systematics: the growth of a symbiotic association. *Taxon* **51:** 637–653.

Armbruster WS. 2014. Floral specialization and angiosperm diversity: phenotypic divergence, fitness trade-offs and realized pollination accuracy. *AoB PLANTS* **6:** plu003.

Armbruster WS. 2017. The specialization continuum in pollination systems: diversity of concepts and implications for ecology, evolution and conservation. *Functional Ecology* **31:** 88–100.

Armbruster WS, **Baldwin BG. 1998.** Switch from specialized to generalized pollination. *Nature* **394:** 632.

Armbruster WS, **Edwards ME**, **Debevec EM. 1994.** Floral character displacement generates assemblage structure of Western Australian triggerplants (*Stylidium*). *Ecology* **75:** 315–329.

Barrett S. 2010. Understanding plant reproductive diversity. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365:** 99–109.

Barrett S. 2013. The evolution of plant reproductive systems: how often are transitions irreversible? *Proceedings of the Royal Society B: Biological Sciences* **280:** 20130913.

Bolker B, **R Development Team**. **2008.** *bbmle: tools for general maximum likelihood estimation. R package version 1.0.20*. Available at: https://cran.r-project.org/web/packages/bbmle/index.html

Chauveau O, **Eggers L**, **Souza-Chies TT**, **Nadot S. 2012.** Oil-producing flowers within the Iridoideae (Iridaceae): evolutionary trends in the flowers of the New World genera. *Annals of Botany* **110:** 713–729.

Dafni A, **Kevan PG**, **Husband BC. 2005.** *Practical pollination biology*. Ontario: Enviroquest Ltd.

Devoto M, **Medan D. 2004.** Effects of grazing disturbance on the reproduction of a perennial herb, *Cypella herbertii* (Lindl.) Herb. (Iridaceae). *Plant Systematics and Evolution* **243:** 165–173.

Erdtman G. 1952. *Pollen morphology and plant taxonomy - Angiosperms*. Stockholm: Almqvist & Wiksell.

Eggers L, **Gil A**, **Lovo J**, **Chukr N. 2015.** *Iridaceae*. In *Lista de Espécies da Flora do Brasil*. Jardim Botânico do Rio de Janeiro. Available at: http://floradobrasil.jbrj.gov.br

Fenster CB, **Armbruster SW**, **Wilson P**, **Dubash MR. 2004.** Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* **35:** 375–403.

Fenster CB, **Martén-Rodríguez S. 2007.** Reproductive assurance and the evolution of pollination specialization. *International Journal of Plant Sciences* **168:** 215–228.

Ferreiro G, **Baranzeli MC**, **Sérsic AN**, **Cocucci AA. 2019.** Patterns of phenotypic selection for oil and nectar in *Monttea aphylla* (Plantaginaceae) in a geographic mosaic of interactions with pollinators. *Flora* **232:** 47–55.

Foxe JP, **Slotte T**, **Stahl EA**, **Neuffer B**, **Hurka H**, **Wright SI. 2009.** Recent speciation associated with the evolution of selfing in *Capsella*. *Proceedings of the National Academy of Sciences of the United States of America* **106:** 5241–5245.

Freitas L, **Sazima M. 2003.** Floral biology and pollination mechanisms in two *Viola* species—from nectar to pollen flowers? *Annals of Botany* **91:** 311–317.

Goldblatt P, **Manning JC. 2006.** Radiation of pollination systems in the Iridaceae of sub-Saharan Africa. *Annals of Botany* **97:** 317–344.

Goldblatt P, **Rodriguez A**, **Powell MP**, **Davies TJ**, **Manning JC**, **Van der Bank M**, **Savolainen V. 2008.** Iridaceae 'Out of Australasia'? Phylogeny, biogeography, and divergence time based on plastid DNA sequences. *Systematic Botany* **33:** 495–508.

Igic B, **Lande R**, **Kohn JR. 2008.** Loss of self-incompatibility and its evolutionary consequences. *International Journal of Plant Sciences* **169:** 93–104.

Johnson SD. 2010. The pollination niche and its role in the diversification and maintenance of the southern African flora. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365:** 499–516.

King C, **Ballantyne G**, **Willmer PG. 2013.** Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology and Evolution* **4:** 811–818.

Knight TM, **Steets JA**, **Vamosi JC**, **Mazer SJ**, **Burd M**, **Campbell DR**, **Dudash MR**, **Johnston MO**, **Mitchell RJ**, **Ashman TL. 2005.** Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution, and Systematics* **36:** 467–497.

Krakos K, **Fabricant SA. 2014.** Generalized versus specialized pollination systems in *Oenothera* (Onagraceae). *Journal of Pollination Ecology* **14:** 235–243.

Lloyd DG. 1965. Evolution of self-compatibility and racial differentiation in *Leavenworthia* (Cruciferae). *Contributions from Gray Herbarium of Harvard University* **195:** 3–134.

Louveaux J, **Maurizio A**, **Vorwohl G. 1978.** Methods of melissopalynology. *Bee World* **59:** 139–157.

Manning JC, **Goldblatt P. 2002.** The pollination of *Tritoniopsis parviflora* (Iridaceae) by the oil-collecting bee *Rediviva gigas*

(Hymenoptera: Melittidae): the first record of oil-secretion in African Iridaceae. *South African Journal of Botany* **68:** 171–176.

Martins AC, **Alves-dos-Santos I. 2013.** Floral-oil-producing Plantaginaceae species: geographical distribution, pollinator rewards and interactions with oil-collecting bees. *Biota Neotropica* **13:** 77–89.

Martins AC, **Melo GAR**, **Renner SS. 2015.** Gain and loss of specialization in two oil-bee lineages, *Centris* and *Epicharis* (Apidae). *Evolution* **69:** 1835–1844.

Ministério do Meio Ambiente. **2007.** *Áreas prioritárias para a conservação, uso sustentável e repartição de benefícios da biodiversidade brasileira: Atualização-Portaria MMA n° 09, de 23 de Janeiro de 2007***.** Brasil. Available at: http://www.mma.gov.br/estruturas/chm/_arquivos/biodiversidade31.pdf

Moeller DA, **Geber MA. 2005.** Ecological context of the evolution of self-pollination in *Clarkia xantiana*: population size, plant communities, and reproductive assurance. *Evolution* **59:** 786–799.

Monty A, **Saad L**, **Mahy G. 2006.** Bimodal pollination system in rare endemic *Oncocyclus irises* (Iridaceae) of Lebanon. *Canadian Journal of Botany* **84:** 1327–1338.

Muchhala N, **Potts MD. 2007.** Character displacement among bat-pollinated flowers of the genus *Burmeistera*: analysis of mechanism, process and pattern. *Proceedings of the Royal Society B: Biological Sciences* **274:** 2731–2737.

Ne'eman G, **Jürgens A**, **Newstrom-Lloyd L**, **Potts SG**, **Dafni A. 2010.** A framework for comparing pollinator performance: effectiveness and efficiency. *Biological reviews of the Cambridge Philosophical Society* **85:** 435–451.

Oleques SS, **Overbeck GE**, **Avila RS Jr**. **2016.** Flowering phenology and plant-pollinator interactions in a grassland community of Southern Brazil. *Flora* **229:** 141–146.

Overbeck GE, **Müller C**, **Fidelis A**, **Pfadenhauer J**, **Pillar VD**, **Blanco CC**, **Boldrini II**, **Both R**, **Forneck ED. 2007.** Brazil's neglected biome: the South Brazilian *Campos*. *Perspectives in Plant Ecology, Evolution and Systematics* **9:** 101–116.

Palacios JAP, **Soteras F**, **Cocucci AA. 2019.** Mechanical fit between flower and pollinators in relation to realized precision and accuracy in the hummingbird-pollinated *Dolichandra cynanchoides*. *Biological Journal of the Linnean Society* **126:** 655–665.

Pinheiro MT, **Brandão BE**, **Harter-Marques B**, **Miotto STS. 2008.** Floral resources used by insects in a grassland community in Southern Brazil. *Brazilian Journal of Botany* **31:** 469–489.

R Core Team. **2015.** *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: https://www.R-project.org/

Raduski AR, **Haney EB**, **Igić B. 2012.** The expression of self-incompatibility in angiosperms is bimodal. *Evolution; international journal of organic evolution* **66:** 1275–1283.

Rambo B. 1956. *A fisionomia do Rio Grande do Sul*, *2nd edn*. Porto Alegre: Selbach.

Ramírez SR, **Eltz T**, **Fujiwara MK**, **Gerlach G**, **Goldman-Huertas B**, **Tsutsui ND**, **Pierce NE. 2011.** Asynchronous diversification in a specialized plant–pollinator mutualism. *Science* **333:** 1742–1746.

Rhodes MK, **Fant BJ**, **Krissa AS. 2017.** Pollinator identity and spatial isolation influence multiple paternity in an annual plant. *Molecular Ecology* **26:** 4296–4308.

Renner SS, **Schaefer H. 2010.** The evolution and loss of oil-offering flowers: new insights from dated phylogenies for angiosperms and bees. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365:** 423–435.

Ruchisansakun S, **Tangtorwongsakul P**, **Cozien RJ**, **Smets EF**, **Van der Niet T. 2016.** Floral specialization for different pollinators and divergent use of the same pollinator among co-occurring *Impatiens* species (Balsaminaceae) from Southeast Asia. *Botanical Journal of the Linnean Society* **181:** 651–666.

Schlindwein C. 1998. Frequent oligolecty characterizing a diverse bee–plant community in a xerophytic bushland of Subtropical Brazil. *Studies in Neotropical Fauna and Environment* **33:** 46–59.

Shuttleworth A, **Johnson SD. 2008.** Bimodal pollination by wasps and beetles in the African milkweed *Xysmalobium undulatum*. *Biotropica* **40:** 568–574.

Schäffler I, **Steiner KE**, **Haid M**, **van Berkel SS**, **Gerlach G**, **Johnson SD**, **Ludger Wessjohann L**, **Dötterl S. 2015.** Diacetin, a reliable cue and private communication channel in a specialized pollination system. *Scientific Reports* **5:** 12779.

Sigrist MR, **Sazima M. 2004.** Pollination and reproductive biology of twelve species of neotropical Malpighiaceae: stigma morphology and its implications for the breeding system. *Annals of Botany* **94:** 33–41.

Souza-Chies TT, **Kaltchuk Dos Santos E**, **Eggers L**, **Flores AM**, **Alves EM**, **Fachinetto J**, **Lustosa J**, **Corrêa LB**, **Tacuatiá LO**, **Piccoli P**, **Miz RB. 2012.** Studies on diversity and evolution of Iridaceae species in southern Brazil. *Genetics and Molecular Biology* **35:** 1027–1035.

Stebbins GL. 1970. Adaptive radiation of reproductive characteristics in angiosperms, I: pollination nechanisms. *Annual Reviews* **1:** 307–326.

Stiehl-Alves E, **Kaltchuck-Santos E**, **Eggers L**, **Souza-Chies TT. 2017.** Using a population genetics approach for a preliminary investigation concerning species boundaries in *Herbertia* (Iridaceae). *International Journal of Plant Science* **179:** 439–449.

Stout JC. 2007. Pollination of invasive *Rhododendron ponticum* (Ericaceae) in Ireland. *Apidologie* **38:** 198–206.

Thébault E, **Fontaine C. 2010.** Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science* **13:** 853–856.

Van der Niet T, **Peakall R**, **Johnson SD. 2014.** Pollinator-driven ecological speciation in plants: new evidence and future perspectives. *Annals of Botany* **113:** 199–211.

Waser N. 2006. Specialization and generalization in plant-pollinatoror interactions: a historical perspective. In: Waser NM, Ollerton J, eds. *Plant-pollinator interactions from specialization to generalization*. Chicago: 3–17.

Waser NM, **Chittka L**, **Price MV**, **Williams NM**, **Ollerton J. 1996.** Generalization in pollination systems, and why it matters. *Ecology* **77:** 1043–1060.

Wolowski M, **Ashman TL**, **Freitas L. 2014.** Meta-analysis of pollen limitation reveals the relevance of pollination generalization in the Atlantic forest of Brazil. *PLoS ONE* **9:** e89498.

Wolowski M, **Cavalheiro LG**, **Freitas L. 2016.** Influence of plant–pollinator interactions on the assembly of plant and hummingbird communities. *Journal of Ecology* **105:** 332–334.

SUPPORTING INFORMATION

Appendices are available as online resources on 10.6084/m9.figshare.8188391.

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. List of all plant populations studied, with their respective collectors, herbarium voucher, localization and coordinates.
Appendix S2. Distribution of studied plant populations in the State of Rio Grande do Sul, Brazil: (1) *Calydorea alba*; (2) *Cypella herbertii* subsp. *brevicristata* + *Cypella herbertii* subsp. *herbertii* + *Kelissa brasiliensis*; (3) *Cypella pusilla*; (4) *Cypella amplimaculata*; (5) *Herbertia pulchella* + *K. brasiliensis*; (6) *Cypella amplimaculata* + *H. pulchella*.
Appendix S3. Fruit set (mean ± SE) from hand self-pollination. Different letters indicate significant differences among sampled taxa.
Appendix S4. Mainly pollen types are found on the body of bees. Pollens from Iridaceae are indicated in bold. The pollen grains are organized evolutionarily according to Angiosperm Phylogeny Group IV. Numbers in the legend are related to pollen images.
Appendix S5. List of pollen types identified in the oil-collecting functional group of bees, with their respective relative abundance and classification.
Appendix S6. List of pollen types identified in the pollen-collecting functional group of bees, with their respective relative abundance and classification.