

Bat and bee pollination in *Psittacanthus* mistletoes, a genus regarded as exclusively hummingbird-pollinated

Mistletoes are aerial parasitic plants of the sandalwood order (Santalales), composed of ~1,500–1,600 species worldwide (Nickrent et al. 2010). Some temperate European and North-American species are folkloric and mystic, but the beauty of colors and the variety of forms are almost exclusively found in the tropical species in South and Central Americas, Africa, Australia, and New Zealand. Loranthaceae, the showy mistletoes, is the largest family on these continents. Within it, *Psittacanthus* is one of the most spectacular and species-rich genus (~119 species), occurring from Baja California, Mexico to northern Argentina (Kuijt 2009). The large radiation of species within *Psittacanthus* has been related to interactions with birds, as the genus is regarded as entirely hummingbird-pollinated and bird-dispersed (Restrepo et al. 2002, Vidal-Russell and Nickrent 2008). Nonetheless, while studying the pollination of *Psittacanthus* species in the Brazilian Pantanal and Amazon, we found *P. acinarius* and *P. eucalyptifolius* to be bat- and bee-pollinated, respectively (Fig. 1). Flower characteristics of these species do not provide cues for hummingbird pollination, such as a narrow and long tubular corolla, absence of odor, and a combination of red, yellow, and orange colors. Instead, *P. acinarius* has brush flowers that exhale an unpleasant odor and are inconspicuously greenish and *P. eucalyptifolius* has curved buds and slightly zygomorphic flowers, sweetly scented and vividly yellow. Based on these floral traits, bat pollination in *P. acinarius* was previously assumed by Araujo and Sazima (2003) and suspected by Kuijt (2009), who also pointed out the potential of bat pollination in *P. macrantherus* and insect pollination in *P. eucalyptifolius*.

Throughout several years of continuous fieldwork in the region of Pantanal of Miranda (19°34' S; 57°00' W), we found *P. acinarius* individuals flowering yearly from January to August (Araujo and Sazima 2003). Each plant produced ~1–10 inflorescences and 10–150 buds in total, and opened 1–20 flowers per night. Because its buds and pedicels are thicker, inflorescences are stronger than those of other *Psittacanthus* species that are pollinated by hummingbirds. *P. acinarius* flowers open at early evening (18:30–19:00) when the six petals (60 mm length) separate, exposing the central style (55 mm length) with a simple stigma. One stamen is attached to each petal, so the six anthers are peripheral and introrse (dehiscence facing inward). These traits contrast with hummingbird-pollinated *Psittacanthus* species whose petals often curl in open flowers and stamens are

central with extrorse anthers (facing outward). On April 27, 2000, between 19:00 and 20:00, we extracted an average of 15.2 μ L of nectar with a mean sugar concentration of 16.5% from each of four flowers of different *P. acinarius* individuals. This is a sugar concentration expected for bat-pollinated flowers in the Neotropics (Ornelas et al. 2007).

On three *P. acinarius* individuals, we observed (on different nights, 21 h total) short hovering visits (<1 s; $n = 118$) by small bats throughout the night and, at the beginning of anthesis, longer perching visits (>1 s; $n = 5$) by large bats. On other nights, we mist-netted bats nearby these plants and captured the nectar-feeding bats *Glossophaga soricina* (14 g) and *Phyllostomus discolor* (34 g), which typically hover and perch, respectively, when visiting flowers (Fischer 1992). On two further occasions, we programmed a camera to take one picture per minute throughout the night focused on *P. acinarius* flowers, and recorded visits of *G. soricina* (Fig. 1; see also Fischer et al. 2013: Fig. 2b). To drink nectar, both bat species inserted the head into the flower and contacted anthers and stigma with their anterior parts (head, neck, thorax, and shoulders). Furthermore, pollen of *P. acinarius* has been found in feces of *G. soricina* and *P. discolor*, as well as of *Platyrrhinus lineatus*, *Carollia perspicillata*, and *Phyllostomus*



FIG. 1. The bat *Glossophaga soricina* visiting *Psittacanthus acinarius* in the Pantanal (upper, photo by P. R. de Souza); and the bee *Centris* cf. *flavifrons* visiting *Psittacanthus eucalyptifolius* in the Amazon (bottom, photo by R. F. Fadini).

hastatus, other flower-visiting bats in the Pantanal (Munin et al. 2012). We recorded 60 visits of *Hylocharis chrysura* (Gilded Hummingbird) in buds of *P. acinarius*, when they are about to open (between 17:00 and 18:00). However, these visits were recorded as “illegitimate” because the flowers were still in opening and anthers were not exposed. Bat pollination may be advantageous for *P. acinarius* in the Pantanal, as hummingbirds are less abundant than flower visiting bats and perch-visiting *Phyllostomus* spp. can disperse pollen for long distances among widely spread clumps of *P. acinarius* (E. Fischer and A. C. Araujo, *personal observation*).

In November 2016, we marked flowers of *P. eucalyptifolius* in an Amazonian patch of savanna near to Alter do Chão (2°31' S; 59°00' W) to study its mating system. Flowering individuals produced 1,000 to 10,000 flowers, and opened 2–3% of them daily. Anthesis started at early morning with a sudden honey-suckle odor. Buds of *P. eucalyptifolius* are S-shaped, and the stigma and anthers are curved upward in open flowers, which are unique characteristics among *Psittacanthus* species. Nectar extracted from 70 flowers (10 individuals) bagged 1 day before averaged 11.4 μ L with a sugar concentration of 23.9%, this last value being greater than the average sugar concentration of nectar from other Neotropical loranthaceous mistletoes (Ornelas et al. 2007).

In December 2016, we filmed *P. eucalyptifolius* flowers during 31.15 hours with video cameras on tripods, and recorded 142 flower visits of at least five bee species: *Centris (Ptilotopus) denudans*, *Centris (Melacentris) atriventris*, *Centris (Centris) flavifrons*, *Centris (Centris) aenea*, and *Xylocopa (Neoxylocopa) frontalis*. The hummingbird *Amazilia fimbriata* (Glittering-throated Emerald) visited flowers in one occasion, but we could not determine if it touched their reproductive parts. Style and stamen filaments of *P. eucalyptifolius* formed a platform for small to medium visiting bees, such as *C. aenea* and *C. flavifrons*, which pressed the anthers and stigma against their abdomens (Fig. 1), while serving as “rapier-style swords” (as in Olympic fencing) for large bees (all other species), with anthers hitting the pollen into the animals’ parts, and the style, which is tougher, lying on them (Video S1). For a massive flowering plant like *P. eucalyptifolius*, pollination by species of *Centris* and *Xylocopa* are expected to be effective for inter-plant pollen flow, preventing a potentially high geitonogamy that territorial hummingbirds (Trochilinae) might produce in clumped flowers (Linhart 1987, Betts et al. 2015).

Floral traits and the high frequency of visits support the hypothesis that *P. acinarius* and *P. eucalyptifolius* evolved to facilitate pollination by bats and bees, respectively. Other *Psittacanthus* species have been reported to bear similar floral traits (Kuijt 2009). For instance, flowers of the Amazonian *P. carnosus* are similar to those of *P. acinarius* (R. F. Fadini, *personal observation*), indicating another possible bat-pollinated species. In addition, flowers of *P. robustus* are vividly yellow and exhale a pleasant scent, like we found for *P. eucalyptifolius* flowers. A working molecular phylogeny by J. F. Ornelas and G. Amico (*unpublished data*) so far suggests that these species with yellow vivid flowers are closely related

to each other. In fact, the study of Ornelas and Amico indicates that *P. eucalyptifolius*, *P. acinarius*, and *P. robustus* form a highly supported clade that is more recent than hummingbird-pollinated lineages of *Psittacanthus*. Meanwhile, our observations on pollination by bats or bees raise novel issues to be addressed through ecological and evolutionary frameworks. Why do the two phylogenetically and closely related *P. acinarius* and *P. eucalyptifolius* employ contrasting pollination systems? Are there different lineages of *Psittacanthus* that independently evolved bat or bee pollination? Are flower morphologies in *Psittacanthus* best explained by pollinator-mediated selection? How much did floral shifts to bat or bee pollination determine the diversification of the species-rich *Psittacanthus*? Although we are just scratching the edge of the story, the facts suggest that the reign of hummingbirds as the unique pollinators of *Psittacanthus* seems to have ended. Our findings additionally indicate that increased caution is needed against the common assumption of relatively fixed pollination syndromes within certain genera.

ACKNOWLEDGMENTS

To Arlison Castro and Natália Araújo for helping in the fieldwork, and to Marcio Luiz de Oliveira for identifying bee species. To David Watson for helpful suggestions that improved the manuscript, and to John Pastor for editorial suggestions that clarified the text. To FUNDECT for E. Fischer and A. C. Araujo grants, CNPq for E. Fischer researcher grant (307016/2015-3), and CAPES for S. J. Castro fellowship. This study is authorized under license SISBIO (10615-1 and 57180-1). This is publication 24 of Parasitic Plants Research Group Technical Series.

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Manuscript received 11 October 2017; revised 14 December 2017; accepted 18 December 2017. Corresponding Editor: John Pastor.

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