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Nora Rossana Maguina Conde
*University of Missouri-St. Louis,* nrmaguina@gmail.com

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**Nectar bats and their flowers across cloud forests of Ecuador and the effect of artificial nectar feeders**

Nora Rossana Maguiña Conde
Biology/B.S.
B.S. Science and Philosophy, Universidad Peruana Cayetano Heredia, 2010

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Nathan Muchhala, Ph.D.
Chairperson

Aimee Dunlap, Ph.D.

Robert Marquis, Ph.D.

Peter Stevens, Ph.D.

## ABSTRACT

Plant-pollinator interactions are important because they determine the reproductive success of animal-pollinated plant species, and contribute to speciation in plants and to the maintenance of biodiversity in the ecosystems. Plants pollinated by bats possess unique characteristics to attract them in order to be successfully pollinated. Also, pollinator bats have evolved several adaptations to feed from nectar and pollen. It is important to register and study these interactions, but also to analyze which activities could threaten them, in order to conduct optimal management decisions for their conservation.

Our study addressed two topics related to bat-plant interactions. The first chapter is about bat-plant interactions across a high biodiversity ecosystem, like the cloud forests of Ecuador, with an emphasis on the super-tongued bat species *Anoura fistulata*, a poorly studied species. And the second chapter is about the effect of artificial nectar feeders on bat-plant interactions also in an Ecuadorian cloud forest, a topic that has never been studied before.

Regarding the first chapter, we described the diet of *A. fistulata* in a broader distribution range of the species, adding 13 new plant species to its known diet. We found that *A. fistulata* is the only visitor to the extremely long flowers of the species *Centropogon nigricans* and *Marcgravia williamsii*, whose nectar is inaccessible to other bat species with shorter tongues. However, *A. fistulata* does not have a more specialized diet than other nectar-bats, and it can occur in sites that lack long flower species. Finally, we found a geographic trait covariation between the tongue length of *A. fistulata* and the nectar depth of the species with the longest flower it consumes, supporting the geographic mosaic theory of coevolution. Regarding the second chapter, our results showed that there is an effect of artificial nectar feeders in the relative abundance of nectar bats, with closer areas to feeders presenting a higher bat relative abundance than further areas. However, in spite of this, the presence of feeders does not affect other aspects of bat-plant interactions, such as, pollen loads carried by bats or the flower visitation rate and breeding success of the bat-pollinated species *Burmeistera glabrata*.

**CHAPTER 1**

**Interactions between nectar bats and their flowers across cloud forests of Ecuador, with an emphasis on the super-tongued bat *Anoura fistulata***

**ABSTRACT**

Nectarivorous bats have evolved various adaptations to feed from nectar, such as long, extensible tongues. The tongue length champion, *Anoura fistulata*, can feed from flowers more than 80 mm deep. However, little is known about its ecology and coevolution with its floral guild. In this study we analyzed the diet of *A. fistulata* and co-occurring nectar-bats in seven sites across Ecuador to evaluate whether *A. fistulata* has a more specialized diet due to its extremely long tongue. We measured bat tongues and depths of bat-pollinated flowers to test whether the occurrence of *A. fistulata* in an area depend on the occurrence of long-tubed flowers in the same area. Finally, because it is known that *A. fistulata* coevolved with the long-tubed flowers of the species *Centropogon nigricans* we evaluated evolutionary outcomes across space testing the geographic mosaic theory of coevolution, testing whether there is a trait covariation between the tongue length of *A. fistulata* and the flower lengths of species it feeds on in the sites it occurs. Our results added 13 new plant species to the known diet of *A. fistulata*, including both long and short-tubed flowers. We found that *A. fistulata* is the only visitor to the extremely long flowers of *C. nigricans* and *Marcgravia williamsii*, whose nectar is inaccessible to co-occurring bat species with shorter tongues. However, *A. fistulata* does not have a more specialized diet than other nectar-bats, and it can occur in sites that lack long-tubed flowers. Finally, we found a geographic trait covariation between the tongue length of *A. fistulata* and the nectar depth of the species with the longest flower it consumes in the different sites in which it occurs, suggesting that natural selection is playing a role in the adaptation of *A. fistulata* to the local floral community, and also supporting the geographic mosaic theory of coevolution.

**Key words:** *Centropogon nigricans*, coevolution, diet, ecology, geographic mosaic, *Marcgravia williamsii*.

**INTRODUCTION**

Nectarivorous bats present a series of adaptations (morphological, behavioral, ecological and physiological) to feed from nectar and pollen (Datzmann et al., 2010). However, among them their long and extensible tongues stand out, resembling the tongues of important pollinators such as hummingbirds, flies or moths (Winter & von Helversen, 2003). Among nectarivorous bats, the champion of tongue length is the species *Anoura fistulata*, with a maximum tongue extension more than 80 mm (equivalent to 150% of its body length), and special adaptations to store the tongue in its rib cage (Muchhala, 2006). *A. fistulata* represents an extreme outlier among nectar bats, resembling striking examples in other pollinator groups, such as the sword-billed hummingbirds (*Ensifera ensifera*) with a 10 cm long bill, which also exceeds its body length (Snow & Snow, 1980), the mega-nosed fly (*Moegistorynchus longirostris*) with a 5.7 cm long proboscis (Johnson & Steiner, 1997) and the giant hawkmoth (*Xanthopan morganii praedicta*) with a 25 cm long proboscis (Nilsson et al., 1985).

The tube-lipped bat *A. fistulata* was first discovered in the cloud forest of the Ecuadorian Andes (Muchhala et al., 2005). Its known distribution extends from Central Colombia (Mantilla-Meluk et al., 2014) to southern Peru (Gárate-Bernardo & Carrasco-Rueda, 2011), and it occurs in montane and pre-montane forest as well as arid and semiarid environments along its distribution range (Mantilla-Meluk et al., 2014). Muchhala et al. (2005) included notes on the diet of *A. fistulata* in montane forest of northern Ecuador; the bats carried pollen on their fur from long-tubed flowers such as *Centropogon nigricans*, *Markea*, *Marcgravia* and bromeliads, while the pollen of the short-tubed flowers of the genus *Burmeistera* were conspicuously absent. Previous studies on nectar-bats' diets showed that they specialize in the consumption of nectar and pollen, but they do not specialize on a limited number of plant species (Muchhala & Jarrín-V, 2002). On the contrary, they use a large number of species within their habitats (for instance, 16 for *Hylonycteris underwoodi*, 15 for *Glossophaga commissarisi* (Tschapka, 2004), 11 for *A. caudifer* and 10 for *A. geoffroyi* (Muchhala & Jarrín-V, 2002)). However, the known diet of *A. fistulata* suggests it could be specialized in long-tubed flowers, as it apparently did not visit short-tubed *Burmeistera* flowers present in their habitats (Muchhala, 2005). However, current information of *A. fistulata*'s diet is only based in a handful of samples from a specific

area of its wide distribution in northern Ecuador (Muchhala et al., 2005; Muchhala, 2006). In addition, it is unknown if *A. fistulata* always co-occurs with long flowered species throughout its geographical distribution.

As regards the pollinator role of *A. fistulata*, Muchhala (2006) reported that it was the only pollinator of the 8-9 cm long flowers of *C. nigricans* in northern Ecuador. What is more, Muchhala and Thomson (2009) showed that the long tongue of *A. fistulata* likely evolved in a coevolutionary race with the long-tubed flowers of *C. nigricans*, with selective pressures acting on longer tongues, because they could get access to more nectar, and on longer tubed-flowers, because they could export and receive more pollen. The theory of coevolution suggests evaluating evolutionary outcomes not only over time (e.g., Slatkin and Maynard Smith 1979) but also across space (Thompson 1994; Nuismer et al. 1999; Gomulkiewicz et al. 2000; Thompson 2005; Gomulkiewicz et al. 2007), due to selective pressures could vary geographically in accordance to local phenotypes, population sizes, biotic and abiotic factors (Gomulkiewicz et al. 2000; Thompson 2005). Thus, a geographic mosaic for the coevolutionary process is one possible result (Thompson, 2005). Previous studies testing the geographical mosaic of coevolution showed geographic trait covariation between the lengths of floral reproductive parts and the lengths of pollinator's body traits involved in effective pollination (for instance mouthparts or legs). Trait covariation was observed between single pollinator species or pollinator guilds and single plant species or floral guilds (Anderson & Johnson, 2008; Cosacov et al., 2013; Boberg et al., 2014; Newman et al., 2014). We suspect that *A. fistulata* and its floral guild could show a similar pattern of trait covariation, with longer and shorter tongues in sites with local longer and shorter flowers, respectively.

In this study we analyzed the diet of *A. fistulata* over a large portion of its geographical range, as well as the diet of co-occurring nectar bats, in order to evaluate 1) what does *A. fistulata* feed on in other areas of its range and 2) if there is a difference in diet specialization between *A. fistulata* and other nectar bats due to differences in their adaptations to feed from flowers. The long tongue of *A. fistulata* could allow it to specialize and limit its diet to long-tubed flowers that co-occurring bat species cannot use; alternatively, it may lead to a more generalized diet by allowing it to feed from both long and short flowers. We also measured bat tongue lengths

and depths of bat-pollinated flowers to test 3) if the occurrence of *A. fistulata* in an area depends on the occurrence of long-tubed flowers in the same area and 4) if there is trait covariation between the tongue length of *A. fistulata* and the flowers' depths of the species it feeds on in the sites it occurs.

## METHODOLOGY

*Study sites:* We collected samples in seven cloud forest sites along both sides of the Andes in central Ecuador. The elevation in the different sites varied from 1000 to 3500 masl, and in each site we captured between two and four species of nectar-bats (Table 1). The authors conducted fieldwork from October 2009 to December 2010 (N.M) and June 2014 to January 2016 (R.M).

*Diet analysis:* To analyze their diet, we captured nectar-bats using mist-nets (2, 6, 9, 12 m x 3, 2.5 m) placed close to known or suspected bat-adapted flowers. Nets were opened at dusk from 1800 to 0100 hrs. We collected pollen and fecal samples from the bats. We collected pollen from bats' fur by touching transparent adhesive tape against different bat body areas (mainly head, belly and inner wing patagium) and then placing the tape on a glass microscope for later inspection and identification of the pollen. We held the bats in cloth bags for two hours to collect fecal samples, which were spread on a glass microscope and covered with adhesive tape. We inspected the fur and fecal samples using a SWIFT light microscope (SWIFT Microscope World, USA), scanning the tape at 100X magnification, switching to 400X as needed to identify and photograph the pollen grains. For each fecal sample we categorized the components as pollen, vegetative tissue, seeds and insects. For all samples (fur and fecal) we registered the presence or absence of pollen (pollen frequency), and when present we identified all pollen types to the lowest taxonomic level possible and estimated the abundance in percentage per pollen type. We identified the pollen using our pollen reference collection taken directly from flowers during fieldwork. For each bat species in each site, we estimated diet richness as number of flower species visited, diet diversity using the Shannon-Wiener (H') diversity index and the Levin's standardized Index ($B_A$) which is a measure of niche breadth. $B_A$

ranges from 0 (only one resource used) to 1 (all consumed resources used evenly) indicating a narrow or wide niche breadth, respectively (Krebs, 1999). We also calculated the overall average diet richness, H' and $B_A$ per bat species across all sites. We used one-way ANOVA to compare the average diet richness, H' and $B_A$ index among bat species.

***Tongue and flower measurements:*** For a subset of the bats captured, after collecting pollen and fecal samples we held them individually in an experimental tent in order to measure tongue length. First, we trained the bat to feed from a plastic test tube (12 mm diameter) filled with a 1:4 sugar-water solution. If the individual did not feed from the tube after two hours, it was immediately released. For those that fed, we then offered the bat a modified drinking straw filled with sugar-water with a 6 mm diameter, this prevented the bat from inserting its snout, allowing us to isolate tongue length. Every 30 minutes we measured the depth of water-sugar consumed since the last check and then replaced approximately half of this. When the depth of water-sugar was the same for three consecutive visits, we considered this to be the bat's maximum tongue length extension (sensu Muchhala, 2006). We also measured nectar depth for all bat-visited flowers in each reserve. We collected between one and ten flowers per species to measure corolla length; in the case of *Marcgravia* species, we collected nectaries and measured nectary depth. Our goal was to measure the corolla/nectary functional depth of the flowers, which represents the length a bat has to extend its tongue in order to reach the nectar. For tubular corolla flowers we measured from the top of corolla opening to the base of corolla, while for campanulate corolla flowers we measured from the top corolla edge to the base of corolla and then subtracted the average cranium length of each bat species, to account for the fact that bats insert their heads into these flowers before extending their tongues. Cranium measurements were taken from Muchhala et al. (2005). We complemented our field data with corolla length measurements of herbarium specimens (Missouri Botanical Garden) collected in the corresponding reserve sites, or from species descriptions when necessary. For statistical analyses we used Pearson's correlation to measure the linear correlation between the average tongue length of *A. fistulata* per site and the average functional depth of the flowers consumed by the species in each site. We also selected the plant species with the longest measurement that was consumed by *A. fistulata* in each site and we correlated these measurements with the average tongue length, given that only the longest

flowers would be expected to select on tongue length. We performed the same statistical analyses for *A. caudifer* and the plant species it consumed, but did not apply these analyses for other species given their low sample sizes. Finally, we applied a linear mixed-effects model (LMM) with flower depth as the fixed effect and bat species as a random effect.

## RESULTS

***Diet analysis:*** We captured a total of 323 nectar bats from six species in the seven Ecuadorian sites we visited: *Anoura caudifer* (N=145), *A. cultrata* (N=17), *A. fistulata* (N=25), *A. geoffroyi* (N=42), *Lonchophylla robusta* (N=91)*,* and *L. thomasi* (N=3)*.* We captured *A. fistulata* in three of the seven sites; namely Domono, Guajalito and Yanayacu. The diet of *A. fistulata* was composed of pollen (present in 96% of the samples) and insects (present in 66% of the samples, Table 2). No vegetative tissues were present in the samples of *A. fistulata*, unlike those of the other species of *Anoura* and *L. robusta*; however, the number of samples from *A. fistulata* is lower than the one of other species of *Anoura* and *L. robusta*. No seeds were present in any of the samples (Table 2). The insect remains in *A. fistulata* samples belonged to the orders Coleoptera and Hymenoptera, and we also recorded scales of the order Lepidoptera in other species of *Anoura* and *L. robusta*. We were not able to identify the vegetative tissues but we suspect that they came from fruit consumption. Pollen presented the highest frequency among all the components in all bat species. These results indicate that *A. fistulata* feed mainly on flowers (nectar and pollen) and supplements their diets with insects; other species of *Anoura* and *L. robusta* also feed mainly on flowers and supplement their diets with insects and likely fruits.  The plant species/genera/families identified corresponded to 51 different taxa, 16 occurred in *A. fistulata* samples, adding 13 new records to the known diet of this species (Table 3). We found that *A. fistulata* feeds from long and short-tubed flowers (including species of *Burmeistera*); but it is the only species that was feeding from the long flowers of *C. nigricans* and *Marcgravia williamsii*. For further analyses of diet specialization we only included results from the species that were captured in two or more different sites and from which we collected at least five samples per site (fur or fecal samples, Table 2). The significance tests showed non-significant difference for diet richness, H' and $B_A$ (one-way

ANOVA test, p>0.05, Table 4) among nectar bat species, indicating that the diet specialization of these bats is similar. These results show that *A. fistulata* does not have a more specialized diet relative to that of other co-occurring nectar bat species.

***Tongue and flower measurements:*** We obtained measurements of bats' tongues and flowers' nectar depths in six and seven sites, respectively (Supplementary material, Tables 2 and 3). We obtained measurements of the tongue length of *A. fistulata* from the three sites where we captured the species, and we also obtained flower measurements from at least three plant species in each of those sites (Table 5). The shortest average tongue length of *A. fistulata* was found in Yanayacu (81.5 ± 4.4), and the longest average was found in Guajalito (87.7 ± 1.1). The corolla/nectary functional depth of flowers consumed by *A. fistulata* ranged from 83.5 mm to 0 mm (Table 3). We found that *A. fistulata* occurs in sites with long flowers, such as Guajalito with *C. nigricans* and Domono with *M. williamsii*, however it also occurs in Yanayacu where the longest flower (*Marcgravia brownei)* does not exceed 50 mm. The Pearson test showed a non-significant correlation between the tongue length of *A. fistulata* per site and the average corolla/nectary functional depth of the plant species it consumed in each site ($R^2$ = 0.58, p = 0.21), however, we did find a significant positive correlation with plant species with the longest measurement consumed by *A. fistulata* ($R^2$ = 0.99, coef = 0.99, p = 0.01, Fig. 1). Similarly, the Pearson test showed a non-significant correlation between the tongue length of *A. caudifer* per site and the average corolla/nectary functional depth of the plant species it consumed in each site ($R^2$ = 0.24, p = 0.25), yet a marginally significant positive correlation with plant species with the longest measurement consumed by *A. caudifer* ($R^2$ = 0.94, coef = 0.79, p = 0.07, Fig. 1). The LMM, with flower depth as fixed effect and bat species as a random effect, also showed a marginally significant positive relationship (p = 0.05). These results show that the tongue length of *A. fistulata* and *A. caudifer* varies accordingly with the longest flower depth of the plant species they consume in the sites they occur.

**DISCUSSION**

This study greatly expands our knowledge of bat-plant interactions, adding 13 new plant species to the diet of *A. fistulata*. *A. fistulata* feeds from long and short-tubed flowers (including species of *Burmeistera*); but it is the only species that feeds from the extremely long flowers of *C. nigricans* and *M. williamsii*, which are inaccessible to co-occurring bat species with their shorter tongues. However, *A. fistulata* does not have a more specialized diet; there is no significant difference in dietary specialization relative to the other nectar bat species. Also, we found that the occurrence of *A. fistulata* in a site is independent of the occurrence of long flowers species in the same site. Finally, we found a geographic trait covariation between the tongue length of *A. fistulata* and the corolla/nectary functional depth of the longest flowers species it consumes in the sites it occurs, suggesting that natural selection is playing a role in the adaptation of *A. fistulata* to the local floral community, supporting the geographic mosaic theory of coevolution.

Before our study, the known diet of *A. fistulata* included only five plant species: *C. nigricans*, *Marcgravia coriaceae*, *Aphelandra acanthus*, *Pitcairnia brogniartiana* and *Meriania tomentosa* (Muchhala et al., 2005). Our study adds 13 new species to this list, presenting information from a wide area of its geographical distribution at both sides of the Andes in central Ecuador. We found that *A. fistulata* fed from both long and short-tubed flowers at the three sites we caught it. Two flowers were clearly specialized to *A. fistulata*: *C. nigricans*, with an average of 83.2 mm-long corollas, and *M. williamsii,* with an average of 68.6 mm-long nectaries; since *A. fistulata* was the only species in which we found pollen of these plant species. Although the species *Trianaea nobilis* presents a long corolla functional depth (74.3 mm) after we subtracted cranium length of bats, *A. fistulata* was not the only species feeding from it; we also found pollen of *T. nobilis* in samples from *A. geoffroyi* with an average tongue length of 43.3 mm. The wide campanulate corolla and long nectar chamber (~40 mm length) of *T. nobilis* flowers would allow access to nectar to short-tongued bats, however, only *A. fistulata* would reach the base of the long nectar chamber to get all nectar available (N.M., pers. obs.). The exclusive feeding on *C. nigricans* and *M. williamsii* by *A. fistulata* indicates that its long tongue confers it an advantage to feed from long flowers that other bat species cannot reach. These interactions

resemble those of other nectarivorous animals with extremely long mouthparts, for instance, the sword-billed hummingbird (*Ensifera ensifera*) that exclusively pollinates around 37 species of *Passiflora* in the Andes mountains (Abrahamczyk et al., 2014; Lindberg & Olesen, 2001) and the mega-nosed fly (*Moegistorynchus longirostris*) that is the sole pollinator of different species of orchids in the Cape Drakensberg mountains (Johnson & Steiner, 1997). What is more, all these interactions are asymmetrical in thus the plant species exclusively depend on a single pollinator to successfully reproduce but the pollinator does not depend on a single plant species to obtain food resources (Johnson & Steiner, 1997).

In spite of the fact that *A. fistulata* can feed from short flowers, we noticed that the frequencies and percentages of abundance of pollen grains belonging to short flowers species were lower compared to species with longer flowers (Supplementary material, Fig. 1, Chi-squared, p<0.01 in Yanayacu, p<0.01 in Guajalito). For instance, in Yanayacu, pollen of species *M. brownei* with 47.2 mm functional depth was present in 93% of the samples of *A. fistulata* and registered 83% of abundance, whereas pollen of short flowers species of *Burmeistera* was present in 40% of the samples and registered 15% of abundance or lower values. When we looked at the frequencies and abundances of the same plants in other (short-tongued) nectar bats, we always found higher values for *Burmeistera* species. For instance, *A. caudifer* in Yanayacu presented pollen of *M. brownei* in 81% of the samples, which registered 22% of abundance, whereas pollen of *Burmeistera* was present in 100% of the samples and registered 70% of abundance. We observed similar percentages for long (*C. nigricans*) and short (*Burmeistera* spp.) flowers in Guajalito (Supplementary material, Fig. 1, Chi-squared, p<0.01). These results indicate that *A. fistulata* could be mainly feeding from long flowers species (when they are present in an area), whereas the other nectar bats would consume species of *Burmeistera* more frequently than *A. fistulata*. Our findings concur with previous records about different frequency of visits to flowers in montane forest between species *A. caudifer* and *A. geoffroyi*, the larger bat *A. geoffroyi* visited longer flowers more frequently, whereas the shorter bat *A. caudifer* visited shorter flowers more frequently (Muchhala & Jarrín-V, 2002). It is likely that there is a partition of resources among nectar bats in montane forest habitats. Additionally, we estimated the percentage of diet overlap between *A. fistulata* and co-occurring nectar bat species, and the results showed percentages of diet overlap of only 17.6% (based on pollen

frequencies) and 25.7% (based on pollen abundance). Interestingly, the percentages of diet overlap between other species of *Anoura*, do not exceed 20% (based on pollen frequencies) and 36.5% (based on pollen abundance) of overlap (Supplementary material, Table 2). These results support our previous idea about partition of resources. Finally, an important result from the diet analysis is that all bat species showed similar values of diet richness, H' and $B_A$, indicating that *A. fistulata* does not have a more specialized diet compared with co-occurring nectar-bats. Therefore, the striking morphological specialization of *A. fistulata* led it to generalization by allowing it to feed from a wider variety of food resources.

The measurements we took from flowers along the visited sites showed that the occurrence of *A. fistulata* in an area does not depend on the occurrence of long-tubed flowers in the same area. These findings line up with our results about the ability of *A. fistulata* at feeding from a wide variety of resources, in terms of a wide depth range of the flowers it visits (from 0 mm to 83.5 mm). Also, we found a geographic trait covariation between the tongue length of *A. fistulata* and the functional depth of the longest flower it feeds on in the sites it occurs. These findings suggest, that natural selection is playing a role in the adaptation of *A. fistulata* to the local floral community; and that the outcomes of the coevolution of *A. fistulata* and its floral guild concur with the geographic mosaic theory of coevolution. Previous studies found similar patterns of geographic trait covariation between plants and different pollinator guilds (Boberg et al., 2014; Cosacov et al., 2013; Newman et al., 2013; Nattero et al., 2011; Anderson & Johnson, 2008). In regards to hummingbirds, Nattero et al. (2011) found a correlation between the corolla length of *Nicotiana glauca* and the bill length of its most frequent hummingbird pollinator across a wide geographic scale along the mountain Andes of Bolivia and Argentina. In the case of flies, correlation was found between the proboscis length of flies and the corolla tube length of one mutualistic plant species (Anderson and Johnson, 2008) or the corolla tube length of a guild of plant species (Newman et al., 2013). Finally, in the case of moths, a recent study by Boberg et al. (2014) found a correlation between the spur length of the orchid *Platanthera bifolia* and the proboscis length of its local moth pollinators in northwestern Europe. As mentioned in previous studies about bats and other pollinator guilds, it is expected that selection will favor longer mouthparts because they confer more efficiency at extracting nectar from long-tubed flowers (Nattero et al., 2011; Muchhala & Thomson, 2009; Anderson &

Johnson, 2008) and longer tubed-flowers because they can export and receive more pollen (Muchhala & Thomson, 2009). Taken together, these studies indicate the influence of geographical variation in creating changing selective pressures for the coevolution of pollinators and floral traits. For future studies, it would be interesting to examine the variation in tongue length of *A. fistulata* and traits of its floral guild with relation to other factors that could constraint or promote an increase in length of the mentioned traits in particular locations, such as abiotic variables, other floral traits or vegetative traits, population sizes, and presence of short-tubed species (Cosacov et al., 2013; Newman et al., 2013; Nattero et al., 2011; Anderson & Johnson, 2008).

## CONCLUSIONS

*A. fistulata* feeds from a wide variety of plant species, including species with short and long flowers, however its long tongue allows it to exclusively feed from the longest flower species *C. nigricans* and *M. williamsii*, which other nectar bats cannot reach. In spite of this, *A. fistulata* does not have a more specialized diet compared to other nectar bats. In addition, the occurrence of *A. fistulata* in a site is independent of the occurrence of long-tubed flowers species in the same site, which makes sense because diet is not restricted to these species. Finally, the geographic trait covariation between the tongue length of *A. fistulata* and the corolla/nectary functional depth of the longest flowers species it consumes supports the geographic mosaic theory of coevolution, therefore different selective pressures along the geographic distribution of these species are shaping coevolutionary outcomes.

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**TABLES AND FIGURES**

**Table 1.** Study sites information, fieldwork dates and bat species captured per site.

| Reserve (Province) | Location | Elevation (m.a.s.l) | Fieldwork dates | Bat species captured |
|---|---|---|---|---|
| Bosque **Domono**, Macas (Morona Santiago) | 02°07'S, 78°08'W | 1000 - 1300 | Apr 2010 | *A. caudifer, A. fistulata, A. geoffroyi, Lonchophylla thomasi* |
| Reserva Floristica Rio **Guajalito** (Pichincha) | 00°14'S, 78°49'W | 1800 - 2200 | Jun, Dec 2010 | *Anoura caudifer, A. fistulata, A. geoffroyi* |
| **San Francisco** Scientific Station (Loja) | 03°58'S, 79°04'W | 1900 - 2200 | Mar-Apr 2010 | *A. caudifer, A. geoffroyi* |
| **Siempre Verde** Reserve (Imabura) | 00°18'N, 78°16'W | 1200 - 3300 | Oct 2009 | *A. caudifer, A. geoffroyi* |
| **Tapichalaca** Biological Reserve (Zamora Chinchipe) | 04°30'S, 79°10'W | 1800 - 3500 | Nov 2010 | *A. caudifer, A. geoffroyi* |
| **Yanayacu** Biological Station (Napo) | 00°35' S, 77°53'W | 2000 - 2500 | Nov 2009, Jun-Jul, Oct 2010, Jul 2014 | *A. caudifer, A. fistulata, A. geoffroyi* |
| **Wildsumaco** Wildlife Sanctuary (Napo) | 00°40'S, 77°35'W | 1400 - 1700 | May 2010, Jun-Aug 2014, Jun-Jul 2015, Jan 2016 | *A. caudifer, A. cultrata, A. geoffroyi, L. robusta* |

**Table 2.** Total sample size per bat species and frequencies of pollen, insect and vegetative tissue in samples per species and sites (numbers show the frequency of items over the total number of samples per species in each site).

| Bat species | N | Domono | Guajalito | San Francisco | Siempre Verde | Tapichalaca | Yanayacu | Wildsumaco |
|---|---|---|---|---|---|---|---|---|
| *Pollen results (fur and fecal samples)* | | | | | | | | |
| *Anoura caudifer* | 145 | 1/1 | 14/14 | 10/10 | 6/6 | 11/11 | 16/16 | 86/87 |
| *Anoura cultrata* | 17 | - | - | - | - | - | - | 17/17 |
| *Anoura fistulata* | 25 | 5/5 | 5/5 | - | - | - | 14/15 | - |
| *Anoura geoffroyi* | 42 | 2/2 | 10/10 | 2/2 | 19/19 | 5/5 | 2/2 | 2/2 |
| *Lonchophylla robusta* | 91 | - | - | - | - | - | - | 82/91 |
| *Lonchophylla thomasi* | 3 | 2/3 | - | - | - | - | - | - |
| *Insects (top) and vegetative tissues (bottom) results* | | | | | | | | |
| *Anoura caudifer* | 41 | - | - | 3/5 | 0/2 | 0/3 | 1/1 | 24/30 |
| | | - | - | 0/5 | 0/2 | 0/3 | 0/1 | 5/30 |
| *Anoura cultrata* | 9 | - | - | - | - | - | - | 9/9 |
| | | - | - | - | - | - | - | 4/9 |
| *Anoura fistulata* | 3 | 1/1 | 0/1 | - | - | - | 1/1 | - |
| | | 0/1 | 0/1 | - | - | - | 0/1 | - |
| *Anoura geoffroyi* | 15 | 0/1 | 1/1 | 1/1 | 8/9 | 2/2 | 0/1 | - |
| | | 1/1 | 0/1 | 0/1 | 0/9 | 0/2 | 1/1 | - |
| *Lonchophylla robusta* | 17 | - | - | - | - | - | - | 12/17 |
| | | - | - | - | - | - | - | 1/17 |
| *Lonchophylla thomasi* | 1 | 1/1 | - | - | - | - | - | - |
| | | 0/1 | - | - | - | - | - | - |

**Table 3.** Plant species recorded in *A. fistulata* samples and their corolla/nectary functional depths.

| Plant Species/Genera/Families | Corolla tube/nectary functional length (mm) | Bellavista * N = 5 | Yanayacu N = 14 | Domono N = 5 | Guajalito N = 5 |
|---|---|---|---|---|---|
| *Centropogon nigricans* | 83.23 | x | | | x |
| *Marcgravia coriaceae* | - | x | | | |
| *Aphelandra acanthus* | 38.73 | x | | | |
| *Pitcairnia brogniartiana* | 85.9 | x | | | |
| *Meriania tomentosa* | 0 | x | | | x |
| *Markea sp.* | - | x | | | |
| Bromeliads | - | x | x | x | x |
| *Marcgravia brownei* | 47.19 | | x | | x |
| *Marcgravia williamsii* | 68.68 | | | x | |
| *Marcgravia sp.* | - | | | x | |
| *Trianaea nobilis* | 74.37 ** | | | | x |
| *Trianaea speciosa* | 31.43 | | x | x | |
| *Ochroma-Ceiba* | - | | | x | x |
| *Passiflora unipetala* | 9.9 | | | | x |
| *Burmeistera borjensis* | 17.59 | | x | | |
| *Burmeistera ceratocarpa* | 21.49 | | x | | |
| *Burmeistera cylindrocarpa* | - | | | | x |
| *Burmeistera lutosa* | 17.84 | | | | x |
| *Burmeistera sodiroana* | 19.71 | | x | | |
| *Burmeistera succulenta* | 17.15 | | x | | |

\* Results from Muchhala et al. (2005) from Bellavista Reserve, northern Ecuador

\*\* Likely overestimated functional depth due to extend corolla width

**Table 4.** Diet richness, diet diversity (H') and Levins' index ($B_A$) results (± SE).

| Bat species | Diet richness | H' | $B_A$ (pollen frenquecy) | $B_A$ (pollen abundance) |
|---|---|---|---|---|
| *A. caudifer* | 4.13 ± 0.8 | 1.88 ± 0.26 | 0.57 ± 0.15 | 0.26 ± 0.14 |
| *A. fistulata* | 4.07 ± 1.6 | 1.63 ± 0.25 | 0.68 ± 0.14 | 0.27 ± 0.17 |
| *A. geoffroyi* | 4.35 ± 0.45 | 1.98 ± 0.11 | 0.68 ±0.12 | 0.29 ± 0.02 |
| Significance test (one-way ANOVA) | p=0.95 | p=0.21 | p=0.42 | p=0.93 |

**Table 5.** Measurements of tongue length and corolla/nectary functional depth of the longest flowers; ± SE and sample size between parenthesis.

| | Domono | Guajalito | Yanayacu | Wildsumaco | Pearson's correlation |
|---|---|---|---|---|---|
| Longest flower | *Marcgravia williamsii* | *Centropogon nigricans* | *Marcgravia brownei* | - | |
| | 68.68 ± 7.59 (6) | 83.51 ± 3.75 (5) | 49.73 ± 7.44 (23) | - | $p = 0.01$ |
| *Anoura fistulata* | 84.73 ± 2.35 (3) | 87.73 ± 1.19 (3) | 81.5 ± 4.41 (3) | - | |
| Longest flower | - | *Marcgravia brownei* | *Marcgravia brownei* | *Burmeistera glabrata* | |
| | - | 41.22 ± 1.2 (2) | 49.73 ± 7.44 (23) | 17.11 ± 1.39 (10) | $p = 0.07$ |
| *Anoura caudifer* | - | 35.26 ± 0.57 (3) | 36.2 (1) | 34.44 ± 2.34 (9) | |
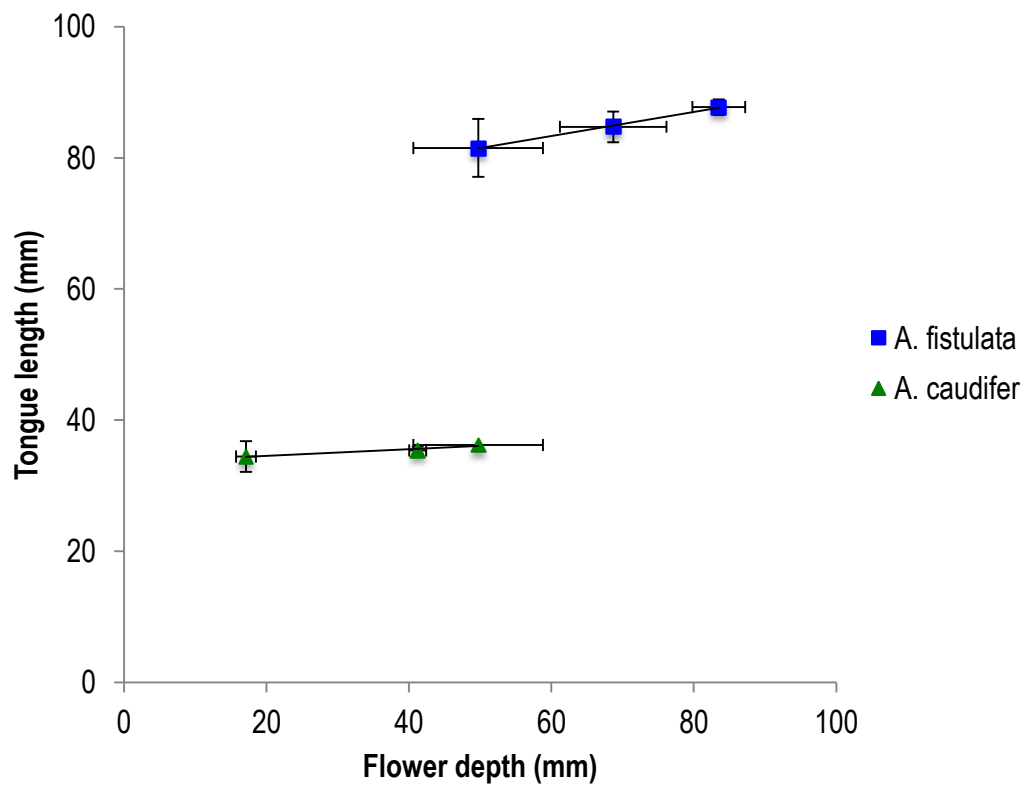


**Figure 1.** Correlations between the average tongue length per site and longest flower depth consumed per site.

**Table 1.** Pollen carried by nectar bats in seven different Ecuadorian cloud forest sites; each cell gives number of bats with that type of pollen on their fur or in their feces. Acau = *A. caudifer*; Acul = *A. cultrata*; Afis = *A. fistulata*; Ageo = *A. geoffroyi*; Lrob = *L. robusta*; Ltho = *L. thomasi*.

| Plant list | Yanayacu | | | Guajalito | | | Domono | | | | Wildsumaco | | | | Siempre Verde | | Tapichalaca | | San Francisco | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Acau* N=16 | *Afis* N=15 | *Ageo* N=2 | *Acau* N=14 | *Afis* N=5 | *Ageo* N=10 | *Acau* N=1 | *Afis* N=5 | *Ageo* N=2 | *Ltho* N=3 | *Acau* N=87 | *Acul* N=17 | *Ageo* N=2 | *Lrob* N=91 | *Acau* N=6 | *Ageo* N=19 | *Acau* N=11 | *Ageo* N=5 | *Acau* N=10 | *Ageo* N=2 |
| Acanthaceae | | | | | | | | | | | | | | | | | | | | |
| Aphelandra acanthus | | | | | | | | | | | | | | | 1 | 15 | | | | |
| Bignoniaceae | | | | | | | | | | | | | | | | | | | | |
| Bignoniaceae sp1. | | | | | | | | | | | 1 | | | 2 | | | | | | |
| Bromeliaceae | | | | | | | | | | | | | | | | | | | | |
| Pitcairnia fusca | | | | | | | | | | | | | | | | 4 | | | | |
| Weriauhia gladioliflora | | | | | | | 1 | 5 | 2 | 2 | | | | | | | | | | |
| Bromeliaceae sp1. | | | | | | | | | | | | | | | | | | | 6 | |
| Bromeliaceae sp2. | | | | | | | | | | | | | | | | | | | 5 | |
| Bromeliaceae spp. | 10 | 4 | | 8 | 5 | 6 | | | | | 60 | 7 | | 17 | | | 11 | 5 | | |
| Campanulaceae | | | | | | | | | | | | | | | | | | | | |
| Burmeistera borjensis | 14 | 6 | 2 | | | | | | | | | | | | | | | | | |
| Burmeistera cylindrocarpa | | | | | | | | | | | | | | | 4 | 15 | | | | |
| Burmeistera glabrata | | | | | | | | | | | 82 | 11 | | 47 | | | | | | |
| Burmeistera sodiroana | 5 | | 1 | | | | | | | | | | | | 6 | 22 | | | | |
| Burmeistera ceratocarpa/succulenta | 12 | 2 | 1 | | | | | | | | | | | | | | | | | |
| Burmeistera cylindrocarpa/lutosa/succulenta | | | | 8 | 3 | 7 | | | | | | | | | | | | | | |
| Burmeistera draconis/zamorensis | | | | | | | | | | | | | | | | | 9 | 3 | | |
| Burmeistera truncata | | | | 13 | | 5 | | | | | | | | | | | | | | |
| Centropogon nigricans | | | | 5 | | | | | | | | | | | | | | | | |
| Syphocampylus sp. | | | | | | | | | | | | | | | | | 5 | 1 | | |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cucurbitaceae | | | | | | | | | | | | | | | | | | | | |
| *Cayaponia sp.* | | | | | | | | | | | 2 | | | 21 | | | | | | |
| *Calycophysum sp.* | | | | | | | | | | | 2 | | | | | | | | | |
| *Gurania sp.* | | | | | | | | | | | 1 | 1 | | | | | | | | |
| Cucurbitaceae sp1. | | | | | | | | | | | 3 | | | 1 | | | | | | |
| Fabaceae | | | | | | | | | | | | | | | | | | | | |
| Fabaceae sp1. | | | | | | | | | | | | | | 5 | | | | | | |
| Gesneriaceae | | | | | | | | | | | | | | | | | | | | |
| *Capanea grandiflora* | | | | | | | | | | | | | | | | | | | 4 | |
| Gesneriacea sp1. | | | | | | | | | | | 1 | | | 4 | | | | | | |
| Macrocarpaceae | | | | | | | | | | | | | | | | | | | | |
| *Macrocarpea harlingii* | | | | | | | | | | | 7 | 1 | | 9 | | | | | 10 | 2 |
| *Macrocapea sp.* | | | | | | | | | | | | | | | | | | | 5 | 1 |
| Malvaceae | | | | | | | | | | | | | | | | | | | | |
| *Abutilon pictum* | | | | | | | | | | | 43 | 4 | | 10 | | | | | | |
| *Ochroma-Ceiba* | | | 1 | 1 | 4 | | | 1 | 1 | 14 | 1 | | 39 | | | | | | | |
| Marcgraviaceae | | | | | | | | | | | | | | | | | | | | |
| *Marcgravia brownei* | 13 | 14 | 1 | 14 | 5 | 10 | | | | | | | | | | 6 | | | | |
| *Marcgravia williamsii* | | | | | | | | 2 | | | | | | | | | | | | |
| *Marcgravia sp.* | | | | | | | 1 | 3 | 2 | | 76 | 16 | 2 | 55 | | 10 | 5 | 6 | | |
| Melastomataceae | | | | | | | | | | | | | | | | | | | | |
| *Meriania tomentosa* | | | | 2 | | | | | | 5 | | | 5 | 6 | 21 | 6 | 2 | | | |
| Onagraceae | | | | | | | | | | | | | | | | | | | | |
| Onagraceae sp1. | | | | | | | | | | | | | | | | | 1 | | | |
| Passifloraceae | | | | | | | | | | | | | | | | | | | | |
| *Passiflora unipetala* | | | 2 | 3 | 5 | | | | | | | | | | | | | | | |
| *Passiflora spp.* | | | | | | | | | | | 1 | | 2 | | | | 1 | | | |
| Polemoniaceae | | | | | | | | | | | | | | | | | | | | |
| *Cobaea trianae* | | | | | | | | | | | | | | 1 | 18 | | | | | |
| Rubiaceae | | | | | | | | | | | | | | | | | | | | |
| Rubiaceae sp1. | | | | | | | | | | | 2 | | | 3 | | | | | | |

| | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Solanaceae** | | | | | | | | | | | | |
| *Trianaea nobilis* | | | 1 | 2 | | | | 2 | | | | |
| *Trianaea speciosa* | 9 | 4 | | | | | | | | | | |
| *Trianaea sp.* | | | | | 24 | 1 | 4 | | 11 | 4 | 8 | |
| Solanaceae spp. | | | | | | | | | | | 4 | |
| **Urticaceae** | | | | | | | | | | | | |
| *Cecropia sp.* | | | | | | | 2 | | | | | |
| Others (Asteraceae, Euphorbiaceae, Moraceae, Poaceae, Urticaceae) | | | | | | | 7 | 1 | 2 | 1 | | |

**Table 2.** Percentages of diet overlap based on pollen frequencies (below grey cells) and pollen abundances (above grey cells). Acau = *A. caudifer*; Acul = *A. cultrata*; Afis = *A. fistulata*; Ageo = *A. geoffroyi*; Lrob = *L. robusta*.

| Guajalito | Acau | Afis | Ageo |
|---|---|---|---|
| Acau | | 12.60% | 24.70% |
| Afis | 11.70% | | 23.80% |
| Ageo | 17.20% | 12.50% | |
| **Yanayacu** | Acau | Afis | |
| Acau | | 25.70% | |
| Afis | 17.60% | | |
| **Wildsumaco** | Acau | Acul | Lrob |
| Acau | | 36.5% | 19.7% |
| Acul | 19.2% | | 30.1% |
| Lrob | 13.1% | 15.3% | |
| **Siempre Verde** | Acau | Ageo | |
| Acau | | 24.30% | |
| Ageo | 17.30% | | |
| **Tapichalaca** | Acau | Ageo | |
| Acau | | 20.80% | |
| Ageo | 14.60% | | |
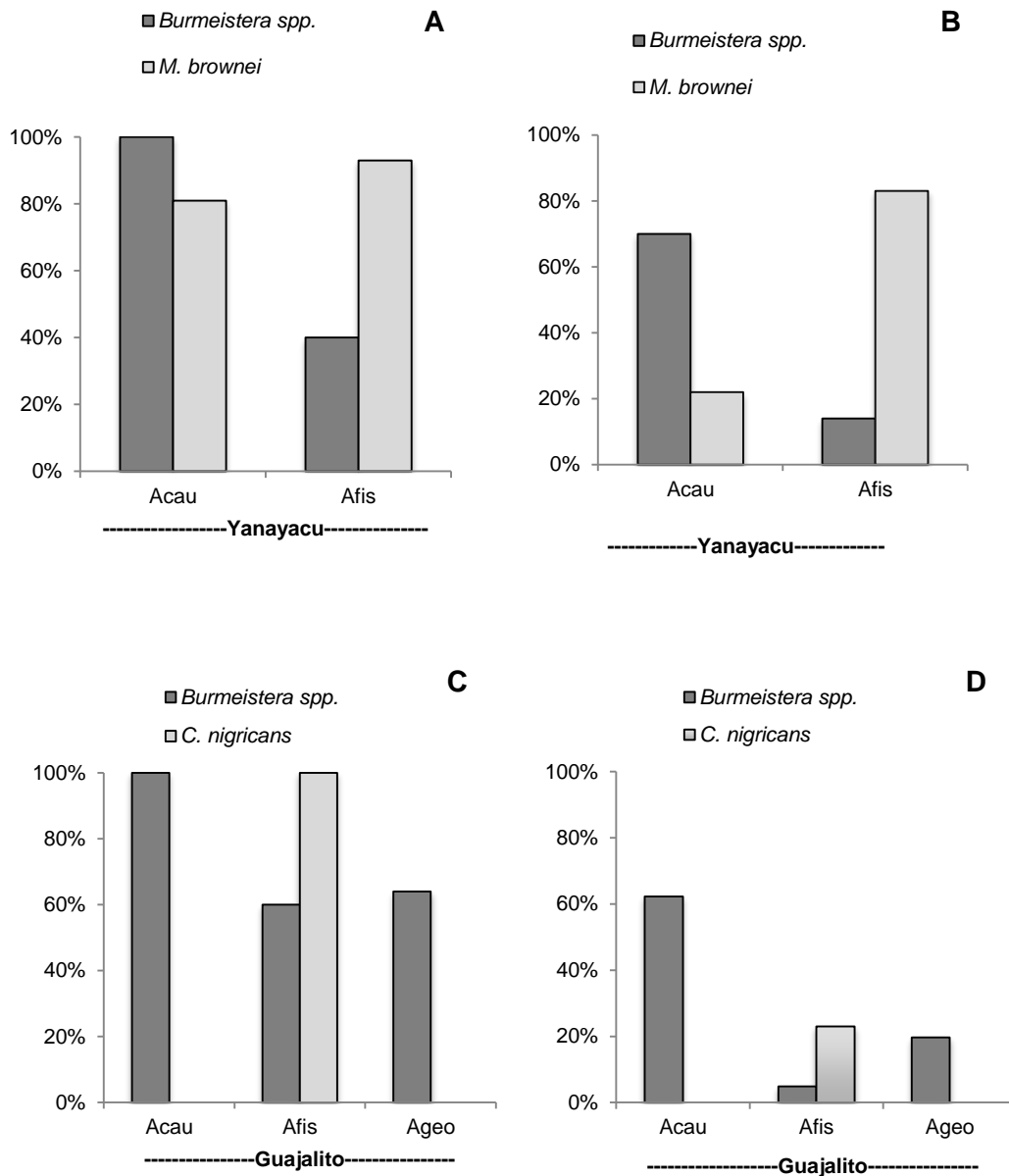
**Figure 1**. Pollen frequencies (A) and abundance (B) of *Burmeistera* spp. and *Marcgravia brownei* in nectar bat samples of Yanayacu (Chi-squared, p<0.01), and pollen frequencies (C) and abundance (D) of Burmeistera spp. and Centropogon nigricans in nectar bat samples of Guajalito (Chi-squared, p<0.01). Acau = *A. caudifer*; Afis = *A. fistulata*; Ageo = *A. geoffroyi*.
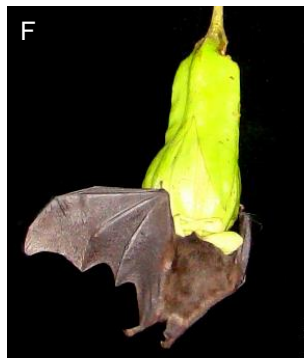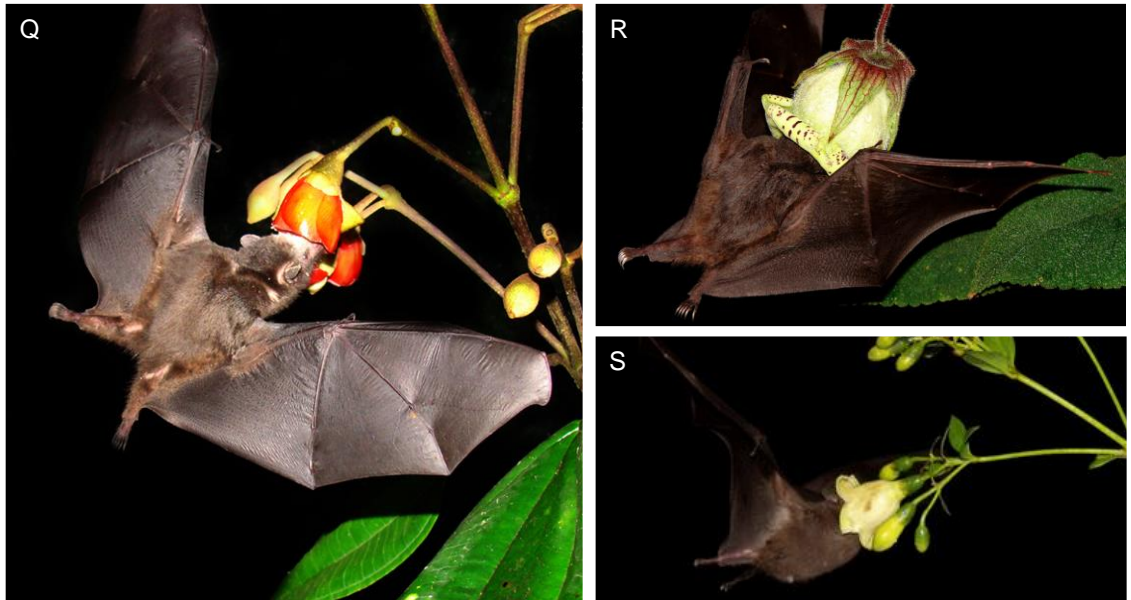
**Figure 2.** Nectar bats visiting flowers in the study sites; Yanayacu: A. *Anoura fistulata* and *Marcgravia brownei*. B. *A. geoffroyi* and *Burmeistera sodiroana*, C. *A. caudifer* and *B. ceratocarpa*, D. *A. caudifer* and *B. borjensis*; Guajalito: E. *A. fistulata* and *Centropogon nigricans*, F. *A. fistulata* and *Trianaea nobilis*, G. *A. fistulata* and *Passiflora unipetala*, H. *A. caudifer* and *B. succulenta*, I. *A. geoffroyi* and *B. truncata*, J. *A. caudifer* and *B. lutosa*; Domono: K. *A. fistulata* and *Werauhia gladioliflora*, L. *A. fistulata* and *M. williamsii*; Wildsumaco: M. *A. cultrata* and *Burmeistera* glabrata, N. *A. cultrata* and *Marcgravia sp.*; Siempre Verde: O. *A. geoffroyi* and *Cobaea trianae*, P. *A. geoffroyi* and *B. cylindrocarpa*; Tapichalaca: Q. *A. caudifer* and *Meriania tomentosa*; San Francisco: R. *A. geoffroyi* and *Capanea grandiflora,* S. *A. geoffroyi* and *Macrocarpea harlingii.* Picture credits: N. Muchhala.

# CHAPTER 2

**Do artificial nectar feeders affect bat-plant interactions in an Ecuadorian cloud forest?**

**ABSTRACT**

Bat-pollinated plants rely on bats for their reproduction, and have evolved various traits to attract them, however, when artificial nectar feeders are available in the area; these interactions could be disturbed if bats are drawn away from the plants. In this study we tested the effects of feeders in a cloud forest of eastern Ecuador on four aspects of bat-plant interactions: (1) bat relative abundance, (2) pollen loads carried by bats, (3) visitation rates to bat-pollinated plants and (4) breeding success of these plants. We divided the study area in close (~30m) and far (~500m) areas from three different feeders site. In each distance area we captured nectar-bats (*Anoura caudifer*, *A. cultrata* and *Lonchophylla robusta*) using mist-nets to estimate their relative abundance and to collect pollen samples from them to record pollen frequency, abundance and diversity. We also videotaped flowers to estimate the visitation rate by bats, and recorded different breeding success variables (based on fruit and seed numbers and fruit abortion rates) of the bat-pollinated species *Burmeistera glabrata*. Our results showed that there is an effect of artificial nectar feeders in the relative abundance of nectar bats, with closer areas to feeders presenting a higher bat relative abundance than further areas by a factor of 40. However, in spite of this, the presence of feeders does not affect pollen loads carried by bats or the flower visitation rate and breeding success of the bat-pollinated species *B. glabrata*. Additionally, we found differences in pollen loads between the three species of bats captured in closer areas from feeders, and particular observations about *L. robusta* activity suggested that feeders could draw bats away from long distances.

**Key words:** nectar bats, pollen loads, flower visitation rate, plant-breeding success, *Burmeistera glabrata*

**INTRODUCTION**

Plant-pollinator interactions determine the reproductive success of animal-pollinated plant species, and more generally they can contribute to speciation in plants and to the maintenance of biodiversity (Fontaine et al., 2006; Gegear & Burns, 2007 Fleming et al., 2009). Bats as pollinators provide specific benefits to the plants they pollinate, different from other pollinators; bats can deposit large amounts of pollen and a variety of pollen genotypes on plant stigmas, and can deliver pollen long distances (Muchhala & Thomson, 2010).

Plants pollinated by bats possess a unique combination of characteristics to attract them, including nocturnal anthesis, drab coloration, musty smell, flowers positioned well-exposed beyond the foliage, high volume of nectar, wide bell-shaped flowers or brush morphology (Faegri & van der Pijl, 1979, Helversen, 1993, Muchhala & Jarrin-P, 2002, Garibaldi et al., 2011;) and long blooming periods (Sazima et al., 1999). However, when artificial nectar feeders are available in an area, there exists the possibility that these traits are no longer sufficient to attract bats, due to the feeders supplying enormous and predictable quantities of artificial nectar that could draw bats away from plants (Arizmendi et al., 2007; Brockmeyer & Schaefer, 2012). For instance, for hummingbirds, one feeder can provide the equivalent of 2000 to 5000 flower visits (True, 1993 cited in Arizmendi et al. 2007), and for bats we estimated an equivalent of 800 to 4000 visits.

In many South American countries there is a growing market for ecotourism, and bird watching is a very popular activity for these tourists (Brockmeyer & Schaefer, 2012). Hummingbirds are particularly appreciated, and in order to allow prolonged observation of them there has been an increasing use of artificial nectar feeders in many ecolodges, including nature reserves that protect important remnants of endangered ecosystems (Brockmeyer & Schaefer, 2012). The reasons for establishing feeders range from purely economical to the general belief that they will benefit the hummingbirds (Avalos et al., 2012). Since the feeders typically remain functional during the night they not only attract to hummingbirds, they may also attract bats (pers. obs.).

It is not clear how artificial nectar feeders affect plant–pollinator interactions (Brockmeyer &

Schaefer, 2012; Avalos et al., 2012), in terms of animal behavior or reproductive success of their associated plants (Arizmendi et a., 2007). Such information is necessary in order to implement optimal management decisions for conservation (Ghazoul, 2005). Most of our knowledge about the effects of feeders on plant-animal interactions is derived from studies of plant-hummingbird interactions, and this literature shows contrasting results. For instance, Brockmeyer and Schaefer (2012) found that there are no overall effects of feeders on hummingbird's visitation to flowers in the Ecuadorian Andes, while Sonne et al. (2015) found hummingbird abundance and visitation rates to flowers of *Psychotria nuda* increased near feeders (<100m radius) in a Brazilian Atlantic rainforest. Other studies concluded that artificial feeders attract hummingbirds across considerable distances (3 km. radius), drawing them away from flowers and decreasing visitation rates (Inouye et al., 1991; Arizmendi et al., 2007; Avalos et al., 2012). Furthermore, hummingbirds captured close to feeders carried very low abundance and diversity of pollen on their bodies (Avalos et al., 2012). In addition, Arizmendi et al. (2007) found a significant decrease of plant reproductive success of the native *Salvia fulgens* in areas close to feeders within an urban park in Mexico.

In the case of bat-plant interactions the impact of artificial feeders is unknown, as no previous studies have addressed the topic. Bat biologists frequently mist-net bats near hummingbird feeders to increase capture success (pers. obs.), which suggests that bats are also using the feeder as nectar resources. For this study we experimentally tested the effect of artificial nectar feeders on bat-plant interactions in a cloud forest of eastern Ecuador. While previous studies analyzed only one to three aspects of pollinator-plant interactions (Arizmendi et al., 2007; Brockmeyer & Schaefer, 2012; Avalos et al., 2012; Sonne et al., 2015), we analyzed the effect of feeders on four aspects of bat-plant interactions: (1) bat relative abundance, (2) pollen loads carried by bats, (3) visitation rates to bat-pollinated plants and (4) breeding success of these plants. We expect that bat relative abundance will be higher in areas closer to the feeders, which could either lead to an increase or decrease in pollination of nearby flowers. Bats could prefer feeding from feeders rather than nearby flowers, causing lower visitation rate, pollen loads and plant breeding success in closer than further areas. Alternatively, bats could feed from feeders and nearby flowers, and the higher number of bats would thus increase flower visitation rate and plant breeding success in those areas, although pollen loads would likely decrease.

**METHODOLOGY**

***Study site and focal species:*** We conducted this study in a cloud forest of the eastern Ecuadorian Andes, in the private protected area of Wildsumaco Wildlife Sanctuary (0° 40.3' S, 77° 35.5' W). This area is located in the Napo province at elevations between 1310 and 1480 m., adjacent to the Sumaco-Napo Galeras National Park, and harbors a wide diversity of flora and fauna, with more than 500 species of birds and 100 species of mammals, including 22 bat species (Coloma-Santos, 2007). Wildsumaco has a tourist lodge, a biological research station, and eight well-maintained trails. It maintains three areas with hummingbird feeders: one near the lodge, one near the biological station, and one along a trail.

We focused our work on the nectarivorous bats species *Anoura caudifer*, *A. cultrata* and *Lonchophylla robusta*, previously recorded in the area. Our focal plant species, *Burmeistera glabrata* (Campanulaceae), is an understory free-standing or hemi-epiphytic subshrub (Jeppesen, 1981). Species of *Burmeistera* are primarily adapted to pollination by bats (Muchhala, 2006), and *B. glabrata* presents floral traits corresponding to chiropterophilous pollination syndrome (Helversen, 1993): the flowers are white and emit odor, they are well exposed on long pedicels, anthesis is nocturnal (lasting between 4-6 days), and the individuals remain in flower throughout the year with 1-4 flowers open at a time. We chose this species as our focal plant because it is very abundant and easy to find at the study site.

***Experimental design:*** To measure the effect of artificial nectar feeders on bat-plant interactions, we divided each of the three nectar feeder areas into one region within a 30 m radius of the feeders and a second, paired region approximately ~500m away, for a total of 6 sites. Each feeder area contained 8 to 10 feeders, which reserve employees fill every morning with a 1:4 sugar-water solution. At night, bats feed on remaining nectar. Fieldwork was conducted during June 12-July 12, 2015 and January 12-February 3, 2016. Each night, we worked simultaneously in a 'closer' feeder area and its paired 'further' area to avoid any temporal biases in sampling. We employed four methods to study bat-plant interactions in these areas:

1. Bats relative abundance: We captured bats in each distance area using mist nets (2, 6, 9, 12 m x 3, 2.5 m). We set up the nets closer to bat-pollinated plant species, 35 and 76 mist

nets in close and far distance areas, respectively. We opened the mist nets from 1900 to 2400 hrs, 1900 hr being one hour after dusk which would allow bats to feed before we caught them, and thus increase chances of obtaining pollen in fur and fecal samples. We calculated bat relative abundance as the number of bats captured per mist net per hour.

2. Pollen loads: We collected pollen from the fur of captured bats by gently pressing transparent adhesive tape against three different bat body areas: head, belly and inner wing patagium. We placed the tape on a microscope slide for later analysis in the lab. Bats were then placed in cloth bags for two hours to collect fecal samples, which were preserved in 70% ethanol. In the lab, we mounted fecal samples on a glass microscope and covered them with adhesive tape for analysis. We inspected the fur and fecal samples using a SWIFT microscope (SWIFT Microscope World, USA), conducting a scanning of the tape at 100X, and switching to 400X to identify and photograph the pollen grains. For each sample we registered the presence or absence of pollen (pollen frequency); when it was present we also estimated the pollen abundance under the categories rare (<= 100 pollen grains) and abundant (> 100 pollen grains); as well as pollen diversity counting the number of pollen types present in the samples. Additionally, we recorded the presence or absence of only *B. glabrata* pollen (*B. glabrata* pollen frequency). We identified the pollen using our pollen reference collection compiled during previous research conducted at the same area.

3. Flowers visitation rate: To obtain *B. glabrata* flower visitation rates, we videotaped flowers using a SONY camcorder with the Night Vision function (SONY Corporation of America, USA) from 1800 to 2300 hrs. We did not videotape flowers the same day that we mist-netted bats to avoid affecting the results. For each flower that we videotaped, we counted all open flowers from the focal individual and from all individuals in the 5m radius to estimate local floral patch size. Additionally we measured the relative abundance of *B. glabrata* in both distance areas using circular plots (5m radius), four per distance area in each feeder site.

4. Plant breeding success: We measured the breeding success of *B. glabrata* using three different approaches. First, we selected and marked between 5 and 10 individuals of similar sizes in each distance area, and counted the number of fruits present on each individual to estimate the average number of fruits in each distance area. Second, we collected between one and seven fruits per individual and counted the number of seeds

produced per fruit. Finally, after flowers fell we tracked the remaining hypanthia (which eventually form the fruits) in order to track fruit abortion. We counted and marked the hypanthia present on each individual and after five days we recounted the marked hypanthia that remained on each individual. That same day we counted and marked new hypanthia present and after five more days we recounted the new marked hypanthia. We calculated the percentage of fallen hypanthia per individual in each distance area. Additionally, we multiplied the average number of seeds per fruit by the proportion of remaining hypanthia and marked hypanthia to calculate the seed set per flower in each distance area.

**Statistical tests**: To assess whether bats relative abundance, flower visitation rate and breeding success variables differ between close and far distance areas we applied a Linear Mixed-effects Model (LMM) using distance area as a fixed effect and feeder site as a random effect. We transformed the non-parametric data (bat relative abundance, flower visitation rate, percentage of fallen hypanthia) using adjusted ranks (Leys & Schumann, 2010). To compare *B. glabrata* relative abundance we applied a T-test. To compare pollen load variables we applied Chi-squared for normal distributed data and Mann-Whitney U and Kruskall-Wallis for non-parametric data. Also, we used a Spearman's correlation to determine if the number of open flowers from the individual recorded and from the nearby individuals (5m radius) was related to the flower visitation rate in each distance area. All statistical analyses were performed with R 3.2.2.


## RESULTS


**Bats relative abundance:** We captured 91 and 8 bats in closer and further areas from feeders, respectively, including the species *A. caudifer*, *A. cultrata* and *L. robusta*. However, *A. cultrata* and *L. robusta* were only captured in closer areas. The LMM showed that bat relative abundance was significantly higher in closer (0.9 ± 1.7 bats per mist-net/hour) than in further areas (0.02 ± 0.06 bats per mist-net/hour) from feeders ($p<0.01$, Table 1).

***Pollen loads:*** We collected pollen samples from 91 and 8 bats in closer and further areas from feeders, respectively. Pollen was present on 89 (97.81%) and 8 (100%) samples for closer and further areas, respectively; there was no significant difference between them (Chi-squared, p=0.42, Figure 1A). Further areas had a significantly (Chi-squared, p<0.01) greater proportion of samples categorized as having abundant pollen (>100 grains), with 8 (100%) versus 58 (63.73%), as well as a significantly higher pollen diversity with 4.3 ± 1.6 pollen types/sample versus 3.3 ± 1.2 pollen types/sample (Mann-Whitney U-test, p=0.02, Figure 2A). Because the species *A. cultrata* and *L. robusta* were captured only in closer areas (as mentioned before), we tested only *A. caudifer* samples for pollen presence, abundance and diversity in the two areas. The new sample set was 36 and 8 in closer and further areas, respectively. In this case we did not find significant difference between any of the variables (Chi-squared, p=1, Figure 1B, Mann-Whitney U-test, p=0.73, Figure 2B). Additionally, we also tested whether frequency, abundance and diversity of pollen loads differ between the three captured species considering only the closer areas samples. Results showed significant difference among bat species for abundance (Chi-squared test, p<0.01, Figure 3) and diversity of pollen loads (Kruskall-Walllis test, p<0.01, Figure 4), *A. caudifer* and *A. cultrata* presented higher pollen abundance than *L. robusta*, and *A. caudifer* presented the highest pollen diversity among the three species. Finally, *B. glabrata* pollen frequency was significantly higher in further than closer areas (Chi-squared, full data set, p<0.01 and only *A. caudifer* samples, p=0.05, Figure 5).

***Flower visitation rate:*** We videotaped 30 and 44 flowers of *B. glabrata* in closer and further areas from feeders, respectively. The LMM showed that flower visitation rate was not significantly different between closer (0.276 ± 0.4 visits per flower/hour) and further (0.313 ± 0.53 visits per flower/hour) areas (p=0.31, Table 2). The relative abundance of *B. glabrata* was also not significantly different between closer (2.58 ± 2.23 individuals) and further (2.23 ± 1.87 individuals) areas (T-test, p=0.674, Table 2). The flower visitation rate was not related to the number of open flowers from the individual recorded in closer (Spearman's correlation, $R^2$=0.01, p>0.05) or further areas (Spearman's correlation, $R^2$=0.01, p>0.05) or to the number of open flowers from all the nearby individuals in closer (Spearman's correlation, $R^2$=0.01, p>0.05) or further areas (Spearman's correlation, $R^2$=0.04, p>0.05).

***Plant-breeding success:*** We took measurements of plant-breeding success variables from an average of 19.2 and 26.8 plant individuals in closer and further areas from feeders, respectively. We did not always achieve taking all measurements from all plant individuals (Table 2). The LMM showed no significant differences between distance areas, close or far from feeders, for the four plant-breeding success variables: number of fruits, number of seeds, percentage of fallen hypanthia or seeds set per flower (Table 2).

## DISCUSSION

This study found an effect of artificial nectar feeders in the relative abundance of nectar bats in the study area; bats are more abundant in closer areas to the feeders than further from them. Despite this, we found no effect of feeders on pollen loads, flower visitation rate and the breeding success of the bat-pollinated species *B. glabrata*. The only exception was the pollen frequency of *B. glabrata*, which was higher in further than closer areas, although this did not affect the breeding success of that species in those areas. Additionally, we found differences in pollen loads between the three species of bats in closer areas to the feeders.

***Bats relative abundance:*** We found a higher relative abundance of bats in areas closer to the feeders by a factor of 40. These results agree with previous studies that found a higher relative abundance of hummingbirds in areas closer to feeders (Sonne et al., 2015). Therefore, the presence of feeders during the day and night will similarly attract and affect the local distribution of hummingbirds and bats. Additionally, we found that the species *L. robusta* and *A. cultrata* were only captured in areas closer to the feeders. Previous captures conducted at Wildsumaco were equally poorly successful in capturing *L. robusta* with mist-nets located at further distances from the feeders (one capture in 8 nights using 4 mist-nets in July 2015). However, in the case of *A. cultrata*, captures were successful setting up mist-nets closer to plant species of the genus *Marcgravia*, regardless their location according to the feeders (pers. obs.). We suspect that the altitude of Wildsumaco (~1400 m) was close to the altitudinal limit of the distribution range of *L. robusta* (Davalos et al., 2015), contrary to the two species of *Anoura* collected, which altitudinal distributions reach up to 1500 m (*A. caudifer*, Bejarano-Bonilla et

al., 2007) and 2600 m (*A. cultrata*, Tamsitt and Nagorsen, 1982). Previous studies suggested that *L. robusta* might roost at medium altitude and cover a larger altitudinal gradient to opportunistically feed from high-energy density resources, such as species that produce high nectar volumes (Tschapka, 2004). Tschapka (2004) observed that *L. robusta* individuals appeared regularly 1 hr later than smaller bat species when mistnetting, and that they were captured in large numbers at the same place (even in different years) next to a high-energy density plant; suggesting that they fly daily since sunset (*c.* 30 km. in 1 hr) to distant feeding areas with predictable and rich resources. Similarly, we captured *L. robusta* individuals late at night and only next to feeders (a high energy-density resources with large amounts of artificial nectar), which suggest that they might be conducting a similar feeding behavior observed by Tschapka (2004) at La Selva Biological Station (Costa Rica). These observations also suggest that feeders can attract bats from far away, rather than only locally.

*Pollen loads:* We did not find any effect of feeders on pollen loads carried by the species *A. caudifer* in Wildsumaco. It is worth noting that our analyses showed contrasting results when we included different sample sets. Results using the full sample set showed higher abundance and diversity of pollen in bats captured in further areas, however, results including only *A. caudifer* sample set showed similar values of pollen frequency, abundance and diversity in both distance areas. Thus the low pollen loads carried by *L. robusta* and *A. cultrata* appear to be driving this pattern. We suspect that there might be differences in feeding behavior among bat species in terms of flower preferences or feeding activity patterns. The pollen frequency of *B. glabrata* was higher in further than closer areas in both analyses, however our other results (below) suggest that this does not affect the breeding success of the species. Ours is the first study to statistically compare pollen loads between pollinators flying close and far from feeders, although Avalos et at. (2012) anecdotally noted that hummingbirds captured close to feeders carried little or no pollen on their bodies (low frequency and abundance) and the majority of those with pollen had only one type (low diversity).

*Flower visitation rate:* There was no effect of feeders on *B. glabrata* flower visitation rates by bats in Wildsumaco. Our results concur with previous studies that found no significant differences in hummingbird visitation rate to flowers located at different distances to feeders

(Brockmeyer and Schaefer, 2012), but contrast with others that found an increase (Sonne et al., 2015) or a decrease (Arizmendi et al., 2007) in visitation rates in plant individuals located closer to the feeders. Sonne et al. (2015) stated that the increase in flower visitation rate is due to higher hummingbird abundance in the area, however, although we found higher bat abundance closer to feeders we did not find a higher visitation rate. This could be due to unknown behavioral differences among these two pollinator guilds, or to differences in sugar concentration among hummingbird and bat pollinated plant species (Perret et al., 2001) that in the case of *B. glabrata*, could make it be less attractive than the feeders. Regarding the study of Arizmendi et al. (2007), they applied a different methodology that could be the principal cause of contrasting results with our study (and that of Sonne et al., 2015). They designed an experiment with control and treatment areas containing 10 individuals of hummingbird-pollinated plants. At the treatment areas they presented two feeders during one day and they registered flower visitation rate at both areas. Our experimental design considered feeders that were presented at specific locations for at least 7 years, allowing time for the pollinators to adjust their distribution to the presence of feeders (Sonnet et al., 2015). The sudden presence of feeders would likely attract many hummingbirds during the day, drawing them away from further areas, increasing flower visitation rates in closer areas and decreasing it in further areas (Arizmendi et al., 2007). In the case of bats, a similar experiment to the one of Arizmendi et al. (2007) would probably fail because it could take multiple nights for bats to find the new feeder resources.

*Plant-breeding success:* We did not find an effect of feeders on the reproductive success of *B. glabrata.* There were similar values for number of fruits, number of seeds, percentage of fallen hypanthia and seed set per flower in both closer and further areas from feeders. Our results are consistent with findings of Sonne et al. (2015), that collecting and counting pollen deposited on flower stigmas of one hummingbird-pollinated species did not find differences in the amount of pollen deposited on flower stigmas (another variable to measure plant-breeding success) of individual located along a distance gradient from feeders. Although Arizmendi et al. (2007) conducted a different experimental design (as mentioned before), it is worth noting that they obtained different breeding success results for each of the plant species they worked with. Seed production of *Salvia fulgens* was lower in individuals exposed to the feeders, whereas

seed production of *S. mexicana* was similar between exposed and non-exposed individuals (Arizmendi et al., 2007), suggesting that effects may be species-specific. In our study we did not find a negative effect of feeders on the breeding success of *B. glabrata*, but we note that this is the most abundant bat-pollinated species in the area; feeders might be expected to have a larger negative impact on rare plant species (McCaffrey & Wethington, 2008).

**CONCLUSIONS**

We conclude that feeders are highly attractive to bats, increasing their relative abundance close to the feeders by a factor of 40. Despite the increase in bats relative abundance, the presence of feeders does not affect pollen loads carried by bats, flower visitation rate by bats, and breeding success of the bat-pollinated species *B. glabrata*. Additionally, we found differences in pollen loads between the three species of bats captured close to the feeders, suggesting that there might be differences in feeding behavior among bat species in terms of flower preferences or feeding activity patterns. Particular observations about the time and place of capture of the species *L. robusta* suggest that feeders could attract bats from long distances. Further studies on the feeding behavior of different bat species under the presence of feeders as well as analyzing the effect of feeders on the breeding success of other bat-pollinated species, in a variety of habitats than the cloud forest of eastern Ecuador, are needed.

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**TABLES AND FIGURES**


**Table 1.** Bat relative abundance in close and far areas to the feeders.

| Bat species | Close areas | | Far areas | |
|---|---|---|---|---|
| | Captured individuals | Mean relative abundance * | Captured individuals | Mean relative abundance * |
| *Anoura caudifer* | 36 | 0.5 ± 1.1 | 8 | 0.03 ± 0.07 |
| *Anoura cultrata* | 4 | 0.07 ± 0.3 | 0 | 0 |
| *Lonchophylla robusta* | 51 | 0.43 ± 0.7 | 0 | 0 |
| **Total** | 91 | 0.907 ± 1.7 bats/mist-net/hour | 8 | 0.023 ± 0.06 bats/mist-net/hour |

* ± SE, LMM, Estimate (SE) = 26.84 (5.3), *df* = 107, t = 5.01, **p < 0.01**

**Table 2.** Results summary for flower visitation rate and breeding success variables of *Burmeistera glabrata.*

| Variables | N | Mean ± SE | Sample unit | Fixed effect | | | | Random effect (SD) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Estimate (SE) | df | t value | p value | Intercept | Residual |
| *Flower visitation rate* * | | | | | | | | | |
|     Close areas | 14 | 0.276 ± 0.4 visits/hr | plant individual | -4.95(4.9) | 70 | -1 | 0.32 | 6.46 | 20.7 |
|     Far areas | 12 | 0.313 ± 0.53 visits/hr | | | | | | | |
| *Average number of fruits*** | | | | | | | | | |
|     Close areas | 23 | 16.78 ± 16.8 fruits | plant individual | -0.45(0.3) | 47 | -1.62 | 0.11 | $2.96 \times 10^{-5}$ | 0.98 |
|     Far areas | 28 | 7.67 ± 6.09 fruits | | | | | | | |
| *Average number of seeds** | | | | | | | | | |
|     Close areas | 25 | 1166.22 ± 374.01 seeds | plant individual | 29.77(80.4) | 54 | 0.37 | 0.71 | 141.43 | 292.17 |
|     Far areas | 33 | 1192.87 ± 323.04 seeds | | | | | | | |
| *Percentage of fallen hypanthia (%fh)*** | | | | | | | | | |
|     Close areas | 16 | 35.86 ± 33.18 %fh | plant individual | -0.19(4) | 39 | -0.04 | 0.96 | 15.35 | 26.2 |
|     Far areas | 27 | 48.66 ± 36.99 %fh | | | | | | | |
| *Seeds set per flower* ** | | | | | | | | | |
|     Close areas | 13 | 832.83 ± 433.82 seeds/flower | plant individual | -194.79(166.5) | 28 | -1.16 | 0.25 | 0.02 | 462.59 |
|     Far areas | 19 | 638.03 ± 480.82 seeds/flower | | | | | | | |

* Sampled in 2015 and 2016, ** Sampled only in 2016

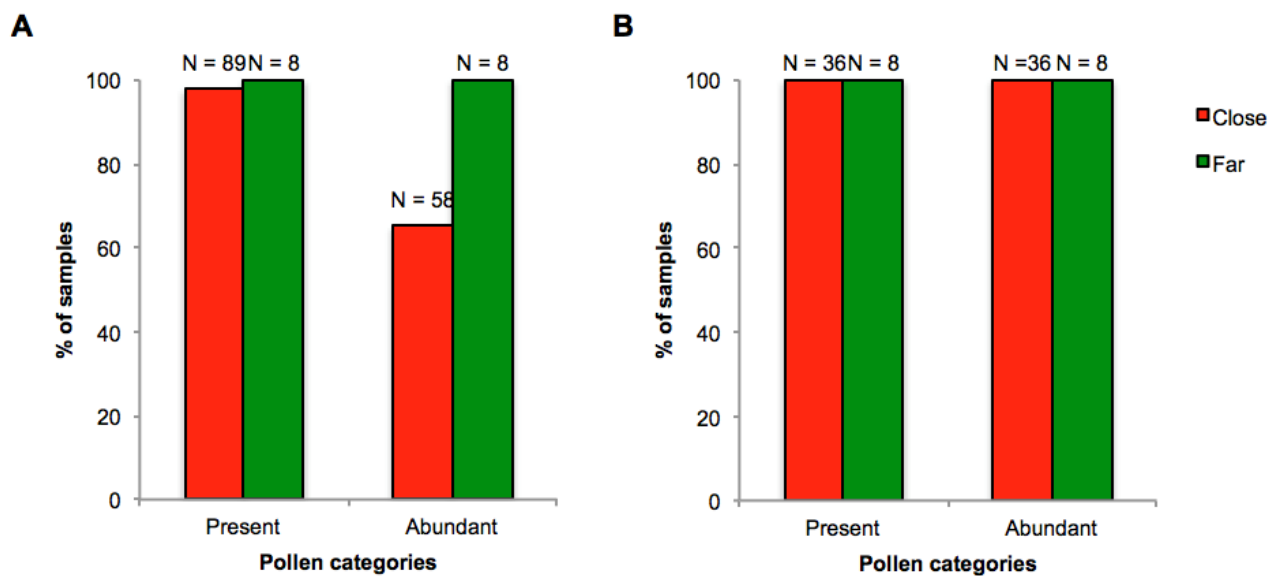SE = Standard error, SD = Standar deviation

**Figure 1.** Percentage of samples with pollen and abundant pollen based on full sample set (A) and only *A. caudifer* sample set (B).
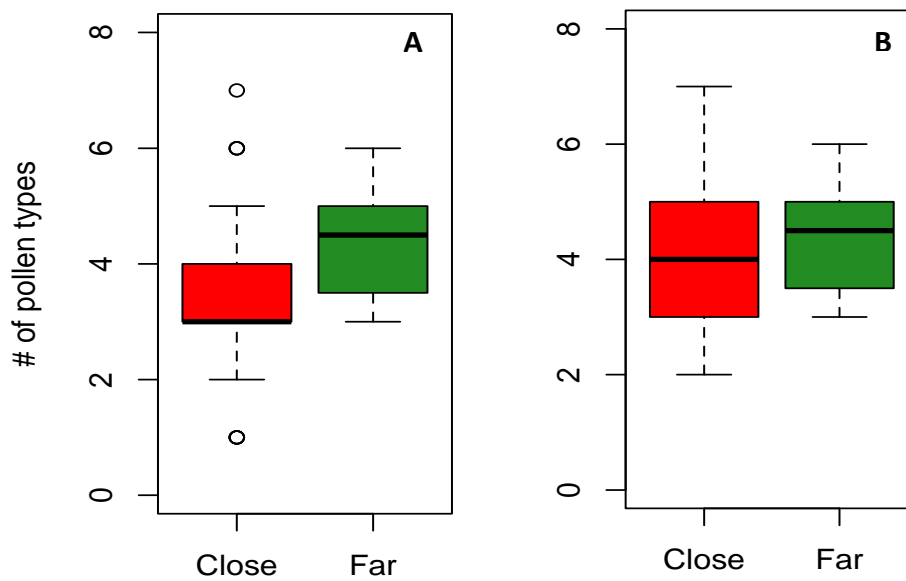


**Figure 2.** Pollen diversity in close and far distance areas based on full sample set (A) and only *A. caudifer* sample set (B).
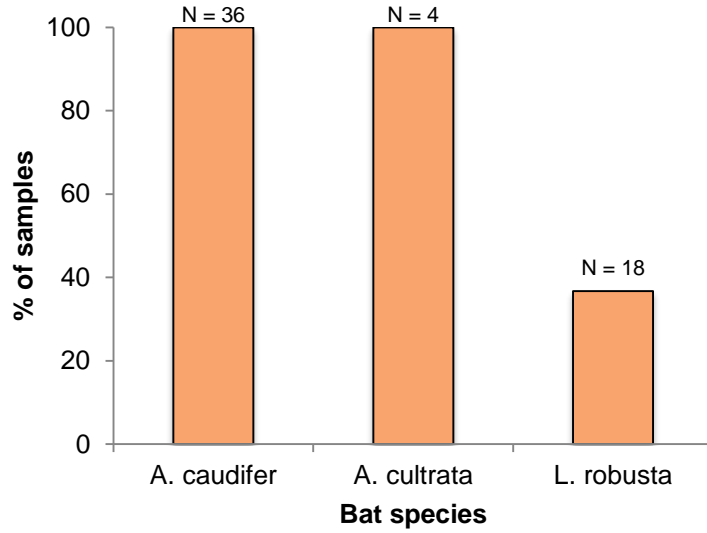
**Figure 3.** Percentage of samples with abundant pollen in the three bat species analyzed in close areas to feeders.
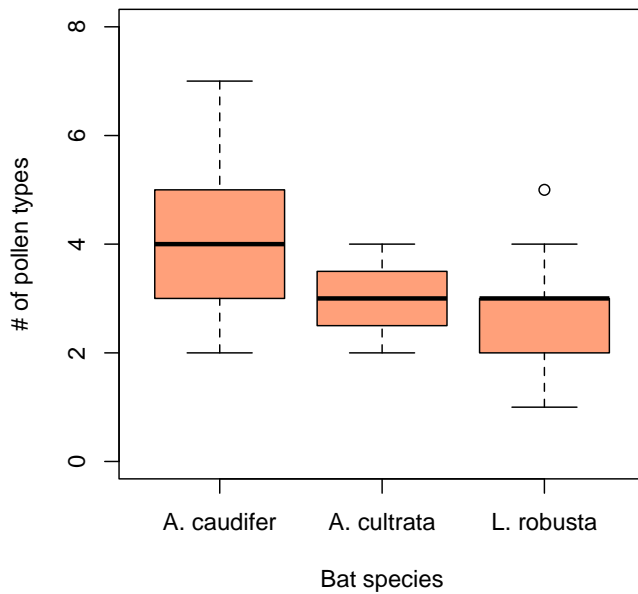


**Figure 4.** Pollen diversity in the three bat species analyzed in close areas to feeders.
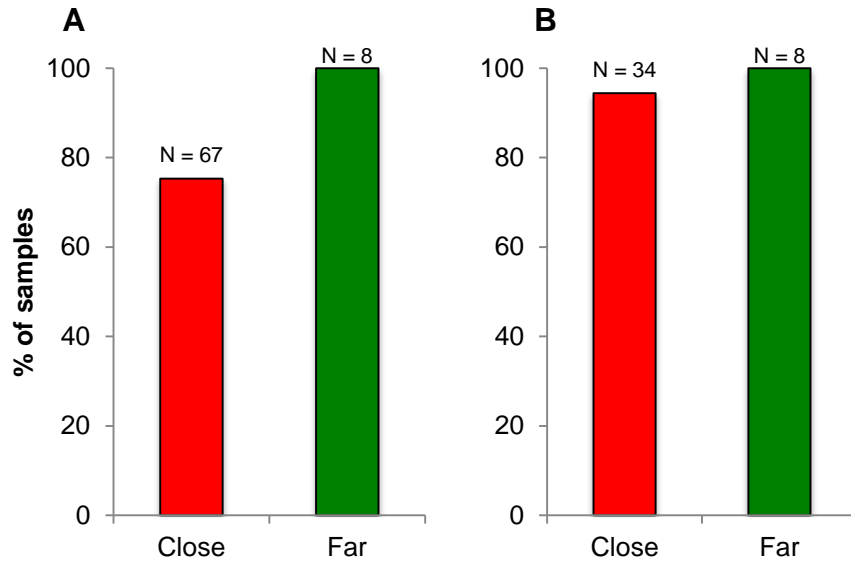
**Figure 5.** Pollen frequency of *Burmeistera glabrata* in close and far distance areas based on full sample set (A) and only *A. caudifer* sample set (B)