ARTICLE
Ecosphere Naturalist

ECOSPHERE
AN ESA OPEN ACCESS JOURNAL

Bat–flower trait matching: Extreme phenotypic specialization affects diet preferences but not diet breadth

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Abstract

Nectarivorous bats have evolved various adaptations to feeding from flowers, such as long, extensible tongues and the ability to hover. The champion of tongue length, *Anoura fistulata*, can extend its tongue to 150% of its body length, yet little is known about its interactions with flowers in the wild. Here we analyzed the diet of *A. fistulata* and co-occurring nectar bats in eight sites across Ecuador. Results demonstrate that, despite its phenotypic specialization, *A. fistulata* is no more ecologically specialized in its dietary breadth than co-occurring nectar bats. However, it prefers deeper flowers, and is the sole visitor to two species (*Centropogon nigricans* and *Marcgravia williamsii*) whose extremely deep flowers make their nectar inaccessible to other bats. Furthermore, *A. fistulata* only occurred in sites with at least one flower deeper than the tongue length of other nectar bats, suggesting it needs such a guaranteed nectar source to maintain a population. Finally, we found strong covariation across sites between the local tongue length of *A. fistulata* and the depth of the deepest flowers it visits. This suggests that the coevolutionary race that selected for the exceptional tongue length of this bat over time is also playing out in a geographic mosaic across space.

KEYWORDS
Anoura fistulata, *Centropogon nigricans*, chiropterophily, coevolution, geographic mosaic, *Marcgravia williamsii*, pollination

INTRODUCTION

Organisms that use other species for resources are continuously selected to better exploit their partner. In particularly specialized interactions, this can cause an evolutionary race involving reciprocal adaptations (Darwin, 1862; Slatkin & Smith, 1979) and the evolution of extreme traits over time, such as the 2-cm-long rostrum of seed-predatory weevils (Toju, 2008) or the 25-cm-long proboscis of the giant hawkmoth (Nilsson, 1988; Rothschild, 1903). One assumes that the costs of enhancing a particular aspect of phenotype will place an upper bound to extreme traits, which should quickly devolve when no longer needed. If true, extreme traits should covary closely across space and time, leading to a geographic mosaic of local adaptations tightly matching local selective pressures (Anderson & Johnson, 2009; Gomulkiewicz et al., 2000; Nuismer et al., 1999; Thompson, 2005; Zhang et al., 2013).

While all nectar bats have long, extensible tongues that allow them to better exploit flowers (Winter & von Helversen, 2003), the Tube-Lipped Nectar Bat *Anoura fistulata* (Phyllostomidae) has a tongue more than twice as long as that of any other species, which, at 8.5 cm, is more than 150% of its own body length (Muchhala, 2006). Previous work showed that *A. fistulata* is the sole pollinator of the 8- to 9-cm-long flowers of *Centropogon nigricans* (Campanulaceae, Muchhala, 2006), and that tongue and flower length likely evolved in a coevolutionary race (Muchhala & Thomson, 2009). Specifically, selection favors longer tongues because they allow access to more nectar, and favors longer flowers because pollinators must probe deeper into the flowers, increasing contact between bat and floral reproductive structures, and leading to greater pollen export and receipt (Muchhala & Thomson, 2009). But *C. nigricans* only occurs in a very narrow range in the Andes of northwestern Ecuador—what happens to tongue length over the rest of the range of *A. fistulata*?

In the time since its discovery in northwestern Ecuador (Muchhala et al., 2005), *A. fistulata* has been captured throughout both slopes of the Ecuadorian Andes, as far north as central Colombia, and as far south as the southern border of Peru (Calderón-Acevedo & Muchhala, 2018; Gárate-Bernardo & Carrasco-Rueda, 2016; Mantilla-Meluk et al., 2009). Muchhala et al. (2005) included notes on its diet from a single site, where it carried pollen on its fur from long-tubed flowers such as *C. nigricans*, *Markea* spp. (Solanaceae), *Marcgravia* spp. (Marcgraviaceae), and bromeliads, while the pollen of the short-tubed flowers of the genus *Burmeistera* (Campanulaceae) was conspicuously absent. Previous work on the diets of nectar bats shows that they typically visit many plant species within their habitats (for instance, 16 species for *Hylonycteris underwoodi*, 15 for *Glossophaga commissarisi* [Tschapka, 2004], 11 for *A. caudifer*, and 10 for *A. geoffroyi* [Muchhala & Jarrín-V, 2002]). Given its exceptional tongue morphology, *A. fistulata* might be more specialized to a smaller set of long-tubed flowers, that is, its phenotypic specialization (sensu Fleming & Muchhala, 2008; Ollerton et al., 2007) might lead to a corresponding ecological specialization (sensu Armbruster, 2017; Futuyma & Moreno, 1988) in terms of reduced dietary niche breadth (for further discussion of terminology, also see Ferry-Graham et al., 2002; Forister et al., 2012). Alternatively, a long tongue may allow them to have an even more generalized diet, enabling them to use both short- and long-tubed flowers. A related question is whether *A. fistulata* requires long-tubed flowers to maintain a local population, or whether it can be found in sites without them.

In this study, we compiled more than a decade of fieldwork to analyze the diet of *A. fistulata* and co-occurring nectar bats in eight cloud forest sites across Ecuador. We captured bats and identified the pollen they carried, and measured tongue lengths and flower depths at each site. Our primary aims were to determine (1) whether the specialized tongue morphology of *A. fistulata* is associated with a more specialized diet, in terms of either niche breadth or food plant morphology, (2) whether the occurrence of *A. fistulata* in an area depends on the occurrence of long-tubed flowers; and (3) whether the local tongue length of *A. fistulata* covaries with the depth of the flowers it visits across the sites. Along the way, we added many new records of the relatively poorly studied interaction between nectar bats and the flowers they pollinate.

METHODS

Study sites

We collected samples in eight cloud forest sites throughout both sides of the Andes of Ecuador. The elevations of the sites varied from 1000 to 3500 m above sea level, and in each site, we captured two to four species of nectar bats (Appendix S1: Table S1 and Appendix S2: Figure S2). The authors conducted fieldwork in 28 site visits (typically 10–14 days each visit) from April 2003 to June 2005 (NM and AC), October 2009 to December 2010 (NM and DP), and June 2014 to January 2016 (RM).

Diet analysis

Bats were captured with mist-nets (2, 6, 9, or 12 m in length by 2.5 or 3 m in height) placed along trails. Nets were opened at dusk and remained open from 1800 to 0100 h. 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 slide for later inspection and identification. Additionally, we held the bats in cloth bags for 2 h to collect fecal samples, which were then spread on a slide and covered with adhesive tape. Fur and fecal samples were stained with gelatin cubes containing fuchsin dye (Kearns & Inouye, 1993) and inspected using a SWIFT light microscope (SWIFT Microscope World, USA) at 100×, switching to 400× when necessary to identify and photograph the pollen grains. We categorized the components of each fecal sample as pollen, vegetative tissue, seeds, or insects. For all samples (fur and fecal) we identified all pollen types to

the lowest taxonomic level using our pollen reference collection taken directly from flowers during fieldwork, and recorded the presence/absence of each pollen type in each sample. For each bat species in each site, excluding instances when fewer than five individuals were captured in the site, we estimated diet richness as the number of flower species visited, diet diversity with the Shannon–Wiener (H') diversity index, and niche breadth with Levin's standardized index (B_A). The H' index yields a value greater than zero, with larger values indicating increasing generalization, while B_A ranges from 0 (only one resource used) to 1 (all available resources used evenly; Krebs, 1999). To address our first aim of examining whether functional specialization of the species is associated with dietary specialization, we used one-way ANOVA to compare the average diet richness, H', and B_A index among bat species.

Tongue and flower measurements

To measure tongue length, we held a subset of the captured bats individually in experimental tents. First, we trained the bat to feed from a plastic test tube (12-mm diameter) filled with a 1:4 sugar–water solution. Individuals that did not feed within 2 h were released. Those that fed were offered the sugar solution in a modified drinking straw, which was sealed at the base to hold the liquid. The narrow opening of the straw (6-mm diameter) prevented the bat from inserting its snout, allowing us to isolate tongue length. After every 30 min, we measured the depth of the sugar solution consumed since the last check and then replaced approximately half of this. When the distance to the surface of the solution was the same for three consecutive visits, we considered this to be the bat's maximum tongue extension (sensu Muchhala, 2006); hereafter we refer to this as "tongue length." We measured tongue length for 24 *Anoura caudifer*, 17 *A. fistulata*, 11 *A. geoffroyi*, 6 *A. cultrata*, and 11 *Lonchophylla robusta*, and tested for differences in tongue extension with a linear mixed model (LMM), with tongue length as the dependent variable, bat species as a fixed factor, and site as a random factor.

We also measured the depth of all bat-visited flowers in each reserve. Our goal was to measure the functional depth of the flowers, which represents the length a bat has to extend its tongue to reach the nectar (hereafter referred to as simply "flower depth"). Accordingly, we used photos and videos of bat pollination to determine whether bats also insert their heads into the flowers. For *Marcgravia* species, which have nectaries with narrow openings that only allow tongue insertion (see Figure 1 and Appendix S2: Figure S1A,L), we measured the distance from nectary opening to base. For flowers with campanulate corollas that allow bats to insert their heads before extending their tongues, we measured corolla length and then subtracted the average cranium length of each bat species (also see Winter & von Helversen, 2003). Cranium measurements were taken from Muchhala et al. (2005). For flowers with a narrow tubular base and flaring corolla lobes (*Burmeistera* spp. and *Aphelandra acanthus* [Acanthaceae]; see Appendix S2: Figure S1), we measured solely the tubular portion without subtracting the cranium length. We complemented our field data with measurements of herbarium specimens (MO) collected in the corresponding reserve sites. As our aim was to examine across-site covariation between tongue length and flower depth, we excluded two species that only occurred in a single site (*A. cultrata* and *L. robusta*) and focused further analyses on the other three species (see Appendix S1: Table S1). For each of these, we excluded sites where we were unable to measure tongue length or where we captured less than five individuals, giving us sample sizes of $N = 6$ sites for *A. caudifer*, $N = 4$ sites for *A. geoffroyi*, and $N = 4$ sites for *A. fistulata*. For each of these three species at each site, we quantified two variables to characterize flower depth in their local diet for further statistical analyses: (1) the greatest flower depth among all plant species visited in that site, and (2) the average flower depth among these plant species. We would expect any patterns of covariation to be more pronounced for the former, given that maximum tongue extension should be most influenced by the deepest flower in that site, while the latter would provide a better picture of the overall depths the bats were utilizing. For this latter value, we used a weighted average, weighing each flower depth by the proportional use by bats in that site (e.g., if *Pitcairnia fusca* [Bromeliaceae] pollen was found on 7 of 10 *A. fistulata* bats captured, it would be weighted by 0.7, while a species found on all individuals would be weighted by 1.0). We tested for differences in flower depth used by the different species across our study sites with a one-way ANOVA that included bat species as a fixed factor and either greatest flower depth or average flower depth (i.e., one value per bat per site) as the dependent variable.

Finally, to address our third aim, we used LMMs to test the overall relationships between tongue lengths (dependent variable) and flower depths (covariate) across the study sites for the three species of bat (fixed factor) that occurred in more than one study site (i.e., *A. caudifer*, *A. geoffroyi*, and *A. fistulata*). We performed one LMM for the greatest flower depth as the covariate and a second for average flower depth as the covariate. For significant LMMs, we used Pearson's correlations to further decompose the relationship, correlating
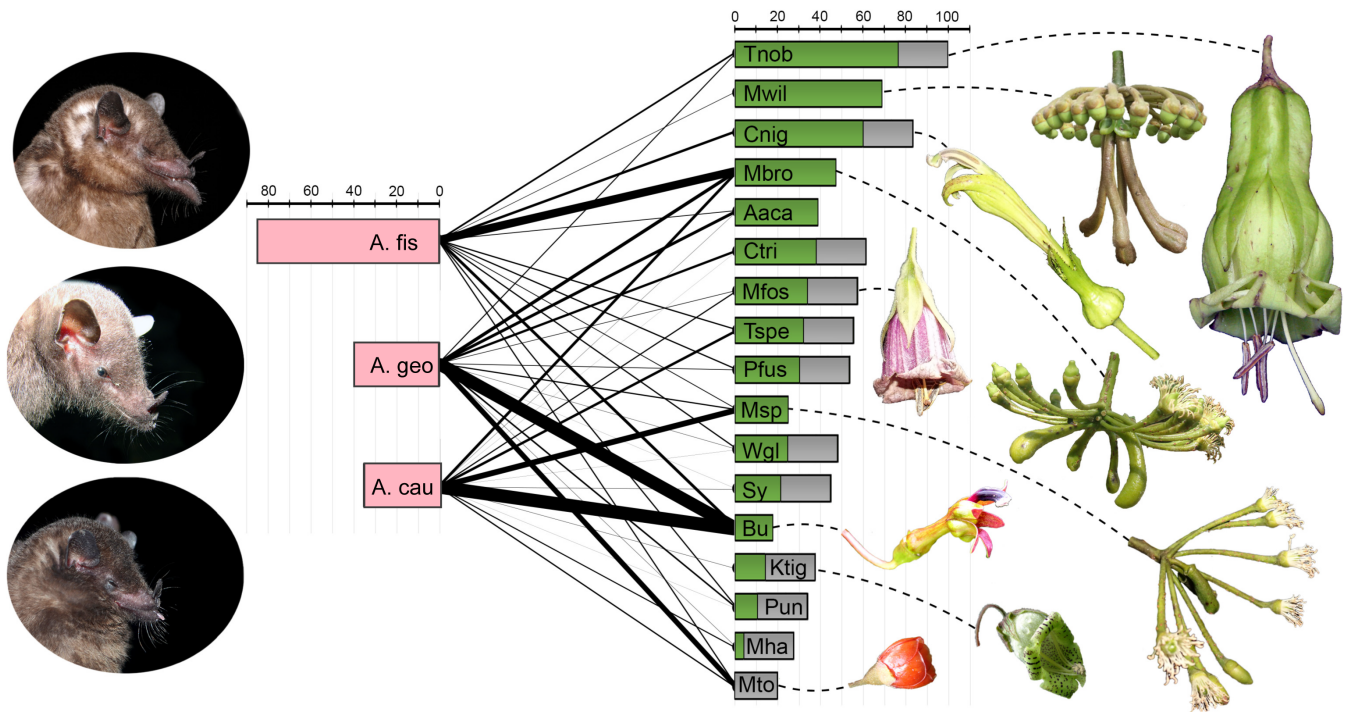
FIGURE 1 Summary of interactions between common nectar-feeding bats in Ecuadorian cloud forests and the flowers they pollinate: combines data from all eight study sites for *Anoura fistulata*, *A. geoffroyi*, and *A. caudifer*, the three bat species regularly captured in these sites (does not include *Lonchophylla robusta* or *A. cultrata*, which only occurred in one site). Pink bars show tongue lengths, while the combined green and gray bars show total flower depths. Green alone shows functional flower depth, with head length (gray bars) of *A. fistulata* (which has an intermediate skull length) subtracted in those cases where bats insert their heads into the flowers during visits. Scale bars apply to all tongue lengths and flower depths as well as flower photos (bat photos are not to scale), and the thickness of lines connecting bats and flowers corresponds to the proportion of bat diet. A. cau, *A. caudifer*; A. fis, *A. fistulata*; A. geo *A. geoffroyi*; Aaca, *Aphelandra acanthus*; Bu, *Burmeistera* spp.; Cnig, *Centropogon nigricans*; Ctri, *Cobaea trianae*; Ktig, *Kohleria tigridia* (=*Capanea grandiflora*); Mbro, *Marcgravia brownei*; Mfos, *Markea fosbergii*; Mha, *Macrocarpea harlingii*; Msp, *Marcgravia* sp.; Mto, *Meriania tomentosa*; Mwil, *Marcgravia williamsii*; Pfus, *Pitcairnia fusca*; Pun, *Passiflora unipetala*; Sy, *Symbolanthus* sp.; Tnob, *Trianaea nobilis*; Tspe, *Trianaea speciosa*; Wgl, *Werauhia gladioliflora*. Photos by Jorge Brito (*A. fistulata*), Camilo Calderón-Acevedo (*A. caudifer*), and N. Muchhala (all others).

tongue length with flower depth for each species separately. These and all other statistical analyses were performed in SPSS version 24.0.

RESULTS

Diet analysis

We captured 428 nectar bats of five species in the eight Ecuadorian sites we visited: *Anoura caudifer* ($N = 182$), *A. cultrata* ($N = 17$), *A. fistulata* ($N = 46$), *A. geoffroyi* ($N = 92$), and *Lonchophylla robusta* ($N = 91$). We captured *A. fistulata* in four of the eight sites: Bellavista, Domono, Guajalito, and Yanayacu. Based on fecal samples, its diet was composed of pollen (found in 89% of the samples), insects (71% of the samples), and vegetative tissues (43% of the samples; Appendix S1: Table S2). The

insect remains in *A. fistulata* samples belonged to the orders Coleoptera and Hymenoptera, and we also recorded scales of the order Lepidoptera in the other species of *Anoura* and *L. robusta*. We were not able to identify the vegetative tissues but suspect that they represent pulp from fruit consumption. Pollen had the highest frequency among all the components in all bat species. These results indicate that *A. fistulata* feeds mainly on flowers (nectar and pollen) and supplements its diet with insects and likely fruits. The other species of *Anoura* and *L. robusta* similarly feed mainly on flowers and supplement their diets with insects and fruits. The pollen identified in fecal and fur samples corresponded to 51 different plant taxa. These results greatly expand our knowledge of bat–plant interactions, adding numerous links to the known web of interactions between nectar-feeding bats and bat-adapted flowers in Andean cloud forests (Figure 1).

In general, the floral diets of the species of nectar bats overlap broadly where they co-occur (Figure 1; Appendix S1: Tables S3 and S4). Notable exceptions include three species with very deep flowers, two of which (*Centropogon nigricans* and *Marcgravia williamsii*) were only visited by *A. fistulata*, and the third (*Trianaea nobilis*, Solanaceae) was visited only by *A. fistulata* and *A. geoffroyi* (and not by the shorter-tongued *A. caudifer*). The only pollen found on other bats but not on *A. fistulata* were those of several species of *Burmeistera* (including *B. sodiroana* and *B. truncata*), which have some of the shortest corolla tubes (although we note that *A. fistulata* did occasionally carry pollen of other species of *Burmeistera*, including *B. ceratocarpa* and *B. borjensis*). For our statistical analyses of diet diversity for the three species that occurred in more than one site (*A. fistulata*, *A. caudifer*, and *A. geoffroyi*; see Appendix S1: Table S1), one-way ANOVAs revealed no significant differences among them for either diet richness, diet diversity (H'), or niche breadth (B_A; Table 1). Thus, despite its specialized tongue morphology, *A. fistulata* does not have an ecologically more specialized diet, in terms of numbers of species visited or overall niche breadth, than that of co-occurring nectar bat species. However, results below show that they do have a more specialized diet in terms of the specific subset of flowers they prefer to visit, in that they tend to visit deeper flowers.

Tongue and flower measurements

An LMM showed significant differences in tongue length across the five species of nectar bats (MS = 7179.8, $F_{4,64} = 785.9$, $p < 0.0001$, Figure 2). For the three species that occurred in multiple sites, a one-way ANOVA showed significant differences in the flower depths they preferred to visit across their study sites, both in terms of greatest depth (MS = 1213.4, $F_{2,11} = 6.2$, $p = 0.016$) and average depth (MS = 334.2, $F_{2,11} = 12.4$, $p = 0.002$), with *A. fistulata* tending to visit deeper flowers (Figure 3). Additionally, *A. fistulata* was only captured in four sites with particularly deep flowers, including (with species name and average flower depth in parentheses) Bellavista (*Trianaea nobilis*, 7.3 cm), Yanayacu (*Marcgravia brownei*,

5.0 cm), Guajalito (*Trianaea nobilis*, 7.9 cm), and Domono (*Marcgravia williamsii*, 6.9 cm). In three of the four sites where it did not occur, the deepest flower was much shorter, including Tapichalaca (*Markea fosbergii*, 3.3 cm), Wildsumaco (*Trianaea speciosa*, 3.1 cm), and San Francisco (*Symbolanthus* sp., 2.1 cm, Gentianaceae). The final site (Siempre Verde) is an exception, as *Trianaea nobilis* occurs here, albeit with a shorter functional depth (5.8 cm) than at the other sites mentioned above.

LMM tests of the effects of flower depth (covariate) and bat species (fixed factor) on tongue length (dependent variable) across the study sites revealed a significant correlation when the greatest flower depth was used as the covariate ($F_{1,10} = 5.2$, $p = 0.046$; with a significant main effect of bat species, $F_{2,10} = 453.4$, $p < 0.001$). The same analysis with average flower depth as the covariate failed to produce a significant correlation ($F_{1,10} = 1.8$, $p = 0.212$; with a significant main effect of species, $F_{2,10} = 161.1$, $p < 0.001$). Thus, tongue length covaries to some extent with the greatest flower depth but not with average depth. To further explore the former result, we performed a separate Pearson's correlation of the greatest flower depth and tongue length among sites for each species. A significant positive correlation was found for *A. fistulata* ($R^2 = 0.99$, $p = 0.012$, $N = 4$ sites; Figure 4), while correlations were not significant for either *A. caudifer* ($R^2 = 0.09$, $p = 0.57$, $N = 6$ sites) or *A. geoffroyi* ($R^2 = 0.17$, $p = 0.59$, $N = 4$ sites). In sum, our results show that *A. fistulata* visits deeper flowers than the other bats, and that its local tongue length covaries with the length of the deepest flower it consumes in the sites where it occurs.

DISCUSSION

Our results show that all nectar bats in our eight study sites visit a wide range of flowers, with substantial overlap in their diets. Despite phenotypic specialization in terms of its extremely long tongue, *Anoura fistulata* is no more ecologically specialized in its niche breadth than co-occurring nectar bats. However, it does rely more heavily on a different subset of available flowers, favoring

T A B L E 1 Diet richness, diet diversity (Shannon–Weiner H'), and niche breadth (Levins' index B_A) estimated for each bat species within each site (N) and then averaged ($\pm$SE).

Bat species	N (sites)	Diet richness	H'	B_A (pollen frequency)
Anoura caudifer	7	8 ± 1.27	1.73 ± 0.08	0.65 ± 0.06
Anoura geoffroyi	5	7.6 ± 0.97	1.78 ± 0.12	0.68 ± 0.06
Anoura fistulata	4	6.25 ± 1.03	1.55 ± 0.23	0.64 ± 0.08

Note: One-way ANOVA found no significant difference among the species for diet richness ($F_{2,13} = 0.519$, $p = 0.61$), diversity ($F_{2,13} = 0.639$, $p = 0.544$), or breadth ($F_{2,13} = 0.1$, $p = 0.905$).
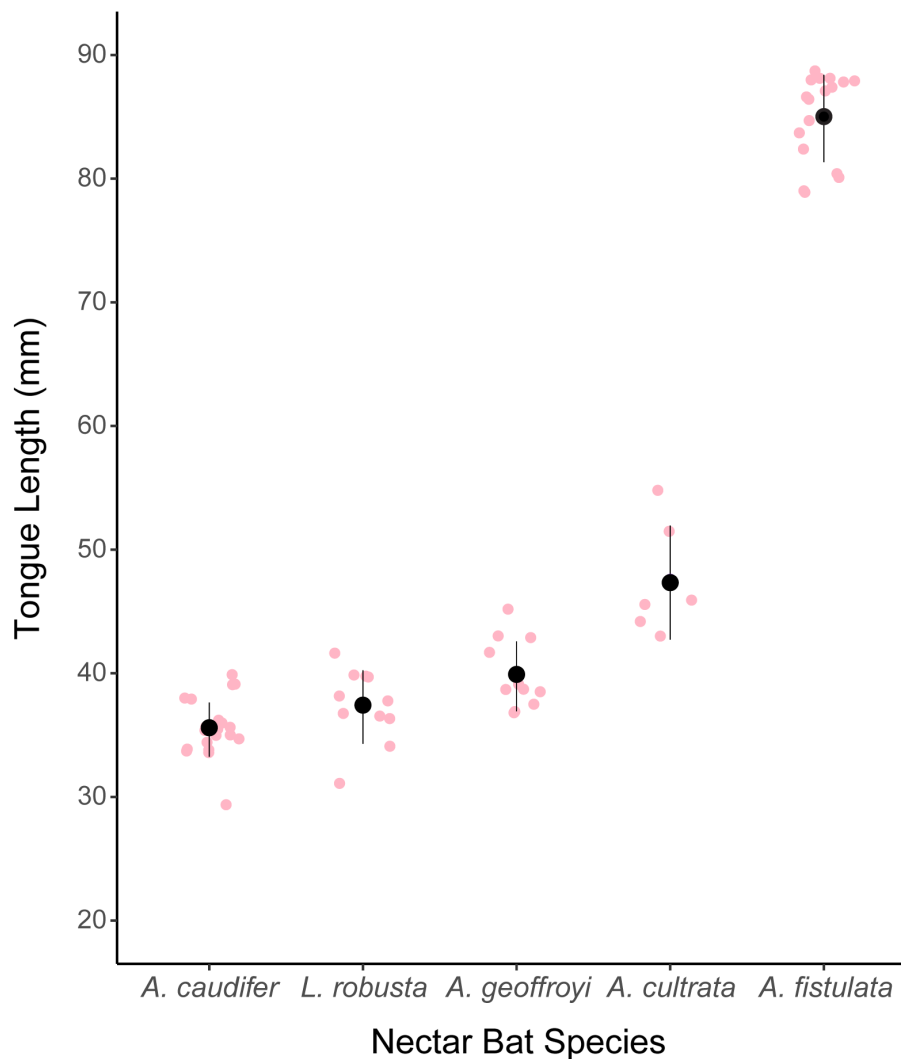
FIGURE 2 Tongue lengths of adult nectar bats captured in Ecuadorian cloud forests, including *Anoura caudifer* (N = 24), *Lonchophylla robusta* (N = 11), *A. geoffroyi* (N = 11), *A. cultrata* (N = 6), and *A. fistulata* (N = 17). Each colored dot represents a single individual, black dots represent the means, and vertical lines represent the SD.

those species with greater corolla or nectary depth, and it is not found in sites that lack deep flowers. Two of the deep-flowered species (*Centropogon nigricans* and *Marcgravia williamsii*) are visited exclusively by *A. fistulata*, while a third (*Trianaea nobilis*) is also occasionally exploited by *Anoura geoffroyi*. Finally, in line with the geographic mosaic theory of coevolution (Thompson, 2005), we find that the local tongue length of *A. fistulata* covaries closely with the functional depth of the deepest flowers it visits in each site. Below we explore each of these findings in greater detail.

Dietary specialization of *Anoura fistulata*

In terms of niche breadth, *A. fistulata* is no more specialized ecologically than co-occurring nectar bats. The

three species of *Anoura* did not differ significantly in any of our measures of diet specialization (richness, diversity, or niche breadth; Table 1). In fact, of all the pollen found on *A. geoffroyi* and *A. caudifer*, the only type not also found on *A. fistulata* was that of two species of *Burmeistera* with short tubes (*B. sodiroana* and *B. truncata*, although *A. fistulata* occasionally carried pollen from three other *Burmeistera* species). Thus, despite its phenotypic specialization in tongue morphology, *A. fistulata* uses a broad range of floral resources, similar to that of other bat species (Figure 1). However, the composition of the diet did differ among bat species. Specifically, *A. fistulata* tended to visit deeper flowers, both in terms of the deepest flower it visits in each site, and the weighted average of flower depth for all of the species it visits (Figure 3). This was due to a heavier reliance on long-tubed
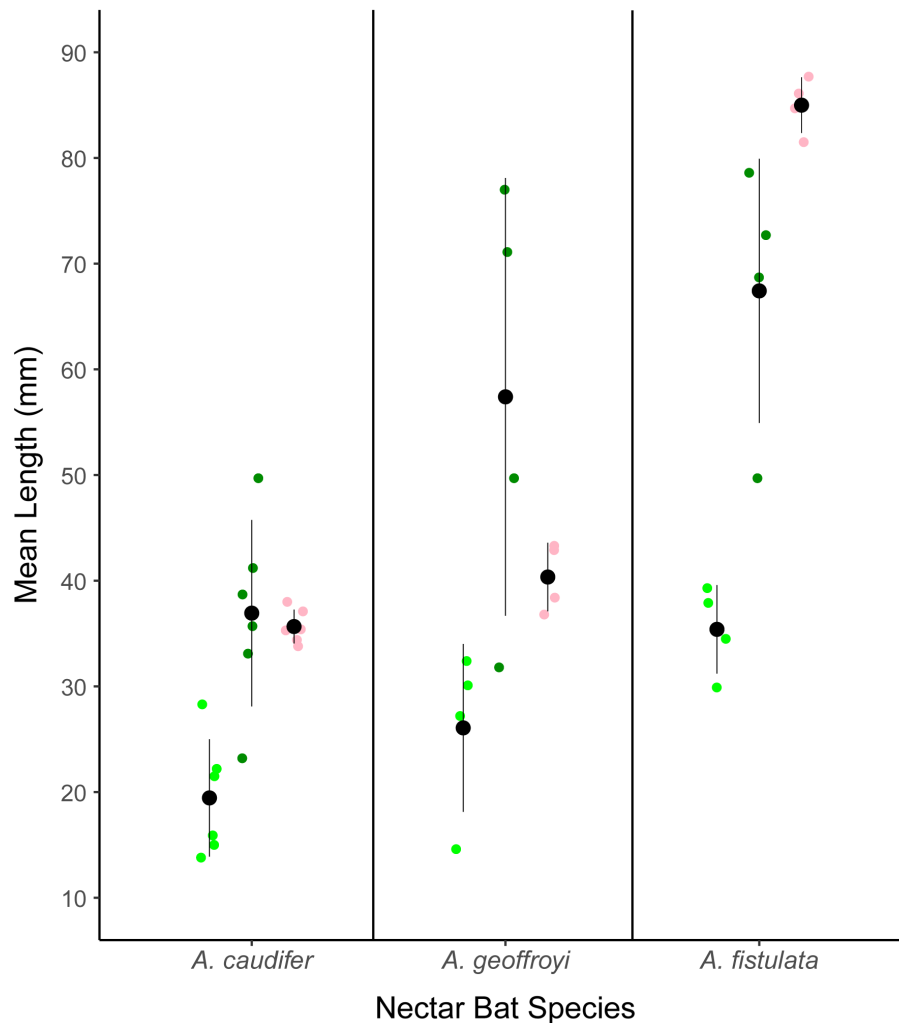
FIGURE 3 Mean lengths across study sites for flowers (green) and tongues (pink), including functional depths of all flowers (light green) and the deepest flowers (dark green) in the local diet of three species of nectar bat, and bat tongue lengths (pink). Bat species include *Anoura caudifer* (*N* = 6 sites), *A. geoffroyi* (*N* = 4 sites), and *A. fistulata* (*N* = 4 sites). Each colored dot represents a site, black dots represent the means, and vertical lines represent the SD.

flowers and less reliance on short-tubed flowers such as those of *Burmeistera* and *Meriania tomentosa* (Melastomataceae). We also found a difference between the diets of *A. geoffroyi* and *A. caudifer*, with the longer tongue of the former species (means: 4.0 cm ± 0.3 SD vs. 3.6 cm ± 0.2 SD) corresponding to longer flowers (2.4 cm ± 0.6 SD vs. 2.1 cm ± 0.5 SD; Figure 3), corroborating results found previously (Muchhala & Jarrín-V, 2002).

Differences in diet composition are further demonstrated in our analyses of niche overlap between co-occurring nectar bat species. Percentages of diet overlap between *A. fistulata* and the other species were only 4.1%–23.9%, and overlap among the other species was similarly low, never exceeding 21.2% (Appendix S1: Table S4). This suggests that, despite broad overlap in the range of plant species they will feed from, *Anoura* display

some underlying niche partitioning in their actual use of different flowers. Similar values of niche overlap were obtained for co-occurring hummingbird species in an Ecuadorian cloud forest, with hummingbirds tending to visit flowers that matched their bill lengths (Weinstein & Graham, 2017).

Interestingly, despite being able to visit a wide range of flowers (from flower depths of 0–7.9 cm), *A. fistulata* has low population sizes throughout its range. This can be seen in its rarity in museum collections relative to other *Anoura* (personal observation) and in its low capture rates over the course of this study (46 individuals, vs. 183 for *A. caudifer* and 88 for *A. geoffroyi*), as well as its absence from four of the eight focal sites. In fact, it was captured only in sites with flowers deeper than the tongue length of other nectar bats. Of the four sites where it occurs, three had
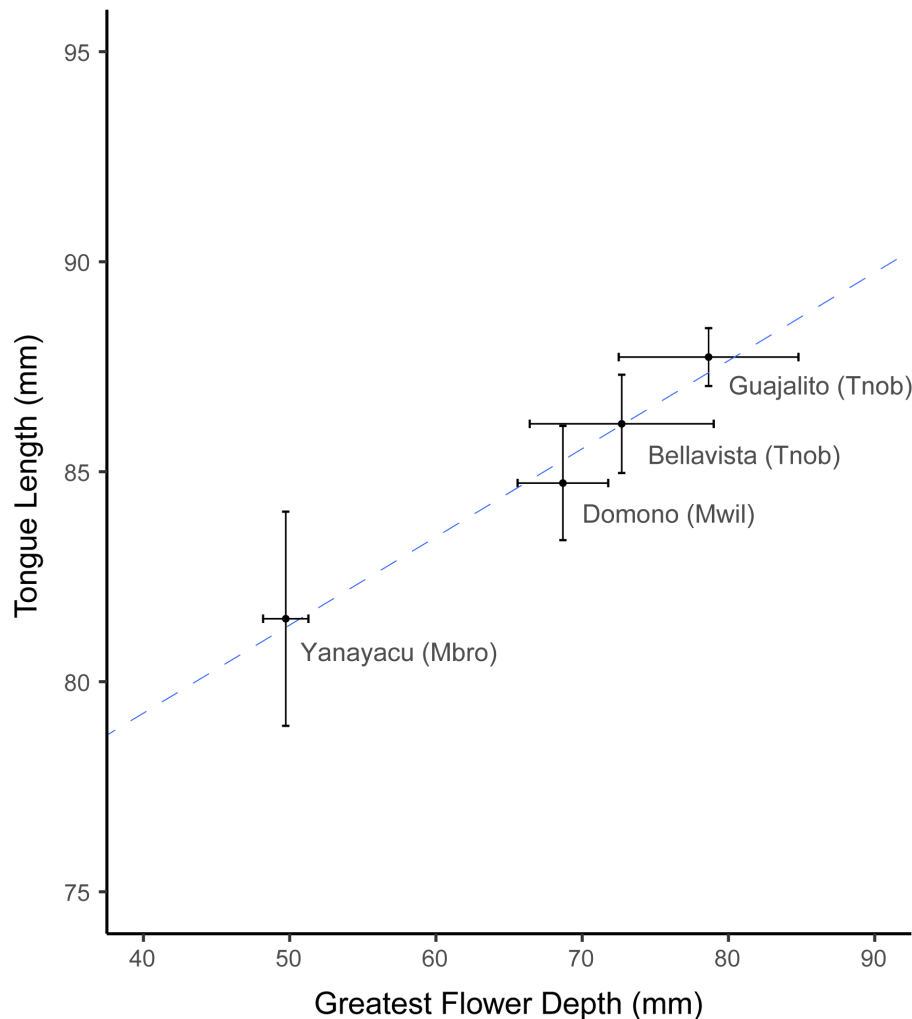
FIGURE 4 Relationship between the tongue length of *Anoura fistulata* and flower depth of the greatest flower in its local diet across four study sites (means and ±SE were calculated from multiple flower individuals and bat individuals per site), with corresponding site and plant species below each point (Mbro, *Marcgravia brownei*; Mwil, *Marcgravia williamsii*; Tnob, *Trianaea nobilis*).

flowers much longer (6.9–7.9 cm) than other nectar bat tongues (~4 cm), while the fourth site had a species with 5.0 cm long nectaries (*Marcgravia brownei*). At this site, all three *Anoura* species visited its inflorescences, but *A. caudifer* and *A. geoffroyi* likely cannot completely empty the nectaries, as their local tongue extensions are only 3.7 and 4.3 cm, respectively. We failed to capture *A. fistulata* at a fifth site with deep flowers (Siempre Verde, 8.2 cm). At the final three sites where we did not capture *A. fistulata*, the greatest flower depth was only 2.3–3.6 cm, and thus the flower resources could be fully exploited by other nectar bats. Although our sample size is low, with only eight sites, and our failure to capture *A. fistulata* does not necessarily mean it does not occur in a site, our results overall suggest that *A. fistulata* requires deep flowers to maintain a population and may be outcompeted by other nectar bats at sites without such flowers.

Flowers specialized to *Anoura fistulata*

Our study identified three particularly long-tubed flowers that are specialized to some degree on pollination by *A. fistulata*. The first, *Centropogon nigricans*, has corolla tubes 8.3 cm long on average and was previously documented to be exclusively dependent on *A. fistulata* (Muchhala, 2006). The second, *Marcgravia williamsii*, has 6.9 cm long nectaries (which bats cannot push their heads into; Appendix S2: Figure S1L) and is similarly exclusively dependent on *A. fistulata*. *Marcgravia williamsii* occurs in Domono, where only *A. fistulata* was found to carry its pollen, while pollen from a co-occurring, unidentified species of *Marcgravia* with 2.5 cm long nectaries (Appendix S2: Figure S1N) was found on all three locally occurring *Anoura* species. Interestingly, based on herbarium collections (BRIT), *M. williamsii* is also found in Peru (Cusco Region,

Quispicanchis Province) where it has much shorter nectaries (approximately 5 cm); perhaps, *A. fistulata* has a shorter tongue or does not occur in these areas. We note that both of these long-tubed species have relatively narrow nectaries (*M. williamsii*) or corollas (*C. nigricans*); thus, when unvisited, the flowers may fill with nectar such that other nectar bats can feed from them, providing a possible fail-safe mechanism to still allow pollination in the absence of *A. fistulata*. The third species, *Trianaea nobilis*, has the longest corolla tubes of any flower in our study, measuring 10.2 cm (Figure 1). However, the functional depth of this flower is less due to their relatively wide, bell-shaped openings; videotaping demonstrated that *Anoura* launch themselves into the corolla of these pendulous flowers from below, hooking on with their thumb claws and inserting their heads up to their shoulders as they feed (Appendix S2: Figure S1F). Likely only *A. fistulata* can access nectar at the very top of the nectar chamber. However, as nectar is produced and drips down the corolla, it may become accessible to bats with shorter tongues. While the short-tongued *A. caudifer* was never found carrying *Trianaea* pollen, *A. geoffroyi* occasionally was.

These specialized interactions with deep flowers resemble those of other nectarivorous animals with extremely long mouthparts. For instance, the sword-billed hummingbird (*Ensifera ensifera*) is the sole pollinator of long-tubed *Passiflora* in the Andes (Abrahamczyk et al., 2014; Lindberg & Olesen, 2001), the mega-nosed fly (*Moegistorynchus longirostris*) is the sole pollinator of different species of orchids in western South Africa (Goldblatt & Manning, 2000; Johnson & Steiner, 1997), and the long-tongued hawkmoth *Agrius convolvuli* is the sole pollinator of many long-tubed flowers in eastern South Africa (Johnson & Raguso, 2016). These interactions are also similar in that they are highly asymmetrical, with the plant species typically completely dependent on the one species of pollinator for its reproduction, while the specialized phenotypes of the pollinators still allow them to have a generalized diet (Johnson et al., 2017; Vázquez & Aizen, 2004).

Trait matching

Across all sites and all species of nectar bat, there was a significant covariation between local tongue length and the deepest flower in the local diet of each bat. This pattern appears to be driven primarily by differences between the bat species in the flowers they visit, as well as by a tight correlation between the local tongue length of *A. fistulata* and the depth of the longest flowers in its local diet. When analyzed separately for *A. fistulata*, this correlation is significant, with a very high coefficient of determination ($R^2 = 0.99$, $p = 0.012$, $N = 4$ sites; Figure 4). Physiological and functional costs likely are associated with maintaining a tongue longer than the body, and these costs impose an adaptive trade-off such that greater length is favored only in sites where it is needed based on local diet. Note that across this covariation, tongue length varies less than flower length (8.1–8.8 cm vs. 5.0–7.9 cm, respectively), which makes sense given that gene flow likely limits local adaptation in the bat, while the local flower depth corresponds to various different flower species (Figure 4). However, flower depth can also vary across sites, for example, the functional depth (subtracting head length) of *Trianaea nobilis* is 7.9 cm in the site where *A. fistulata* demonstrates the longest tongue extension (Guajalito), 7.3 cm in a site with a shorter tongue extension (Bellavista), and only 5.8 cm in a site where we did not capture *A. fistulata* (Siempre Verde). Similarly, *Marcgravia brownei* has short nectaries (3.2 cm) on the western slopes of the Andes, where it co-occurs with the long-tubed *C. nigricans* and *T. nobilis*, and long nectaries (4.9 cm) on the eastern slopes, where it is the deepest flower visited by *A. fistulata*. Thus, while the majority of the detected pattern of trait matching may be due to one-sided evolution of *A. fistulata* tongues in response to local flowers, some evidence points to reciprocal evolution (e.g., coevolution) between bats and flowers.

Previous studies have found similar patterns of geographic trait covariation between plants and pollinator guilds. For hummingbird pollination, a correlation was found between the corolla length of *Nicotiana glauca* and the bill length of its most frequent hummingbird pollinator at each site across the Andes of Bolivia and Argentina (Nattero et al., 2011). For fly pollination, strong evidence of trait matching was found across the geographic range of focal long-tongued flies (Anderson & Johnson, 2008, 2009; Newman et al., 2014) as well as across the range of focal long-tubed, fly-adapted flowers (Anderson et al., 2014; Newman et al., 2015; Pauw et al., 2009). Finally, for moths, researchers found a correlation between the spur length of the orchid *Platanthera bifolia* and the proboscis length of its local moth pollinators in northwestern Europe (Boberg et al., 2014). Ours is the first demonstration of trait matching for bat pollination. Notably, these studies all represent taxa with extreme trait lengths compared with typical members of their guild. Although this may reflect bias in the choice of study organism, or greater ease in detecting patterns at the extremes, we argue that it is primarily due to the strong costs associated with such extreme morphologies, which makes them particularly susceptible to evolutionary decreases in length when they are not needed.

CONCLUSIONS

Our results show that *Anoura fistulata* feeds from a wide variety of flowers throughout its range in the cloud forests of Ecuador, including 14 species from 9 genera, with functional flower depths from 0 to 7.9 cm. Compared with co-occurring nectar bats, it relies more heavily on flowers with longer tubes, and its extreme tongue extension makes it the only bat able to feed from two species with particularly deep flowers (*C. nigricans* and *M. williamsii*). Furthermore, it only occurs at sites with flowers longer than the tongues of other bats. Despite its greater reliance on deeper flowers, *A. fistulata* is not more ecologically specialized in terms of diet breadth than co-occurring bats. Close trait matching between its local tongue length and flower depth across sites suggests that the coevolutionary race that led to its extremely derived tongue length over time also plays out in a geographic mosaic across space, with local selective pressures differentially influencing coevolutionary outcomes. It would be useful to extend the geographic scope of this work even further across the range of *A. fistulata*, to determine which other plant species have evolved deep flowers specialized to this bat, and to confirm whether it requires such deep flowers to occur in a given site.

AUTHOR CONTRIBUTIONS

Nathan Muchhala conceived the study. All authors collected data. Rossana Maguiña-Conde and Angelica Caiza analyzed the samples. Nathan Muchhala and Rossana Maguiña-Conde performed statistical analyses. Rossana Maguiña-Conde wrote an initial draft. Nathan Muchhala performed extensive revisions, and all authors contributed to the final revisions.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data (Maguiña-Conde, 2024) are available from Dryad: https://doi.org/10.7291/D1QX26.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.