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Hovering among flowers: understanding patterns of biodiversity through plant-hummingbird interactions

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Costa Rica, Ecuador and Brazil: epicenters of biodiversity

Costa Rica, a small piece of land of only 51.000 km^2 and five million people, extends through valleys and volcanoes shaping dream landscapes that peek out between the Pacific and Atlantic oceans in the central American isthmus. Ecuador, prided for its unique geographical location at the middle of the world, extends from the sea to the snow-capped tips of the Andes Mountains at more than 6.200 m of altitude, harboring an ancient culture in the heart of the Americas. Brazil, the largest country of the Neotropics, holds the greatest extension of rainforest on Earth and the last remnants of untouched, pristine habitat in times where forests are disappearing at an alarming pace. All three countries seem to be quite dissimilar, but they have something in common - their rich biodiversity. All three countries host an impressive diversity of flora and fauna with countless species constantly interacting with each other, and enabling the dynamic balance of life in the Neotropical forests.

This vast number of species is interwoven in an intricate web of interactions. Species interactions, such as those between plants and nectar feeding animals, are vital to the integrity of ecosystems. These interactions often result in pollination, a fundamental process in both natural and human managed terrestrial habitats. Somewhere between 75% and 95% of all flowering plants on our planet rely on pollinators for their reproduction where small animals carry pollen grains from one flower to another, mostly being rewarded with nectar or pollen in return (Ollerton et al. 2011). An estimated 35% of global crop production volume depends on animal pollination (IPBES 2016) and without this nearly invisible service, there would be little we could do to prevent the loss of many interconnected species, ultimately leading to the collapse of ecosystems.

In the Neotropics, one fascinating pollination system is that between plants and hummingbirds, the most specialized group of nectar-feeding birds (Feinsinger & Colwell 1978). With their hallmark of hovering up and down and forward and backward to feed on flowers and tiny insects, it is not surprising that hummingbirds have an extremely fast metabolism that is unique among vertebrates (Figure 1). Red, orange, fuchsia, purple, blue, pink… this is the colorful world where hummingbirds fuel their little bodies constantly with nectar (Figure 2, 3, 4). Day after day hummingbirds visit a great number of flowers, meanwhile acting as pollen carriers where both the bird and the plant benefit in a mutualistic relationship (Stiles 1995; Waser et al. 2018).

A threatened network of interactions

The need to obtain copious volumes of precious sugary liquid from flowers means hummingbirds interact with a great number of plant species, and thus are important pollinators in a variety of Neotropical ecosystems. By being the protagonists in plant reproduction, the crucial role hummingbirds play in maintaining biodiversity needs no further explanation. However, many forests and other ecosystems in Central and South America where hummingbirds and their food plants live have long been altered and threatened by human activities.

Deforestation, changes in land-use, urbanization, livestock farming, and extreme weather events caused by human-induced climate change, have only intensified over the past decades, posing major challenges for the conservation of habitats where hummingbirds thrive (Figure 5). Although hummingbirds are often considered as opportunists and can be seen foraging in open disturbed habitats, a recent study found that species that live predominantly in forest ecosystems are at a greater risk of extinction (Leimberger et al. 2021). This finding indicates that certain hummingbird species are not responding well to human-induced environmental changes. Ten percent of 366 hummingbird species that inhabit the Americas are considered globally threatened and 60% are in decline (IUCN 2020). Both population decline and the loss of hummingbird species may lead to changes in patterns of interactions and the pollination service these birds provide (Potts et al. 2010).

In Southern Costa Rica there are several hummingbird species that only occur in that region and northwestern Panama, such as the Talamanca Hummingbird *Eugenes spectabilis* and the White-tailed Emerald *Microchera chionura*. Because of their very local distribution, populations of these species are inherently sensitive to decline and might face conservation difficulties in the near future. In Brazil, the Hook-billed Hermit *Glaucis dohrnii*, is endemic of central Atlantic rainforest and is nowadays classified as Vulnerable (IUCN 2020). In Ecuador eight species are listed in some threat category and five are endemic. The recently discovered Blue-throated Hillstar *Oreotrochilus cyanolaemus*, whose population is estimated to be between 250-750 individuals, has been assessed as Critically Endangered (BirdLife International 2022). In this discouraging scenario it is mandatory that scientists do their best to generate first-hand knowledge that can be used in conservation actions. For example, identify critical food plants that hummingbirds require to guide resource guardians and managers in habitat enrichment and restoration processes.

The EPHI project: an overview

With this aim in mind, in 2017 we initiated our research on the ecology of plant-hummingbird interactions (EPHI) in Costa Rica, Ecuador and Brazil; a project led by the Swiss Federal Research Institute (WSL) in collaboration with the Universidad Estatal a Distancia, Aves y Conservación and Universidade Federal do Paraná, among other organizations (Figure 6). Inspired by the extraordinary diversity of hummingbirds and their food plants in the three countries, we are documenting the temporal and spatial variation of plant-hummingbird interactions. We use this information to build interaction networks showing which hummingbirds use which plant species (Figure 7). From these networks we can gain a deeper understanding of hummingbird communities, and identify which flower resources are key in a hummingbird's diet or which

hummingbirds are specialized, foraging on flowers from only a handful of available plant species.

Determining how specialized species are in networks is important for conservation efforts and for advancing our scientific understanding of how so many similar species can live in one place. There is a classic long-standing hypothesis in ecology that resource specialization is important for maintaining high species diversity. The idea is that if all species living in the same place use exactly the same resources, then one species would be a slightly better competitor and over time would outcompete other species. As a result, diversity would be lower and only the best competitors would persist. However, if hummingbirds are specialized and use different resources, competition is lower and more species can persist in the same place because each species uses different resources (Tilman 1982, MacArthur 1984).

Specialization is also important from a conservation perspective. This information is very relevant for restoration initiatives because the right mix of flowers will provide resources to both generalist hummingbirds, that forage on a broad array of flowers, and specialist hummingbirds, that require specific plant species. If certain species are highly specialized and use only a few flower resources, then it is essential to make sure that these resources are protected. The same is valid from the plant perspective, if it is pollinated by only a few hummingbirds, it is crucial to safeguard these hummingbirds as well. Given the importance of specialization in conservation and ecology we evaluate how it differs among our three biodiverse regions.

How do scientists measure specialization?

To find out which hummingbirds use which flowers we first needed to create pollination networks like the one depicted in figure 7. This required 22 people actively collecting data from 32 sites across the three countries (Figure 8)! We established transects along an elevation gradient from about sea level to 3,500 m of altitude, where we recorded hummingbirds visiting plants, flower abundance and species morphology for more than two years to account for temporal variation.

We used time-lapse cameras combined with MotionMeerkat software to catch hummingbirds red-handed, just when they visit the flowers. This approach enabled us to obtain valuable data through thousands of pictures and thus, identify which plant species are the most and least favorite repast of hummingbirds. We also photographed hummingbird-visited flowers and hummingbird bills to later use sophisticated computer measurement tools to collect morphological data. These data allowed us to determine if hummingbirds preferentially forage on flower species whose flower tubes are the same length as their bills, a phenomenon called trait-matching. Such matching is a fascinating outcome of coevolution, a process that molds which species interact during long periods of time, often leading to specialization. Finally, we have counted thousands of flowers on transects at our study sites to quantify the availability and flowering period of hummingbird food resources so that we can detect whether variation in the abundance or diversity of hummingbirds mirrors variation in their resources.

To estimate specialization for each hummingbird species at each site, in each country, we calculated a specialization index (Blüthgen et al. 2006). This metric integrates information on the interaction frequency between species e.g., how often a hummingbird visits a plant. This is an important step since the frequency of interactions can influence the delivery of an ecosystem function, in our case pollination. The index can take values from 0 (generalist) to 1 (specialist). According to this specialization index, in a given community a hummingbird species is considered highly specialized when it uses few and uncommon plant species that are not visited by other hummingbird species, and very generalist when it visits different and common plant species that are also visited by many other hummingbirds.

What did we find?

During our intensive fieldwork we recorded approximately 330,000 hours of video footage. From which we extracted 68,037 interactions between hummingbirds and plants, representing 72 hummingbird species (20% out of total diversity of hummingbirds) interacting with 471 plant species. This effort, and the resulting information we obtained about interactions, would have been inconceivable with only human observers.

Ecuador had the highest number of interacting hummingbird (41) and plant species (258), followed by Costa Rica (24 hummingbirds, 145 plants) and Brazil (15 hummingbirds and 82 plants). The pattern of species richness for hummingbirds and plants varied across elevation and these elevation patterns were different in each country. Hummingbird species richness was greater at the lowest elevations and only at high elevations the number of species dropped off (Figure 9). The richness of hummingbird-visited plants showed a similar pattern across elevation to that of hummingbird richness in Brazil, i.e., more species in the lowlands. In contrast, both Ecuador and Costa Rica had the greatest number of plant species at mid-elevations (Figure 9). These patterns of richness across elevation - either a decline with elevation or mid-elevation hump - are typical of many tropical communities (McCain and Grytnes 2010).

Despite the three countries having dramatically different numbers of species and different species compositions (CR and EC share 14 plant and 8 hummingbird species and Brazil shares no hummingbird species with the other countries), hummingbird specialization varied little across sites (Figure 10). These results indicate that across these very different regions hummingbird communities are similarly structured; some species are quite specialized, preferring only a subset of plants available while others forage on many different species.

We now turn to the question of what do these specialist hummingbirds look like? In particular, do they have some morphological traits, e.g., long or curved bill that makes them particularly efficient at foraging on a subset of flowers that also have long or highly curved flower tubes? It turns out that many of the most specialized hummingbirds do have characteristics that make them rather distinct among hummingbirds. For instance, the two most specialized hummingbirds Purple-throated Woodstar *Calliphlox mitchellii* from Ecuador and Frilled Coquette *Lophornis magnificus* from Brazil, are really rather tiny, weighing respectively 3g and 2g while the average hummingbird weighs 5.5g. Several other species that are very specialized have either very long (Sword-billed Hummingbird), very short (Rainbow-bearded Thornbill

Chalcostigma herrani), or very curved (White-tipped Sicklebill *Eutoxeres aquila*) bills. These hummingbirds tend to forage on flowers that match their bills and they even extract nectar more efficiently when there is a good match (Maglianesi et al. 2014, Weinstein & Graham 2017). However, there are also some species that are relatively specialized, but that do not seem to have distinctive characteristics, such as Versicolored Emerald *Chrysuronia versicolor* and Western Emerald *Chlorostilbon melanorhynchus*. For these species, we do not yet know why they are so specialized. Further research to learn more about what makes a species a specialist is needed. Specialist species can be at risk because they highly depend on few other species, something not often considered in conservation assessments. With the data we are collecting in EPHI we hope to reveal new information on the vulnerability of hummingbirds and their respective food plants. Thus, we can find out what are the characteristics that could make these species more sensitive to environmental disturbances. For example, some hummingbird species may prefer to visit plants with specific flower morphologies as we mentioned before or particular nectar properties, which could increase their dependence on such plants. Therefore, human-caused environmental disturbances that negatively affect those plants will end up affecting the visiting hummingbirds as well.

Looking into the future:

Space and time, these two dimensions of hummingbirds' lives can be combined to decipher why they interact with certain plant species and not with others and thus, we can better understand patterns of biodiversity and the underlying processes that result in well-functioning ecosystems. In addition, we can start to anticipate the potential responses of hummingbirds to human-induced threats and try to mitigate these threats. For instance, we can augment regenerating lands with those plant species that hummingbirds rely on. We have taken this approach for a pilot project in a high elevation site in Ecuador where we trained a group of women on native plant propagation and restoration (Figure 11). The strategy was to plant species that are used by many hummingbirds as well as those used by the specialist hummingbird species. This was no easy task because not all native plants from the forest thrive in greenhouse conditions. We had to use many propagation techniques and identify the environmental conditions where plants would not only survive but produce copious flowers! With hard work and patience, we are now succeeding as our ultimate goal is to move from basic research to management and conservation. In this way we can continue to enjoy watching colorful acrobatic hummingbirds hovering among the flowers.

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Figure legends for the article:

Hovering among flowers: understanding patterns of biodiversity through plant-hummingbird interactions.

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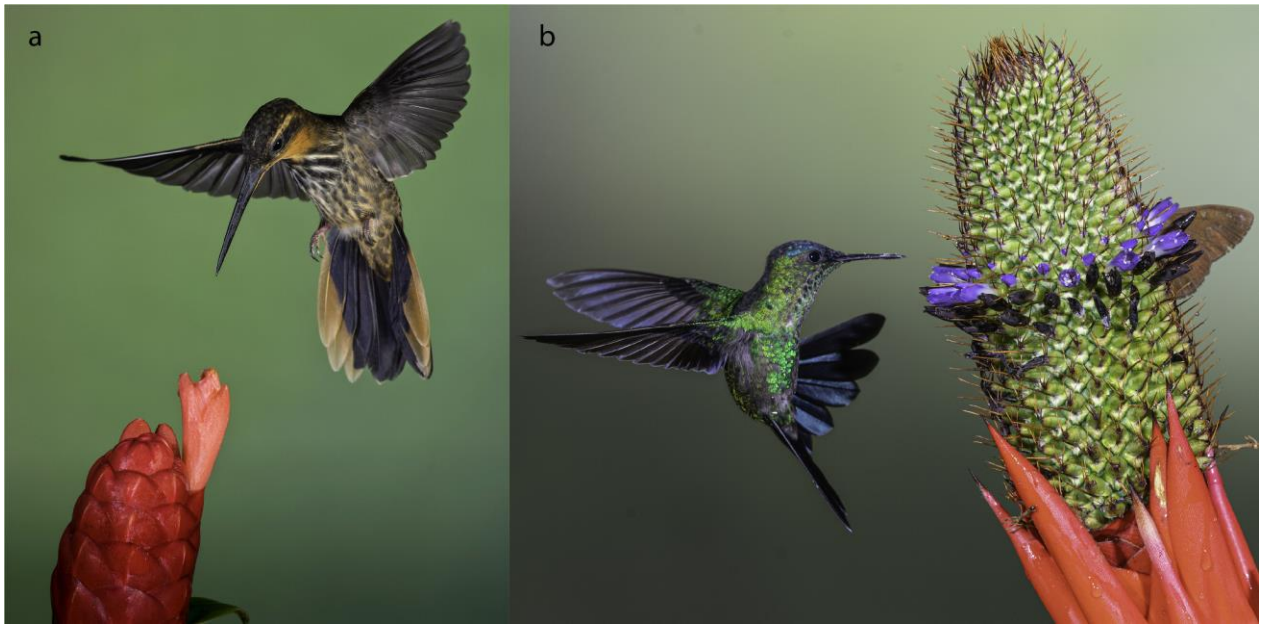
1. Hummingbirds have long fascinated people with their small size, colorful plumage, unique flight and remarkable bill lengths and shapes. Photo credits: (a) Scale-throated Hermit *Phaethornis eurynome* (Sergio Gregorio), (b) Sword-billed Hummingbird *Ensifera ensifera* and (d) Purple-backed Thornbill *Ramphomicron microrhynchum* (Santiago Salazar - Neotropical Nature & Birding Trips), (c) Green-crowned Plovercrest *Stephanoxis lalandi* (Cauã Galeazzi).



2. No other bird can compare to a hummingbird. Through a refined design of its wing anatomy, hummingbirds can hover in one spot, move amazingly fast backwards, straight up and down, side to side, and even upside-down, proving that they are exceptional acrobats. Photo credits: (a) Saw-billed Hermit *Ramphodon naevius* and (b) Violet-capped Woodnymph *Thalurania glaucopis* (Sergio Gregorio).

3. Due to their exceptional way of life, hummingbirds have special adaptations to meet their energetical needs and obtain a sweet reward on many different flowers, with a variety of shapes. Pictured are flowers from the high elevation site, Verdecocha in Ecuador, that are regularly used by hummingbirds. Some species have long (a) or curved flowers (e) that can only be reached by long-billed hummingbirds while others (b, d) are more open and might thus be shared with other types of organisms, such as bees. (a) *Passiflora cumbalensis*, (b) *Berberis paniculata*, (c,g) *Heppiella repens*, (d) *Palicourea fuchsioides*, (e) *Centropogon pichinchensis*, (f) *Salvia sagittata*, (h) *Disterigma noyesiae*. Photo credits: Francisco Tobar.

4. Hummingbirds do not always access the nectar from flowers through their opening but sometimes they use an alternative strategy: they drill a hole or use an existing hole in the base of the flower. In this case hummingbirds act as cheaters since they do not touch the reproductive organs of the plants stealing the nectar resource without any benefit for the plants. Photo credits: (a) Speckled Hummingbird *Adelomyia melanogenys* (Miguel Hipo - Quinde Huasi Birding) and (b) Volcano Hummingbird *Selasphorus flammula* (Emanuel Brenes Rodríguez).

5. Some hummingbird-visited plant species are threatened as the result of human activities, for example (a) *Schlumbergera opuntioides* (Vulnerable) in the family Cactaceae inhabits only the coastal mountains of south-eastern Brazil. (b) *Griffinia hyacinthina* (Endangered) in the family

Amaryllidaceae is also an endemic species from Brazil. Photo credits: (a) Ephi Brazil and (b) Ana Paula Caron.

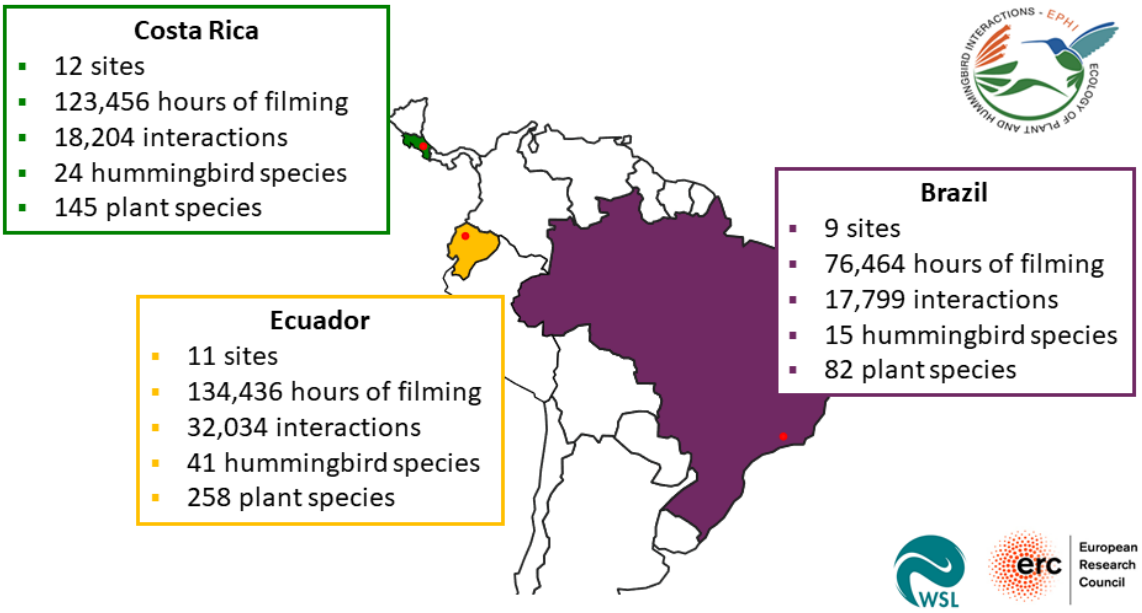


Figure 6. The project Ecology of Plant-Hummingbird Interactions (EPHI) is led by the Swiss Federal Research Institute (WSL) and takes place in Central and South America, more specifically in southern Costa Rica, in Pichincha forest of Northwestern Ecuador and in the Atlantic forest of southeastern Brazil.

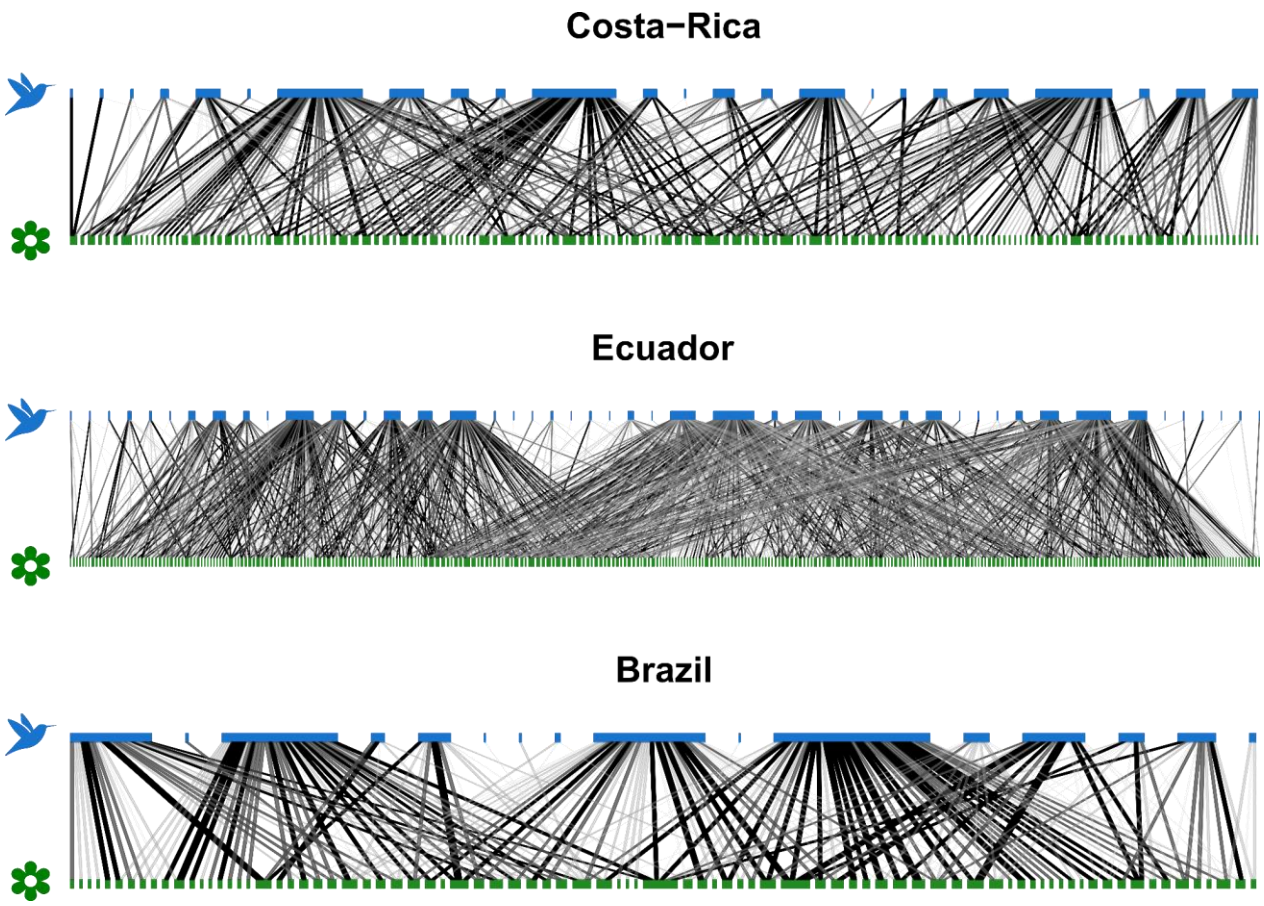


Figure 7. Pollination networks where the lines depict interactions between hummingbird (blue boxes) and plant species (green boxes). Within each meta-network, line color and thickness are proportional to interaction frequency, from light gray and thin lines for rare interactions to black and thick for frequent interactions. Similarly, the width of boxes representing hummingbird (blue top boxes) and plant (green bottom boxes) is proportional to the number of interactions observed for that species. We recorded a total of 68,000 interactions between plants and hummingbirds during the EPHI project. Ecuador has the largest network of the three countries.

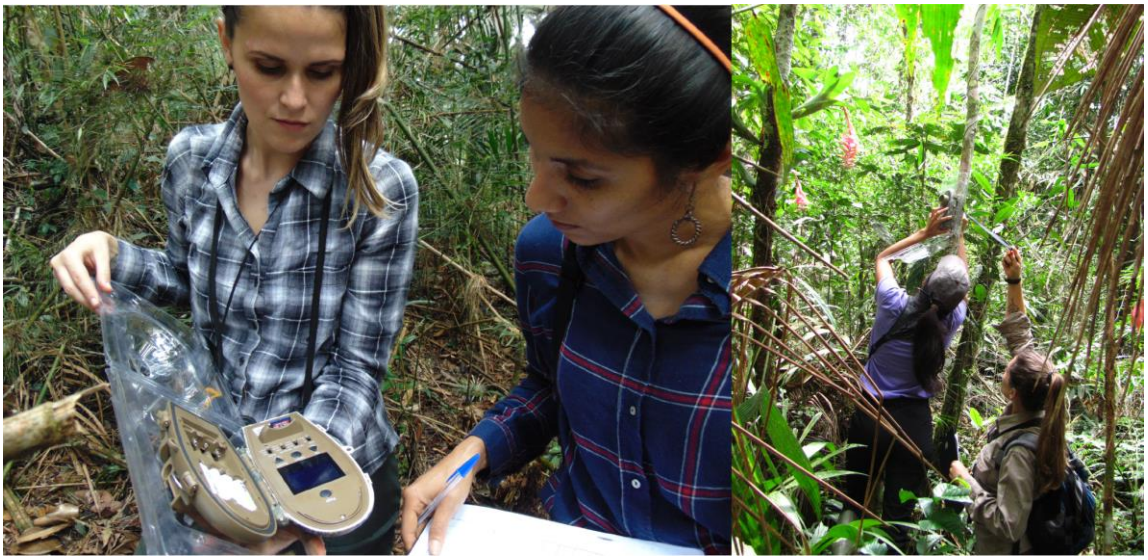


Figure 8. Time-lapse cameras are a very efficient method that allow us to see hummingbirds in action with more than 68,000 visits to plants recorded in Costa Rica, Ecuador and Brazil.

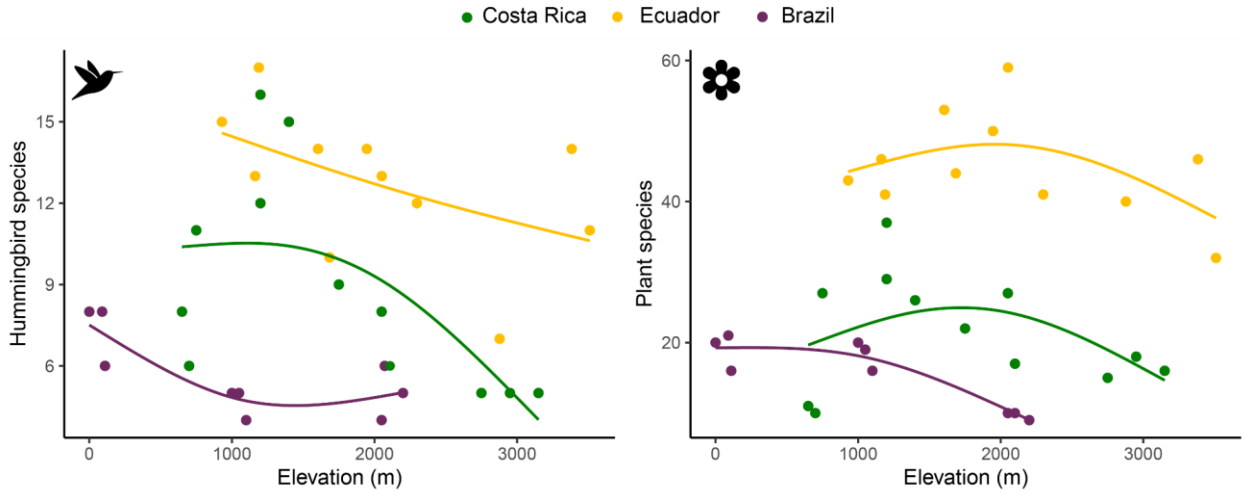


Figure 9. Variation in the number of species of hummingbirds (left) and plants (right) across elevation in all study sites in each country.

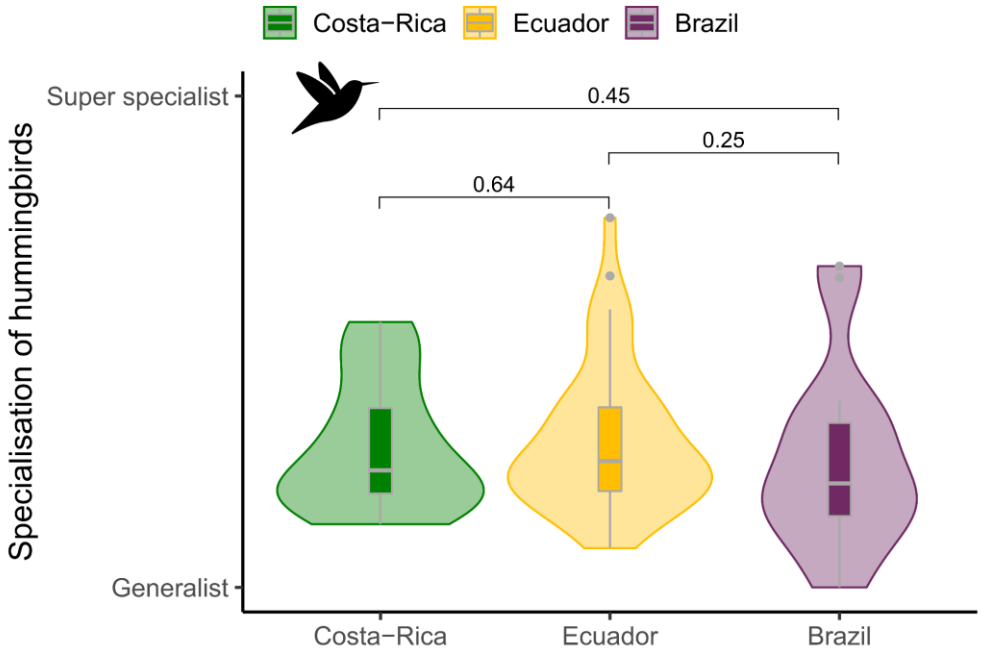


Figure 10. Hummingbird specialization across countries. Here, specialization is calculated on meta-networks presented in Figure 7. The width of the colored area represents the density (the wider it is, more species exhibit this specialization value), while the boxplot inside represent (from bottom to top): the minimum, 25% quartile, 50% quartile (median), 75% quartile and the maximum (excepting outliers). The number above the distributions show the p-value of pairwise Wilcoxon tests, and they indicate that there is no statistical difference between countries.

Figure 11. The group of women in the Alambi community in Ecuador are propagating native plant species as *Macleania rupestris* (down left) used by the endangered Black-breasted Puffleg (top left) and other hummingbirds.