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**Higher abundance and species richness at lower elevations in Monteverde  
Altitudinal Survey on Euglossine bee diversity**

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**ABSTRACT**

In many neotropical areas, Orchid bees are the primary bee pollinators, so knowing their biogeographical and altitudinal distribution in Monteverde, Costa Rica could be useful for understanding ecosystem health. The Orchid bees' optical uniqueness, large biodiversity and accessibility serve as useful and easily identifiable bioindicators of farm and ecosystem health. The biogeographical distribution of Orchid bees has been thoroughly researched, but there are few scientific papers conducting surveys on their altitudinal distribution. I caught a total of 138 bees from six different species: *Euglossa viridissima*, *Euglossa imperialis*, *Euglossa mixta*, *Euglossa maculilabris*, *Exaerete smaragdina* and *Eulaema bombiformis* using methyl salicylate, cineole and eugenol in four different locations of altitudes ranging from about 1200 m to 1800 m. I spent two mornings and three hours each day at Bajo del tigre, the Crandell reserve, la Estacion Biologica and the TV towers. The region of lowest elevation had the highest abundance and species richness. I found five species and 66 individuals at Bajo del Tigre and only two species and six individuals at the TV towers. Surprisingly, a site at medium elevation, la Estacion Biologica had the highest Shannon diversity due to possessing the most heterogenous species composition and a presence of four species. Since my results show that bee biodiversity does vary with altitude, they would make useful indicators of ecosystem change over time as climate change causes locations of lower elevations to heat up and become less habitable.

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**Mayor abundancia y riqueza de especies de abejas euglosinas en las elevaciones más bajas de Monteverde**

**RESUMEN**

En muchas áreas neotropicales, las abejas de las orquídeas o euglosinas son los polinizadores primarios de este grupo de insectos. Por lo tanto conocer su distribución biogeográfica y altitudinal en Monteverde, Costa Rica podría ser útil para entender el salud del ecosistema. La singularidad óptica de las abejas euglosinas, su gran biodiversidad y su accesibilidad, les permiten servir como bioindicadores útiles y identificables fáciles de la salud de las granjas y de la salud del ecosistema. La distribución biogeográfica de las abejas de la orquídea ha sido investigada a fondo, pero hay pocos documentos científicos que realizan encuestas sobre su distribución altitudinal. Se capturaron un total de 138 abejas de seis especies diferentes: *Euglossa viridissima*, *Euglossa imperialis*, *Euglossa mixta*, *Euglossa maculilabris*,

*Exaerete smaragdina* y *Eulaema bombiformis* usando salicilato de metilo, cineol y eugenol en cuatro ubicaciones diferentes de altitudes de aproximadamente 1200 m a 1800 m. Pasé dos mañanas y tres horas cada día en Bajo del tigre, la reserva de Crandell, la Estación Biológica y las torres de TV. La región de menor elevación tuvo la mayor abundancia y riqueza de especies. Encontré 5 especies y 66 individuos en Bajo del Tigre y sólo dos especies y seis individuos en las torres de TV. Sorprendentemente, un sitio en la elevación media, la Estación Biologica tenía la diversidad más alta del Shannon debido a poseer la composición más heterogénea de la especie y una presencia de cuatro especies. Como mis resultados muestran que las abejas tienen una preferencia altitudes menores. Este grupo de especies pueden resultar indicadores útiles de la salud del ecosistema. El cambio climático podría provocar que a raíz del calentamiento global, estas abejas suban a altitudes mayores como respuesta.

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## INTRODUCTION

A critical aspect of ecological restoration and conservation is understanding species distributions and abundance over biogeographical boundaries. In many neotropical forests, the Euglossine tribe comprises the main bee component. Therefore, having knowledge of their population's distribution is essential for the preservation of their role as pollinators (Roubik and Hanson, 2004). It is predicted that insects will be very important bioindicators of ecosystem changes along the altitudinal gradient as climate change variably affects these regions (Morochz, 2015). For example, global warming will cause many species to migrate to higher altitudes in search of cooler conditions. This has been seen with Grassland Butterflies of the Sierra de Guadarrama, where the average occupancy of elevation increased by about 150 meters over 31 years (Gutierrez, 2005). Euglossine bees are also accurate bio-indicators of farm health (Hedstrom, 2005). For example, the number of euglossine bees on organic farms is on average 3 times higher than on conventional farms.

The biogeography of Euglossine bees has been studied latitudinally and grouped into six geographical zones, including Mexico to Nicaragua and Panama to Costa Rica. Despite widespread knowledge of their latitudinal species distribution, little is known about their altitudinal species distribution and abundance in each region. However, research was conducted on the pacific and atlantic slopes of Costa Rica and in San Luis by UCEAP students showing that the bees' biodiversity is higher at lower elevations (Christie, 2008).

The bees' geographical distribution is affected by climate, vegetation and intraspecific and interspecific competition (Roubik and Hanson, 2004). Similar factors could also contribute to species diversity and abundance distribution changes across elevation, such as temperature, resources and floral distribution (Uehara-Prado, 2006). Costa Rica has 70 species of orchid bees, but their altitudinal distribution is unknown (Roubik and Hanson, 2004). One study determined that species abundance of *Eufriesea violacea* varies between 700 and 1,100 meters in Pindamonhangaba, Brazil (Uehara-Prado, 2006). If one species of orchid bees has a preference, it is likely that other species will exhibit a similar behavior. To pursue this idea further, I did a

survey of most orchid bee species present in Monteverde between 1,100 and 1,800 meters, providing a biogeographical overview of the tribe Euglossini that is more general than Uehara-Prado's research.

Monteverde, specifically the area between Bajo del Tigre and the TV towers, is an ideal place to conduct a survey on the species distribution of Euglossine bees across the altitudinal gradient because of the steep rise in altitude within a small area. The latitudinal linear distance between the TV towers and my area of study in Bajo del Tigre is around 3,800 meters while the altitudinal difference in distance was 617 meters. Since altitude changes rapidly relative to latitude between the locations tested, changes in species distribution and abundance can be attributed to altitudinal variation and potentially habitat fragmentation instead of latitudinal variation. I addressed: how does Euglossine bee distribution and abundance vary with altitude?

## METHODS

My research was conducted in Monteverde, Costa Rica between May 18 and May 26, 2017. I spent two mornings doing bee surveys from 7:30 am to 10:30 am for each of the locations. My locations were Bajo del tigre at 1,216 meters, the Crandell Memorial Reserve at 1,378 meters, la Estacion Biologica, at 1,517 meters, and the TV towers at 1,833 meters. Bajo del tigre is a drier variety of forest, called rain shadow forest, while the TV towers, the Crandell Memorial reserve and la Estacion Biologica are cloud forest. I used the attractants Cineole, Eugenol and Methyl Salicylate to pipette 0.5 ml of each fragrance on one paper towel sheet hung 1.5 meters from the ground and one meter apart from each other using string. I reapplied with 0.25 ml of fragrance every 30 minutes. I captured bees using a butterfly net and identified them in the field using the book 'The Orchid bees of Tropical America Biology and Field Guide' (Roubik and Hanson, 2004). I marked specimens by painting a dot of white-out on their scutellum, so that I would not count them twice if re-captured.

## RESULTS

A total of 138 bees were captured and identified. Species were found in the genera *Euglossa*, *Eulaema* and *Exaerete*.

### *Species richness and abundance at every site*

My Euglossine bee survey showed that there is a slight shift in the species present and a large shift in the abundance between locations of different altitudes. I found 11 times as many individuals in Bajo del Tigre than TV towers (Table 1, Fig. 1). Bajo del Tigre had 5 species present including *Euglossa viridissima*, *Eulaema bombiformis*, *Euglossa imperialis*, *Euglossa mixta*, *Exaerete smaragdina*, and *Euglossa maculilabris*. The Crandell reserve had 3 species present, including *Euglossa viridissima*, *Eulaema bombiformis* and *Euglossa imperialis*. La Estacion Biologica had 4 species, including *Euglossa viridissima*, *Eulaema bombiformis*, *Euglossa imperialis* and *Euglossa mixta*. TV towers had 2 species, including *Eulaema bombiformis* and *Exaerete smaragdina* (Table 1). *Eulaema bombiformis* was the only species

that I found at every location. I found that two species were specific to one location; I only found *Euglossa maculilabris* at 1833 m and I only found *Exaerete* at 1216 meters (Table 1). However, many more mornings of research would have to be done at the TV towers and Bajo del Tigre to definitively determine this. The Shannon diversities were  $H=0.83$  at Bajo del Tigre,  $H=0.62$  at the Crandell Reserve,  $H=1.01$  at la Estacion Biologica and  $H=0.64$  at the TV towers.

**Table 1: The number of individuals per Euglossine bee species present at each location.**

	<b>Bajo del Tigre</b>	<b>Crandell Reserve</b>	<b>Estacion Biologica</b>	<b>TV towers</b>
Altitude (m)	1216	1378	1517	1833
<i>Euglossa viridissima</i>	55	37	12	0
<i>Eulaema bombiformis</i>	3	6	4	4
<i>Euglossa imperialis</i>	3	3	1	0
<i>Euglossa mixta</i>	5	0	2	0
<i>Exaerete smaragdina</i>	1	0	0	0
<i>Euglossa maculilabris</i>	0	0	0	2
TOTAL	67	46	19	6

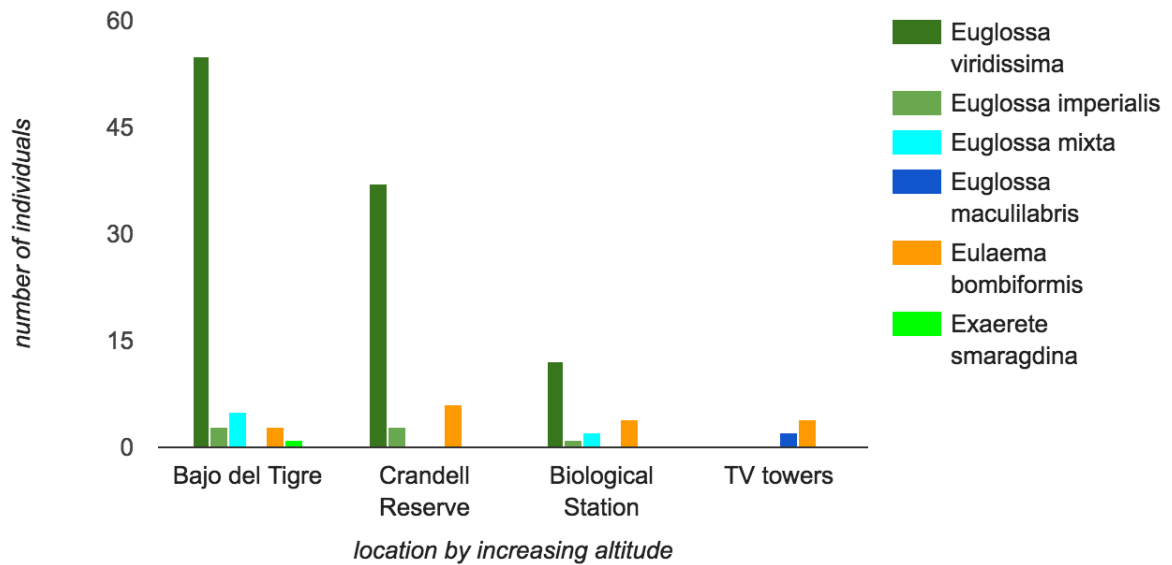


Fig. 1: The number of individuals per species present at each location.

### *Altitudinal range of my species compared with the literature*

According to my results, the guide's altitudinal population limits for *Euglossa viridissima*, *Euglossa maculilabris* and *Eulaema bombiformis* are too low. For example, I found the species *Euglossa maculilabris* 833 meters higher than the guide suggested (Table 2).

**Table 2: The altitudinal distribution of Orchid bee species I found according to the Orchid bees of tropical America Biology and field guide vs. the distribution I found during my research.**

Species	Published altitudinal range	Project's range
<i>Euglossa viridissima</i>	Lowlands-1200 m	1215 m- 1517 m
<i>Eulaema bombiformis</i>	Lowlands- 1700 m	1215 m- 1833 m
<i>Euglossa imperialis</i>	Lowlands-1700 m	1216 m- 1517 m
<i>Euglossa mixta</i>	Lowlands- 1700 m	1216 m- 1517 m
<i>Exaerete smaragdina</i>	Lowlands- 2600 m	1216 m
<i>Euglossa maculilabris</i>	Lowlands- 1000 m	1833 m

### *Orchid bee presence varies between open and forested areas*

There was a surprisingly large disparity in the abundance and richness between the open and forested areas. Five bee species were present in the forested area, whereas only two were present in the open area (Table 3). Also, more than twice as many total individuals were caught in the forested area than in the open area. These locations were very close to each other biogeographically and only one meter apart in altitude (Table 3). The only environmental factors that varied between the two locations is that the open area had more wind and sun and was not under tree cover.

**Table 3: Number of individuals of each species in two different habitats within the same reserve: a sunny, open, windy lookout spot and a forested area.**

<b>Bajo del Tigre</b>	<i>Euglossa viridissima</i>	<i>Eulaema bombiformis</i>	<i>Euglossa imperialis</i>	<i>Euglossa mixta</i>	<i>Exaerete smaragdina</i>	<i>Euglossa maculilabris</i>	<b>TOTAL</b>
Forested	35	3	3	3	1	0	45
Open	20	0	0	2	0	0	22

## **DISCUSSION**

### *Species richness and abundance at every site*

A previous UCEAP study on Euglossine bee biodiversity was conducted in San Luis, Costa Rica which is only a couple miles away from the sites I sampled. 13 species of orchid bees were found with an approximate Shannon diversity index  $H = 2.04$  between 1,100 and 1240 meters (Asarian, 1999). In my study, at an elevation of 1517 meters,  $H = 1.01$  and at 1833 m,  $H = 0.64$ . Comparing my diversities of highest elevation with Asarian's data gives some indication that regions of low elevation have higher diversity than higher regions when comparing similar habitats. Also, at all of my sites combined, I was able to find six species total, whereas 13 species were found in San Luis. Since San Luis is only about 100 meters lower than Bajo del Tigre and seven more species were found in San Luis, it is unlikely that altitude was the only contributing factor. Forest fragmentation, such as roads separating habitats, types of forest and time sampling was also likely a factor. The relatively similar species composition coincides with the short distance between my and Asarian's sampling locations. In San Luis, the most common species was *Euglossa imperialis* and *Euglossa viridissima* was the second most common. Furthermore, in Monteverde I found *Euglossa viridissima* to be the most common species and *Euglossa imperialis* to be the third most common species.

There is a limitation to comparing my data with Asarian's data because he performed his tests 18 years ago and did not compare species along an altitudinal gradient within San Luis. It is more accurate to compare my results with an elevational study of one region at a certain time. Another UCEAP student found higher abundance and richness, or number of species in low regions when she measured eight species at 800 m and one species at 1450 m along the Pacific

slope of the Tilaran mountains(Christie, 2008). This pattern is apparent not just in tropical regions but temperate regions as well. When the species distribution of bees was tested along the altitudinal gradient in the German Alps, only seven species were found above 1500 m and 34 species were found below 1000 m (Hoiss, 2012). Christie also found that *Euglossa imperialis* and *Euglossa viridissima* were in the top three most abundant species on the Pacific slope. Other species we both found are *Euglossa mixta* and *Exaerete smaragdina*.

It is evident that altitude is correlated with species distribution when comparing my own data from the elevational extremes in Table 1. In Bajo del Tigre, 5 species and 66 individuals were found and only two species and six individuals at the TV towers. Despite there being 11 times as many individuals in Bajo del Tigre than TV towers, Bajo del tigre had a Shannon diversities that was only a 0.19 higher than that of the TV towers. I expected that the Shannon diversities would increase linearly with an increase in elevation but this was not the case. The Shannon diversities show that there was higher diversity at la Estacion Biologica ( $H=1.01$ ) than at Bajo del Tigre ( $H=0.83$ ), even though the lower elevation had more than three times as many individuals and 1 more species present. This was a species specific to Bajo del Tigre, *Exaerete smaragdina* (Table 1). These unexpected Shannon diversities are explained by the relative abundance of species; my sampling from 1517 meters and 1833 meters was much more heterogenous than my sampling from 1216 m. A more heterogenous population is one with similar abundances of the different species. Specifically, at 1216 meters 82% of the population was comprised of *Euglossa viridissima* whereas at 1517 meters only 63% of the population was comprised of *E. viridissima*. The Shannon diversity preferentially measures for heterogeneity as an indicator of biodiversity, rather than species richness and total population number.

There are many potential ecological causes for differential biodiversity and abundance along the altitudinal gradient, such as temperature, radiation, precipitation, wind and niche breadth (Hodkinson, 2005). Temperature usually decreases by about six degrees celsius for every 1000 meters of ascent. Short-wave radiation, wind speed and precipitation increases with altitude. This can lead to nutrient leaching where rainfall rates are high. The atmospheric partial pressure decreases with an increase in elevation, which can affect flight performance and slow down energy processing in insects.

Orchid bees are species-specifically dependent on orchids. They harvest nectar, pollen and perfumes and consequently the bees' distribution is affected by the biogeographical and elevational spread of orchids. I could not find data on altitudinal orchid diversity in Costa Rica but studies have been done about this around the world. In one study in Nepal, no correlation between species richness and elevation was found between 900 m and 2500 m (Rai,2015). Alternatively, in Yunnan, China, a bell curve of species richness along the altitudinal gradient was found, with the highest species richness at medium elevations (Zhang, 2015). Zhang deduced that the mid-domain effect and climate were two of the largest contributing factors to this gradient. A study from Colombia agrees that orchid distribution is dependent on climate and studies from Peru, Venezuela and Colombia predict that due to climate change there will be an increase in altitudinal distribution of 378 m by 2050 for 12 focal species of orchids (Reina-



Rodriguez, 2017). If this pattern is true for species that Orchid bees pollinate then we can also predict that bee populations would shift upwards in altitude as well. In Reunion island, there was a decrease of orchid species with an increase in altitude and a large difference in species composition between low and high regions (Jacquemyn, 2006). If Reunion island's orchid distribution reflected that of Monteverde's, I would not have seen such similar species compositions among my sites. Orchid bee species turnover would have been higher, since orchid preferences are species specific (Roubik and Hanson, 2004). Even though altitudinal patterns of orchid richness have been observed around the world, there are many contradicting patterns of distribution and there is no such information about the orchid species pollinated by orchid bees in Monteverde and therefore I cannot deduce how orchid distribution affects the range of the bees I sampled.

### *Altitudinal range of my species compared with the literature*

The Orchid Bees of Tropical America Biology and Field guide is one of the only guides for identifying and understanding Orchid bee. The altitudinal distribution it depicts for three out of the six species I identified is different than the altitudinal range I observed. According to my results, the guide's altitudinal population limits for *Euglossa viridissima*, *Euglossa maculilabris* and *Eulaema bombiformis* are too low (Table 2). This shows that either the authors of this book simply did not analyze the altitudinal distribution of these species thoroughly enough or that due to environmental changes, such as climate change, bees have migrated to higher elevations since the publishing of this book 21 years ago. For example, a study from Colorado concluded that climate change caused an average altitudinal increase of 317 meters for queen and worker Bumblebees between the years 1974 and 2007 (Pyke, 2016). It is important to know what species are present at which elevations now so that in the future the same studies can be continued in order to measure ecosystem health and changes over time. Understanding the biogeographical and altitudinal distribution of bees is important for maintaining their population levels. Bees, particularly Orchid bees in tropical rainforests are significant contributors to ecosystem diversity since they are the main pollinators of many plants (Morochz, 2015). It is important to monitor Euglossine population levels and their overall distributions closely since they are likely to change in the future with current orchid extinction rates being 1,000 times higher than background rates (Reina-Rodriguez, 2017, Swarts, 2009).

### *Orchid bee presence varies between open and forested areas*

When taking samples from the same biogeographical region it is important to choose locations with the same environmental conditions. A previous study discovered that the abundance of many species is lower in cleared areas than forested areas and that species biodiversity is directly proportional to forest fragment size even if the spaces between fragments are very narrow (Powell and Powell, 1987). In Powell's study, *Eulaema bombiformis* was not affected by fragment size, which may explain why I found this species in all the habitats I surveyed. I sampled two different spots in Bajo del Tigre and found a much higher abundance of

orchid bees in the completely forested area than in the open area (Table 3). The opening was small and still within the depths of the forest but it allowed for much more wind and sun. Perhaps in the open area the bees had trouble finding the origin of the scent due to the strong wind or due to behavioral preferences, never venture away from under tree cover. It is important to note the huge difference in abundance and species between these areas, because it may have skewed my results. If I had taken data in the forested area twice instead of in the open area once, my Shannon diversity for the lowest elevation would have most likely been higher. I would have captured more bees in the forested area on day two and potentially found more species as well. This change in methods has likely resulted in a greater difference between high and low elevational biodiversities. This more consistent method would have obtained a more accurate estimate of the biodiversity of orchid bees, because every other region I surveyed was within the forest and not in a clearing.

According to my results, Euglossine bee biodiversity is specific to altitude in Monteverde and changes over a range of 617 meters, with more species and more individuals present at lower elevations. Observing the changes of their altitudinal biodiversity over time could indicate ecosystem health and orchid abundance and diversity and may help researchers find ways of maintaining this unique group of bee's population levels.

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