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The Taxonomy, Reproduction, and Distribution of Rare Plants: A Study of Magnolia sp. in the Río Zuñac Reserve, Ecuador

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Running Head: The Taxonomy, Reproduction, and Distribution of Rare Plants

The Taxonomy, Reproduction, and Distribution of Rare Plants: A Study of Magnolia sp. in the Río Zuñac Reserve, Ecuador

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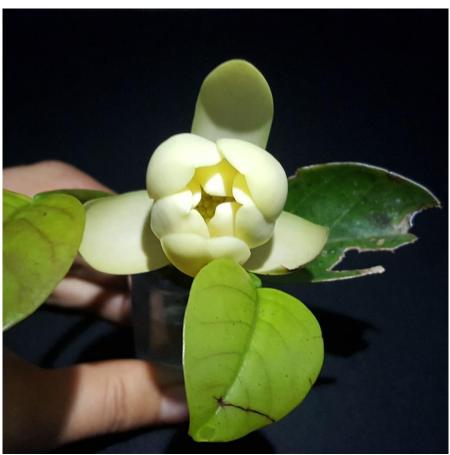


Photo by author.

Colby College Environmental Studies and Spanish

South America, Ecuador, Tungurahua, El Topo

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Abstract.

Ecuadorian cloud forests are biodiversity hotspots and centers of unprecedented levels of endemism, but they are also under-researched and under-protected. This study took place in the Río Zuñac Reserve, which is a reserve of about 850 ha located in the Tungurahua province of Ecuador, and is home to at least 20 endemic plant species. Three new species of Magnolia have been discovered in this reserve in the past five years: Magnolia llanganates, Magnolia vargasiana, and one species that has yet to be formally described. This study dealt with accurately locating and describing new individuals of these species over the course of three weeks. Twelve individuals of M. vargasiana were discovered, increasing the total number of registered individuals to 18. Seven new individuals of the newly discovered species were found, raising the total number to nine. Nine juvenile individuals were found, two of which were identified as being of the new species and five of which were M. vargasiana. A map of the distributions of the three species was created using GoogleEarth, which can be used by a drone to locate the individuals for remote research. The elevation range of *M. llanganatensis* was significantly lower than the other two species, which both overlap in their elevation ranges almost entirely. Visually, the leaf shapes of the three species are markedly different, which was supported by leaf ratio measurements of length to width. Flowers provide better data for comparison between Magnolia sp., but this study took place outside of the peak flowering season for all three species. Five flowers/buds were collected from the newly discovered species and observational comparisons were made between these and flower statistics of the other two species. Herbivory on magnolias between two of the ridges sampled from was significantly different and was also significantly higher in M. vargasiana than in the other two species.

Resumen.

Los bosques nublados de Ecuador son áreas de alta biodiversidad y de niveles altos de endemismo de especies, pero a pesar de eso hay una falta de investigaciones científicas y de protección. Este estudio tuvo lugar en la Reserva Río Zuñac, la cual es una reserva de alrededor de 850 ha ubicada en Tungurahua, Ecuador y habitada por por lo menos 20 especies endémicas de plantas. Tres especies de Magnolia han sido descubiertas en los últimos cinco años: Magnolia llanganatensis, Magnolia vargasiana, y una especie que todavía no ha sido descrita. El estudio se ocupó de ubicar y describir individuos nuevos de estas tres especies durante tres semanas. Diez individuos de M. vargasiana fueron descubiertos, y ahora hay un total de 16 individuos registrados. Se encontraron siete individuos de la especie nueva para hacer un total de nueve individuos registrados. Nueve juveniles fueron descubiertos, de los cuales dos son de la nueva especie y cinco de M. vargasiana. Un mapa de la distribución de las tres especies fue creado con GoogleEarth, el cual puede ser usado por un drone para ubicar los individuos para hacer investigaciones remotas. La distribución de elevación de *M. llanganatensis* fue significativamente más baja que las otras dos especies, estas casi totalmente se sobreponen. Visualmente, las formas de las hojas de las tres especies son marcadamente diferentes, y esto es comprobado por las medidas del ratio de largo y ancho de las hojas. Sus flores proveen datos mejores para comparar entre las especies., pero este estudio ocurrió fuera de la estación de florecimiento de todas las especies. Cinco flores/botones fueron recolectados de la especie nueva y comparaciones observacionales fueron hechas entre estas flores y las estadísticas en cuanto a las flores de las otras especies. El nivel de herbivoría en las magnolias entre dos cuchillos en la reserva fue significativamente diferente y también fue significativamente más alta en M. vargasiana que en las otras dos especies.

ISP Topic Codes: 601, 613, 620 **Keywords:** taxonomy, botany, endemism, magnolia

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Introduction.

The eastern slopes of the Andes host some of the most diverse ecosystems on Earth, especially in Ecuador, a country home to 4000 endemic species of plants alone (Jost, 2008). On these very slopes, a non-governmental organization called the EcoMinga Foundation has been working since 2005 to raise money to conserve land between two national parks: Llanganates and Sangay. One of the major goals of the organization is to conserve the land before it is converted to agricultural land and pasture in order to connect these two national parks and create a wildlife corridor between them, while also conserving species that are hyper-endemic to these relatively small areas.

The precise combination of altitudinal change provided by the montane environment with the cooler refugia that they provide makes these montane cloud forests of high conservation priority. During the last glacial maximum, all of Amazonia cooled and caused the extinction or migration of many species. This cooling, along with a more temporally consistent moisture regime than most of Amazonia, allowed cloud forests species that are usually restricted to the tops of mountains to migrate downward and expand their ranges. When Amazonia warmed again at the end of the Ice Age, many species shifted their ranges once again to higher elevations, where the temperature regime was cooler. The climatic consistence over thousands of years has allowed the biodiversity along the eastern side of the Andes to flourish (Urrego, Silman, & Bush, 2005).



Figure 1. A map of the Río Zuñac Reserve, outlined in yellow. The reserve straddles the Ecuadorian province of Tungurahua to the West and Pastaza to the East. It is located just South and West of Llanganates National Park, and North of Sangay National Park.

EcoMinga's Río Zuñac Reserve in the Cordillera Llanganates of the Tungurahua province of Ecuador was established in 2005 and has steadily grown over the past twelve years to its current size of about 850 ha (pers. comm. Lou Jost; Figure 1), much of which has been possible thanks to donations from the World Land Trust (World Land Trust 2014). This relatively small reserve is home to at least 20 plant species that have been found nowhere else in the world, and that number is growing by the year (World Land Trust 2014). Lying on the wet and windy frontier between the Amazon Basin and montane cloud forest, this reserve receives the brunt of Amazonian moisture, which condenses with orthographic lifting to bring over 4000 millimeters of rainfall annually (Figure 2; Galeas & Guevara, 2012). This high level of rainfall supports the growth of over 800 trees of diameter at breast height of greater than 10 cm per hectare (Vázquez-García, Neill, Asanza, & Recalde, 2015).

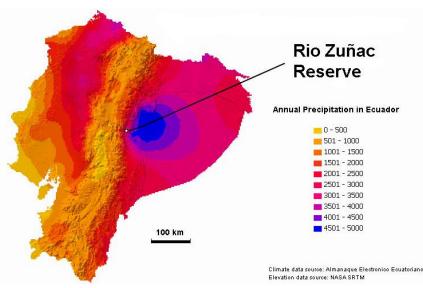


Figure 2. Precipitation map of Ecuador in millimeters per year, with the Río Zuñac Reserve labeled as a white dot (Jost, n.d.).

Unsurprisingly, much of the diversity in these trees remains relatively unexplored by science, and with so many hyper-endemic species in the area, there are still giant species that are just now being discovered and described: the magnolias. The plant family Magnoliaceae is thought to be one of the oldest families of flowering plants, and they are highly diverse (Rose & Dosmann, 2014). There are at least 270 species in the family in between one and six genera (this number is hotly debated among botanists; Magnoliaceae, 2013) ranging from large shrubs to large trees and from temperate and deciduous to tropical and evergreen species. This paper follows the classification system described by Figlar and Nooteboom (2004).

Within Ecuador there are 18 recognized species of *Magnolia* (Magnoliaceae), 14 of which are endemic to the country. Additionally, there are five undetermined species of *Magnolia* currently under review, and quite possibly more that have not yet been discovered (Vázquez-García et al. 2015). Within the Río Zuñac Reserve, there are two described species of *Magnolia* (Vázquez-García et al., 2015), *Magnolia llanganatensis* and *Magnolia vargasiana*, and one species that was recently discovered and has yet to be formally described. The two described species (and probably the newly discovered species) are of the subsection *Talauma* within the section *Talauma*.

There are many reasons that discovering and describing new species is important, primarily because discovering endemic species can garner support for conservation. This is because increased numbers of endemic species indicates that the area is biologically unique and that it may also be a favorable conservation area for vertebrates (Lamoreux et al. 2006). Specifically in the case of the EcoMinga Foundation, new species provide attractive talking points for speakers trying to attract funding from major donors (pers. comm. Lou Jost).

Discovering new plant species can also have important implications for medicine. Without basic knowledge of the plant species that exist, it is impossible to research their possible uses in medicine. Over 700 plants are known to have medicinal use worldwide, and with continued research this number continues to grow (Crellin & Philpott, 1990). In fact, members of the *Magnolia* genus have in the past been shown to have anti-angiogenic and anti-tumor properties (Amblard, Delinsky, Arbiser, & Schinazi, 2011). The magnolia trees of Río Zuñac certainly have the potential to have medicinal properties, but we must first understand the basic characteristics, reproduction, and distribution of these species before we will be able to research their potential uses in medicine.

Describing new species is also useful solely for the sake of adding to the pool of scientific knowledge and increasing the potential for making connections between species and systems. For example, the plant life on the ridgetops of the Río Zuñac Reserve have shown that these ridges act almost as climatically-isolated islands, following aspects of MacArthur's theory of island biogeography (Bos, 2016). Islands have been on the tops of conservation priority lists for decades (Brooks et al., 2006), but it is important to recognize that effectively, islands do not only exist in the oceans, but rather can manifest themselves as habitat pockets in terrestrial systems, where the same biogeographical processes still apply. This island-like pattern of montane cloud forests is part of what puts them at risk, and part of what allows for such high levels of endemism. Ecological patterns such as this may shed light on evolution and species distributions in similar ecosystems.

Where it has been shown that cloud forests create islands for plants, there are probably other communities affected by this process as well, such as insect communities. Insect communities are extremely under-studied in Ecuador, especially given their high level of diversity (Dangles, Barragán, Cárdenas, Onore, & Keil, 2009). This is due to challenges such as lack of financial and human resources, as well as the difficulty of sampling and identifying the incredibly high number of species that exist in complex community structures and

oftentimes remote habitats. Herbivorous insects are especially important to study when considering the population structure and regulation of plant species (Crawley, 1989), and along with pollinator insects they are vital for understanding rare and endemic species.

The first priority of this project was to search for more individuals of a newly discovered species of *Magnolia* which has not yet been described. At the beginning of this study there were only two known individuals, and in order to be confident that it is truly a new species, more individuals were needed for full analysis and comparison between other similar species. The study also deals with furthering scientific knowledge on all of the *Magnolia* species of Río Zuñac Reserve. This included documenting juveniles of all species (defined as having a DBH of less than 20 cm), which is important for understanding the recruitment of these species. Data was also gathered on variation between individuals of the species *Magnolia vargasiana* because experts are beginning to doubt whether it is actually a different species than *Magnolia mercedesiarum* (soon to be described), which has been found on a nearby mountain range. When possible, seeds of the *Magnolia* species were collected to send to the Quito Botanical Gardens to be able to reproduce the trees in a lab setting. The *Magnolia* species with drones. In addition, levels of herbivory were monitored to begin to understand herbivorous insects' effects on these rare, endemic tree species.

Methods and Materials.

Field Components

As one of the primary objectives of this study was to identify, locate, and sample new individuals of *M. vargasiana* and of the newly discovered species of *Magnolia*, the Río Zuñac Reserve was systematically searched over the course of three weeks (April 17-May 5) for individuals of these two species. Previously, there were two documented individuals of M. vargasiana (Vázquez-García et al., 2015) and only one documented individual of the newly discovered species (pers. comm. Lou Jost), and the locations and altitudes of these individuals were used to determine search-areas. Searches were conducted on three different neighboring ridges, one of which falls about 1 km from the border between Río Zuñac Reserve and Llanganates National Park, another immediately to the South, and the other across the valley to the South through which Río Lou runs (Figure 2). Both ridges fall in what is known as the Cordillera Abitagua. Searches were limited to areas within a maximum of 20m from the trail, as vegetation is dense and slopes along these ridges are between 30 and 60 degrees, making off-trail trekking nearly impossible at times. Differentiating individuals of different species of Magnolia involved the use of the descriptions and taxonomy guides provided by Vázquez-García et al. (2015) and Pérez Castañeda (2015), along with personal communications with Lou Jost, Fausto Recalde, and Santiago Recalde in order to differentiate the newly discovered species, which has not yet been formally described.

Samples were taken when possible from each individual of *Magnolia* encountered of three different species: *M. vargasiana*, *M. llanganatensis*, and the newly discovered species. This was done by climbing the tree or a neighboring tree with an elongated pruner in order to snip branches from the magnolias when it was safe to do so. This was done in order to bolster the existing but limited data on the two described species and to test whether there are significant phenotypic differences between these species and the newly discovered species. Leaves were collected from all trees with the exception of five individuals which were not safe to sample from, and were measured for length and width of leaf blade. Leaves were also measured for the extent to which they were affected by herbivory, measured as the percent of the leaf surface eaten away or damaged clearly by herbivores (estimated using a grid of 0.25 cm² squares held up to the light in front of the leaf).

Flowers provide more robust information on the taxonomy of these species of *Magnolia* (Pérez Castañeda, 2015), however, this study did not align with the flowering season of *M. vargasiana* (late August to late February; Vázquez-García 2015) or *M. llanganatensis* (around November; pers. comm. Fausto Recalde). This being the case, buds and flowers were collected when possible from individuals of the newly discovered species to compare to statistics on the other two species provided by Vázquez-García et al. (2015) and Vázquez-García et al. (2016). Even then, the peak of the flowering season of the newly discovered species occurs in December (pers. comm. Fausto Recalde), which meant that the flowers that were there were few and far between, making statistical analyses unlikely.

In addition to being sampled, each individual of *Magnolia* was measured for diameter at breast height (DBH) at a height of 1.37 m on the uphill side of the tree. DBH was often measured around lianas, mosses, and other epiphytes due to difficulty and possible ecological harm that would be caused by removing the epiphytes from the magnolias. When large epiphytes such as bromeliads were present at 1.37 m, the DBH measurement was shifted up by up to 40 cm to avoid unnecessary overestimates. This shifting probably did not have a great effect on DBH accuracy, as magnolia trunks tend to be rather uniform in diameter regardless of height. Tree height was also estimated with the use of a clinometer.

One of the objectives of this study was to describe the reproductive success of these rare tree species, which in the case of this short-term study meant searching for seedlings and saplings of the three species of *Magnolia* present on the ridges. These data were used to get a sense of the age distribution of the Río Zuñac *Magnolia* community overall and to cursorily assess their reproductive success. Juvenile trees were identified to species level when possible, however, leaves on young trees are often rather dissimilar to leaves of their adult counterparts, making species-level identification difficult. When trees were identified to the species-level, distance and direction to parent tree, assumed to be the nearest mature tree of the same species, was measured. GPS coordinates of all juveniles were also recorded.

Slope, aspect, and elevation around each individual were also measured to determine the basic habitat preferences of each of the three species. Slope was measured with a clinometer in the direction of the deepest downhill slope around the tree, determined visually. Aspect was measured with a compass in the same direction as slope. Elevation was determined by using a Garmin GPSMap64. Coordinates of each tree were also determined by use of the GPS, although the thick canopy prevented precise measurement. In order to circumvent this issue, methods described by Bos (2016) were followed using referenced formations of *Dictyocaryum* palms, which are distinguishable on the aerial image of the reserve, to precisely locate magnolias. Unfortunately, the upper limit in terms of elevation of *Dictyocaryum* is near the middle range of *M. vargasiana* and of the newly discovered species, which prevented the accurate georeferencing of many of the magnolias.

Analytical Components

A database in GoogleEarth was created by entering the GPS coordinates of all adult and juvenile trees. This was combined with photographs of each tree to facilitate the EcoMinga drone being able to locate each tree. Because of the complications with georeferencing with *Dictyocaryum*, these locations are often not exact and give more of a general distribution of the *Magnolia* community within the reserve. The elevation ranges of the three species were compared using an analysis of variance (ANOVA) test in R Studio (v. 3.3.1).

Leaf lengths and widths were combined to create leaf ratio measurements (length:width), which allows for comparability between leaves of different ages and sizes. Leaf ratios were then compared across individuals of the same species using an ANOVA test. Leaf ratios were also compared between the three species using an ANOVA test. Leaves were also compared observationally for information on leaf shape using photographs of samples.

Herbivory measurements were likewise analyzed using ANOVA tests to compare 1) between ridges, marking different sub-populations of trees, and 2) between the three different species regardless of location. All ANOVA tests were followed up with a post-hoc Tukey's Honestly Significant Difference test (Abdi & Williams, 2010), a stepwise multiple comparisons procedure to identify which sample means were significantly different from each other. Herbivory was also analyzed in relation to elevation using a linear regression test in R.

As too few flowers were collected to make statistical analyses, flowers were photographed and described for information on the basic biological processes, and were also dissected to count sepals, petals, and stamens, the latter of which often helps to differentiate between species of *Magnolia* in Ecuador.

Results.

Over the course of this study, the number of registered individuals (found and located with GPS) of *M. vargasiana* increased by 12, from 6 to a total of 18 across three ridges all in the Río Zuñac Reserve within an area of about 3.2 km^2 (Figure 3). The number of registered individuals of the newly discovered species increased by 7, from 2 to a total of 9 individuals. Four already registered individuals of *M. llanganatensis* were encountered and included in the study for the purpose of comparison with the other two species, although a more extensive study on this species was done already by Bos (2016). Individuals of all species were found on three different ridges within the Río Zuñac Reserve.

Nine juvenile individuals were found, three of which were identified as being of the newly discovered species and five of which were identified as being *M. vargasiana*. The remaining juvenile could not successfully be identified to species level, which was largely due to not being able to collect enough samples from these individuals to be able to make a confident identification. All of the juvenile identifications are tentative, because many of these individuals were too small or young to collect more than a few leaves without causing significant damage to the individual. However, based on the elevations of the unidentified individual, it is most likely either a member of the newly discovered species or of *M. vargasiana*.



Figure 3. A map of all GPS referenced individuals of Magnolia found during this study. Yellow pinpoints denote individuals of M. llanganatensis; blue pinpoints denote individuals of M. vargasiana; red pinpoints denote individuals of the newly discovered species; white pinpoints denote juvenile individuals (some of which were tentatively identified to the species level). The yellow line indicates the border of the Río Zuñac Reserve, and the gray line marks the border between the provinces of Tungurahua to the West and Pastaza to the East.

The elevation range of *M. llanganatensis* (Table 1) was significantly lower than that of both *M. vargasiana* (p < 0.001) and the newly discovered species (p < 0.001; Figure 4). However, elevation range is not significantly different between *M. vargasiana* and the newly discovered species.

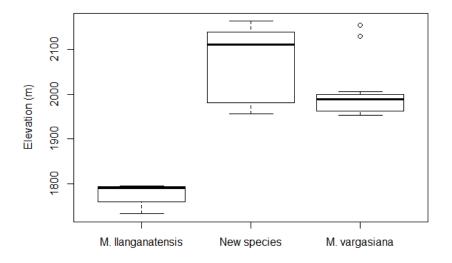


Figure 4. Side-by-side boxplots comparing the distribution of three different species of Magnolia based on elevation in meters above sea level ($n_{llanganatensis} = 4$, $n_{new} = 8$, $n_{vargasiana} = 13$).

The ratios of leaf length to leaf width were significantly different between all three species (p < 0.001 in all cases; Figure 5). Although there is a significant difference between the newly discovered species and *M. vargasiana*, there is also a considerable amount of overlap in the leaf ratios between these two species, which both show a considerable amount of variation within each species.

When comparing variation within the 11 individuals of *M. vargasiana* from which samples were successfully collected, 42% of the 55 possible combinations showed significant differences between the leaf ratios (p < 0.05). Of the three individuals of *M. llanganatensis* from which samples were collected, one of the three possible combinations of individuals showed a significant difference between the leaf ratios (p < 0.01). Of the nine individuals of the newly discovered species, 47% of the possible combinations showed significant differences between the leaf ratios (p < 0.05).

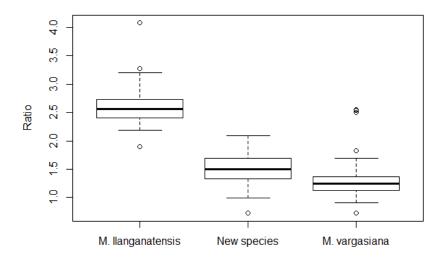


Figure 5. Side-by-side boxplots comparing three different species of Magnolia based on the ratio of leaf length to leaf width ($n_{llanganatensis} = 61$, $n_{new} = 177$, $n_{vargasiana} = 193$).

However, although there is a large amount of variation of leaf ratios within the three species of magnolia, the variation between the species is still greater than the within-species variation, which is also clear when looking at the shapes of the leaves (Figure 6). The leaves of *M. llanganatensis* have been described as elliptic-lanceolate (Vázquez-García, Neill, Recalde, & Asanza, 2016) with acute to acuminate tips. From the photos it is clear that the leaves look quite different from those of *M. vargasiana*, whose leaves have been described as suborbicular, broader toward the bottom half, and subcordate to cordate at the base, with obtuse to emarginate apices (Vázquez-García et al., 2015). The newly discovered species has yet to be described, but the leaves are generally longer than those of *M. vargasiana*, and often has acute to obtuse apices and rarely emarginate apices.



Figure 6. Leaves of the three species of Magnolia examined in this study. From left to right: M. llanganatensis, M. vargasiana, newly discovered species (photos taken by author).

Two flowers and three buds were found over the course of the study, all from individuals of the newly discovered species. This did not yield enough data to compare statistically with the other two species, however observations can still be made. The flowers and buds of the new species all had three sepals, two had seven petals and three had eight petals, and they had an average of 36.6 stamens with a range of between 32 and 43 stamens (Table 1).

	M. llanganatensis	M. vargasiana	New species
Tree height (m)	11 – 27	11 – 26	10 - 30
DBH (m)	14 - 70	20-70	16 - 86
Leaf length (cm)	5.1 - 13.0	4.2 - 20.7	3.0 - 23.0
Leaf width (cm)	1.1 – 5.3	4.0 - 16.2	2.3 - 13.5
Leaf ratio	1.9 – 4.1	0.7 – 2.5	0.7 – 2.1
(length:width)			
Leaf shape	elliptic-lanceolate, apex acute to acuminate	suborbicular, subcordate to cordate base, apex obtuse to emarginate	ovate, apex obtuse to acute and rarely emarginate
Flowering period	around November	August to late February	Around December
Number Sepals	3	Х	3
Number Petals	8	6	7-8
Number Stamens	50 - 54	20-25	32 - 43
Elevation (m)	1730 - 1800	1960 - 2160	1950 - 2170

Table 1. A comparison between various characteristics of the three magnolia species involved in the study.

Only one of the two flower buds of the new species that were found over the course of the study actually opened. This did not allow for statistical analysis regarding the biology of the flower opening process, but allowed for observational data nonetheless (Figure 7). As with the other two species of magnolia involved in the study, the flower opened only partially on the first day that the sample was collected. It opened for about three hours, beginning at around 16:30 in the afternoon. According to Vázquez-García et al. (2015), this is to receive incoming flea beetle pollinators, which are then trapped within the flower until the flower opens again the following afternoon, when the stamens also fall from the flower. At this point the gynoecium begins to mature and accept pollen. This process aids in avoiding self-pollination, and is common across many species of *Magnolia* (Rose and Dosmann 2014).



Figure 7. Photos of the stages of the opening of the newly discovered species of Magnolia. From left to right, starting on the top row, the first photo is of the bud freshly sampled from the tree at 10h55; in the second photo, taken the same day at 16h33, the sepals are opening; in the third photo the petals are beginning to open, taken at 17h06; the fourth photo, also taken the first day, shows the maximum amount that the petals open on the first day, taken at 18h16. The final two photos were taken the following day, when the flower opens fully. The fifth photo was taken the second day at 16h05, and shows the stamens falling into the concave petals as they open. The sixth photo, taken at 16h58, shows the flower fully open, after all of the stamens have fallen. Over the next 24 hours, all of the petals also fell from the flower, leaving only the gynoecium. (Photos taken by author)

Due to a combination of the reactionary nature of the herbivory data collection and adverse weather conditions, leaves were only measured for the level to which they are affected by herbivory from the second and third ridges that were sampled from, which excludes the northernmost ridge from this part of the study (Figure 2). Based purely on observation, it was clear that the magnolia trees from the northernmost ridge were most predated upon by herbivores by far, followed then by the middle ridge, leaving the southern ridge with the least amount of herbivory. Since the middle and southern ridges were sampled for herbivory measurements, it is possible to compare these two areas using statistical techniques.

Across all three species of magnolia involved in the study, the middle ridge showed a significantly greater level of herbivory on the leaf blades than the southern ridge (p < 0.01; Figure 8). If the northernmost ridge had been included in this analysis, it is estimated that it would have a significantly greater amount of herbivory on the leaves than both the middle and southern ridges.

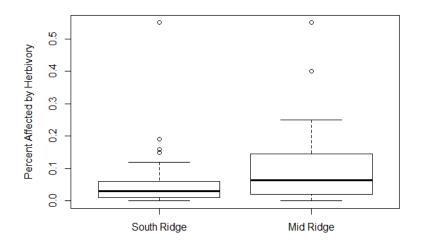


Figure 8. Side-by-side boxplots comparing the level of herbivory, measured as percent of the leaf blade that was absent or severely damaged, between two of the three ridges involved in the study, the southernmost ridge and the middle ridge.

The level of the effects of herbivory on the leaf blades was also compared between species, regardless of location. While there was not a significant difference between the level of herbivory on *M. llanganatensis* and the newly discovered species, the level of herbivory on *M. vargasiana* was significantly higher than on both *M. llanganatensis* (p = 0.002) and the newly discovered species (p < 0.01; Figure 9). It is possible that with more data on herbivory of these three species, the results may be different, and would definitely be more refined.

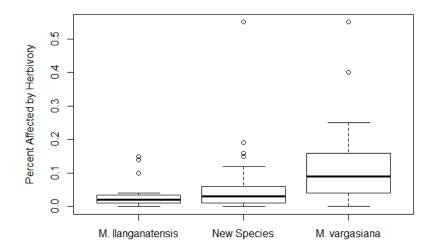


Figure 9. Side-by-side boxplots comparing the level of herbivory, measured as the percent of the leaf-blade that was absent or severely damaged, between the three species of Magnolia involved in the study.

The level of herbivory was also compared with change in elevation, which resulted in an insignificant weak positive trend (Figure 10). That is, it seems that herbivory increases with increased elevation, but the relationship is not significant under the standard 95% confidence interval (p = 0.067).

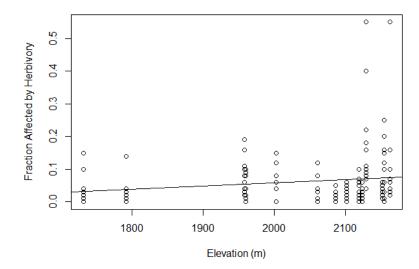


Figure 10. A scatterplot of the level of effect of herbivory by elevation, in meters above sea level, with a fitted linear model ($R^2 = 0.02$).

The average DBH of the newly discovered species was 0.35 m, ranging from 0.06 m to 0.86 m. The distribution of DBH values for this species is skewed to the right (Figure 11), although the dataset is very small, with only seven individuals. For *M. vargasiana*, the average DBH was 0.52 m, ranging from 0.19 m to 0.95 m. The distribution of DBH values for *M. vargiasiana* is not normal (Figure 12), but it is possible that with more data points, the distribution may be different. When all individuals of *Magnolia* were aggregated, the resulting distribution of DBH is more normal (Figure 13).

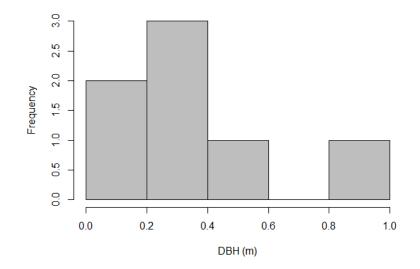


Figure 11. A histogram of the diameter at breast height (cm) of the newly discovered species (n = 7).

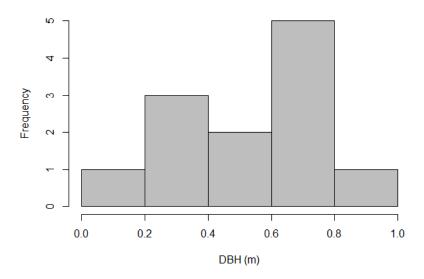


Figure 12. A histogram of the diameter at breast height (cm) of M. vargasiana (n = 12).

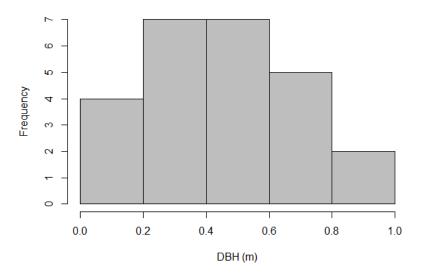


Figure 13. A histogram of the diameter at breast height (cm) of all Magnolia species in the study (n = 25).

When the tree height data of all individuals of all three species in the study were combined, the resulting histogram yielded the expected distribution of being skewed to the right (Figure 14). This indicates that the majority of the individuals between these three species are relatively young and there are fewer older individuals.

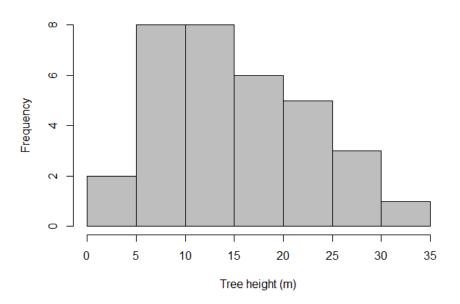


Figure 14. A histogram of tree heights in meters of all individuals of Magnolia (of all three species) for which height was recorded (n = 33).

Discussion & Conclusions.

Over the course of this relatively short study, the information regarding two rare, hyper-endemic species to the Río Zuñac Reserve increased substantially. In total, 25 individuals of *Magnolia* were discovered during this study between two species: the newly discovered species, and the relatively recently-discovered *M. vargasiana*. This more than doubles the number of registered individuals for each of these species and provides the opportunity for botanists and researchers to more easily access these individuals. This lays preliminary groundwork for being able to target more easily where to search for even more individuals to add to the database while also informing the statistics that already exist on these species.

Before this study took place, there was no information on juveniles or recruitment of the three *Magnolia* species in the reserve. Locating juvenile magnolias was difficult due to the highly steep terrain and impracticality of wandering aimlessly off of the established trails. Even so, nine juveniles were located over the course of this study, all located directly next to the trail. Despite the small number of juveniles found, it is likely that searching among the understory off-trail would have yielded a much higher number and a more accurate representation of the recruitment of these species. It is also worth mentioning that no juveniles of *M. llanganatensis* were located, and it is unclear why this would be the case. More research regarding their recruitment is needed regardless.

However, from this data it can be concluded nonetheless that the newly discovered species and *M. vargasiana* are both reproducing. Whether or not this is at sustainable rates is unclear, but it may allow scientists to begin to form an informed decision as to the conservation status to these species. As of the writing of this paper, of the three species only *M. vargasiana* appears on the IUCN Red List of Threatened Species, and it is classified as Data Deficient (Rivers 2016). The authors of the original paper written to describe *M. vargasiana* suggested that the species be classified by the IUCN as Vulnerable, since there were at the time only two known mature individuals and no known recruits (Vázquez-García et al., 2015). Perhaps now that the number of registered individuals has increased by eight

times and includes five juveniles, experts will be able to better determine this species' conservation status. It is possible that *M. llanganatensis* does not appear on the IUCN Red List because the original paper published describing the species is in Spanish, and the language barrier may have prevented its addition to the website. Obviously, the newly discovered species does not appear on the IUCN Red List because it has not yet been described and does not even have a scientific name yet.

Between this study and the one conducted by Bos (2016) on *M. llanganatensis*, there is now a considerable amount of information regarding the distribution of these three species of magnolia trees. The distribution information from this study, which can be seen in Figure 3, is part of an interactive map in the GoogleEarth program. Each pinpoint locates the GPS coordinates of an individual magnolia tree, the color marking the relative age or the species. Each pinpoint is also labeled with a unique ID code for each tree, along with photos, DBH measurements, height measurements, and the elevation as recorded by the GPS. Hopefully the combination of all of this information will provide the resources needed by EcoMinga to be able to locate these individuals with their drone, which will greatly simplify the monitoring and research on these trees, which are highly remotely located and not easily reached. In addition, if the drone is able to locate these individuals, this may aid in bolstering information to allow machine learning, whereby the drone might even be able to find new individuals of these species that have not yet been found or referenced.

A pattern that can even be clearly seen on the map is that the range of *M*. *llanganatensis* is significantly lower in altitude than the other two species, which do not differ significantly in altitude. All three species fall within very narrow windows of elevation range, the newly discovered species having the largest range at only 220 m. This makes sense, as rare tropical tree species are typically more aggregated or clustered than common tree species (Condit et al., 2000). However, this also puts these species at a higher risk, as they have limited habitat both in area and in requirements. According to Fausto Recalde, forest ranger and local to the area, precipitation patterns in the region have changed noticeably over the past decade (pers. comm. Fausto Recalde). This, combined with changing temperatures due to global climate change, may play a part in further limiting or shifting where these species are able to persist. More information on juvenile magnolias may be able to shed light on whether these species are reacting to their changing environment, which would present itself as the juveniles growing in different elevation ranges or different habitats otherwise.

Due to the small sample size of all of the species within the study, a result of dealing with rare species, breaking up data to describe each tree by species did not yield any results that can be realistically analyzed using statistical techniques. For example, the distributions of DBH for both the newly discovered species and for *M. vargasiana* yield non-normal results. When all DBH data regardless of species was aggregated to make a histogram, the distribution became much closer to normal. This suggests that with more individuals, the data within each species would become more normal. The somewhat normal distribution of all of the magnolias aggregated suggests that there are few old individuals, many mid-age mature individuals, and few juveniles. However, it is highly probable that there is an underrepresentation of low DBH individuals in this study simply because those individuals are more difficult to spot. The trend of tree heights followed a more expected trend, as there were many short trees, tapering off to very few tall trees, the distribution being fairly strongly skewed to the right. This, as expected, suggests that there are more younger individuals of Magnolia overall than there are old individuals, an age distribution that would make sense based on basic population models. This is also promising for the future of these Magnolias, and suggests that they are not suffering too heavily yet from a changing environment since there are not only older individuals.

It is also important to understand the relationship of characteristics among and between these species of Magnolia to be able to understand the variation within species and the differences between species. The most useful way to do this among Magnolia subsect. Talauma is to compare various aspects of the flowers, however, the fieldwork component of this study did not align with the flowering season of any of the three species. Fortunately enough, two flowers and three buds were collected from the new species nonetheless. Because of the lack of available data regarding flowers, data were compared between leaves of the three species. Individuals were placed into their species identifications by looking at multiple leaves and deciding based on shape of the leaves, which are rather distinct between all species (see Table 1). However, leaf shape is not a quantifiable form of data, so in addition to this, ratios of leaf length to width were calculated to roughly quantify the shape of the leaf. Unsurprisingly, the ratios were significantly different between all three species. It is true that between 30 and 50 percent of the individuals within each of the three species were significantly different from each other as well in terms of leaf ratio, so there is indeed a fairly high degree of within-species variation as well. However, based on the formerly mentioned results, the differences between species were greater than the differences between individuals of the same species, meaning that leaf ratio or leaf shape is a good way to tell these species apart.

Of the five flowers/buds that were found, one flower opened, which is pictured and described in the results section of this paper. The process of the flower opening of this species is very similar to those of the other two Magnolia species, described by Vázquez-García et al. (2015) and Vázquez-García et al. (2016), whereby the flower bud opens only partially on the first day to allow pollinators to enter, which in the case of Magnolia vargasiana is a species of flea beetle in the tribe Alticini. Based on the strong, sweet scent of the flowers of *M. vargasiana*, it is probable that the flea beetle is attracted by the scent to pollinate the flower. On the other hand, facultative and antagonistic visitors of many species of flowering plants are repelled by such floral scents, meaning that it is likely that this species of flea beetle is an obligate pollinator, that is, it may not be the only insect that pollinates M. vargasiana but probably does rely on this species' flowers at least in part to obtain its nutrition (Junker & Blüthgen, 2010). There is currently no data on the pollination of the other two species, M. llanganatensis and the newly discovered species, however, it may be that it is the same species of flea beetle or a similar species that provides this service. It is also worth mentioning that the pollinator data on *M. vargasiana* was a one-time observation, which means that the flea beetle may not even be the only (or even the primary) pollinator of the species.

The flowers then open again on the second day, and rotate their petals past 90 degrees as all of the pollen-covered stamens fall from the flower, leaving the gynoecium to mature and begin to accept pollination. This process of flower maturation allows the *Magnolia* trees to avoid self-pollination by separating the male and female parts of the flower by time instead of by space, as is the case with many species. Although the three species share a common process for pollination, they seem to differ regarding some of the basic physical characteristics of their flowers (see Table 1). The three species all differ in peak flowering period (which is also a simple way to prevent hybridization between similar species), number of petals, and number of stamens. With more data on the flowers of the newly discovered species, more accurate statistics regarding their basic flower characteristics may be reached.

The aspect of this study dealing with herbivory on the magnolia trees was not originally planned on, and so is not a complete dataset. The northernmost ridge was sampled from first, and because of adverse weather conditions, it was not possible to return to that ridge to complete the dataset on herbivory. Therefore, all analyses of herbivory involve only the trees from the middle and southern ridges. Based solely on observations, it was clear that



Figure 15. A leaf of the newly discovered species affected by herbivory.

the magnolias of the northernmost ridge (possibly excluding M. llanganatensis) suffered the most from herbivory. Based on statistical analyses, the magnolias regardless of species were more affected by herbivory on the middle ridge than the southern ridge. Spatially, this would also make sense with the hypothesis that the northernmost ridge is most affected by herbivores, with the level of herbivory increasing in a gradient from South to North. However, at similar altitudes and with similar temperature and precipitation regimes, it is unclear why such a great difference may exist between these three ridges. It is also possible that the same type of hyper-endemism exists in Río Zuñac with herbivorous insects as exists with many of the plant species. Slight differences in herbivorous insect communities between neighboring ridges could allow for the differential levels of herbivory. Currently, little is known regarding insect communities within the reserve (pers. comm. Santiago Recalde), making an analysis on the herbivory of certain plant species difficult. However, caterpillars of an unknown Lepidopteran family have been observed feeding directly on the leaves of M. llanganatensis. Additionally, Ecuadorian cloud forests have been identified as diversity hotspots of Geometridae and quite possibly other Lepidopterans (Brehm et al.

2005), which means that Río Zuñac quite possibly represents an area of hyper-endemism of these herbivorous insects. The patterns of herbivory found on the leaves were mostly grazing and mining, which are both common patterns to Lepidopterans, along with a few other orders of insects such as Diptera and Symphyta (Figure 15). To be able to understand the pattern of differential herbivory across the different ridges in Río Zuñac, more research needs to be done on the most likely highly-complex nature of the herbivorous insect communities within the reserve. According to Brehm et al. (2005), arthropod diversity and communities in the tropics are extremely understudied, especially in comparison to their plant and vertebrate counterparts.

Geographical distances aside, there were also differences in the level of herbivory between different species. Interestingly, although *M. vargasiana* and the newly discovered species fall within very similar elevation ranges, *M. vargasiana* was found to be predated on by herbivores significantly more than the newly discovered species. It is possible that some of the herbivores are specialists that do not cross between the *Magnolia* species. This would explain why similar species occupying very similar habitats would be affected differently by herbivory. *M. llanganatensis* was affected significantly less by herbivory than was *M. vargasiana*, which may also be explained by the possibility that there are species-specific herbivores in this area, or it may also be explained by the possibility that the herbivorous insect communities are significantly different across the elevation gradient, as these two species differ significantly in their altitudinal range. The fact that the lower species, *M. llanganatensis*, suffers less from herbivory may be a clue that the insect communities of Río Zuñac are following worldwide trends of shifting their ranges upward in altitude, retreating from the warming climate (Hodkinson, 2005). It is possible that species that once predated on the leaves of *M. llanganatensis*, or even the newly discovered species, which also showed

low levels of herbivory, have shifted their ranges upward faster than the tree species themselves have been able to migrate. As mentioned previously, it remains unclear as of the writing of this paper whether the *Magnolia* species of the reserve are beginning to shift their distributions as well. If so, it would be interesting to look into whether younger individuals are affected more by herbivory than older individuals, as the younger individuals would more likely remain within the ranges of their herbivores as the species react to the same changes and shift together.

Herbivory was also compared to elevation, and in this case the relationship was a weak positive trend that was found to be insignificant by a slim margin (p = 0.067). With more data, including from the unsampled ridge, it is likely that the trend would be significant. However, it is also very possible that although a significant positive relationship may exist, the species may be a confounding factor. That is, *M. llanganatensis* is only found at significantly lower elevations than the other two species and is affected significantly less by herbivory than *M. vargasiana*, meaning that the linear regression may just be a representation of the different species. It would be interesting to look at within-species trends of herbivory compared to elevation, which would eliminate species as a confounding factor.

In the future it will be important to continue research on these rare trees of the cloud forest, especially focusing on the biology of their reproduction. The Quito Botanical Garden has a program in alliance with Botanic Gardens Conservation International to aid in the conservation of rare and/or threatened plant species in the wild by growing, maintaining, and researching them ex situ as part of The Global Trees Campaign and the Global Seed Conservation Challenge (Botanic Gardens Conservation International, n.d.). These programs seek to place responsibility on botanical gardens to protect and research the plants native to their specific regions, the Magnolia trees of this study pertaining to the area covered by the Quito Botanical Garden (Narváez, 2006). During this study seeds of *M. llanganatensis* were found and sent to the Quito Botanical Garden in hopes of being able to cultivate the species ex situ. Especially in the case of these Magnolia species, they are highly remote and not easy to reach, making it very difficult to study their biology. Of course, in situ research is vital for learning about aspects such as habitat, pollination, herbivory, epiphytes, and other possible interactions with their surrounding environment; however, to be able to learn about the specific biology of these species, such as the biological process of their flowers opening, or to bolster population sizes for restoration and reintroduction programs for threatened species, or even simply to be able to raise public awareness on these rare and endemic species, having individuals growing in a more controlled setting will allow us to better achieve these goals.

There is still much work left to be done both *in situ* and *ex situ* regarding the magnolias of Río Zuñac. Hopefully with the increase in the total number of registered individuals of the newly discovered species experts will be able to formally describe it. It is easier to protect named species with strong population and distribution estimates than those for which we only know of a few individuals or those without names. The data from this study and the future studies for which it paves the way will be able to inform the IUCN and other authorities on threatened species to create awareness for the region, for the reserve, and for EcoMinga. The more money that conservation organizations such as EcoMinga are able to raise, the better able they will be to protect strategic areas when the government has no want or will to do so.

There also remains work to be done with juvenile magnolias in the reserve. There is now more optimism that they are indeed reproducing, as very young trees were found during this study. However, it is unclear whether they are reproducing at a high enough rate to maintain stable population levels, especially with the changing climate. The distribution of these highly particular species may also be affected by the changing climate, which has in recent years altered micro-habitat temperature and precipitation regimes in the region (pers.

comm. Fausto Recalde). Locating and analyzing the distribution of the juvenile *Magnolia* populations should be a top research priority in the reserve. During this study, evidence was found that fallen branches of *Magnolia* may be able to propagate under the right conditions and grow into new trees (pers. comm. Fausto Recalde), and this would be an interesting and potentially useful topic to look into, especially in terms of possible conservation efforts.

Although the number of known individuals of two of the three species involved in this study have more than doubled, these numbers still remain relatively low, meaning that even though they are located within protected land in a reserve next to a national park, they are still at risk. There are known threats to these species, such as high levels of herbivory and even predation on flower buds, which both may compromise their recruitment. There are quite likely even more giants such as these magnolias that have yet to be discovered in the cloud forests of Ecuador within the thin but rich stretch that separates the Andes from the Amazon Basin. With funding, manpower, and sheer will to protect and increase scientific knowledge on Ecuadorian cloud forests, information on these species and others will continue to grow in the future.

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