UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Ciencias Biológicas y Ambientales

Spatial distribution and microhabitat use of species of geckos in San Cristobal Island, Galapagos

Artículo académico

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Biología

Trabajo de titulación presentado como requisito
para la obtención del título de
Licenciada en Biología

Quito, 23 de mayo de 2019

UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

COLEGIO DE CIENCIAS BIOLÓGICAS Y AMBIENTALES

HOJA DE CALIFICACIÓN
DE TRABAJO DE TITULACIÓN

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Quito, 23 de mayo de 2019

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Lugar y fecha: Quito, 23 de mayo de 2019

DEDICATORIA

A todos quienes me han apoyado y ayudado a lo largo de esta hermosa carrera.
A la biología y a la vida.

Agradecimientos

Gracias a mi familia, por su apoyo incondicional y por inconscientemente inculcarme el amor a la biología desde el inicio de mi vida. A mi madre, Sara María Paz porque sé que siempre podré contar con ella y por ser un ejemplo de perseverancia. A mi padre, Ángel Oleas por sus consejos, y por motivarme siempre a tener curiosidad por el mundo. A mi hermana, Anahí Oleas por sus sonrisas que me alegran día a día. A mi nana, Dito por todo el amor y compañía que me ha dado durante estos años. A Mateos Berrú por darme paz y por hacer de mis días alegres con su espontaneidad. También quiero agradecer a mis compañeros de carrera por estos años de aprendizaje juntos, en especial a Xavier Chavarría por su amistad y paciencia. Agradezco a todos mis profesores por el tiempo dedicado a transmitir sus conocimientos. Gracias a Diego Cisneros por darme la oportunidad de participar en este proyecto y por el apoyo brindado.

RESUMEN

Los reptiles nativos de islas están adaptados a las condiciones propias de los ecosistemas que habitan. Se conoce poco acerca del estado de las poblaciones de reptiles de islas tropicales, y se ha determinado que una de las amenazas más grandes que enfrentan son las especies introducidas. Los gecos son reptiles que comúnmente son introducidos accidentalmente en nuevos hábitats, y muchas de estas especies cuentan con una facilidad de colonización impresionante, provocando así el desplazamiento de las especies nativas. La introducción de gecos en las Islas Galápagos se ha dado en diferentes ocasiones por la expansión humana hacia el archipiélago y por el transporte de productos. En San Cristóbal existen cuatro especies introducidas de gecos y dos especies endémicas. La información que existe acerca de la distribución espacial, uso de microhábitats y preferencia de sustratos de las especies de gecos de la isla es escasa, por esa razón en este estudio se han investigado estos tres parámetros mediante censos visuales y experimentos con sustratos. Se determinó la composición de la comunidad de gecos en diferentes ecosistemas de la isla: bosques siempreverdes estacionales (84,8% *G. caudiscutatus*, 15,2% *P. leei*), bosque deciduo (bosque seco) (58.8% *G. caudiscutatus*, 9,3% *H. frenatus* , 7,7% *L. lugubris*, 2,2% *P. reissi* , 15,4% *P. leei*, 6,6% *P. darwini*), tierras agrícolas (99,4% *G. caudiscutatus*, 0,6% *L. lugubris*) y áreas urbanas (19,9% *G. caudiscutatus*, 48,7% *H. frenatus*, 20,3% *L. lugubris*, 3,5% *P. reissi* , 7,6% *P. darwini*). Se determinó que los microhábitats más utilizados por gecos estaban compuestos por madera lacada, bloques, empaste de interiores, corteza de árbol, opuntia y roca volcánica. Se encontró que las especies introducidas tienen una velocidad de caminata menor en sustratos rugosos que en sustratos lisos, con ciertas excepciones. La especie más frecuentemente encontrada en todos los sitios de estudio fue *G. caudiscutatus*, su expansión podría estar relacionada con factores climáticos particulares del año de muestreo (2017), pero no es una amenaza significativa para las especies endémicas debido a su actividad diurna. *L. lugubris* y *P. reissi* son especies introducidas adaptadas a construcciones humanas. La principal amenaza identificada para los gecos endémicos es *H. frenatus*, esta especie cuenta con comportamientos agresivos de competencia y está colonizando microhábitats naturales con mucha rapidez.

Palabras clave: gecos, microhábitat, islas Galápagos, desplazamiento, competencia, preferencia de sustratos, distribución espacial.

ABSTRACT

The native reptiles of islands are adapted to the conditions of the ecosystems they inhabit. Little is known about the status of tropical island reptile populations, and it has been determined that one of the biggest threats they face are the introduced species. Geckos are reptiles that are commonly introduced into new habitats, and many of these species have an impressive capacity of colonization, causing the displacement of native species. The introduction of geckos in the Galapagos Islands has occurred on different occasions due to the human expansion towards the archipelago and the transport of products. In San Cristóbal there are four introduced gecko's species and two endemic species. The information that exists about the spatial distribution, use of microhabitats and preference of substrates of the gecko species of the island is scarce, for this reason in this study these three parameters have been investigated through visual censuses and experiments with substrates. We determinate the composition of the community of geckos in different ecosystems of the island: seasonal evergreen forests (84.8% *G. caudiscutatus*, 15.2% *P. leei*), deciduous forest (58.8% *G. caudiscutatus*, 9.3% *H. frenatus*, 7.7% *L. lugubris*, 2.2% *P. reissi*, 15.4% *P. leei*, 6.6% *P. darwini*), agricultural land (99.4% *G. caudiscutatus*, 0.6% *L. lugubris*) and urban areas (19.9% *G. caudiscutatus*, 48.7% *H. frenatus*, 20.3% *L. lugubris*, 3.5% *P. reissi*, 7.6% *P. darwini*). We found that the microhabitats used by geckos were mostly composed of lacquered wood, concrete bricks, interior filling, tree bark, Opuntia and volcanic rock. We fund that the introduced species have a lower speed on rough substrates than on smooth substrates, with some exceptions. The species most frequently found in all study sites was *G. caudiscutatus*, its expansion could be related to particular climatic conditions of the year of sampling (2017), but it is not a significant threat for endemic species due to its diurnal activity. *L. lugubris* and *P. reissi* are introduced species adapted to human constructions. The main threat identified for endemic geckos is *H. frenatus*; this species has aggressive behavior of competition and is colonizing natural microhabitats very quickly.

Keywords: geckos, microhabitat, Galapagos Islands, displacement, competition, preference of substrates, spatial distribution.

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Spatial distribution and microhabitat use of species of geckos in San Cristobal Island, Galapagos

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ABSTRACT

The growth and expansion of the human population, including the introduction of non-native species has a negative impact in ecosystems. The introduction of reptile species on islands is a serious problem, since the introduced species can quickly displace native or endemic species, also causing the extinction of endemic species. The introduction of geckos in the Galapagos Islands has happened accidentally on different occasions. On San Cristobal Island there are four introduced species of geckos and only two endemic species. We studied the spatial distribution, microhabitat use and substrate preference of the species of geckos on the island. We did visual surveys (spatial distribution and microhabitat use) and substrate experiments (substrate preference). We found the composition of the gecko community in evergreen seasonal forest and shrubland (84,8% *G. caudiscutatus,* 15,2% *P. leei*), deciduous forest (58.8% *G. caudiscutatus,* 9,3% *H. frenatus,* 7.7% *L. lugubris,* 2,2% *P. reissi*, 15,4% *P. leei*, 6,6% *P. darwini*), agricultural lands (99.4% *G. caudiscutatus,* 0,6% *L. lugubris*), and urban areas (19.9% *G. caudiscutatus,* 48,7% *H. frenatus,* 20,3% *L. lugubris*, 3,5% *P. reissi*, 7,6% *P. darwini*). We found that the most common microhabitats were composed of lacquered wood, concrete brick, interior filling, tree bark (*Hippomane mancinella*), opuntia cactus (*Opuntia megasperm*) and volcanic rock. We determined that introduced species walk slower on rugged substrates than on smooth substrates. Our study found that *G. caudiscutatus* is the most frequently found species on the island, its expansion might be related to temperature, humidity and precipitation, yet it is not a major threat due to its diurnal activity. *L. lugubris* is an introduced species mostly adapted to man-made structures. The major threat for endemic geckos is *H. frenatus,* this species displaces others using aggressive behavior and it is colonizing natural microhabitats.

Keywords: competition, displacement, Galapagos islands, geckos, microhabitat use, spatial distribution, substrate preference.

INTRODUCTION:

The diversity of herpetofauna in the Pacific oceanic islands has been studied since the 1970´s. Scientist first believed that Pacific islands shared most of their herpetofauna, yet, with many posterior studies scientists determined that the species where morphologically similar but genetically different (Zug, 2013; Zug and Moon. 1995). Like in the case of *Nactus pelagicus,* this specie was described as one and was found in different islands, yet an exhaustive investigation determined that actually there are two different species that were described as one (Zug and Moon. 1995). Studies in island herpetofauna are mostly carried on temperate weather islands, yet in tropical islands there is an enormous gap of information about this animal group (Fisher. 2011).

The growth and expansion of human population has had negative impacts on natural environments (Vitousek, 1988; Ehrlich and Holdren, 1971; Watson et al., 2010) The principal negative impacts related to the growth and expansion of human population are deforestation, habitat fragmentation, the extinction of species, higher competition between species, the lack of food, and the change in abiotic factors (ex. temperature, humidity) (Ehrlich and Holdren, 1971; Laurance *et al*. 2014; Damme and Banfield, 2011; Du *et al*., 2007). Island ecosystems are more vulnerable to the impacts due to their specific biological composition (Fritts and Rodda, 1998). The introduction of invasive species is a big issue due to their easy colonization of biological niches and the displacement they cause in competition with endemic and native species, also they can trigger changes in the structure and function of an entire ecosystem (Hanley et al., 1998.; Clavero and García-Berthou, 2005; Meyer and Turner, 1992; Usher, 1988; Cole et al., 2005; Dame and Petren, 2006).

Geckos are the most common introduced reptiles in the world, they are able to colonize areas and displace endemic species quickly, they are in new ecosystems (Dame and Petren, 2006; Bauer, 2013; Pearson, et al., 2007). The spatial distribution of the introduced species of geckos on the islands is mainly centered on urban areas and agricultural lands, since they have adapted to anthropogenic constructions, so they move along with human expansion (Case *et al.*, 1994). Additionally, it is well known that many introduced gecko species such as *Hemidactylus frenatus* and *Lepidodactylus lugubris* have an impressive adaptation and colonization facility (Cole *et al.*, 2005, Case and Bolger, 1991; Crombie and Pregill, 1999).

The Galapagos archipelago is not an exception, because the introduction of species is one of the most serious threats for endemic species (Causton et al. 2006). Several cases are known where introduced species have caused irreversible impacts within ecosystems, and the near extinction of many endemic species (Olmedo and Cayot, 1994, Torres-Carvajal and Tapia, 2016). The introduction of reptiles into the Galapagos islands first occurred accidentally via cargo ships from the mainland to the archipelago (Olmedo and Cayot, 1994, Hoogmoed, 1989). The four species of geckos that have been introduced into the archipelago are: *Lepidodactylus lugubris, Gonatodes caudiscutatus, Hemidactylus frenatus* and *Phyllodactylus reissi*. The records of the introduction of species date from different years, and both their spatial distribution and their adaptability to the natural environment, vary according to the species. The first record of *Gonatodes caudiscutatus* was in 1892 on San Cristobal Island, while *Phyllodactylus reissi* was first seen in the mid-1970s. Additionally, there are records of the endemic species of San Cristobal Island, *Phyllodactylus leei* introduced into Isabela island on 1939 (Olmedo and Cayot, 1994, Hoogmoed, 1989; Wood, 1939). As for *Hemidactylus frenatus,* there is a record of its introduction into the archipelago by Torres-Carvajal and Tapia in 2011, found on Santa Cruz island (Torres-Carvajal and Tapia, 2016).

On San Cristobal Island, there have been observations of the four introduced species mentioned, and in addition, this island has two endemic species (*Phyllodactylus leei* and *Phyllodactylus darwini*) (Carvajal-Campos, 2017 a, Carvajal-Campos, 2017 b). Many researchers have proposed that introduced species do not represent a major threat to endemic gecko species because of the different abiotic conditions that they prefer (Olmedo and Cayot, 1994, Hoogmoed, 1989); however, there are no updated data, so it is not known with certainty. There are no studies focused on the determination of threats in particular, nor studies on the population trend of these two species (Cisneros-Heredia and Márquez, 2016)

There are some interactions that have been observed all along the oceanic islands between native and introduced gecko species. The species tend to compete for food and refuge (they will compete for microhabitat) (Cruz et al., 2005; Lara-Resendiz et al., 2013) The anatomy of their feet together with the adherence that they have with each type of substrate will determine if the microhabitat is suitable for a specie (Cole *et al.*, 2005; Guo et al., 2012). Predation is another common interaction, sometimes the introduced species are better predators than native species, thus will cause a reduction in the abundance of the native species (Case and Bolger, 1991). It is also known that some geckos can carry parasites, such as mites, and diseases, such as Salmonella (Arredondo, 2016; Jiménez, 2014).

San Cristóbal is one of the islands of the Galapagos archipelago with the greatest particularity on its geckos community´s structure. This island has two endemic species (*Phyllodactylus leei* and *Phyllodactylus darwini*) and four introduced species (*Gonatodes caudiscutatus, Lepidodactylus lugubris, Hemidactylus frenatus, and Phyllodactylus reissii*). Studying the structure of the community of geckos, their spatial distribution, the use of microhabitats and the preference of substrates will allow us to determine if the introduced species are a threat for the conservation of endemic species. Since there is currently a lack of information, the results obtained in this study will help us to understand what is happening with this group of animals on the islands. Our study's aim is to learn the distribution and use of microhabitats of the community of geckos present in San Cristóbal Island, Galápagos. We want to determine the existence of displacement and competition between native and introduced species of geckos by verifying if the type of substrate of each microhabitat is a factor that limits the presence of species in natural ecosystems.

METHODS:

Study Site:

The study took place in San Cristóbal, Galapagos. This island is located in the eastern part of the archipelago. San Cristóbal has 7,475 inhabitants, who are distributed in the coastal zone (Puerto Baquerizo Moreno, province capital) and the highlands (El Progreso and surroundings) (Tarapues, 2015; Gobierno Autónomo Descentralizado Municipal del Cantón San Cristóbal, 2012). San Cristóbal Island, as well as the other islands of the archipelago, has climatic floors along the altitudinal gradient that gives the characteristic of altitudinal zoning. The altitudinal zoning found in the islands establishes the climatic and biological characteristics of each zone (coastal zone, arid zone, transition zone, Scalesia area, Miconia area and pampa zone) (Lanteri, 2001, Huttel, 1986, Quintanilla, 1983). It is also known that the level of highest endemism on the islands occurs in low elevation areas (Johnson and Raven, 1973). Rivas-Torres *et al.* (2018) mentions that San Cristobal is composed of: agricultural area (15%), urban area (0,3%), evergreen seasonal forest and shrubland (3,3%), evergreen forest and shrubland (0,2%), deciduous forest (67,9%), deciduous shrubland (16,4%), deciduous tallgrass (4,1%), mangrove (0,02%) and coastal humid forest and shrubland (0,002%).

Data Collection:

The study was carried out in different areas, which represent agricultural lands, urban areas, deciduous forest, and evergreen seasonal forest and shrubland (**Table 1 and Figure 1**); in order to have a complete study of the spatial distribution of gecko species on the island. These zones are distributed along the altitudinal gradient in order to obtain data of the entire altitudinal zone due to their different characteristics.

Data was collected during the months of July to December 2017. This period is characterized by mild weather, the presence of winds and light rain and a temperature range of

18 ° C to 25 ° C (Gobierno Autónomo Descentralizado Municipal del Cantón San Cristóbal, 2012). The data collection was divided into two periods, one during July-August 2017, and the second during September-December 2017.

Spatial distribution and use of microhabitat:

Visual surveys were carried out in the agricultural lands, deciduous forests and evergreen seasonal forests by belt transects of at least 500 meters long and 1 meter wide on each side. These were sampled in three different time periods (9h00, 15h00 and 19h00) during two consecutive hours, for three days. Two observers walked slowly through the transect looking for geckos; we looked for this animals on ground substrates (volcanic rock, leaf litter, plants, etc.), on trees (looking on branches, moss, bromeliads, tree bark, etc.) and on the human constructions (trails, handrails, walls, trail information signs, lights if available). We logged the microhabitat and substrate characteristics and structure where the individuals were found and its position (vertical, horizontal or tilted), their activity, their interactions with other species, the geographic reference point and environmental data (including climate and the type of vegetation present).

For the study of the spatial distribution of geckos in urban areas, we applied two methodologies used by Olmedo and Cayot (1994) and Hoogmoed (1989), which consisted of visual surveys throughout the populated area. By using ARCGIS, we divided the urban areas by sectors that covered approximately the same area. Subsequently, a visual survey was carried out in a specific sector of the urban area for two hours, in the same three schedules used in agricultural and natural areas´ survey (9h00, 15h00 and 19h00) until all sectors were covered. Two observers looked for geckos on the walls, streetlights, and every human construction we found. We also looked in ground microhabitats, like volcanic rock, rubble, and wastelands. We logged the same data as in the agricultural and natural areas´ survey.

The study of the microhabitat use was based on the data collection during the surveys of spatial distribution. We registered the microhabitat where each individual was found, and if other species were present as well. We named each microhabitat by its majoritarian substrate (the one that was covering at least the 75% where geckos were found). It is important to mention that a photographic record of the majority of the observed individuals was taken. When photographing the individuals was not possible, we registered morphological characteristics for their correct identification.

Use and preference of substrates:

The aim of this part of the study is to determine if the substrates of the most used microhabitats are limiting factors for the presence of gecko species. This idea is proposed because of the union at a molecular level (by the action of the Van der Waals forces) that geckos have with the substrates where they walk, thus they could have better adhesion on some substrates than others (Autumn et al., 2002; Sun et al., 2005). The successful mobilization of the animal in each substrate will depend on the size and shape of the feet and the size and distribution of some toe structures known as lamellae (Autumn et al., 2000; Sun et al., 2005).

Six different types of substrates were chosen, recognizing them from the study of the microhabitat use, we chose the most common substrates used and with more species present on them (**Table 2**). The three substrates most used by geckos in urban areas (lacquered wood, concrete brick and interior filling), and the three substrates most used by these animals in natural and agricultural areas (tree bark (*Hippomane mancinella*), opuntia cactus (*Opuntia megasperm*) and volcanic rock) (**Table 3**).

The experiment consisted of the design of a material where individuals can walk a meter in each type of substrate. For the construction of the material, we used a wood plank of 50cm x 1m, to which we added strips of 10 cm x 1m of each possible substrate (lacquered wood, interior filling, opuntia and tree bark). We used a piece of anti-mosquito aluminum mesh to prevent the geckos from walking out of the analyzed substrates and escaping. In the case of the volcanic rock and the concrete brick, we obtained a sample of the substrate of at least one meter long and we measured the time it took the animal to walk one meter. In these two cases, we also used the aluminum mesh to control the individuals.

Five adult individuals were collected from each of the introduced species and we made them walk through the different substrates. In order to calculate the velocity, we registered the time it took each animal to walk one meter on each substrate. Other interesting data and observations were also registered, such as the pauses that the animals made before reaching the end, if they walked through the mesh or if they wanted to return to the starting point.

Data analysis:

One-way analysis of variance (ANOVA) tests were performed (use and preference of substrates), and a Tukey test was carried out to find out if the means of the velocities of each substrate were significantly different between them. General linear models (GLM) were used to analyze the spatial distribution and microhabitat use. It is important to mention that the normality and homoscedasticity of the data was checked prior to the statistical analysis. Spatial distribution maps were made for both urban areas with ARCGIS, in order to present a visual result of the number and species of geckos present on each sector of the urban areas. Non-parametric analyses were carried out to know the use of microhabitats and the spatial distribution of the species. Abundance, frequency, and diversity were calculated to understand the differences in gecko communities in each environment (urban and suburban areas, deciduous forest, seasonal evergreen forest and agricultural lands). All the analyses were performed in Past 3.24, MiniTab 18 and ARCGIS 10.4.

RESULTS:

Space distribution:

The non-parametric analyses performed allowed us to observe the number of individuals per species found in each of the studied locations (**Figure 2 and Table 4**). The most common species in Carola was *G. caudiscutatus*, followed by *H. frenatus* , *L. lugubris* , *P. darwini* and finally *P. reissi* . In comparison, on Tijeretas only three species were observed, of which *H. frenatus* had the most reports, followed by the endemic species *P. darwini* and finally, *G. caudiscutatus*. In La Soledad only two species were found.he species that was most abundant was *G. caudiscutatus* with only one report of *L. lugubris.* In La Lobería the species that had the most observations was *L. lugubris*, followed by *G. caudiscutatus*, *H. frenatus*, *P. darwini* and finally *P. reissi*. In the last location sampled with transects, Jardin de las Opuntias, only 2 species, *G. caudiscutatus* and *P. leei* , were observed . The first transect of this locality was carried out in evergreen seasonal forest and shrubland, while the second transect was carried out in deciduous forest. The number of individuals of *G. caudiscutatus* was higher in both transects, however, more individuals of this species were found in the first transect. More individuals of *P. leei* were found in the second transect. It is important to mention that the endemic species, *P. leei*, was found only in this study site.

The Shannon index shows that non ecosystem has a high diversity (Evergreen seasonal forest and shrubland = 0.4262; deciduous forest = 1.282; agricultural areas = 0.05555; urban areas = 1.308), yet the most diverse ones are deciduous forest and urban areas. The Simpson index shows the same results (evergreen seasonal forest and shrubland = 0.25; Deciduous forest = 0.6008; Agricultural areas = 0.01961; Urban areas = 0.6748)

Regarding the results obtained in the urban areas of the island (Puerto Baquerizo Moreno and El Progreso) four species were found that inhabit the locality El Progreso (**figure 3**), *G. caudiscutatus* in a greater percentage, followed by *L. lugubris*, *H. frenatus* and finally *P. darwini*. In Puerto Baquerizo Moreno (**figure 4**) the species that had the most occurrences was *H. frenatus* , followed by *L. lugubris* , *G. caudiscutatus* , *P. darwini* and finally *P. reissi* .

The parametric analysis performed shows that there are significant differences between the species found depending on study site. Two MGLs were carried out, one for the localities of urban areas and the other for the natural ecosystems, due to the difference in the sampling methods of these localities. In the urban localities it was found that the number of individuals observed does not vary significantly according to the locality (El Progreso and Puerto Baquerizo Moreno) ($F = 3.89$; $p > 0.05$), however, there is a significant variance in the individuals of each species ($F = 19.06$, $p < 0.05$). This suggests that the spatial distribution of the species does vary according to the study site (**figure 5**). In the natural ecosystems (La Soledad, La Lobería, Jardin de las Opuntias, Carola and Tijeretas) it was found that the number of individuals observed does not vary significantly according to the species ($F = 2.10$, $p > 0.05$), however, there is a significant variance in the individuals found in each locality ($F = 4.17$, $p < 0.05$). A pairwise Fisher comparison was made with the species data, and the only ones that are significantly different are *P. leei* and *G. caudiscutatus*. This was an expected result, since *G. caudiscutatus* is the species that had the most occurrences in these localities, whereas *P. leei* was only found in one locality (Jardín de las Opuntias).

Use of Microhabitat:

There were 12 identified microhabitats used by geckos: plastic garbage, concrete brick, tree bark (*Hippomane mancinella*), interior filling, introduced plant species, leaf litter, lacquered wood, metal, opuntia (*Opuntia megasperma*), volcanic rock, roof tiles, and glass. It is important to mention that not all the species were found in all the microhabitats listed (**Table 2**). We found that two species had unique microhabitats, *L. lugubris* and *H. frenatus*, in introduced plant species (inside the flowers) and glass, respectively.

The parametric analysis shows there is a significant difference between the species and the microhabitat that they use ($F = 2.75$, $p < 0.05$). This is an expected result since the species were found using different microhabitats, although we found species coexisting on the same substrates, and therefore, they have interactions between each other (**table 5**). Having that, both endemic species share microhabitats with introduced species. In the case of *P. leei, it* was reported that it shares a microhabitat with *G. caudiscutatus*, they were found in volcanic rock, tree bark and plant litter. *P. darwini* was found in the same microhabitat with *H. frenatus*, *P. reissi* and *L. lugubris*. Negative interactions between the species were observed twice, in both, *H. frenatus* adopted an aggressive behavior with other individuals (*P. reissi* and *L. lugubris*).

Substrate preference:

We found that *G. caudiscutatus* had a higher velocity in rugged substrates (volcanic rock, concrete brick and tree bark) and a lower velocity in smooth substrates (lacquered wood, opuntia and interior filling). The highest speed reached by this species was in volcanic rock (0.023 m / s) and its lowest velocity was in interior filling (0.010 m / s). On the other hand, in *H. frenatus* high speeds were found in opuntia, interior filling and lacquered wood; and lower speeds in tree bark, volcanic rock and concrete brick. The highest speed reached by this species was in opuntia and lacquered wood (0.026 m/s and 0.025 m/s respectively), and the lowest speed reached was in volcanic rock and concrete brick (0.011 m / s). As for *L. lugubris*, higher speeds were found in lacquered wood, interior filling and opuntia (smooth substrates); and lower speeds in tree bark, concrete brick and volcanic rock (rugged substrates). The highest speed reached by this species was in lacquered wood (0.021 m / s) and its lowest velocity was in volcanic rock (0.009 m/s). Finally, in *P. reissi* high speeds were found in interior filling and lacquered wood; the speed decreased in tree bark, opuntia, concrete brick and volcanic rock. The highest speed achieved by this species was in lacquered wood and internal filling (0.025 m/s and 24 m/s, respectively) and its lowest velocity was in volcanic rock (0.011 m/s) (**Table 6**).

The parametric analysis carried out suggests that the speed reached by the species varies significantly depending on the substrate. In *G. caudiscutatus* (**figure 6**), we found that the variance in the speeds is statistically different ($F = 36.31$, $p < 0.05$), among the substrates. Those that were statistically similar to each other were: opuntia-wood, opuntia –interior filling, wood-interior filling, wood-concrete brick and tree bark-concrete brick. On the other hand, in *H. frenatus* (**Figure 7**) it was found that the variance in the velocities is statistically different ($F = 62.30$, $p < 0.05$). The substrates which were statistically similar: opuntia-tree bark, rock-tree bark, volcanic rock-concrete brick and Tree bark-concrete brick. As for *L. lugubris* (**Figure 8**) it was found that the variance in the velocities is statistically different ($F = 82.21$, $p < 0.05$), the substrates that were statistically similar were rock-opuntia, tree bark- interior filling, opuntia-tree bark, rock-concrete brick, opuntia-concrete brick, and tree bark-concrete brick. Finally, in *P. reissi* (**Figure 9**) it was found that the variance in the speeds is statistically different ($F = 48.39$, $p < 0.05$), the substrates which were statistically similar were tree bark-interior filling, rock-tree bark, and tree bark-concrete brick.

Additional data was collected during the experiments, where several individuals tried to return to the beginning or walk through the mesh. In *G. caudiscutatus,* three of the five individuals tried to return to the start in the smooth substrates. On the other hand, in *H. frenatus*, four individuals tried to walk through the mesh instead of the volcanic rock and the concrete brick. As for *L. lugubris*, an individual tried to return to the beginning on all substrates, except lacquered wood. Three individuals of *P. reissi* tried to return to the beginning in opuntia; and in volcanic rock and concrete brick two preferred to walk through the mesh.

DISCUSSION:

In San Cristobal island there is definitely a serious problem between endemic and introduced species like in the other Pacific islands. There is a clear expansion of the introduced species along the island, and therefore, a competition between the species. The results obtained regarding the spatial distribution of the species suggest that introduced species are increasingly reaching natural and agricultural areas. *P. reissi* and *P. leei* have remained in deciduous forest, the endemic species was found only in the Jardin de las Opuntias, while *P. reissi* was found in Puerto Baquerizo Moreno and surrounding areas. The other endemic species was found in

Puerto Baquerizo Moreno and surrounding areas, such as El Progreso. Olmedo and Cayot (1994) mention that endemic species prefer arid zones, however, it was observed that they are also present in humid environments such as evergreen seasonal forest,shrublands, and agricultural lands. In studies of the ecology of species of the genus *Phyllodactylus*, which include *P. reissi,* it is found that these animals also inhabit humid areas (de Espinoza *et al.*, 1990; Arizmendi , 2011), so this altitudinal movement of the species is justified. It was observed that *L. lugubris* was found in all the studied sites except Tijeretas and Jardín de las Opuntias. This is a parthenogenic species, and they are successful colonizers in human settlement areas (Short and Petren, 2008), however, it has been seen that the number of individuals of this species declines when there is the presence of *H. frenatus*, as they compete for food and microhabitat (Petren and Case, 1996). The individuals of *L. lugubris* were found in microhabitats related to human constructions (walls, streetlights, lacquered wood, among others), however, it was also found on few occasions in opuntia.

The expansion and colonization of *H. frenatus* is impressive, since it has managed to be distributed in many localities in a very short period of time. The number of individuals of this species found in urban areas is very large. There was a record of up to seven individuals of this species in a wall of a house, around an artificial light. It is known that *H. frenatus* is a successful colonizing species given that it does so through competitive interference "which represents a direct competition of a foreign species with a native one due to aggression or reproductive interference " (Abarca, 2006. Pag. 1). We observed cases in which this species was aggressive with other species causing their displacement (Vences *et al.*, 2004, Abarca 2006). During the study, two aggressive interactions of this species with *P. reissi* and *L. lugubris* were observed, so it is suggested that this species has aggressive interactions with the other species on the island.

A study by Petren and Case (1996) suggests that habitat structure determines the intensity of competition between species. For this reason, it is justified that the geckos of San Cristóbal have managed to use several different microhabitats in order to avoid interspecific competition. The results obtained suggest that each species tends to occupy a different microhabitat at the same time, although in some cases we found that some species coexisted together. There was a unique case in which we saw three species coexist, *H. frenatus*, *L. lugubris* and *P. reissi* on a wall of a house around a light source, this is a common pattern of introduced species since they are adapted to human settlements and the light sources will attract invertebrates that geckos can eat (Pérez and Ochotorena, 2016; Chou *et al.*, 1988; Ceríaco *et al.*, 2011). The complexity of the habitat can affect the species in three different ways, according to the study by Petren and Case (1996), the first is predation, related to the ability to get food; the second is the efficiency of foraging and response to threats; finally, the last one is social interaction. When the habitat is not physically complex the animals tend to compete, we were able to observe this in the two cases of aggressive interaction of the species, since they were in a non-complex microhabitat, the wall of a house.

Substrate experiments show that not all introduced species are able to move through natural substrates, such as volcanic rock and tree bark, as was the case of *L. lugubris*, *H. frenatus* and *P. reissi*. However, *H. frenatus* was found on these two substrates during the surveys. In the study by Cole *et al.* (2005) similar experiments were carried out. The authors investigated not only the speed, but the relationship of the foot morphology, demonstrating that natural substrates are a natural barrier for introduced species, which included *H. frenatus*. This could also be applied in this case with *H. frenatus, P. reissi* and *L. lugubris*, since their speed in rough and loose substrates was lower than the speed in smooth substrates. However, *H.*

frenatus and *G. caudiscutatus* having non-retractable claws are likely to have facilitated mobility on these types of substrates (Russell *et al.*, 2015).

The main threats to the endemic species that have been found is the displacement cause by the colonization and successful expansion of *H. frenatus*. *L. lugubris* and *P. reissi* have not been identified as a great threat to endemic species since they do not show aggressive behavior and are mostly concentrated in urban areas with only a few reports in natural areas. Finally, *G. caudiscutatus* is a species that has colonized the upper part of the island, where the environment is humid. This species does not represent a threat to endemic species because it occupies other microhabitats, and also its activity is diurnal, so there is no competition for food with both endemic species (which are nocturnal) (Olmedo and Cayot, 1994; Torres-Carvajal and Tapia, 2016).

The introduction of gecko species could be a serious problem because they can transmit diseases to other animals and humans. Some of the additional observations that were taken during the sampling period are related to the existence of mites in some gecko species. The highest number of mites was found in *H. frenatus;* there were some individuals of *P. darwini* and *P. reissi* that had mites too. The red mites of gecko skin have been studied in Mexico and Costa Rica, identifying these parasites as organisms belonging to the genus *Geckobia* (Arredondo, 2016; Abarca, 2006). Some species of geckos can become harmful to human health. In Costa Rica, a study has been carried out where individuals of the species *H. frenatus* were defined as Salmonella vectors (Jiménez, 2014).

Not all introduced gecko species are a major threat to the endemic species of San Cristóbal. The introduced species that directly threatens the endemic species is *H. frenatus*. The introduced species of geckos are dispersing in more localities, however, most individuals of *L. lugubris* and *P. reissi* found in natural areas occupied microhabitats of human constructions, such as light poles and wooden rails, amongst others. *G. caudiscutatus* is the only introduced species that was found to coexist with *P. leei*, however this does not represent a threat since it has diurnal activity and is concentrated more in humid areas and with light rain, unlike *P. leei*.

Natural substrates could act as natural barriers for the dispersal of *L. lugubris*, *P. reissi* and *H. frenatus*. This last species was found in natural microhabitats, but according to the study of Cole *et al.*, (2005), it does not tend to colonize natural microhabitats due to the type of rough and loose substrates of which they are composed. *G. caudiscutatus* is a species that has colonized the natural area of the highlands, due to its abiotic characteristics that favors its existence (humidity, and drizzle). The expansion of this species can be related to the temperature, high precipitation and humidity in 2017 caused by a low temperature of the ocean surface on that year (Instituto Oceanográfico de la Armada, n/d).

It is important to mention the limitations of this study to take into consideration for future research. The identification of the individuals in some cases could become difficult due to the height in which the animals were observed, and the poor visibility of key morphological characteristics for their identification, such as the shape of the tubers in the *Phyllodactylus* species. It is important to mention that some observed individuals could not be identified, due to their speed of escape or the low visibility of key characteristics for their identification. Access to areas of the National Park Galápagos at night was restricted, which made night sampling of places like El Tongo difficult. This represents a great limitation because five of the six species of geckos found in San Cristóbal have nocturnal activity. The third limitation of this study was the impediment to carrying out the substrate preference experiments with individuals of the two endemic species, therefore; only comparisons could be made between the introduced species.

G. caudiscutatus expansion is totally related with the weather anomalies of the second semester of 2017. The humidity and constant drizzle allowed this specie to expand into the lowlands, since the specie is commonly found in the highlands. *P. reissi* and *L. lugubris* are introduced species that are completely adapted to human settlements, thus their expansion along the island will depend on human movement. *H. frenatus* is a major threat for endemic species, it is an aggressive specie and a successful colonizer, we determined that the natural substrates tested will not represent a barrier for the expansion of this specie. We only found *P. leei* far away from urban areas, while *P. darwini* was in urban areas but we reported not as many individuals as the ones of the introduced species,

The results obtained in this study will help to plan conservation activities for the endemic species, since a certain level of displacement and competition between the species can be observed. We suggest carrying out similar studies in the future in order to understand and learn how the population of geckos in San Cristóbal behaves, and if there is a change in years, where there are not anomalies in the weather conditions. It is important to keep monitoring the introduced species behavior, especially *G. caudiscutatus and H. frenatus*, which can represent a major threat for endemic species.

AKNOWLEDGEMENT

This project was done under the permission of scientific investigation N° PC-62-17 of the Galapagos National Park (GNP). This study would not have been possible without the collaboration of the Galapagos Science Center (GSC), Universidad San Francisco de Quito (USFQ), INIAP, and Hacienda Tranquila S.A. We thank the members of the Laboratory of Terrestrial Zoology and all our field assistants for the given support.

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Appendix A: Tables

Table 1. Coordinates (DMS), survey dates and ecosystem definition of the sampled sites

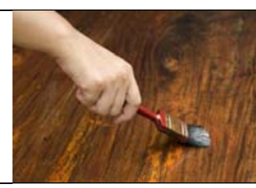
Site		Coordinates (beginning of transect or middle point of urban area)	Coordinates end of transect	Survey dates	Ecosystem type
Jardín de las Opuntias	Highland	0° 55.244'S 89°33.121'O	0°56.095'S 89°32.924'O	August 2017	Evergreen seasonal forest and shrubland
	Lowland	0°55.798'S 89°32.757'O	0° 56.548'S 89°32.990'O	August 2017	Deciduous forest
La Soledad (6 transects)		0°53.149'S 89°32.407'O	0°53.236'S 89°32.385'O	July 2017	Agricultural lands
		0°53.020'S 89°32.348'O	0°52.950'S 89°32.122'O	August 2017	Evergreen seasonal forest and shrubland
		0°53.263'S 89°32.376'O	0°53.288'S 89°33.371'O	July 2017	Agricultural lands
		0°53.217'S 89°32.206'O	0°53.179'S 89°32.113'O	July 2017	Agricultural lands
		0°53.011'S 89°32.391'O	0°52.881'S 89°32.531'O	August 2017	Evergreen seasonal forest and shrubland
		0°53.369'S 89°32.927'O	0°53.376'S 89°33.004'O	July 2017	Agricultural lands
Cerro Tijeretas		0°53.623'S 89°36.569'O	0° 53.291'S 89°36.505'O	September 2017	Deciduous forest
La Lobería		0°55.292'S 89°36.919'O	0°55.623'S 89°36.675'O	September 2017	Deciduous forest
Punta Carola		0°53.703'S 89°36.558'O	0° 53.440'S 89°36.612'O	September 2017	Deciduous forest
El Progreso (3 zones)		0° 54.454'S 89° 33.430'O	-	November - December 2017	Urban and suburban areas
Puerto Barquerizo Moreno (15 zones)		0° 54.162'S 89° 36.515'O	-	November -	Urban and suburban areas

			December 2017	

Table 2. percentage of individuals of the gecko species on each microhabitat that they were found

Microhabitat	***G. caudiscutatus***	***H. frenatus***	***L. lugubris***	***P. reissi***	***P. leei***	***P. darwini***
Plastic garbage	0.22%	2.20%	-	-	-	-
Concrete brick	14.35%	12.28%	-	5.27%	-	4.44%
Tree bark	25.27%	2.64%	2.92%	-	-	8.89%
Interior filling	-	41.67%	39.81%	73.68%	-	33.33%
Flowers	-	-	0.98%	-	-	-
Leaf litter	3.65%	0.88%	-	-	-	-
Lacquered wood	3.43%	13.15%	41.75%	10.52%	-	2.22%
Metal	0.22%	0.44%	-	-	-	-
Opuntia	-	-	11.65%	10.52%	8.51%	11.12%
Volcanic rock	49.47%	25.88%	2.92%	-	91.49%	40%
Roof tile	3.43%	-	-	-	-	-
Glass	-	0.88%	-	-	-	-

Table 3. Detailed description and photos of the substrates used in the experiment

Substrate	**Description**	**Photo / image**
Lacquered wood	Wood with a layer of lacquer, that makes it shiny and hard. Mostly found in streetlights and railings	
Concrete brick	Brick of concrete used to build houses.	
Interior filling	White material used as a base for paint in walls. It is made out of acrylic material	

Tree bark (Hippomane mancinella)	The outer layer of the log of Hippomane mancinella	
Opuntia cactus (Opuntia megasperma)	Opuntia is an endemic cactus specie of the Galapagos Islands	
Volcanic rock	A kind of rock composed of solidified magma.	

Table 4. Abundance and frequency of the species in each studied habitat

Species	Seasonal evergreen forest		Agricultural area		Deciduous Forest		Urban and suburban areas	
	N	%	N	%	N	%	N	%
Gonatodes caudiscutatus	106	84,8%	100	99.4%	107	58,8%	86	19,9%
Hemidactylus frenatus	-	-	-	-	17	9,3%	211	48,7%
Lepidodactylus lugubris	-	-	1	0,6%	14	7,7%	88	20,3%
Phyllodactylus reissi	-	-	-	-	4	2,2%	15	3,5%
Phyllodactylus leei	19	15,2%	-	-	28	15,4%	-	-
Phyllodactylus darwini	-	-	-	-	12	6,6%	33	7,6%
Total	125	100%	169	100%	182	100%	433	100%

Table 5. Interactions between gecko species on San Cristóbal.

Species	**Observed interaction**
G. caudiscutatus	It was observed that this species shared microhabitat with *P. leei* , and *H. frenatus* . No interactions were observed between them
H. frenatus	This species was found in the same microhabitat with all other species except *P. leei* , especially in interior filling, lacquered wood, and litter. On two occasions an aggressive interaction was observed by this species towards *P. reissi* and *L. lugubris* .
L. lugubris	*L. lugubris* was found in the same microhabitat as *H. frenatus* , *P. reissi* and *P. darwini* . No interactions were observed between them.
P. reissi	It was found in the same microhabitat as *H. frenatus* , *L. lugubris* and *P. darwini* . An occasion of an aggressive interaction between this species and *H. frenatus* was reported. Coexistence of *L. lugubris* , *H frenatus* and this species was observed on one occasion.
P. darwini	It was found in the same microhabitat as all other species except *P. leei*. No interactions were reported.
P. leei	It was found in the same microhabitat together with *G. caudiscutatus* , no interactions were reported.

Table 6. Average speeds of each species in each substrate (experiment use of substrates).

		Species			
		G. caudiscutatus	***H. frenatus***	***L. lugubris***	***P. reissi***
Average speed per substrate (m / s)	**Interior paste**	0.010	0.019	0.019	0.024
	Lacquered wood	0.014	0.025	0.021	0.025
	Concrete brick	0.018	0.011	0.013	0.014
	Volcanic rock	0.023	0.011	0.009	0.011
	Tree bark	0.021	0.014	0.011	0.015
	Opuntia	0.011	0.026	0.015	0.013

Appendix B: Figures

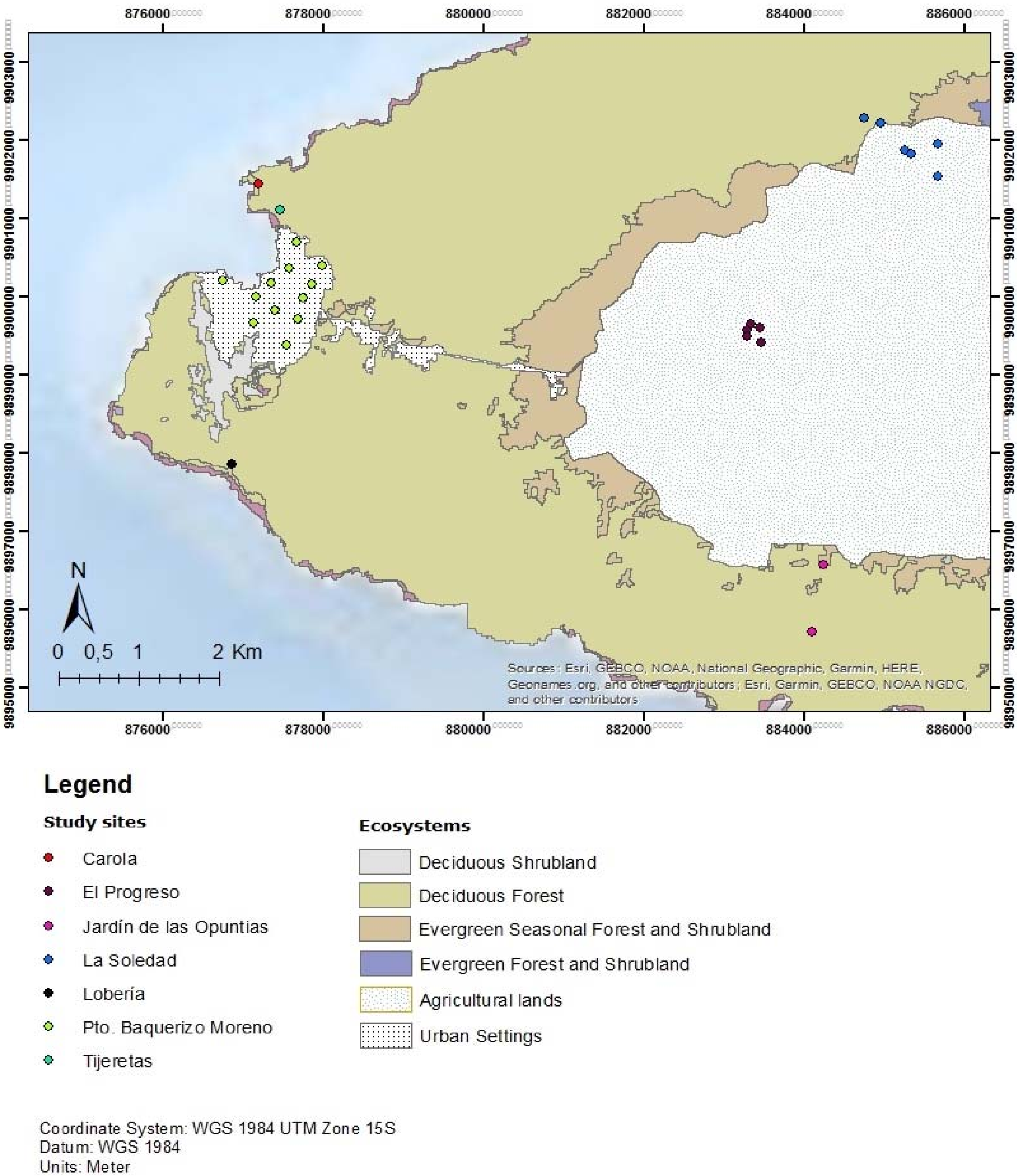


Figure 1. Location of the study sites in San Cristobal.- Map obtained from Rivas-Torres et al. 2018 with the location of the study sites

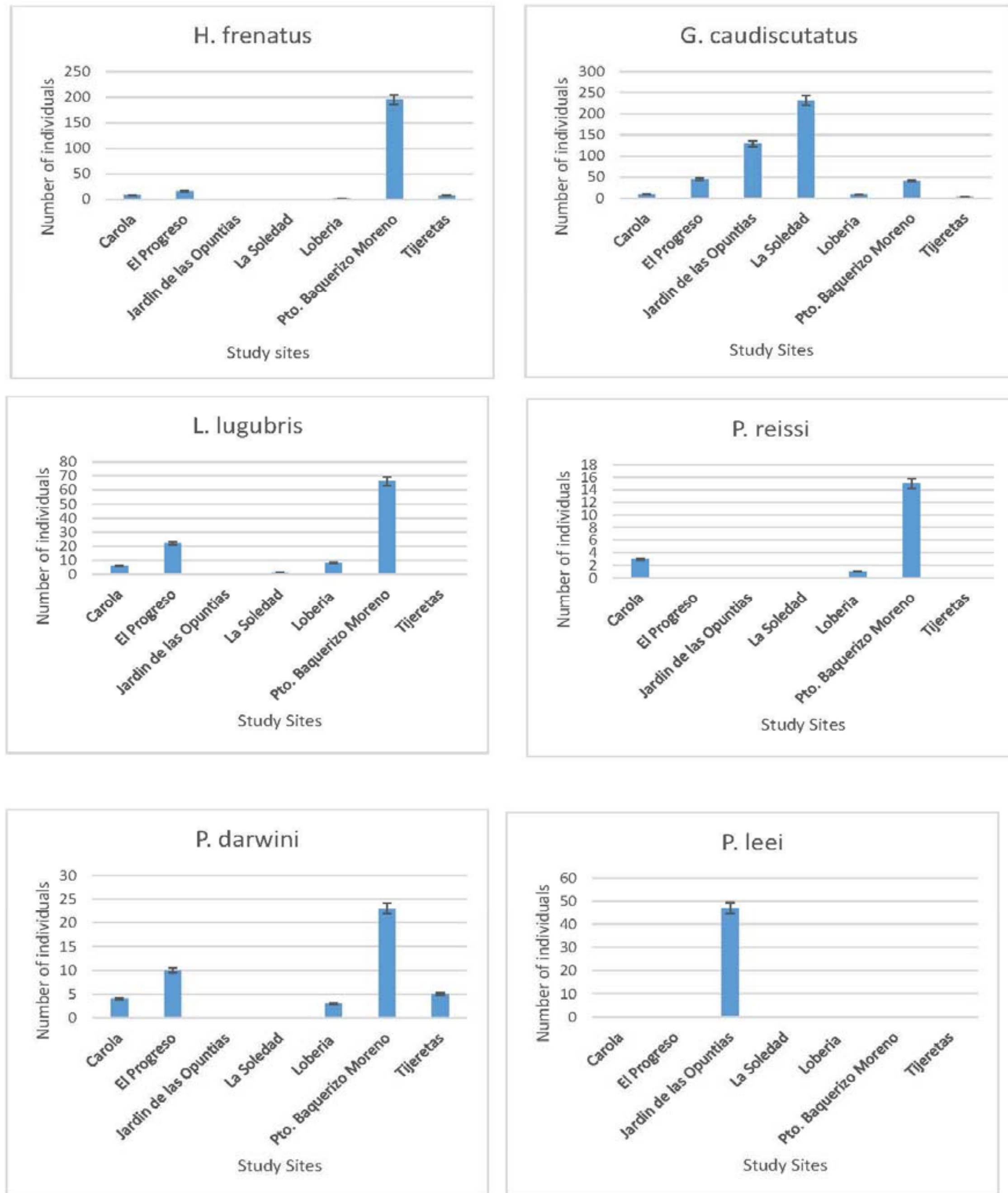
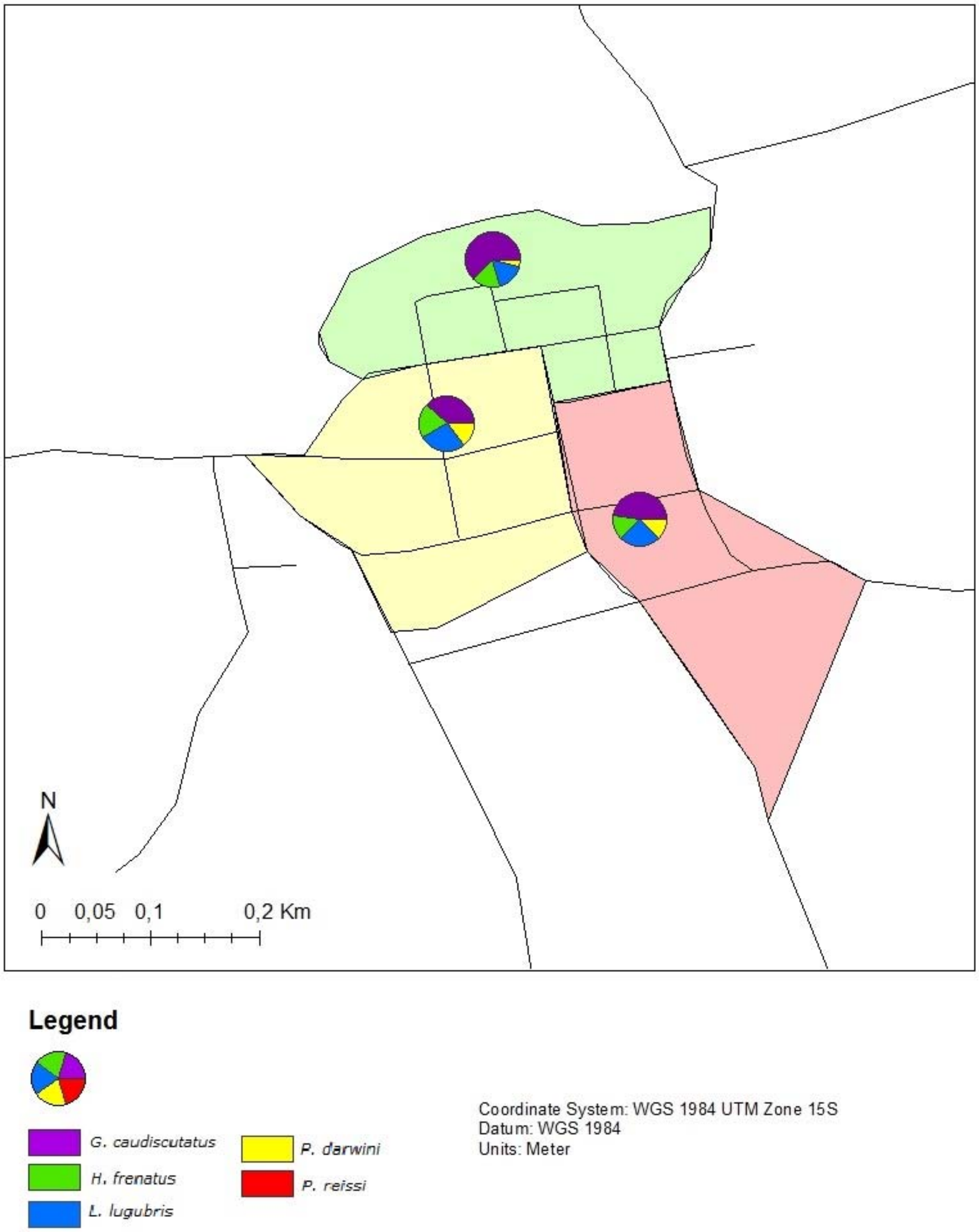
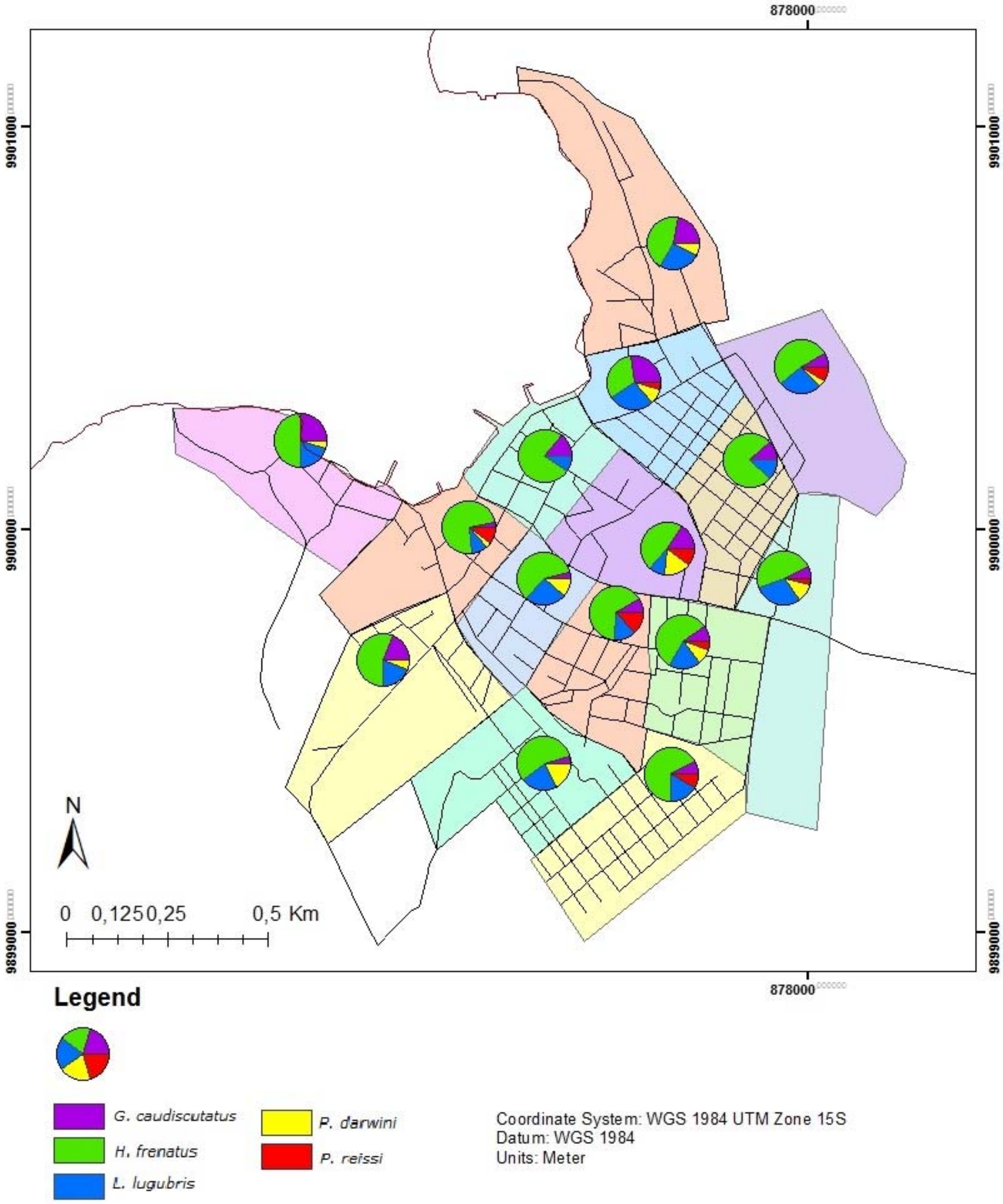


Figure 2. Distribution figures of each specie on all the study sites.- We can observe that *G. caudiscutatus* is the most common specie in all microhabitats. The other 3 introduced species are mostly found in urban areas and surroundings. *P. darwini* is found in study sites located in the lowlands, and *P. leei* is only found in Jardin de las Opuntias



Roads shapefile obtained from INEC and SNI

Figure 3. Spatial distribution map in El Progreso.- We can observe that the most common and abundant specie is *G. caudiscutatus*, followed by *L. lugubris, H. frenatus* and last *P. darwini*



Roads shapefile obtained from INEC and SNI

Figure 4. Spatial distribution map in Puerto Baquerizo Moreno.- We can observe that the most common species found in the urban area of the lowlands are *H. frenatus, L. lugubris* and *G. caudiscutatus*. In some zones, we observed some individuals of the endemic specie *P. darwini*, while *P. reissi* is only found in 7 zones.

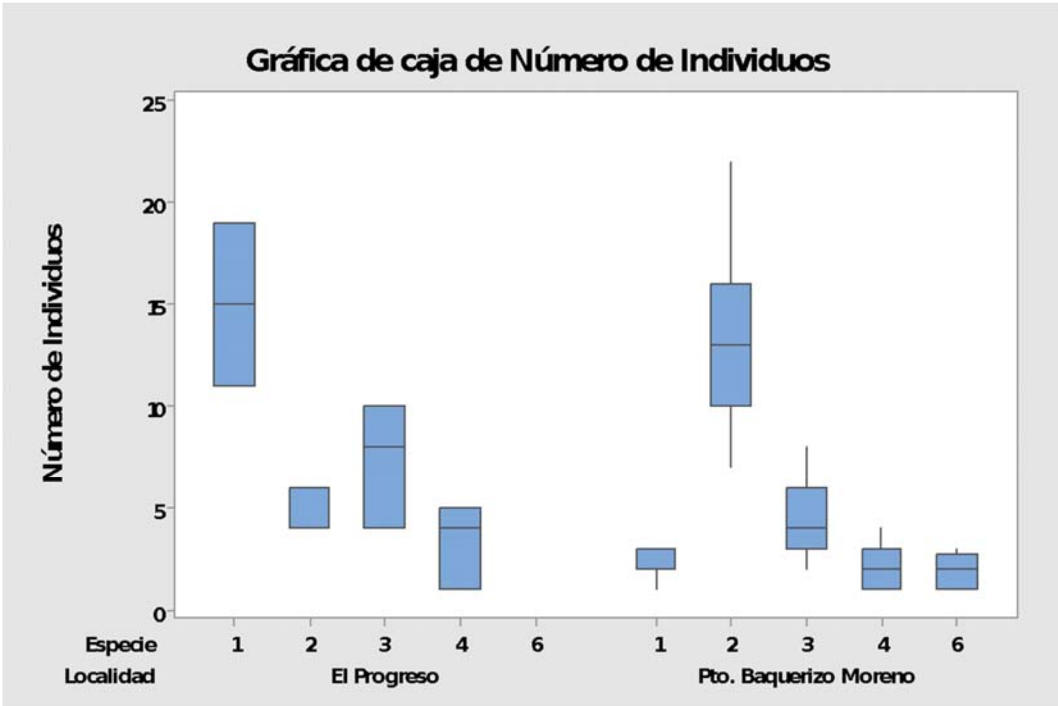


Figure 5.- Number of individuals on urban areas.- Species: 1 = *G. caudiscutatus*; 2 = *H. frenatus*; 3 = *L. lugubris*; 4 = *P. darwini*; 6 = *P. reissi.* We can observe that the most abundant specie in El Progreso is *G. caudiscutatus*, while in Pto. Baquerizo Moreno is *H. frenatus*.

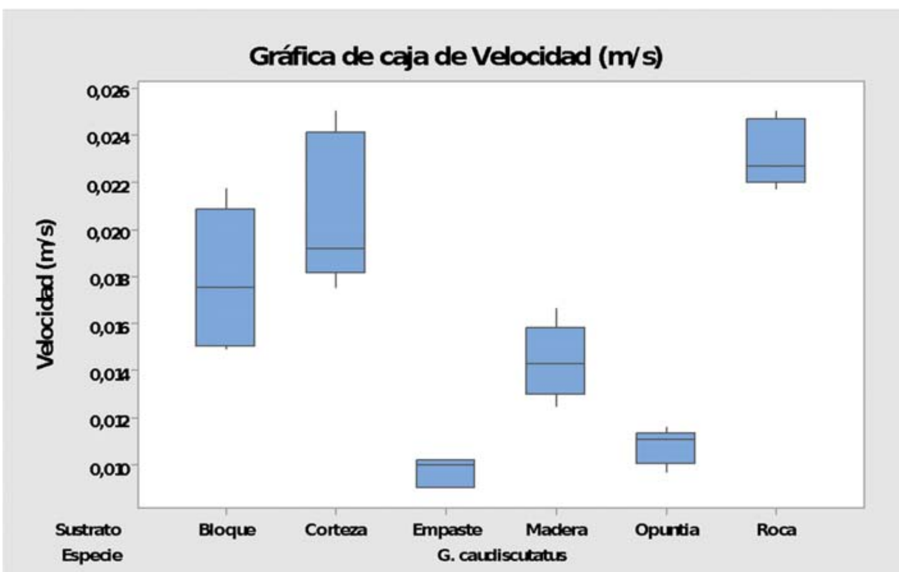


Figure 6. Speed of *G. caudiscutatus*.- We can observe that this specie has a higher velocity in rough substrates (volcanic rock, tree bark and concrete brick)

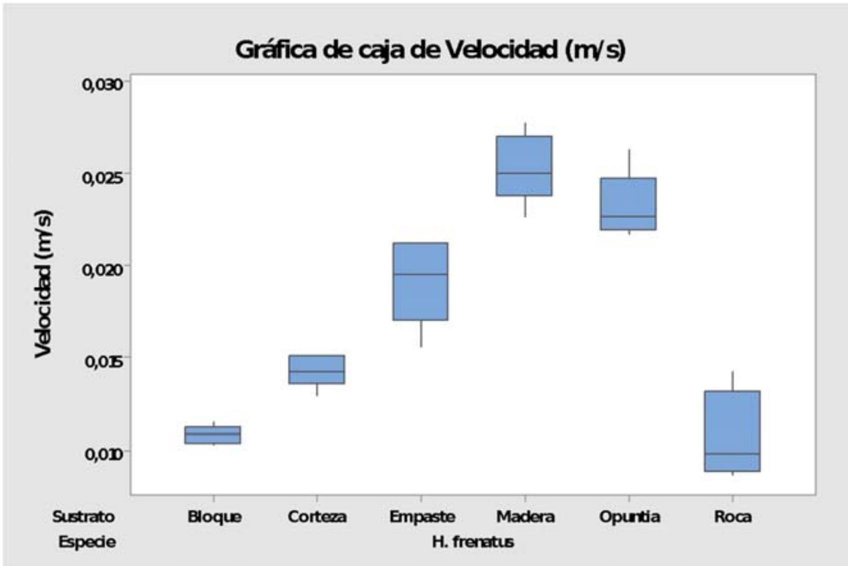


Figure 7. Speed of *H. frenatus*.- We can observe that this specie had a higher velocity in soft substrates (Interior filling, lacquered wood and opuntia cactus), yet we found this species on several occasions on substrates such as volcanic rock and concrete brick.

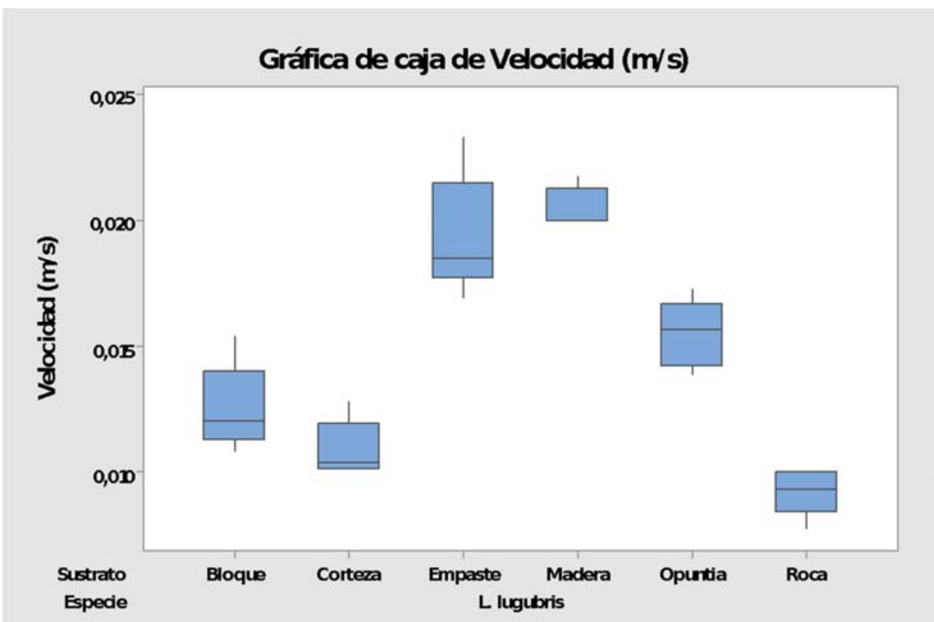


Figure 8. Speed of *L. lugubris*. We can observe that this specie had a higher velocity in soft substrates (Interior filling, lacquered wood and opuntia cactus)

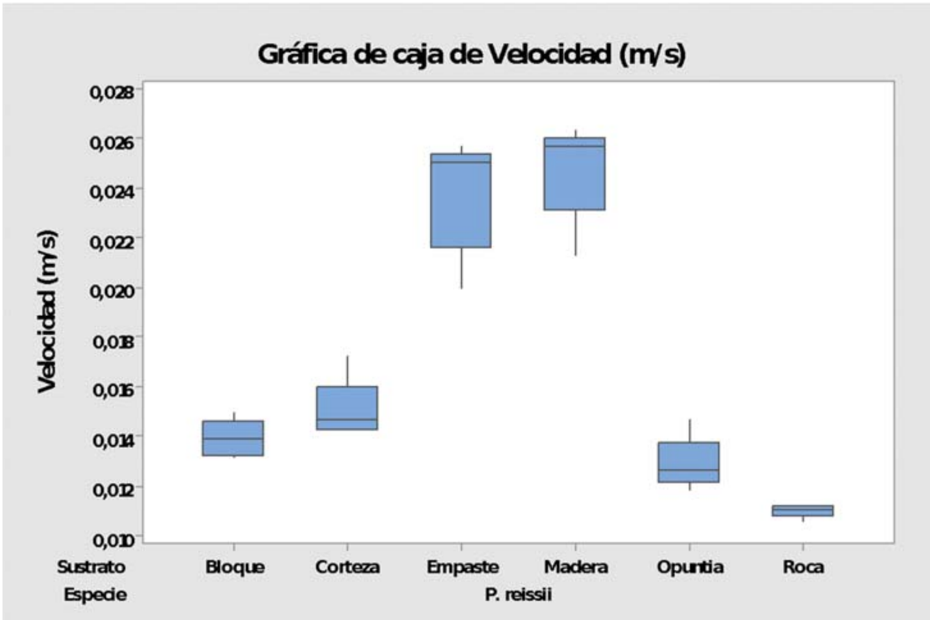
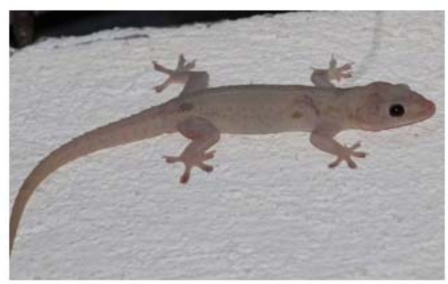


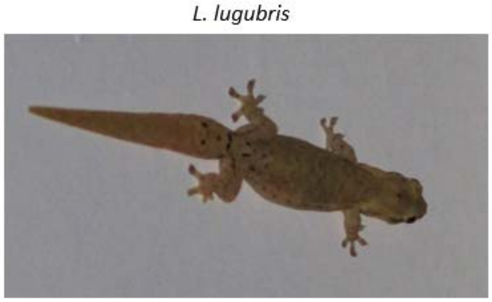
Figure 9.- Speed of *P. reissi*.- We can observe that this specie had a higher velocity in soft substrates (Interior filling and lacquered wood)

G. caudiscutatus

H. frenatus



L. lugubris



P. reissi

a. P. leei

b. P. darwini

Figure 10. Geckos of San Cristobal