Measuring tropical insect diversity: A comparative study of Chrysomelidae richness in *Luehea seemannii*

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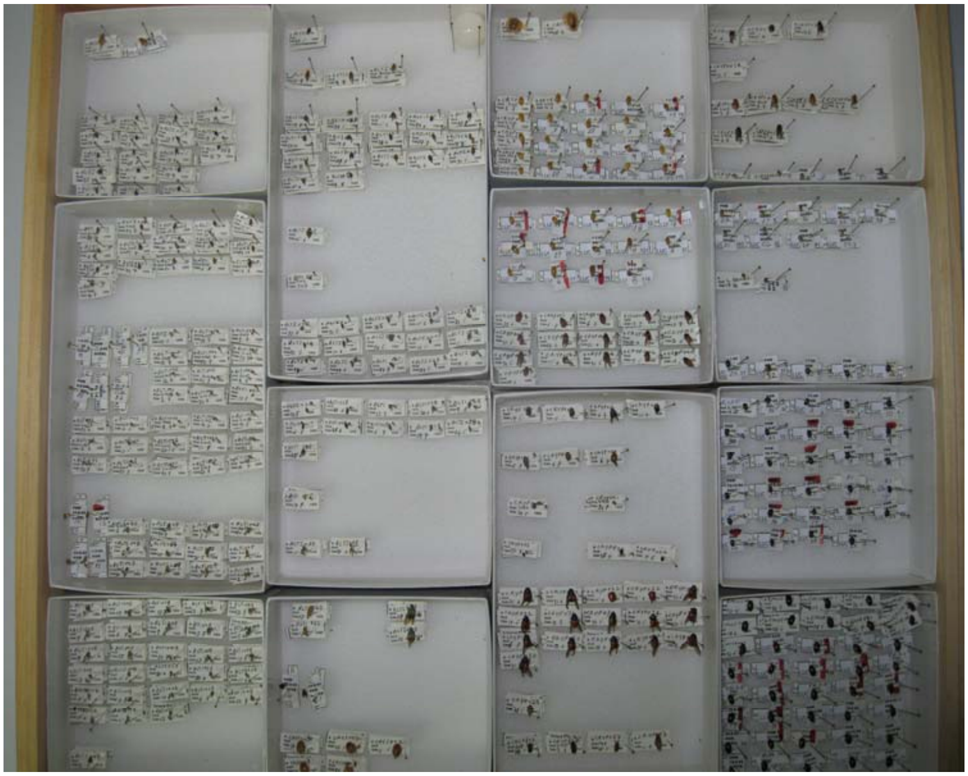

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Internship Report Presented to Dr. Roberto Ibáñez

McGill UNIVERSITY

Smithsonian Tropical Research Institute

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1. Project Summary

i. Executive Summary

Measuring tropical insect diversity: A comparative study of Chrysomelidae richness in *Luehea seemannii*

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Hosts: Dr. Héctor Barrios (Smithsonian Tropical Research Institute Unit 0948 APO, AA 34002-0948) and Dr. Yves Basset (Smithsonian Tropical Research Institute, Apartado 0843-03092, Ancon, Panama)

Estimating tropical insect diversity is an incredibly difficult task, with global estimates over the past 30 years ranging between 2 and 30 million species. Beetles have historically been used as a model system, with measures of beetle diversity in a small portion of a forest extrapolated to estimate the total number of insect species in all tropical forests. Variation in estimates stems from uncertainties with respect to the number of species that remain undescribed, the proportion of species that live in each strata of the forest, variation over temporal and spatial scales, and the applicability of extrapolating information from one study area to the diverse array of tropical forests.

Our objective was to integrate Chrysomelid beetles collected on Luehea seemannii from the collections of three different entomologists in order to create one master collection. A master database was also created, systematically organizing each beetle's information in one easy to access document. This master collection is larger (encompassing a wider time and spatial scale) and more accurate (eliminated redundancies between collections) allowing us to statistically test the assumptions on which diversity estimates are based and investigate variations in the leaf beetle diversity on a tropical tree species.

We worked with the Chang, Charles and Barrios beetle collections in the Entomology Department of the University of Panama, which were collected between 1994-1999. The Chrysomelids from each source were organized into morphospecies, and then compared to the other collections to eliminate redundancies in naming. Each morphospecies was then compared to Henry Stockwell's extensive reference collection, housed at the Smithsonian Tropical Research Institute, in order to find the names of any that have been previously described. The specimens could then be organized in a master database under a new master code. This excel file identifies the morphospecies, name of species, genus, and subfamily, date of collection, source strata, collector, and redundant species.

This information was then used to carry out statistical comparisons and analysis of the Chang, Charles, Barrios and Master collections.

We found much greater specimen redundancy (60.9%) than species redundancy (13.6%) and many singletons in our master collection, indicating that most beetles collected belong to the few most common species. The remaining beetles belong to a wide range of rare species, a common phenomenon in entomology where the collection of new rare species remains high despite huge amounts of sampling effort and large sample sizes (Novotny and Basset 2000). The high evenness and low dominance of the master beetle community are also the result of large numbers of rare species present – as more rare species were collected, the effect of the few common species decreased and drove the evenness value up and the dominance value down. All measures of alpha and beta diversity changed between collections according to sample size, suggesting that the larger samples gave a more accurate reading of true community diversity and composition. Characterizing the species richness using rarefaction curves revealed that large numbers of new species were being found even at 555 samples. Thus, even the master collection was not a comprehensive representation of total Chrysomelid diversity on *Luehea seemannii*. Estimates of spatial and temporal change in diversity and community composition continue to be complicated by this 'mystery of singletons' (Novotny and Basset 2000).

The ratio of canopy to understorey species was determined to be 3.2:1, a result very close to values found in other studies, confirming the validity of its use to estimate diversity (Erwin 1982). However, we found great variation between the numbers of beetles in the canopy over the course of the year, with the least beetles present in this upper stratum from March-May (dry season). This could be because the hotter, dryer climate requires a greater number of beetles to seek a lower and therefore cooler and more humid habitat. Our results that 32.4% of beetles in our sample did not feed on Luehea, and were thus not host-specific to the plant on which they were captured, indicates that samples may contain large numbers of non-specific specimens. These, if included in species counts to estimate diversity by host-specificity, would greatly inflate estimates, indicating that more conservative calculations may be needed.

Overall we found that integrating the collections of Barrios, Charles and Chang increased the accuracy of diversity measures and community composition on spatial and temporal scales. Seeing the effect of increased sample size, and knowing that the master collection is still not large enough has given us a new appreciation for the immense diversity of tropical arthropods and the obstacles that must be overcome when studying them. Exercises like the one we have undertaken would be useful in the future to fill information gaps and to increase the descriptive power of entomological knowledge as a whole.

ii. Resumen Ejecutivo

Estimación de la diversidad de insectos tropicales es una tarea increíblemente difícil, con estimaciones a nivel mundial en los últimos 30 años con una gama entre 2 y 30 millones de especies. Los escarabajos se han utilizado históricamente como un sistema modelo - las medidas de la diversidad de escarabajos en una pequeña parte de un bosque estaban extrapolado para estimar el número total de especies de insectos en todos los bosques tropicales. Variación en los cálculos se debe a las incertidumbres con respecto al número de especies que permanecen sin describir, la proporción de especies que viven en cada estrato de la selva, la variación en escalas temporales y espaciales, y la aplicabilidad de la extrapolación de información de un área de estudio a otros bosque tropicales.

Nuestro objetivo era integrar los escarabajos Crisomélidos recogidos en *Luehea seemannii* de las colecciones de tres entomólogos diferentes con el fin de crear una colección principal. Una base de datos maester también se creó, que organizó sistemáticamente la información de cada escarabajo en un documento. Esta colección principal es más grande (abarca escalas temporales y espaciales más amplios) y más preciso (con las superfluidades entre las colecciones eliminado) lo que nos permite probar estadísticamente los supuestos en que se basan las estimaciones de diversidad e investigar las variaciones en la diversidad de escarabajos de la hoja en una especie de árbol tropical.

Trabajabamos con las colecciones de escarabajos de Chang, Carlos Barrios en el Departamento de Entomología de la Universidad de Panamá, los cuales eran recogidos entre 1994-1999. Los Crisomélidos de cada origen se organizaron en morfoespecies, y luego se compararon con las otras colecciones para eliminar despidos en el nombramiento. Cada morfoespecies fue comparado a la colección de referencia extensa Henry Stockwell, que se encuentra en el Smithsonian Tropical Research Institute, con el fin de encontrar los nombres de los que han sido descritas previamente. Las muestras luego podrían ser organizados en una base de datos maestra en bajo de un nuevo código maestro. Este archivo de Excel identifica el morfoespecies, los nombres del especie, género, y subfamilia, la fecha de recogida, el estrato de origen, el autor, y las especies redundantes. Esta información fue utilizada para realizar comparaciones y análisis estadísticos de los Chang, Charles, Barrios y colecciones Maestro.

Hemos encontrado quel despido entre especímenes es mucho mayor (60,9%) quel despido entre especies (13,6%), y que hay muchos únicos en nuestra colección principal, lo que indica que la mayoría de los escarabajos recolectados pertenecen a las pocas especies más comunes. Los escarabajos restantes pertenecen a una amplia gama de especies raras, un fenómeno común en entomología cuando la colección de muchos nuevas especies raras sigue a pesar de enormes cantidades de esfuerzo y de muestras de tamaño grande (Novotny y Basset, 2000). La alta uniformidad y baja dominancia de la comunidad de escarabajos maestro son también el resultado del

gran número de especies rares - el efecto de los pocos especies comunes disminuyó cuando mas especies raras eran colectados y esto empujó el valor de la uniformidad de arriba y el valor de dominancia de abajo. Todos las medidas de diversidad alfa y beta cambiaron entre los colecciones con el cambio del tamaño de muestreo, sugiriendo que las muestras más grandes dan valores más precisos de la diversidad y composición comunitaria verdadera. Caracterización de la riqueza de especies con curvas de rarefacción reveló que el descubrimiento de nuevas especies continuó después de 555 muestras. Por lo tanto, dejamos ver que la colección principal no era una representación exacta de la diversidad Crisomélido total de *Luehea seemannii*. Las estimaciones de los cambios espaciales y temporales en la composición comunitaria y de la diversidad siguen siendo complicadas por este "misterio de únicos" (Novotny and Basset 2000).

La relación entre los especies del dosel y los del sotobosque era 3,2:1, un resultado muy cercano a los valores encontrados en otros estudios, lo que confirma la validez de su uso para estimar la diversidad (Erwin 1982). Sin embargo, encontramos una gran variación entre el número de escarabajos en el dosel en el transcurso del año, con el menor escarabajos presentes en este estrato superior de marzo a mayo (época seca). Esto podría deberse a que el clima más caliente y seco requiere la mayoría de escarabajos de buscar un hábitat más bajo y por lo tanto más fresco y más húmedo. Encontramos que hay muchos escarabajos que no son específicos a sus acogidas, porque 32,4% de los escarabajos de la muestra no se alimentan de *Luehea*, y por lo tanto no son específicos para la planta en la que fueron capturados. Si se incluyen estos en estimaciones de diversidad, la medida de la riqueza inflaría mucho, lo que indica que los cálculos más conservadores pueden ser necesarios.

En general hemos encontrado que la integración de las colecciones de Barrios, Charles y Chang ha aumentado la precisión de las medidas de la diversidad y la composición comunitaria en las escalas espaciales y temporales. Al ver el efecto del aumento de tamaño de la muestra, y sabiendo que la colección maestra todavía no está suficientemente grande, nos ha dado una nueva apreciación de la inmensa diversidad de artrópodos tropicales y los obstáculos que hay que superar durante el estudio de ellos. Ejercicios como el nuestro podría ser útil en el futuro para llenar vacíos de información y aumentar el poder descriptivo de los conocimientos entomológicos en su conjunto.

2. Host

i. Host Institution

This internship was carried out with the University of Panama, founded October 7th, 1935. The University of Panama is an institution for higher learning, research and professional training consisting of education, business, pharmacology, engineering, law, and natural science faculties. The University aims to support national development, contribute to the eradication of poverty and improve the quality of life of Panamanians (Consejo General Universitario 5-07). We will be working within the faculty of Entomology, which emphasizes Panamanian priorities, seeking to train new Central and South American researchers and scientists.

ii. Supervisors

Our supervisors at the University are:

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Dr. Barrios' and Dr. Basset's research interests lie in biodiversity and host-specificity of insect herbivores as well as community structure and taxonomy of arboreal arthropods.

iii. Study Site

The insect collections are found in the Entomology building of the University of Panama, Octavio Mendez Pereira Main Campus, Panamá City. Reference collections are housed in the entomology storage room of the Smithsonian Tropical Research Institution, Tupper branch, Ancón, Panamá City. The insects were originally collected from *Luehea seemannii* trees between 1994 and 1999 in Parque Natural Metropolitano, Panamá City.

3. Context

i. Literature Review

In 1980, Terry Erwin identified tropical arboreal ecosystems as the last frontier of knowledge with respect to Coleoptera (Erwin and Scott 1980). 30 years later, the species richness of tropical arthropods is still poorly described; the number of species existent is thought to be several orders of magnitude greater than the number of species now named (Odegaard 2003). The proportion of described to undescribed species in tropical samples is highly variable, however, so it is difficult to give an accurate estimate of the number of undescribed species left to identify and thus the total number of species in the world. Estimates of total arthropod species range from 2 to 30 million (Erwin 1982, Miller et al. 2002), with recent estimates between 3 and 5 million (Odegaard 2000, 2003).

These estimates are commonly made using the host specifity of a phytophagous insect, which is defined as the taxonomic range of plant species or plant parts used by the insect or insect community (Odegaard 2003). For example, in Erwin's original ingenious estimates, he first found the number of host-specific beetles on the *Luehea seemannii* tree (Erwin 1982). He then multiplied this by the number of tree species per hectare of forest and extended his estimate to all arthropods using the assumption that beetles make up 40% of all arthropods- Coleoptera are one of the most speciose groups in the canopy (Erwin 1982, Basset 2001) to find the number of arthropod species per hectare of tree canopy. In his estimates Erwin assumed that a 1/3 as many species live in the understorey as in the canopy, and thus added another 1/3 to his total number, allowing him to multiply this by the number of hectares of forest and make a prediction for the total species richness of arthropods in the world- 30 million rather than the 1.5 million previously estimated (Erwin 1982). Though this number is now thought to be an order of magnitude too large, Erwin's original work inspired important scientific controversy and much subsequent research (Miller et al. 2002).

Inconsistency in estimates of species richness often stem from variance in sampling methods (Basset 2001, Odegaard 2003), uncertainty with respect to the proportion of canopy to understorey species, variation in species composition of a forest over temporal and spatial scales (Odegaard 2003), and the extrapolation of data from common trees to the 50,000 tree species of tropical forests (Basset et al. 1996). Estimates using common trees such as *Luehea seemannii* overestimate host-specificity because more species will evolve to be specific to a common tree (Odegaard 2000). Redundancies due to the same species in a different area being specific to an ecologically similar, but taxonomically different, tree also artificially inflate estimates of global species number (Odegaard 2000). Thus, when Erwin multiplied the number of beetle species specific to each tree by the total number of tree species, assuming they had different host-specific inhabitants, he was counting many species twice or more.

The study of beetle diversity is incredibly difficult- Odegaard asserts that "it is difficult to get a representative sample ... from an area smaller than 50 hectares"- a process often constrained by time and resources (Odegaard 2003). Many samples, smaller than ideal size, must thus be analyzed for accuracy. An important measure of representative power of a sample is a species accumulation curve, in which sampling effort is compared with the number of species found (Novotny and Basset 2000). These curves are initially very steep and flatten, approaching an asymptote, as the number of species in the sample starts to reflect total species richness.

There is a split in habitat use between the understorey and the canopy, which has to do with the different biotic and abiotic conditions of the strata (Charles and Basset 2005). The canopy has a higher ambient temperature, more intense solar radiation, and a greater density of fruits, seeds, flowers and young leaves (Basset et al. 2003), all of which allow for increased density and evolutionary specialization of insects (Miller et al. 2002). Taxonomic groups differ between the canopy and understorey, with greater species richness in the canopy (Basset 2001, Charles and Basset 2005). Uncertainties with respect to species composition and density in the different strata further complicate estimates of insect diversity.

ii. Coleoptera Chrysomelidae

This investigation of insect diversity was focused specifically on Chrysomelids, one family of Coleoptera (beetles). The Chrysomelid leaf beetle family is dominant in the canopy of *Luehea seemannii* (Erwin and Scott 1980), and is thus an important group in the study of insect biodiversity. There are 11 subfamilies of Chrysomelid, all of which are phytophagous, feeding mostly on leaves and flowers (Borror et al. 1981).

4. Objectives

i. Main Goal

The University of Panama is home to the Chrysomelid collections of Dr. Elroy Charles, Ing. Rodrgio Chang, and Dr. Héctor Barrios, which contain specimens from *L. seemannii* trees in Parque Natural Metropolitano. Each of these collections has been compiled using a different labeling system and recording slightly different information. Some specimens have been identified to the species level, and some only to the order. Our primary task, therefore, was to compile all of these specimens into one unified master collection, identified to the species, morphospecies or genus level, and create a common labeling methodology and display system. Accompanying this new collection is a master excel database describing all of the information recorded for every specimen in the collection.

Significant redundancies exist between the Chang, Barrios, and Charles collections. Compiling these collections to create a master database will remove these redundancies in the species count and improve the quality of the data, thus increasing the accuracy of estimates of species richness in *L. seemannii*. This collection also indicates variation in species composition and diversity across temporal scales and between strata.

ii. Questions and Hypotheses

Estimates of insect biodiversity are based on a few key assumptions, this database allows us to test a whole set of these assumptions, and our own hypotheses. It then allows us to make estimates of alpha and beta diversity using different indicators.

One assumption to be tested stems from Erwin's original estimate, supported by Dr. Basset's evaluation (Basset et al. 2003), that the ratio of canopy to understorey species is 3:1 (Erwin 1982). We hypothesize that Chrysomelids are three times more species rich in the canopy than in the understorey due to greater plant biomass density and complexity (fruits, seeds, flowers and young leaves) (Erwin and Scott 1980). We also hypothesize that a bit less than a third of Chrysomelid species will be common to both the canopy and understorey, based on the more general finding that trees in the dry forest of PNM share 28% of Coleoptera species between their different strata (Charles and Basset 2005). This distribution of beetles between the canopy and understorey habitats is affected by the abundance of young flowers and foliage (Basset et al. 1996). Since *L. seemannii* flowers in the dry season in Panama, from December to April (Country of Panama Climate Report 2011), there is an expected difference in the species composition dependent on *when* the different samples were collected (Borchert 1996). We predict a more pronounced division of species between the strata during the months when flowers are available, and dominance by different Chrysomelid species over the different seasons.

The phenomenon of 'tourists', insects who are not actually specific to a habitat but just passing through, being collected in samples confounds estimates of diversity further. Testing if a collected specimen feeds on the foliage of the plant it was collected from indicates host-specificity- that the insect is not just a 'tourist'. In both wet and dry tropical forests the most common specimens collected have been those that fed on the plant they were collected from (Charles and Basset 2005), since PNM is a representative dry tropical forest we predict that the same is true for our specimens collected from *L. seemannii*.

Another factor that can either depress or artificially inflate estimates of species richness is the amount of sampling effort (time and specimen number) (Odegaard 2003). We expect to see differences in the species richness of the individual collections here based on sample size. We will investigate how this variance in sample size affects species accumulation in each case. Since we expect to find a number of species unique to each collection, we hypothesize that the largest number of species will be found in our master collection where all the different specimens are aggregated. In this way, the species accumulation curve for the master collection should begin to approach an asymptote, giving us more confidence in its accuracy than in the accuracy of the smaller individual collections.

iii. Justification for Research Question

It is vital that it the factors affecting composition of insect samples are determined in order to accurately estimate insect diversity. The extent to which using different types of traps, collecting at different times of the year, from different strata, and 'tourists' all affect samples needs to be determined in order to compensate for the error they introduce.

Measuring the diversity characteristics of *L. seemannii* is not only of great interest but also very important since it is a model system- a common tree with a history of providing the basis for insect diversity estimates. Understanding the similarities and differences between the three separate collections and master collection is key to understanding variation possible from one type of tree, a characteristic which it is impossible to determine during one sampling session.

5. Methodology

i. Ethical Considerations

Research with established collections of Chrysomelid beetles did not require ethical approval. However, if we were to be doing research with human participants we would have consulted professionals of McGill University and the Smithsonian Tropical Research Institute and the McGill University Policy on the Ethical Conduct of Research Involving Human Subjects (Accessed 2011). This code ensures that no research begins until prospective subjects have been given the opportunity to voluntarily give free and informed consent about participation (Article 2.1). Participants should be provided with all information relevant to the research, such as the purpose, methods, duration, possible risks, and possible uses of results (ex: academic or commercial) (Article 2.4). Relevant information would continue to be provided throughout the time of participation, a participant would have the right to withdraw at any point and, whenever possible, would be given more information after participation (we would give something back to the people we got information from). It is also important that the researcher does not exclude potential participants on the basis of race, religion, culture, sex, mental or physical disability, sexual orientation, ethnicity, sex or age unless there is a valid reason relating to the research for doing so (Article 5.1).

ii. Materials and Tools

The specimens under investigation are organized in wooden display boxes organized by collector, housed either in the Entomology building of the Universidad de Panama or at the Tupper branch of STRI. All taxonomic materials used in compiling the master database and labeling the specimens were provided by the Entomology department. This work required microscopes, tagging and insect mounting materials (pins, styrofoam, tags, naphthalene balls), wooden entomological boxes, *Introduction to the Study of Insect 5th ed.* textbook for reference and identification, and the use of Excel. Dr. Barrios and Dr. Basset were valuable sources of aid and taxonomic knowledge.

iii. Obtaining Data

First, we separated all Chrysomelid species from the three collections that were found on *Luehea Seemannii*. This new sub-collection was then identified and entered into the master database, with each insect's newly given unique specimen number, and collection information. The database includes the specimen code, taxonomic and sample codes, collecting author, date collected, result of feeding test, source strata, genus name, species name, subfamily, and family of each beetle.

After entering our base collection we identified each morphospecies to the subfamily level. This identification was done using the Chrysomelid key from Borror, DeLong and Triplehorn's *Introduction to the Study of Insects 5th ed.* and with the help of Hector Barrios. Each morphospecies' taxacode had been assigned according to a different coding system for each collector. These multiple naming systems created redundancies where the same species was labeled with different taxacodes; for example, morphospecies x may be E-GALE007 in the Charles collection, C-COCHRY73 in the Chang collection, and B-COCHRY26 in the Barrios collection, meaning that it had been counted as 3 different species. Assigning a new Master-code to all of the insects of the same morphospecies across the three collections (the insects from the above example would now all be labeled as MLCOCHRY45) eliminated these redundancies, providing an accurate count of overall species richness in the master collection. After integration of the collections, they were referenced against the extensive collection of Henry Stockwell, located at the Tupper facility of STRI. This allowed us to identify the species name and even genus of morphospecies in our collection. However, even Stockwell's valuable collection does not describe all tropical Chrysomelids (nor does any existing collection), and many species in our collection were not present in the reference. The identification of all Chrysomelids with named counterparts was recorded, but even so, many of our morphospecies remain unnamed to the species or genus level. These undescribed specimens are recognized only by their master code. This is characteristic of insect, and particularly beetle, taxonomy- a field where our knowledge is full of gaps, and the majority of beetle species remain unknown and unnamed.

iv. Analyzing Data

Our main interest was to gauge the quality of the information contained in the master database as compared to the three smaller collections of Barrios, Chang and Charles. To do this, we characterized and compared the species richness and community composition of the three smaller collections and the master collection. Total species richness of every collection and the most common species in the master collection were calculated. We also examined the amount of redundancy between collections, as well as two measures of alpha diversity - Shannon-Wiener's index of heterogeneity and Simpson's dominance index. We also used the Chao-Jaccard estimated abundance based beta diversity index to further describe the similarity of species composition between collections. This modified index was chosen in place of the classic Jaccard because it reduces negative sampling bias by including the effect of unseen shared species (Chao et al 2005). This property is especially important when dealing with small sample size and a large proportion of rare species, as is usually the case with tropical arthropods.

Sample-based rarefaction curves were used to examine how successful our collections were in describing total species richness of Chrysomelids living on *L. Seemannii*. Comparing the master collection with the curves of the three smaller collections allowed us to see the effect of increased sample size, and whether or not the master collection provides a more comprehensive view of the Chrysomelid community. Species rarefaction curves were used instead of accumulation curves because we did not know the order in which all samples were collected, and because rarefaction produces a smoother curve than accumulation (Gotelli and Colwell 2001).

The number of named species and named specimens was compared to the total number of species and specimens in each of the collections to see how much of the assemblage was described by Stockwell's reference collection.

We looked at the relationship between canopy and understorey communities by comparing the number of species present in each habitat and calculating the number of species common to both. The degree of species overlap between canopy and understorey was also described using the Chao-Jaccard estimate abundance based similarity index. These values were compared between collections to see if the results varied highly between studies, and were compared to literature values for arthropod diversity to see if our collection accurately represented previous findings.

Temporal turnover of Chrysomelid communities was assessed using 4 annual time periods - December to February, March to May, June to August, and September to November. The total number of species present and the most common species were recorded for each time period. Chao-Jaccard estimate abundance based index was again used for each combination of time periods to see when Chrysomelid community composition changed the most, and if there was greater stratification between habitats during different seasons. In order to see how the community composition of different strata changed between the wet season and dry season, we calculated the species richness of the canopy and understorey and the number of species common to both communities for each of the four time periods.

The records of feeding tests from Chang's collection were used to ascertain the proportion of host-specific beetles versus tourists on *L. seemannii*. The master database could not be used to answer the question of host specifity because Chang is the only author who recorded which insects fed on *L. seemannii,* and which did not.

v. Limitations to Methodology and Difficulties Encountered

Our lack of familiarity with Chrysomelid taxonomy made it difficult to identify the subfamilies within the collection of Chrysomelids, an activity that is a challenge even to trained taxonomists. In order to become more familiar with the topic and cultivate the skills necessary to even begin working with our collections, we spent the first two weeks learning taxonomy under Héctor Barrios, with the help of several entomology textbooks.

We encountered the most difficulties while attempting to create the Chrysomelid database using existing Coleoptera collections. Many original tags were missing information such as date of capture or host species, and this limited our ability to enter the specimen correctly into our database. Since these samples were all taken more than 10 years ago there was no way to recover the lost tag information, a limitation that decreases our usable sample size and the accuracy of our database. Even when all of the data was present, the writing on the tags was not always legible, making it difficult to interpret the data accurately. Systems of organisation differed between entomologists, so we also had to decipher these differences before integrating the data into a master database. Fortunately we had access to Rodrigo Chang and Hector Barrios, who helped to clarify their individual tagging systems, reducing these interpretation sources of error.

Missing specimens (those present in a collector's database but missing from the physical collection) plagued us throughout the process. Since we needed the physical specimens to put together the final collection, we were required examine every one of 930 specimens, ensuring that all the information on its tag matched an entry, to determine which entries should go in the master database. These missing insects made our master collection significantly smaller than the original database, decreasing our sample size and thus the power of our statistical analyses.

vi. Budget

Our only expense was travel to and from either the University of Panama or the Tupper campus of STRI every day. This was often by bus, costing 50¢ a person each way.

6. Results

The most common beetle species was found almost twice as often as the second most common species; dominant across all collections examined. This beetle was subfamily Eumolpinae, genus rhabdopterus, and was found in both the canopy and the understorey.

We found that, while 13.6% of the species were redundant, almost five times as many of the specimens were members of redundant species (60.9%) (Table 1). The collection containing the most named species was Charles' with 52.9% of species described previously (Table 2). The Chang collection, the only one with complete feeding data, showed that 64.5% of the specimen collected from *L. seemannii* fed on its vegetation (Table 3). The ratio of canopy to understorey specimen we found was 3.2:1, with 19.0% of the species common to both strata (Table 6).

Table 1 - Redundancy

	Number	Percent of Total
Redundant Species	24	13.6
Redundant Specimen	566	60.9

Table 2 - Proportions of Named Species

Collection	% of specimen named	% of species named
Barrios	21.6	18
Chang	51.7	44
Charles	50.7	52.9
Master	43.1	39.2

Table 3 - Feeding Data

Collection	# of	# of	# of	# of	%	% of

	Specimen that Fed	Specimen that did not Feed	Species that Fed	Species that did not Feed	of Specimen that Fed	Species that Fed
Chang	232	111	20	11	67.6	64.5

Table 4 - Indices of Alpha Diversity

Assemblage	Species Count	Shannon-Wiener's Index of Heterogeneity (Mean)	Simpson's Dominance Index
Master	152	4.22	0.024216
Chang	75	3.57	0.0474
Charles	51	3.3	0.051384
Barrios	50	3.3	0.060392

The total species richness found in the collections of Charles, Chang and Barrios were 51, 75 and 50 species respectively. Charles and Barrios, with similar species counts, also had the same level of species evenness at 3.3. The Chang collection produced a Shannon-Wiener index of 3.57, slightly higher, while the master collection showed an even higher value of 4.22. All these values were relatively high (a low species evenness index being around 1.5), but the collections with the greatest sample size showed the greatest evenness. Opposite to Shannon-

Wiener's Index of Heterogeneity, Simpson's Dominance index showed the highest value in the Barrios collection and the lowest value in the master collection. Even the greatest value of 0.06, however, is low compared to the maximum possible value of 1.

Table 5 - Turnover Between Collections

	Chao-Jaccard Estimated Abundance Based Similarity Index
Charles/Chang	0.226
Charles/Barrios	0.14
Chang/Barrios	0.158
Charles/Master	0.59
Chang/Master	0.692
Barrios/Master	0.507

The greatest similarity was calculated between the Charles and Chang collections, with a modified Jaccard index of 0.226. Chang and Barrios were slightly less similar with a value of 0.158, and the Barrios and Charles collections were the least similar, showing a value of only 0.14. Seeing as the maximum value of this index is 1, and the minimum value 0, the similarities between all of these collections is very low. When compared to the master collection, Chang's collection was the most similar and Barrios' the least.

Figure 1: Chang Rarefaction Curve

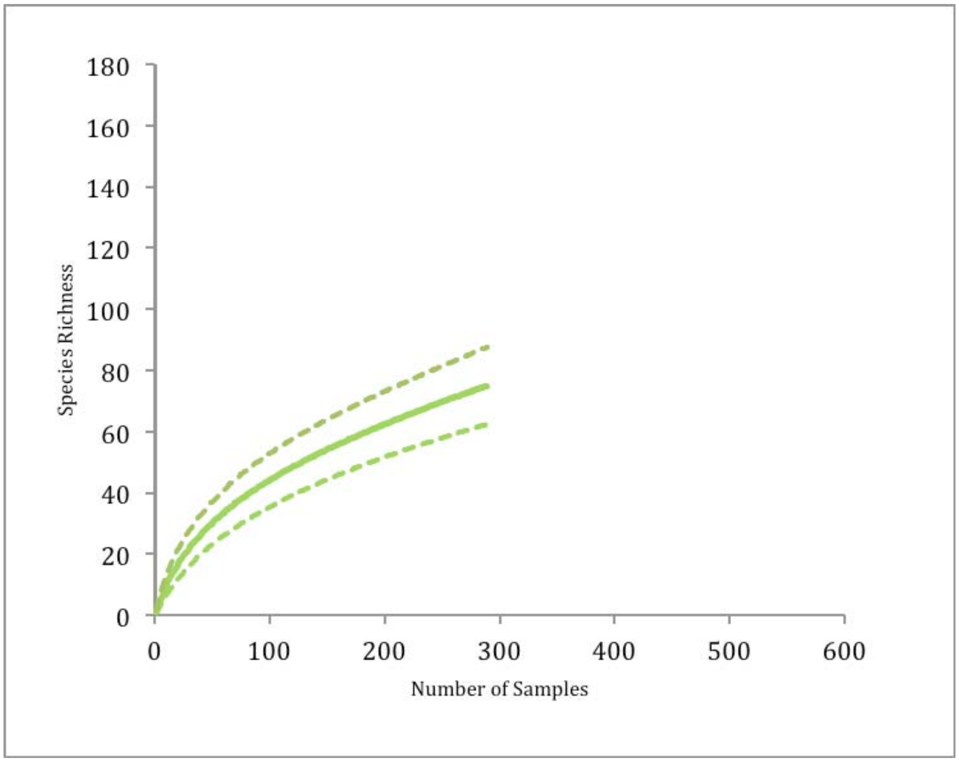

Figure 2: Charles Rarefaction Curve

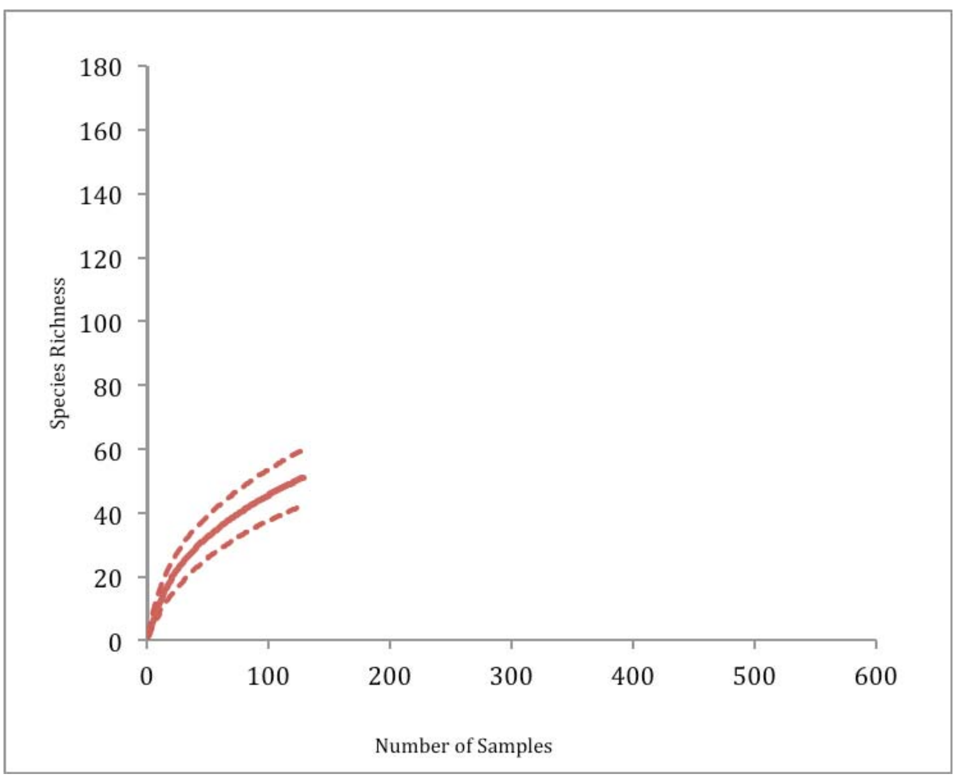

Figure 3: Barrios Rarefaction Curve

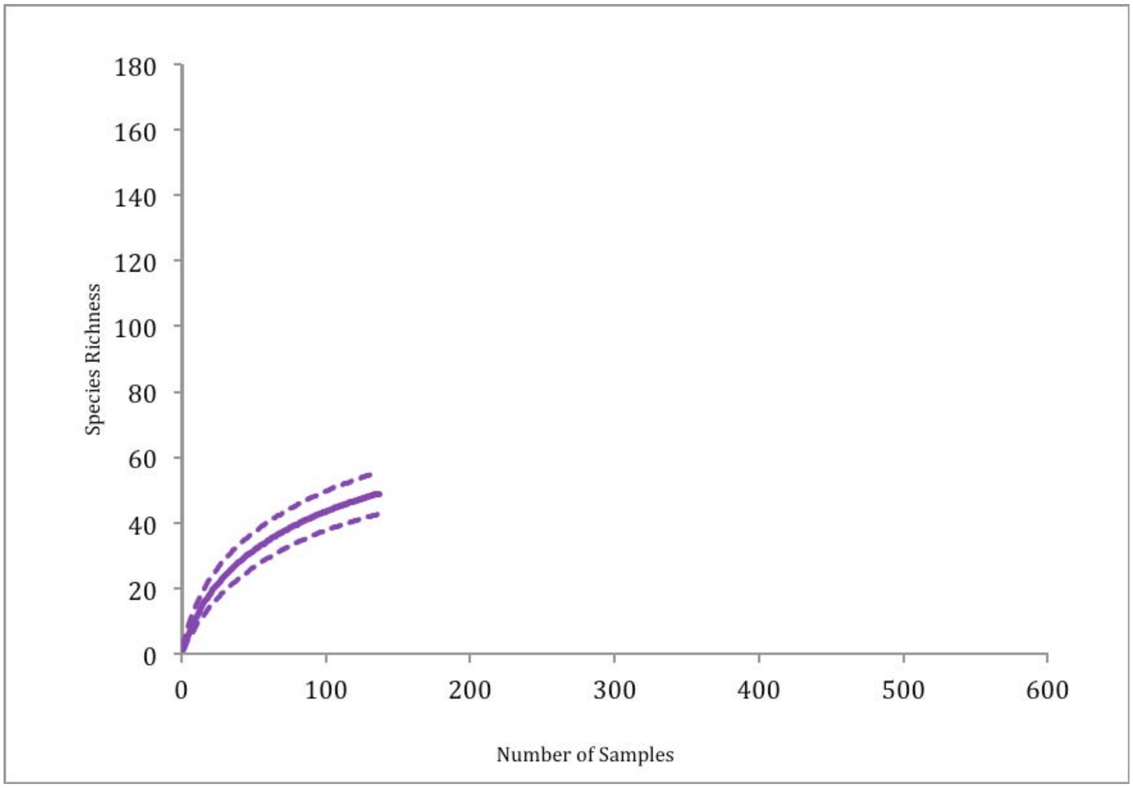

Figure 4: Master Collection Rarefaction Curve

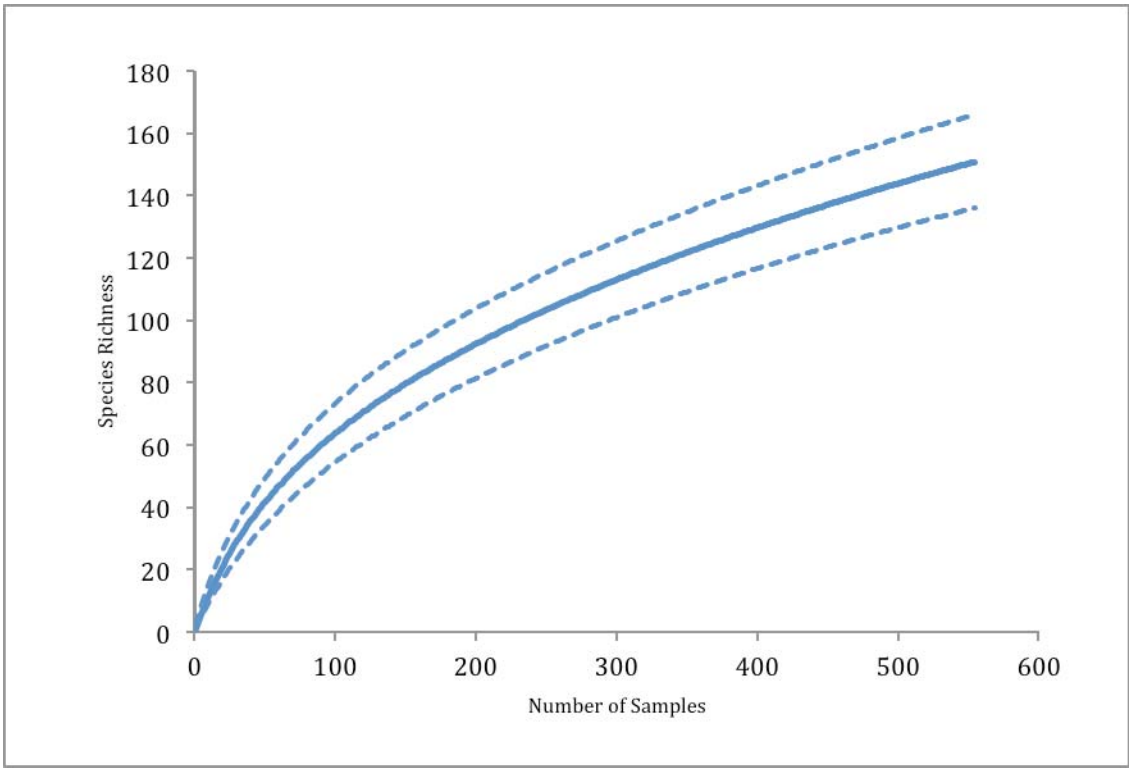

Figure 5: Comparing Rarefaction Curves

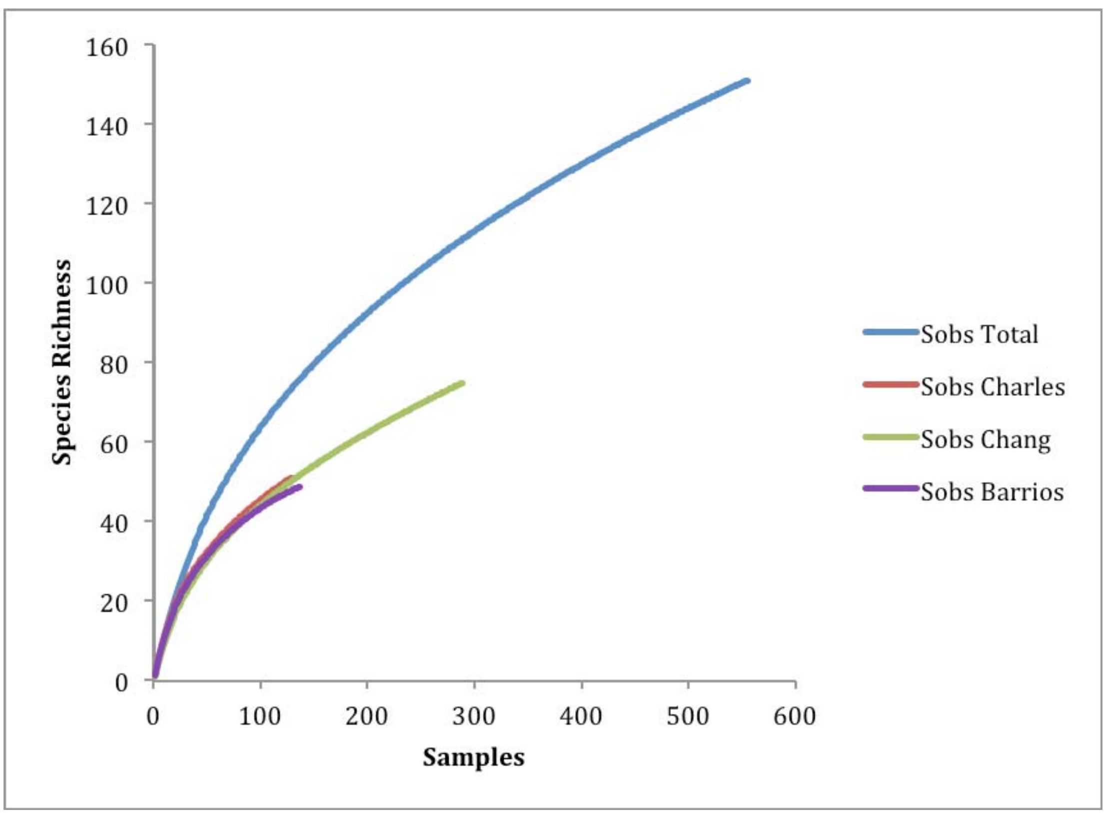

Figure 1, Figure 2, and Figure 3 show respectively the sample-based rarefaction curves of the Chang, Charles and Barrios collections. The middle curve represents the rarefied species richness of the samples, while the two dotted lines represent the upper and lower 95% confidence limits of the rarefaction curve. Figure 4 shows the sample-based rarefaction curve of all three assemblages compiled together (the master curve); Figure 5 displays a comparison between the three smaller assemblages and the master assemblage. Clearly none of these curves comes to an asymptote, not even the master assemblage with 930 specimens and 152 species. The Barrios curve appears to be the closest to leveling off, while the others maintain very steep slope even at the end of the curve. Figure 5 makes it clear how much lower the species richness estimates were for the smaller collections compared to the master collection.

Table 6 - The Canopy and the Understorey

Collection	# of understorey specimen	# of canopy specimen	Ratio Canopy: Understorey	# of species common to understorey and canopy	% of common species
Chang	96	291	3.0 : 1	13	17.3
Charles	64	224	3.5 : 1	11	21.6
Master	160	515	3.2 : 1	24	19.0

Table 7 : Species Turnover Between Strata

	Jaccard Classic	Chao-Jaccard Estimated Abundance Based Similarity Index
Master	0.215	0.767
Charles	0.235	0.537
Chang	0.173	0.681

The greatest stratification between canopy and understorey Chrysomelid communities was in the Charles collection, which showed a Chao-Jaccard estimated abundance based index of 0.537. The Chang collection showed greater similarity between canopy and understorey beetle assemblages. This index could not be calculated for Barrios because this author collected only in the canopy. The master index was higher than either of the Chang or Charles assemblages, at 0.767. The Classic Jaccard index tells a different story altogether – canopy and understorey communities are most similar in the Charles collection and least similar in the Chang, while the master collection shows an intermediate value. This pattern exactly reflects the results from Table 6, which shows there to be more common species between strata in the Charles collection and less in the Chang.

Table 8 - Seasonal Variation

	% of Specimen in the Canopy	% of Species in the Canopy	Most Common Species
March - May	78.8	71.7	MLCOCHRY13
June – August	63.0	78.6	MLCOCHRY40
September – November	81.0	89.7	MLCOCHRY40
December - February	n/a	n/a	MLCOCHRY118

Seasonal variation was measured across all three collections with the most species found in the canopy from September to November and the most in the lower strata from March to May.

Table 9 - Seasonal Similarity

	Chao-Jaccard Estimated Abundance Based Similarity Index
March-May/June-Aug	0.745
March-May/Sept-Nov	0.388
March-May/Dec-Feb	0.346
June-Aug/Sept-Nov	0.830

June-Aug/Dec-Feb	0.387
Sept-Nov/Dec-Feb	0.628

Chrysomelid communities on *L. seemannii* change the least between the June-August and September-November time periods, showing a similarity index of 0.830; March-May versus June-August and September-November versus December-February also have relatively low turnover at 0.745 and 0.628 respectively. All of these are sequential time periods – community composition changed the least between months that were next to each other. The time periods which showed high turnover of Chrysomelid diversity were December-February versus March-May, March-May versus September-November and June-August versus December-February. Two of these low Jaccard values were produced when comparing months from different times of year, but the lowest similarity was calculated for March-May versus December to February.

7. Discussion

Integrating the 3 collections into one master collection with a larger sample size of more specimens and more species has allowed us to do a broader comparison of Chrysomelid diversity on Luehea across a larger time scale and habitat range (both canopy and understorey).

i. Measuring Diversity

We found only moderate *species* redundancy between the collections (13.6% of species were redundant), but a huge amount of redundancy in the *specimens* collected (60.9%) (Table 1). This indicates that majority of specimens collected are from a small number of the most common species. The 24 redundant species that were collected in all collections are probably these common species, and contain 60.9% of the insects in the whole collection. The other 128 species contain only 40.1% of the insects. This means that an average of only 2.9 specimens were collected of each non-redundant species compared to an average of 23.6 specimens collected for each of the redundant species. Thus, as more and more individuals were aggregated into our master collection the number of redundant specimens continued to rise drastically while redundant species remained low – a disproportionate number of specimens belong to these common, redundant species.

This finding echoes a common theme in studies of insect diversity where a large number of species are represented by only one or a few individuals. This is referred to as the 'mystery of singletons'- rare species (those found as single individuals) remain numerous despite huge amounts of sampling effort and large sample sizes (Novotny and Basset, 2000). Our collection contained 65 singletons- meaning that almost half the species collected over this 4-year period consisted of only one individual.

We found that the majority of species in our collection were previously undescribed (Table 2). This was very interesting to see, as it impressed upon us the magnitude of the difficulties involved in estimating insect diversity- the number of insects which have been collected over the years is so large and unmanageable that most of them have never been assigned names, and even this assemblage is only a small fraction compared to the total number of insects existing in tropical forests which have not been sampled at all. Even the reference collection, an extensive and laboriously compiled set of insects collected over the lifetime of Henry Stockwell, contains many species that have not been assigned names.

The change in Shannon-Wiener and Simpson alpha diversity indices between Charles', Chang's and Barrios' original collections and the master collection also points to the fact that the sampling effort made by one scientist is not enough to give a comprehensive diversity measure. The evenness values for the smaller assemblages are all lower than the evenness value for the master assemblage, which suggests that the smaller sample sizes are not sufficient to reflect the true species composition of the Chrysomelid community. Small assemblages seem to disproportionately collect large numbers of a small number of species, depressing the evenness index. As the sample size increases more rare species are collected in smaller numbers, pushing the Shannon-Wiener index up towards its true value. This same trend can be seen using Simpson's Dominance index, which decreases steadily as alpha diversity goes up. The sample size required to properly describe the Chrysomelid community would be indicated when these diversity indices stabilise. In this case, further sampling would have to be done to ascertain whether or not 4.22 and 0.024 are the stable diversity values describing the beetle community on *L. seemannii* or not. What we can tell is that the true evenness value is very high, probably even higher than 4.22, because of the presence of large numbers of rare Chrysomelids on *L. seemannii*.

The low Chao-Jaccard Estimated Abundance Based Index for all assemblages again points to the fact that none of these collections was successful in creating a comprehensive assemblage of Chrysomelids. If a large proportion of the total species present on *L. Seemannii* had been sampled in each of these collections, then in theory they would all have similar species assemblages, and should have had modified Jaccard indices approaching 1. As it is, all the collections are highly dissimilar, which shows that each author managed to sample a different part of the Chrysomelid community present on *L. Seemannii,* but none of them succeeded in sampling the whole. While this does strongly suggest that the sample size of these collections was not large enough, the dissimilarity of the beetle communities described by the Barrios, Chang and Charles collections may also have something to do with the time they were collected and the methods used to collect insects. Barrios sampled during all months of the year, but his work was done between 1994 and 1996, as opposed to the other two authors who sampled in 1999. Charles did most of his sampling in May June and July, with few insects collected in the other months; Chang had a large proportion of samples from June and July, but there was also a significant amount of work done in April, May, August, September, and October. The exact methods of collection are not known for the different authors, but we do know that the beetles were not collected in the same way, which makes it likely that some of the variation between collections is due to collection method and not just to the small number of samples.

The sample-based rarefaction curves displayed in Figures 1 through 5 are the final indication that the original collections did not accurately describe the total species richness of the Chrysomelids. The Barrios, Charles and Chang curves were all lower than the master curve even on a per sample basis – if these curves were extrapolated based on their current trajectory they would come to an asymptote at a much lower point than the master curve reaches. The smaller collections therefore give a false underestimation of true species diversity that can only be seen when comparing them to a larger assemblage. Knowing this, it is impossible to say whether or not the master collection displays an accurate estimation of the true species richness either; creating a rarefaction curve from an even larger sample size of the same community may prove that our master collection estimate is also wrong. The fact that the master curve did not reach an asymptote means that 152 species is definitely a low estimate of species richness. What is clear is that the curve based on a larger sample size is more accurate than the curves based on smaller sample sizes, so creating the master collection signifies a significant improvement in describing the Chrysomelid diversity on *L. Seemannii.*

ii. Host Specificity

The only collector who conducted feeding tests, leaving us with information indicating whether or not each specimen fed on *L. seemannii* leaves was Rodrigo Chang, so the master collection did not improve our understanding of the host specificity of collected insects. The Chang collection showed that 67.6% of specimen fed on *L. seemannii* (Table 3), which supports the hypothesis that the majority of herbivorous insects collected from a tree will be host specific and feed on that species, rather than transient tourists or occupants of the tree for another reason.

However, the relatively large number of insects remaining that did not feed (32.4%) indicates that many samples may also contain a significant portion of tourists. If these are counted in the number of host-specific insect species of a tree, estimates of tropical insect diversity will be greatly inflated. Thus our results support the more recent trend in entomology that Erwin's original estimate was much too high, due in part to the presence of tourists he unwittingly included in his sample (Odegaard 2000).

iii. Understorey versus Canopy

Our results support the assumption for diversity estimates that the ratio of canopy to understorey insects is generally 3:1. In the Chang collection the ratio was 3.0:1, while in the Charles it was further off (3.5:1) (Table 6). The integrated collection thus improved the overall accuracy, giving us a final measure of 3.2:1 for insects collected in the canopy. The results from our collection, however, do not support the hypothesis that around 28% of species are common to both the canopy and the understorey. We found only 19.0% of species in our master database had been found in both habitats (Table 6), a lower estimate than that given for the an average tree species in Parque Natural Metropolitano (Charles and Basset 2005). This could be do due to either error introduced by the unrepresentativeness of our collection, or an actual difference in the specialization of Chrysomelids on *L. seemannii* from that of the average insect-tree pair. Further investigation would be necessary to determine the cause of our result.

The difference between the Classic and modified Jaccard indices for canopy versus understorey species turnover, shown in Table 7, supports the idea that the proportion of shared species found in our master collection may actually be an underestimate. The modified indices are all much higher than the Classic values, indicating that the true similarity between strata is greater than what is indicated by our collection. Since the Chao-Jaccard estimated abundance based index compensates for the effect of unseen shared species, it can be assumed to be more accurate in this case than the Classic index (Chao et al 2005). This means that the proportion of shared species of the true Chrysomelid communities in the understorey and the canopy of L. seemannii is probably closer to the literature value of 28% than it is to the calculated value of 19%.

iv. Seasonal Variation in Community Composition

The number of species in the canopy was highest from September to November and lowest from March to May (Table 8). This contradicts our hypothesis that the highest number of Chrysomelids would live in the canopy during the dry season (December to April). We predicted that the beetles would be drawn to the flowering canopy during this time because of the various food sources that would become available. However, it appears that an alternate factor is determining the distribution of beetles in either habitat. Our data indicates that more species exist lower during the hottest driest months, which makes sense if one of their main goals is staying as cool and moist as possible. As one ascends in the canopy, ambient temperature rises and air humidity decreases, since more sun reaches these levels than lower in the forest (Potvin 2011). This result has thus indicated that finding the most diverse food sources is not a Chrysomelid beetle's top priority during the dry season when seeking the coolest strata seems more important. Since trees and their insect herbivore predators have co-evolved over the years (Agrawal 2004) it is of note that *L. seemannii* flowers during a time when the least phytophagous insects were observed in the canopy. Since flowers and new leaves are the most vulnerable type of vegetation for a tree, It would be interesting to consider that *L. seemannii* may have evolved to flower during a time when there are the least predators in the canopy, decreasing the amount of insect herbivoury on this vulnerable foliage.

According to the similarity indices outlined in Table 9, the greatest turnover of Chrysomelid community composition occurs not between temporally separated periods, but between the end of the dry season and the beginning of the wet season. The beetles that are present on Luehea during the dry season are not the same beetles present when the wet season starts, and they remain very different communities when compared to the June-August period as well. The change between the end of the wet season and the beginning of the dry season is not as pronounced – the similarity index is 0.628; however this value is still less than the March-May/June-August and June-August/September- November similarity indices which indicate the turnover between periods during the wet season. So although the Chrysomelid communities on *Luehea* did not change in the way we expected, these similarity indices confirm that the beetles did respond to the change between the wet season and the dry season.

v. Sources of Error

The expression of the number of species that can be found with a given sampling effort varies between collection methods. For example, sampling individuals randomly and one at a time usually gives a higher estimation of the number of species found per effort than sampling individuals using quadrats because of the effect of patchiness. The collection methods of the three entomologists Chang Charles and Barrios are all unknown, thus it is possible that the comparison of sample-based rarefaction curves between these three collections is not representative of their true relationship. It is also possible that the master curve is not representative of the true species accumulation per unit sample effort because the samples were not all the same size. Some contained only 1 individual and some had as high as 20. This does not change the conclusion that the overall sample size is not large enough to reach an asymptote, but it means that the shape of the curve is not as accurate as it should be and therefore may over- or under-estimate how quickly species would accumulate after the end point of 555 samples.

We know that the estimated species richness is below the true species richness, and this casts a shadow of doubt on all of our other results. The observed stratification of species between the canopy and the understorey and the calculated rate of species turnover between the dry and wet season may be correct - the fact that our results reflect literature values could indicate that only a certain sample size is really necessary to reflect the true Chrysomelid community. However, since our collection does not include a part (probably a large part) of the Chrysomelid population there is also the possibility that are results are inaccurate and that better sampling would in fact end up refuting literature values.

8. Conclusion

Combining the three collections of Hector Barrios, Elroy Charles and Rodrigo Chang created a master collection that was more accurate in measuring diversity and community composition of Chrysomelid beetles on *Luehea seemannii* than any of the original assemblages were on their own. Many conclusions drawn from the smaller collections were dissimilar to those drawn from the master collection, and these inaccuracies were exposed when comparing these results. This allowed us to discover what facts and trends more accurately described the true Chrysomelid community on *L. seemannii*. The inadequacy of the small collections in displaying the extent of Chrysomelid species richness was revealed; comparing the master collection to a reference collection showed how few Chrysomelid species are currently described; trends in increasing species evenness and decreasing community dominance along with increasing sample size were pointed out; the temporal dynamics of community composition during the wet versus the dry seasons were elucidated; the extent of species stratification between the understorey and the canopy was made clear; and host specificity of Chrysomelids on *L. seemannii* was described using the Chang collection. Trends and phenomena that were incorrect or unclear in these smaller collections were clarified when analyzing the master collection.

Assembling this master collection gave us a new appreciation for the immense diversity of tropical arthropods – 930 specimens and we have not even scraped the surface of the richness of one insect family that exists on just one species of tree. The master database was successful in describing the Chrysomelid community more accurately than was done with the original collections, but it also gave us an idea of how much larger the collection would have to be in order to describe the true population of beetles under study. We can see now that the tools available for describing insect diversity are inadequate for such a gigantic undertaking - the reference collections available describe only a small fraction of insect species, the sampling effort made by one scientist is too low. More investigation is certainly required to test the accuracy of insect diversity measures; moving forward, more integration of collections could help overcome these barriers by increasing sample sizes and thus increasing the ability to describe insect populations accurately.

9. Acknowledgments

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11. Appendices

i. Time Spent

Activity	Days (Hours)
Secondary Research	5 (40)
Work with Collections	16 (128)
Statistical Work	7 (56)
Writing of Progress Report and Preparation for Informal Presentation	1 (8)
Preparation for Final Symposium	1 (8)
Writing of Final Report	6 (48)
Total	36 (288) × 2 people = 576 hours

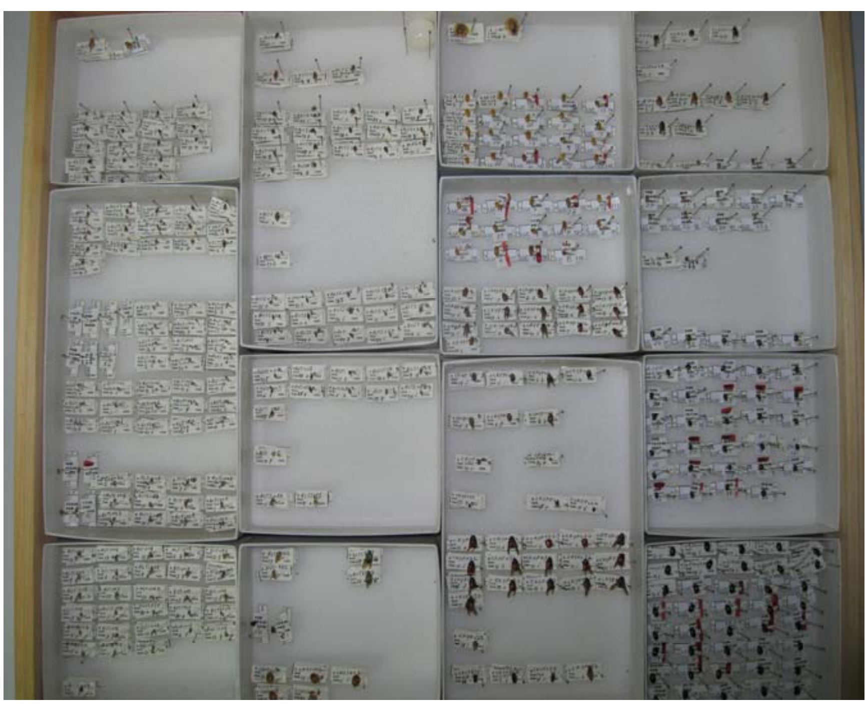
ii. Species and Specimen Numbers for Each Collection

Collection	# of specimen	# of species
Barrios	255	50
Chang	288	51
Charles	387	75
Master	930	152

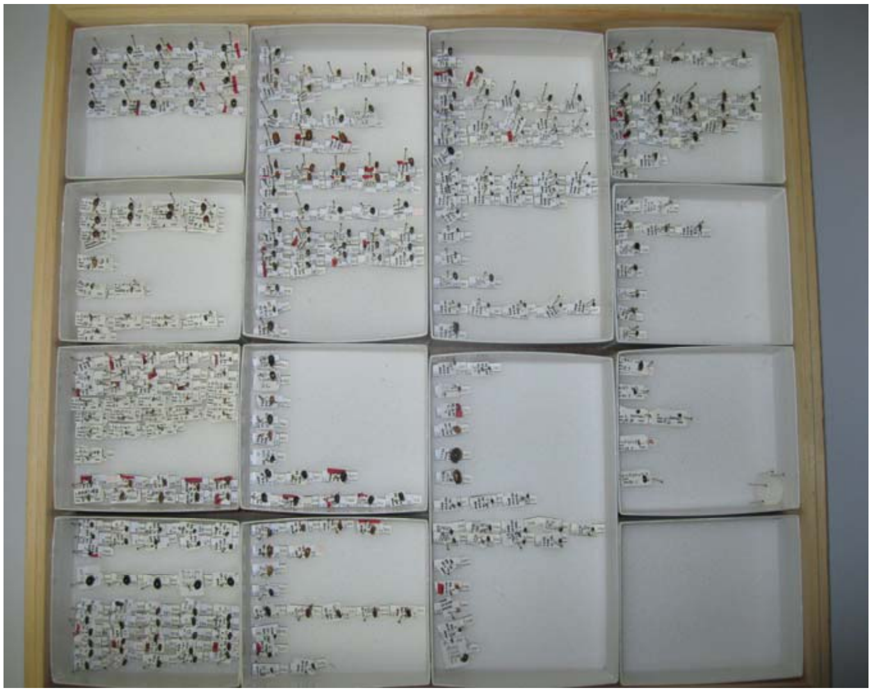
iii. Photographs of the Master Collection

1) *Barrios Box*

2) *Box 1: MLCOCHRY001- MLCOCCHRY040*



3) *Box 2: MLCOCHRY40- MLCOCHRY153*



4) *Total Collection*

5)

6)

7)

8)

9)

10)

5) Kyra holding Box 2