

ARTHROPOD DIVERSITY ESTIMATES FOR THREE NATIVE SUBALPINE PLANT
SPECIES ON THE MAUNAKEA VOLCANO OF HAWAI'I ISLAND

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Abstract

Terrestrial arthropods are among the most abundant and diverse animals on Earth, especially in Hawai‘i where they constitute the vast majority of endemic fauna and play crucial roles in nearly every habitat throughout the islands. Arthropod surveys and inventories are useful methods for documenting arthropod diversity, but studying arthropods can be extremely difficult. Arthropod collection is often taxing because many species are very mobile and exist in harsh climates or on terrain that is difficult to access. Arthropod identification can also be challenging since many arthropods are remarkably small with complex morphologies and diverse life histories. The main goals of this study are to (1) broaden scientific knowledge regarding Hawaiian arthropods by conducting a baseline inventory of the arthropod diversity associated with three endemic Hawaiian plant species in Maunakea’s subalpine region: ‘Āweoweo (*Chenopodium oahuense*), Hinahina (*Geranium cuneatum*); and Māmane, (*Sophora chrysophylla*), and to (2) determine how arthropod diversity and community composition varies between these plant species and various arthropod sampling techniques. Additionally, this study is intended to help the University of Hawai‘i at Hilo’s Office of Maunakea Management (OMKM) fulfill its regulatory need for arthropod inventories, monitoring, and research by demonstrating the use of limited empirical data to develop an alternative, targeted sampling approach that uses species accumulation curves to offset the logistic and taxonomic challenges of arthropod sampling and diversity estimates. Between July and November 2015, we collected over 13,000 arthropods within the University of Hawai‘i (UH) Management Areas and Maunakea Forest Reserve in the subalpine region of the Maunakea Volcano on Hawai‘i Island. For our data analyses, we used R version 3.3.1 (R Development Core Team 2015) for statistical analyses to compare arthropod diversity and community composition between plant species and

sampling techniques. We used EstimateS version 9 (Colwell 2013) to create species accumulation curves to determine the sample size necessary to detect the total estimated arthropod diversity associated with *C. oahuense*, *G. cuneatum*; and *S. chrysophylla*. The results of this study will ultimately increase knowledge and awareness of Hawaiian arthropods and their ecological interactions, and help the OMKM and other land management entities minimize the cost and effort required to conserve native arthropods on Maunakea.

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Chapter 1

Literature Review

Section 1.1 Introduction

Arthropods are some of the most abundant and diverse organisms on Earth. They play critical roles in nearly every terrestrial ecosystem from soil and leaf litter, to forest canopies, and even harsh alpine deserts where no other animals can thrive. Arthropods are particularly prevalent in Hawai‘i where they constitute the vast majority of endemic Hawaiian fauna, but in Hawai‘i and globally many arthropods have yet to be identified and formally named. As such, their diversity, ecosystem functions, and vulnerability to threats are not sufficiently understood. This lack of knowledge is largely due to challenging identification, diverse life histories, complex morphology, and the fact that arthropods are often small and difficult to detect. Fortunately, various statistical techniques may be useful for facilitating arthropod diversity estimates with minimal sampling and taxonomic effort. The purpose of this literature review is to summarize the prevalence of arthropods in Hawaiian ecosystems, the threats to Hawaiian arthropods, the usefulness of arthropods as bioindicators, the need for arthropod conservation, surveys, and diversity estimates, and the various methods for overcoming the associated challenges. The goal is to highlight the intrinsic and functional value of arthropods, and demonstrate how these organisms and their functions can be used as conservation and management tools to indicate environmental changes, ecosystem pressures, ecosystem health, and biodiversity levels.

Section 1.2 Arthropod Prevalence in Hawaiian Ecosystems

Arthropod diversity and abundance far exceeds the variety and numbers and of other animals in many, if not most, terrestrial habitats. Even though they largely go unseen due to their small sizes and inconspicuous behaviors, thousands of arthropod species can be collected by using a single sampling method on only a few trees in the tropics (Stork 1991). Erwin (1982) collected and identified almost 1,000 Coleoptera species from 19 tropical trees canopies and used that data to estimate that there are likely as many as 30 million arthropod species worldwide. Although 30 million species may have been an overestimate, and more recent probabilistic models estimated that arthropod species richness is somewhere between 2.9 - 20 million species (Hamilton et al. 2013), these observations and estimates highlight the extent to which arthropods occupy global ecosystems.

Arthropod diversity is an even more pronounced faunal component on islands than it is in most other geographical locations. Unusually high diversity of arthropod species on islands is due to a variety of factors including island age and degree of isolation, climate, and habitat diversity (Howarth 1990; Gillespie & Roderick 2002). These factors appear to have influenced the arthropod diversity in the Hawaiian Islands which are the most isolated land masses on Earth, positioned nearly 4,000 kilometers from North America, approximately 6,000 kilometers from Japan, and over 8,000 kilometers from Australia (Juvik & Juvik 1998). Perhaps due to this extreme isolation, numerous orders and families of arthropods that are present elsewhere are absent from the endemic fauna of the Hawaiian Islands, but the colonizing species that did arrive in Hawai‘i diversified into extremely numerous species assemblages (Simon et al. 1984; Howarth 1990; Gillespie & Roderick 2002). The 10,000 or more insect species known in Hawai‘i are thought to be the result of the extensive speciation and diversification of about 400

initial colonizing lineages (Howarth 1990). In addition, 50 of the colonizing species became the more than 900 endemic Hawaiian Lepidoptera species that were recognized in 1982 (Gagné 1982), and more than 1,000 endemic Hawaiian Coleoptera species stem from only 18 initial colonist species (Gillespie & Roderick 2002). These types of diverse insects and other arthropods make up a major portion of endemic Hawaiian biota (Roderick & Gillespie 1998; Medeiros et al. 2013). In Hawai‘i, nearly 58% of endemic life form species and 73% of endemic animal species are insects (Eldredge & Evenhuis 2002). When other terrestrial arthropod species are included those percentages increase to 62% and 77%, respectively (Eldredge & Evenhuis 2002).

Both native and non-native arthropods have major influences on ecosystems in Hawai‘i and around the world. The majority of soil fauna are arthropods that provide direct and indirect benefits for humans, and play important ecological roles like freeing available bionutrients (Decaens et al. 2006). For instance, arthropod herbivores and detritivores influence nutrient cycling directly by consuming foliage and producing waste, and indirectly by triggering plant and microbial responses to arthropod feeding (Seastedt & Crossley 1984). Although arthropods impact all aspects of terrestrial ecosystems, perhaps arthropods’ most obvious interactions are with plants.

Native plants provide essential resources and habitats for native arthropods, and many arthropods and plants have coevolved to develop mutualistic relationships (Magnacca 2007; Leblanc et al. 2013). Since many arthropods evolved to physiologically or behaviorally evade specific plant defenses (Tallamy 2004; Burghardt et al. 2010; Burghardt & Tallamy 2013), and predator or parasite pressures can also restrict arthropod host range (Bernays & Graham 1988), many phytophagous arthropods are specialists that utilize only a limited number of specific plant hosts (Bernays & Graham 1988; Tallamy 2004; Bassett et al. 2012). For example, Hawai‘i’s

native *Hylaeus* bees rely almost entirely on native plant resources, and certain native plants such as *Sophora chrysophylla* and *Metrosideros polymorpha* are primarily pollinated by these bees, though the full extent of the function and significance of bees in Hawaiian ecosystem maintenance is not fully understood (Magnacca 2007).

Since arthropods may depend on different plants for habitat and resources, altered flora could affect arthropods in complicated and yet unknown ways. Litt et al. (2014) reviewed nearly 90 published studies and discovered that a large percentage of the studies documented decreases in arthropod abundance and richness in areas impacted by invasive plants. Herbivorous arthropods in the tropics are not likely to be monophagous, such that phylogenetically distinct plant species share some herbivorous arthropod species, yet the degree of sharing decreases as phylogenetic distance between hosts increases (Novotny et al. 2002, 2010). Both specialist and generalist Lepidoptera richness and abundance were reduced on non-native plants, even when the non-native plants were congeners of native plants (Burghardt et al. 2010). Similar results were found for multiple arthropod feeding guilds and life-stages (Burghardt & Tallamy 2013). The effects of altered vegetation on arthropods may also indirectly influence other organisms that depend on arthropods for food or the various ecosystem services that arthropods provide (Tallamy 2004; Burghardt et al. 2010; Burghardt & Tallamy. 2013).

Section 1.3 Threats to Hawaiian Arthropods

Despite their prevalence and diversity, arthropods in Hawai'i were largely ignored or overlooked until Reverend Thomas Blackburn started formally collecting Hawaiian arthropods in 1887, and R.C.L. Perkins began working on a comprehensive arthropod survey in 1892 (Howarth 1990). Blackburn and Perkins collected and described many endemic Hawaiian

arthropod species, but by the time they began their work the arthropod fauna in Hawai‘i had already been altered by unregulated human introductions of non-native plant and animal species into the islands. In 1890 the reigning government of Hawai‘i established the first regulations to prevent the transport of plants or animals into Hawai‘i from other geographic locations, and in 1903 the Board of Commissioners of Agriculture and Forestry was formed and began to study and release beneficial arthropods for biocontrol of pestiferous arthropods and plants (Funasaki et al. 1988). Between the years 1890 and 1985 insects and mites constituted 639 of the 679 organisms released for biocontrol control purposes in Hawai‘i (Funasaki et al. 1988), yet no records were kept of the results or the impacts on native and nontarget biota (Howarth 1990).

Introduced arthropods can have considerable effects on native arthropods and ecosystems, especially on islands. The unique and diverse invertebrate fauna that evolved in the Hawaiian Islands has been greatly reduced in a relatively short time frame due to both non-native invertebrate and vertebrate species and the purposeful release of non-native arthropod predators or parasitoids into novel environments for biocontrol purposes (Asquith 1995). A review of the conservation status of endemic Hawaiian Lepidoptera contracted by the Office of Endangered Species of the United States Fish and Wildlife Service in 1980 reported that biocontrol agents were likely the main cause of endemic Hawaiian Lepidoptera extinctions (Gagné 1982).

Although these records were incomplete and several species have recently been rediscovered (Haines et al. 2004), a separate study found that purposefully released parasitoid wasps made up over 80% of the parasitoids reared from native and non-native Lepidoptera larvae collected in a native forest on Kauai (Henneman & Memmott 2001). While organisms released for biocontrol purposes can negatively impact nontarget native species, the literature clearly indicates that the greatest threats to native arthropods and ecosystems in Hawai‘i are social wasps (Hymenoptera:

Vespidae) and ants (Hymenoptera: Formicidae). Predation by non-native generalist predators such as ants and spiders had a much greater effect on the mortality of endemic Hawaiian koa bugs *Coleotichus blackburniae* (Hemiptera: Scutelleridae), than did parasitism by the biocontrol agents *Trissoclus basalis* (Hymenoptera: Scelionidae) and *Trichopoda pilipes* (Diptera: Tachinidae) that were introduced in Hawai‘i to control the non-native stink bug *Nezara viridula* (Hemiptera: Pentatomidae) (Johnson et al. 2005). Although non-native social arthropods like ants and wasps can negatively impact any novel ecosystem, the threat may be exacerbated in Hawai‘i since endemic Hawaiian flora and fauna evolved without the ecological interaction pressures or influences of social Hymenoptera (Zimmerman 1948; Wilson 1996). Species that have adaptively radiated from a small number of colonizing species on remote oceanic islands exhibit an exceptional range of morphological and ecological diversity (Zimmerman 1948), and these unique species are particularly susceptible to extinction when faced with habitat destruction by humans and competition and predation pressure from non-native species (Simon et al. 1984; Paulay 1994).

Several studies reported that invasive ants caused reduced native arthropod diversity in Hawai‘i (Williams 1927; Cole et al. 1992; Gillespie & Reimer 1993; Krushelnycky & Gillespie 2008; Krushelnycky & Gillespie 2010). Invasive ants *Pheidole megacephala* (Hymenoptera: Formicidae) may have led to the extirpation of the endemic Hawaiian beetle *Colpocaccus tantalus* (Coleoptera: Carabidae) that was frequently collected during surveys on Oahu in the 1890s, but was not observed or collected at all in the 1990s (Liebherr and Polhemus 1997). It is not clear in the literature whether ants directly consume and predate upon such insects or ants are simply better competitors for food and other resources.

Studies have reported that invasive wasps had direct negative impacts on endemic Hawaiian arthropods (Gambino 1992; Wilson & Holway 2010), and indirect negative impacts on endemic Hawaiian plants (Wilson & Holway 2010; Hanna et al. 2013). Endemic Hawaiian *Hylaeus* bees (Hymenoptera: Colletidae) and *Nesodynerus* wasps (Hymenoptera: Vespidae) face predation and competition pressures from *Vespula pensylvanica* (Hymenoptera: Vespidae) invasions in Hawai‘i, and endemic Hawaiian plants such as *Metrosideros polymorpha* may suffer from reduced pollination services as a result of the displacement and reduced foraging of these native Hymenoptera (Wilson and Holway 2010). Furthermore, visitation by native *Hylaeus* bees to native *M. polymorpha* trees has been shown to increase, as does fruit production, when non-native, non-pollinating, predatory wasp populations are decreased (Hanna et al. 2013).

Beardsley (1980) surveyed arthropods in the Haleakala crater district on Maui and determined that 60% of the species collected were endemic, and that the major threats to these endemic arthropods included Argentine ants (*Linepithema humile*), and wasps (*Vespula vulgaris* and *V. pensylvanica*). Subsequent arthropod surveys in Hawai‘i have supported Beardsley’s assertion that non-native wasps and ants threaten endemic Hawaiian arthropods. Gambino (1992) sampled prey items from *V. pensylvanica* in Haleakala and Hawai‘i Volcanoes National Parks and found that, of the identifiable specimens, roughly 66% were endemic and 34% were non-native. Years later, Krushelnycky et al. (2007) also surveyed arthropods on the Haleakala Volcano in Haleakala National Park on Maui, and determined that competition from the Argentine ant (*L. humile*) is the utmost threat to native arthropods in the park, and the spread of this ant within the park will undoubtedly lead to habitats with decreased native arthropod richness and increasing numbers of non-native species. Krushelnycky and Gillespie (2008) also

concluded that habitats with numerous native arthropod species were in danger of sizeable declines in native arthropod species richness when faced with invading ants.

Section 1.4 Arthropods as Bioindicators

Many examples of threats to Hawaiian arthropods can be found in the literature, but the potential use of arthropod sensitivity to these threats and other environmental factors as a proxy for determining ecosystem health has long been overlooked (Medeiros et al. 2013). Soulé et al. (2003) advocated for the conservation of species that have important and ecologically complex interactions with other species. Arthropods are certainly highly interactive animals, and their influential roles in terrestrial ecosystems may make certain arthropod species useful bioindicators. Bioindicators are taxa that can be used to signify or monitor environmental changes, ecosystem pressures, or biodiversity levels (McGeoch 1998; Gerlach et al. 2013), assess ecosystem health and function, and protect threatened habitats and total biodiversity (Kremen et al. 1993). Invertebrates are ideal indicators because their small sizes and rapid generation times may make them more sensitive to subtle variations in local environmental conditions (Gerlach et al. 2013), and characteristics such as large populations, ample species diversity, and rapid growth rates also make arthropods ideal indicators (Kremen et al. 1993).

McGeoch (1998) outlined recommended protocols for effectively using arthropods as bioindicators which include clearly defining what factors are to be tested, and at which spatial and temporal scale to test these factors. Many arthropod groups such as beetles, ants and bees may prove to be useful surrogates for biodiversity to detect environmental changes and evaluate conservation or management actions, especially when used together to increase the types of disturbances that are detectable and decrease chances of confounding factors (Gerlach et al.

2013). In Hawai‘i, the highly specialized nature of certain endemic Hawaiian flies (Diptera: Drosophilidae) may make them ideal indicators of habitat quality, ecosystem health, and total biodiversity (Leblanc et al. 2013).

Section 1.5 Arthropod Conservation

The literature has demonstrated that arthropods may be ideal bioindicators due to their prevalence, diverse functional attributes, and vulnerability to threats in both global and Hawaiian ecosystems. As such, arthropods appear to be deserving candidates for conservation attention. However, invertebrate conservation has only recently started to gain global attention (Wilson 1987; Spector 2008; New 2009). Inventory programs that record the spatial distribution of biological ecosystem components and monitoring programs that track ecosystem changes are two strategies that have been used in arthropod conservation efforts (Kress et al. 1998). Kremen et al. (1993) explained the many ways inventories and monitoring of terrestrial arthropods are useful for conservation planning and natural resource management. More specifically, Roets and Pryke (2013) used the results of a comprehensive arthropod survey on Robbens Island near the coast of South Africa to determine that non-native eucalyptus trees should be removed and native vegetation restored. Furthermore, Stork (2007) referred to the surveys conducted by Dyer et al. (2007) in the Americas from Canada to Brazil, and Novotny et al. (2007) in Papua New Guinea as examples of the types of large-scale sampling efforts that are necessary to begin piecing together an understanding of global diversity and the ecology and risk of extinction of many types of arthropods.

Surveys are also necessary to understand Hawaiian arthropods and the factors threatening arthropod diversity in Hawai‘i (Simon et al. 1984; Liebherr & Polhemus 1997). Williams (1927)

compiled a comprehensive list of native and non-native Hymenoptera in Hawai‘i, Beardsley (1980) surveyed the arthropod fauna on the Haleakala Crater in the dry areas above 1,800 m elevation from 1975-1977, and the Pacific Island Network of the National Park Service Inventory and Monitoring Program has a record of over 60,000 arthropods collected in a survey conducted between 2001 and 2004 on the upper slopes of Haleakala Crater on Maui (Krushelnycky et al. 2007). These surveys improved knowledge of Hawaiian arthropods, but continued arthropod inventories and monitoring are essential for natural resource management plans to detect and track invasive arthropod species as well as their impacts on native arthropods (Medeiros et al. 2013).

In addition to surveys, a variety of actions and additional ecological research are needed to increase knowledge and awareness of Hawaiian arthropods (Howarth 1990; Medeiros et al. 2013). Studies that provide habitat use information for arthropod species are also necessary for developing conservation plans to prevent or counteract anthropogenic impacts on arthropods and their habitats, as was the case for the wēkiu bug on Maunakea in Hawai‘i, (Eiben & Rubinoff 2010; Stephenson et al. 2016). By trapping and identifying Drosophilidae flies on the islands of Hawai‘i and Maui, Leblanc et al. (2013) accumulated occurrence and abundance data for endemic Hawaiian Drosophilidae species across a land use gradient ranging from intact native forest to agricultural land. This type of information is useful for conservation efforts because it may reveal the potential habitat range, and the types and amount of habitat disturbance, that can be endured by native species in Hawai‘i. This study seemed to effectively weigh the ethics of collecting native flies against the need for information that may ultimately protect the flies and their habitat.

Section 1.6 Challenges Associated with Arthropod Surveys and Diversity Estimates

Surveys that monitor arthropod populations can provide copious information that may be needed for arthropod diversity estimates, conservation efforts and natural resource management decisions, but there are many challenges associated with arthropod surveys. Despite arthropods' impressive observed numbers, actual arthropod abundance and diversity is likely far greater than estimated from observed or sampled individuals. This underestimation may be due to factors such as wide-ranging habitats, temporal population fluxes, and broad taxonomic diversity that can make arthropod surveys difficult or expensive to conduct (Gerlach et al. 2013). It has been estimated that as few as 30% of arthropod species (Hamilton et al. 2010) and 5% of insect species on Earth have been collected and identified. This dearth of information is likely due to infrequent or insufficient sampling, and the taxonomic challenge of identifying certain invertebrate species when they are collected (Stork 2007).

Since sampling, rather than census, is the most feasible way to collect most types of biodiversity data (Colwell & Coddington 1994), undersampling bias may preclude accurate estimations of species richness obtained from arthropod surveys, and increasing sampling intensity may be required to make up for this discrepancy (Coscron et al. 2008; Coddington et al. 2009). Up to 72% of species collected in the field are represented by only one individual, a singleton (Coddington et al. 2009). Since the percentage of singletons decreases as sampling intensity increases it is possible that insufficient sampling intensity may be the primary driver of high singleton frequency in arthropod surveys (McGill 2003; Coscron et al. 2008; Coddington et al. 2009). Even with large-scale sampling efforts, both the sampling method and the level of experience of the person sampling may affect richness and diversity estimates of arthropod species (Coscron et al. 2008).

Perhaps even more prohibitive than sampling challenges are the taxonomic limitations that often hinder arthropod diversity estimates. Many arthropod species have yet to be described, and there is a shortage of taxonomic knowledge and specialists to identify even those species that have been described (Howarth 1990; Cardoso et al. 2011). Since trained taxonomists are necessary for proper arthropod identification, the absence of these specialists may result in the usefulness and value of arthropod biodiversity surveys being diminished and arthropod vulnerability being overlooked when it comes to management actions (Howarth 1990; Cardoso et al. 2011; Leblanc et al. 2013). Small arthropods such as mites, mealybugs, and parasitic wasps are particularly difficult to identify, and may only be identified to the level of order or family (Krushelnicky et al. 2007). Since this is also true for other small or cryptic arthropods, arthropod species richness, abundance, and vulnerability may be highly underestimated. Hawaiian arthropods are threatened by non-native species and anthropogenic impacts, but arthropod conservation in Hawai'i has not garnered much attention due to taxonomic limitations that preclude the identification and understanding of many Hawaiian arthropod species (Howarth 1990). Beardsley (1980) mentions that the relative incompleteness of his catalog of arthropods in the Crater District of Haleakala National Park on Maui is due to a scarcity of taxonomic specialists, a scarcity of information regarding certain arthropod groups, and the fact that certain arthropods have yet to be identified. Many undetected and undescribed arthropod species may be at risk of extinction (McKinney 1999), but it is difficult or impossible to develop conservation plans to protect species that are unknown (Howarth 1990; Cardoso et al. 2011).

Section 1.7 Overcoming the Challenges of Arthropod Surveys and Diversity Estimations

Assessing arthropod diversity requires taxonomic identification which can be time-consuming and expensive (Gerlach et al. 2013). However, taxonomic limitations may be mitigated by identifying arthropods with morphospecies designations until they can be further identified by a taxonomic specialist (Oliver & Beattie 1996; Derraik et al. 2002; Cardoso et al. 2011; Morrison et al. 2012). A morphospecies is a description of a specimen's physical or morphological characteristics that is used to informally differentiate dissimilar species for analyses until they can be formally identified. Morphospecies designations allow useful arthropod richness information to be extrapolated from surveys or collections that may include specimens that are difficult to identify, or have yet to be identified and formally named. Although morphospecies can help offset taxonomic deficiencies and reduce the time and cost of specimen identification in biodiversity studies, there are limitations to this approach. A worker with little or no taxonomy experience can sort arthropod specimens into morphospecies with minimal species overestimation, but these results may actually be due to nearly equal occurrences of species overestimations (splitting) and underestimations (lumping) nearly canceling each other out (Derraik et al. 2002).

In addition to morphospecies designations, there are other methods to counteract the taxonomic limitations of accurately quantifying arthropod diversity. Kress et al. (1998) showed that even though Amazonian taxa have never been exhaustively surveyed, specimens in museum collections can be used to direct conservation efforts to areas with known concentrations of biodiversity. Online and electronic resources and databases may also help resolve some of the challenges of estimating arthropod biodiversity (Meier & Dikow 2004; Miller et al. 2014). For instance, Meier and Dikow (2004) made cost-effective diversity estimates of a predatory fly

(Diptera: Asilidae: *Euscelidia*) using only published revisions and monographs. Eiben and Rubinoff (2010) showed how temperature data and arthropod development and population growth information can be useful for understanding and conserving the endemic Hawaiian wēkiu bug. Eiben and Rubinoff (2014) also demonstrated how degree day models, developed for agricultural use, can be used to improve the timing and efficiency of field surveys to monitor the wēkiu bug in its harsh alpine habitat.

Statistical methods and population estimations by extrapolation are also useful for arthropod surveys and diversity estimates (Colwell & Coddington 1994), and for planning subsequent biodiversity monitoring and assessment activities (Oliver & Beattie 1996). Direct observation data and remotely sensed, large-scale biological data can be incorporated into models that determine areas of high conservation priority (Ferrier 2002, 2011; Ferrier et al. 2004; Stephenson et al. 2016), and quantitative models such as species-area curves may be necessary for estimating endemic diversity (Green & Ostling 2003) and total diversity (He & Legendre 2002) over a variety of spatial scales. These methods are particularly helpful for arthropods that may have varied species-specific distributions for which ample empirical data are either unavailable or difficult to obtain. Species accumulation curves can be effective for comparing richness between different communities since the estimates are based on sampling completeness, which is the point when additional species are not expected with additional sampling effort, rather than sample size (Colwell et al. 2004; Chao & Jost 2012). Colwell et al. (2012) used data from several empirical studies to demonstrate how mathematical models can link interpolated (rarefaction) curves and extrapolated curves at an observed reference point. Leblanc et al. (2013) used species accumulation curves to compare the completeness of endemic Hawaiian *Drosophilidae* fly collections between different sampling methods. The ability to assess and

compare sampling completeness, and estimate diversity with limited empirical data, is necessary to effectively monitor and protect organisms such as arthropods that may be difficult or impossible to exhaustively survey.

Section 1.8 Conclusion

In Hawai‘i and elsewhere in the world, arthropod species dominate faunal species richness. As major constituents in nearly every terrestrial habitat, arthropods have been the focus of many studies that have investigated their prevalence and functional roles in global ecosystems as well as the potential to use these animals as indicators of overall biodiversity and ecosystem health. The need for arthropod conservation has also recently become increasingly evident in the literature, and emphasis has been placed on identifying and understanding threats to native and endemic arthropods from habitat alteration and non-native species. Hawaiian arthropods are especially susceptible to these threats and have likely been impacted even before the major Hawaiian arthropod collections and research began in the late 19th century. Even in the 21st century much is still unknown about endemic Hawaiian arthropods. Due to sampling and taxonomic limitations, the full extent of arthropods’ value and ecosystem services has yet to be thoroughly understood or appreciated. Many studies have begun to highlight arthropods’ expansive diversity and important roles in ecosystem health, but more research is needed, especially in Hawai‘i where many arthropod species, and the organisms that interact with them, are endemic with no direct taxon or ecological interaction replacements in any other location on earth. Sampling and taxonomic challenges should not prevent arthropod surveys, but rather models and extrapolation methods should be utilized to overcome these limitations. Arthropods

should be studied and protected for their inherent value, and doing so may also prove to be a valuable tool for broader biodiversity conservation.

Chapter 2

Arthropod Diversity Estimates for Three Native Subalpine Plant Species on Hawai‘i Island’s Maunakea Volcano

Section 2.1 Introduction

Approximately 75% of endemic animal species in Hawai‘i are arthropods (Medeiros et al. 2013), and these endemic arthropods play important ecological roles throughout the Hawaiian Islands as they do in most terrestrial and aquatic habitats on Earth. This is especially true in the subalpine region of Maunakea which, despite the presence of humans and non-native biota, is populated by many species of endemic plants and animals that have mutualistic relationships with endemic arthropods. For instance, arthropods pollinate Māmane (*Sophora chrysophylla*) (Magnacca 2007) which is the most predominant of the 33 endemic plant species in Maunakea’s subalpine region, also referred to as the Māmane Woodland (Gerrish 2013). *S. chrysophylla* is an essential food and nesting resource for the endangered endemic Hawaiian honeycreeper (*Loxioides bailleui*) that feeds on both *S. chrysophylla* seeds (Juvik & Juvik 1984) and the endemic *Cydia* (Tortricidae) moth larvae that are in the *S. chrysophylla* seed pods (Banko et al. 2002; MKCMP 2009). The endangered endemic Maunakea silversword (*Argyroxiphium sandwicensis*) and other endemic plant species also rely on arthropods such as the endemic yellow-faced bee (*Hylaeus flaviceps*) for pollination and reproduction (MKCMP 2009). Unfortunately, unique plant and animal species on remote islands such as the Hawaiian archipelago are particularly susceptible to extinction when faced with competition and predation pressure from non-native species (Simon et al. 1984; Paulay 1994). The University of Hawai‘i at Hilo’s Office of Maunakea Management (OMKM) has determined there is regulatory need for recurrent arthropod inventories and monitoring in Maunakea’s relatively intact subalpine and

alpine regions within the University of Hawai‘i (UH) Management Areas to conserve native arthropods and to detect and prevent the arrival and establishment of potentially harmful non-native arthropods (MKCMP 2009). For this study, we surveyed arthropods in the subalpine region of Maunakea as part of a baseline arthropod inventory, and used our data to determine how arthropod diversity varies between three native plant species: ‘Āweoweo (*Chenopodium oahuense* (Meyen) Allen Caryophyllales: Chenopodiaceae); Hinahina (*Geranium cuneatum* Hook. subsp. *Hololeucum* Geraniales: Geraniaceae); and Māmane (*Sophora chrysophylla* (Salisb.) Seem. Fabales: Fabaceae) (Figure 1). We sought to advance overall knowledge and awareness of Maunakea’s arthropods, and determine how arthropod diversity varies between these three native plant species and between extensive and intensive sampling efforts. By conducting a baseline inventory of Maunakea’s arthropod diversity, and creating a statistical model to guide annual arthropod diversity comparisons that help conserve native arthropods, our research results will ultimately help the OMKM and other land management entities achieve natural resource management goals.

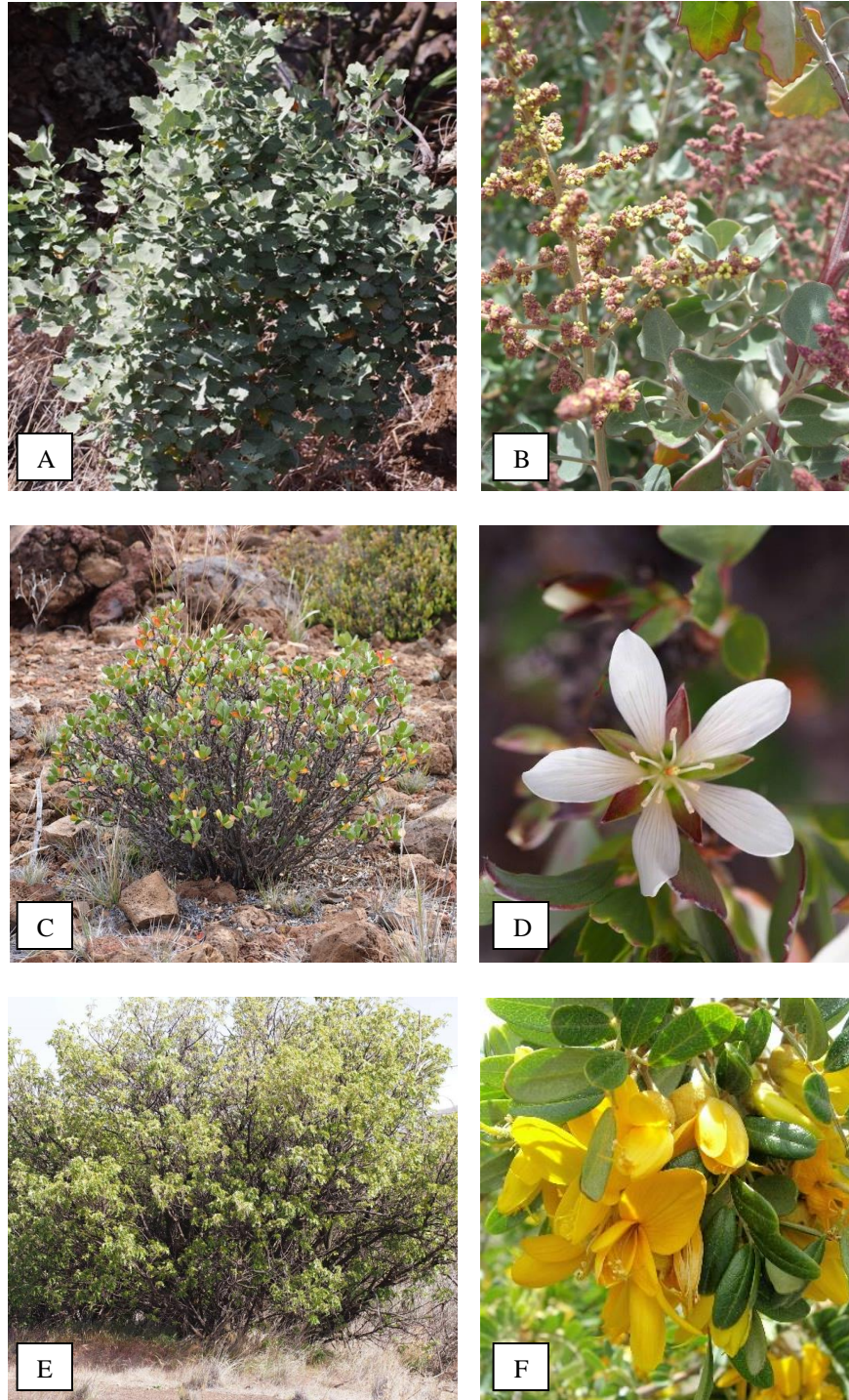


Figure 1. Photos of *Chenopodium oahuense* (‘Āweoweo), *Geranium cuneatum* (Hinahina), and *Sophora chrysophylla* (Māmane). (A) *C. oahuense* plant; (B) *C. oahuense* seeds & flowers; (C) *G. cuneatum* plant; (D) *G. cuneatum* flower; (E) *S. chrysophylla* plant; (F) *S. chrysophylla* flowers.

Natural resource management and conservation efforts are essential due to human-induced ecosystem changes, especially in locations that are populated and visited by many endemic species, such as in Maunakea's subalpine region where the arrival of non-native species, especially non-native arthropod species, can negatively impact ecosystem functions and biodiversity. Banko et al. (2002) documented parasitism and predation pressures along an elevation gradient on Maunakea by non-native arthropods on the *Cydia* and *Scotorythra* moth larvae that are major food sources for *L. bailleui*. The endemic moth *Uresephita polygonalis virescens* (Crambidae) was also once was a prey source for *L. bailleui* on Maunakea, but this moth species is now uncommon possibly due to parasitism pressures (Banko et al. 2002). While parasitism can negatively impact native arthropod species, the impacts of social Hymenoptera on native arthropods and overall biodiversity may be particularly detrimental in Hawai'i since there are no native or endemic social Hymenoptera species in Hawai'i and their competitive pressures are novel for all endemic Hawaiian taxa (Williams 1927; Beardsley 1980; Cole et al. 1992; Gambino 1992; Gillespie & Reimer 1993; Liebherr & Polhemus 1997; Krushelnycky et al. 2007; Krushelnycky & Gillespie 2008; Wilson & Holway 2010). The severity of social Hymenoptera impacts in Hawai'i is because Hawaiian floral and faunal species adaptively radiated from a small number of original colonizers in the absence of social insects (Zimmerman 1948; Wilson 1996). Wilson (1996) suggested that special attention should be paid to the effects of ants on endemic Hawaiian biodiversity, and both Beardsley (1980) and Krushelnycky et al. (2007) determined that Argentine ants (*Linepithema humile*) are a major threat to endemic Hawaiian arthropods on Maui's Haleakala crater. Wasps (Hymenoptera: Vespidae) also compete with and prey upon Hawaiian arthropods (Beardsley 1980; Gambino 1992), and in particular endemic

Hylaeus bees (Hymenoptera: Colletidae) face predation and competition pressures from *Vespula pensylvanica* (Wilson & Holway 2010).

Although ants and social wasps are established in many locations throughout the Hawaiian Islands, neither appear to be established in the subalpine or alpine regions of the UH Management Areas on Maunakea. Banko et al. (2002) found that ants were not detected above 2,800 m on Maunakea, and the rate of *Cydia* and *Scotorythra* parasitism by non-native wasps (Ichneumonidae and Braconidae) and flies (Tachinidae) decreased with increasing elevation. Krushelnycky et al. (2005) concluded that introductions of new ant species seems to have slowed, but the species that are already in Hawai‘i are major threats to native biodiversity, and methods to control, eradicate, or at least detect and prevent the spread of invasive ants into new habitats should be carefully implemented. Our study is part of an arthropod diversity project started by Jesse Eiben and Dan Rubinoff in 2012 to comprehensively document Maunakea’s alpine and subalpine arthropod diversity, and address the OMKM’s regulatory need of establishing a baseline inventory of native and non-native arthropod diversity for native arthropod conservation.

Arthropod diversity at high elevations on the Haleakala Crater on Maui has been relatively well documented thanks to multiple arthropod inventories (Blackburn & Sharp 1885, Beardsley 1980, Krushelnycky et al. 2007), but until the Maunakea arthropod diversity project began in 2012, there has never been a comprehensive arthropod inventory conducted in high elevation habitats on Maunakea. Biodiversity surveys can establish a baseline against which conservation efforts or management decisions can be made and evaluated (Bull et al. 2014). Since arthropods play diverse and important roles in terrestrial ecosystems, arthropod biodiversity data can be useful for evaluating the consequences of management actions or inactions. Evaluation of the effectiveness of conservation actions is necessary to ensure that

conservation funding is used as effectively as possible; moreover, it may encourage and justify future financial investments for conservation efforts (Ferraro & Pattanayak 2006).

Baseline inventories and regular monitoring are two actions recommended by the OMKM to help conserve Maunakea's native arthropods and other natural resources (MKCMP 2009). The results of our study include a baseline arthropod inventory that documents the arthropod diversity associated with *C. oahuense*, *G. cuneatum*, and *S. chrysophylla* in the subalpine region of Maunakea, and species accumulation curves to help determine the minimum amount of sampling effort necessary to detect all estimated arthropod diversity. Here we describe an alternative, targeted sampling approach using species accumulation curves that can be created with limited empirical data, scaled up, and used by the OMKM to surmount the challenges associated with arthropod sampling and diversity estimates in the subalpine region of Hawai'i Island's Maunakea Volcano. Species accumulation curves are useful management tools that can help minimize fieldwork effort and cost and increase the accuracy of biodiversity assessments by indicating, at the point where the curve reaches a plateau, the sample size necessary to be confident that observed species richness will not increase with additional samples (Colwell et al. 2004; Chao et al. 2009). Accurately estimating arthropod diversity with efficient and cost-effective sampling will allow entomologists, ecologists, and natural resource managers, on Maunakea and elsewhere, to effectively monitor arthropod diversity which may also be an indicator of overall biodiversity.

Section 2.2 Study Location

The location for this study is within the Halepōhaku and Road Corridor management units in the subalpine region of the UH Management Areas on the Maunakea Volcano of Hawai'i

Island (Figure 2). Maunakea is a dormant volcano and the tallest mountain in the Hawaiian Archipelago reaching 4,205 m elevation (Juvik & Juvik 1984; Gerrish 2013). Maunakea's subalpine region ranges from 1,700 - 3,000 m elevation, and Halepōhaku is a 7.8 hectare parcel on the southern slope in the upper portion of the subalpine region starting at about 2,800 m elevation (MKCMP 2009). The UH Management Areas on Maunakea are entirely above the trade wind inversion layer that is generally present between 1,500 - 2,700 m elevation, and buffers Halepōhaku from lower moist air and pollutants (MKCMP 2009). The mean annual rainfall at Halepōhaku is 66 cm (Frazier et al. 2016). Infrastructure in the Halepōhaku area consists of a portion of the 26 km long Summit Access Road within the 366 m wide road corridor, and mid-level support facility buildings including the Visitor Information Station, the Onizuka Center for International Astronomy, and various construction laborer camp buildings (MKCMP 2009). The ground substrate in the area consists of bare soil interspersed with fine cinder and lava rock fragments, and medium to large lava rock fragments and aeolian dust particles on the slopes of the area's three cinder cones (MKCMP 2009).

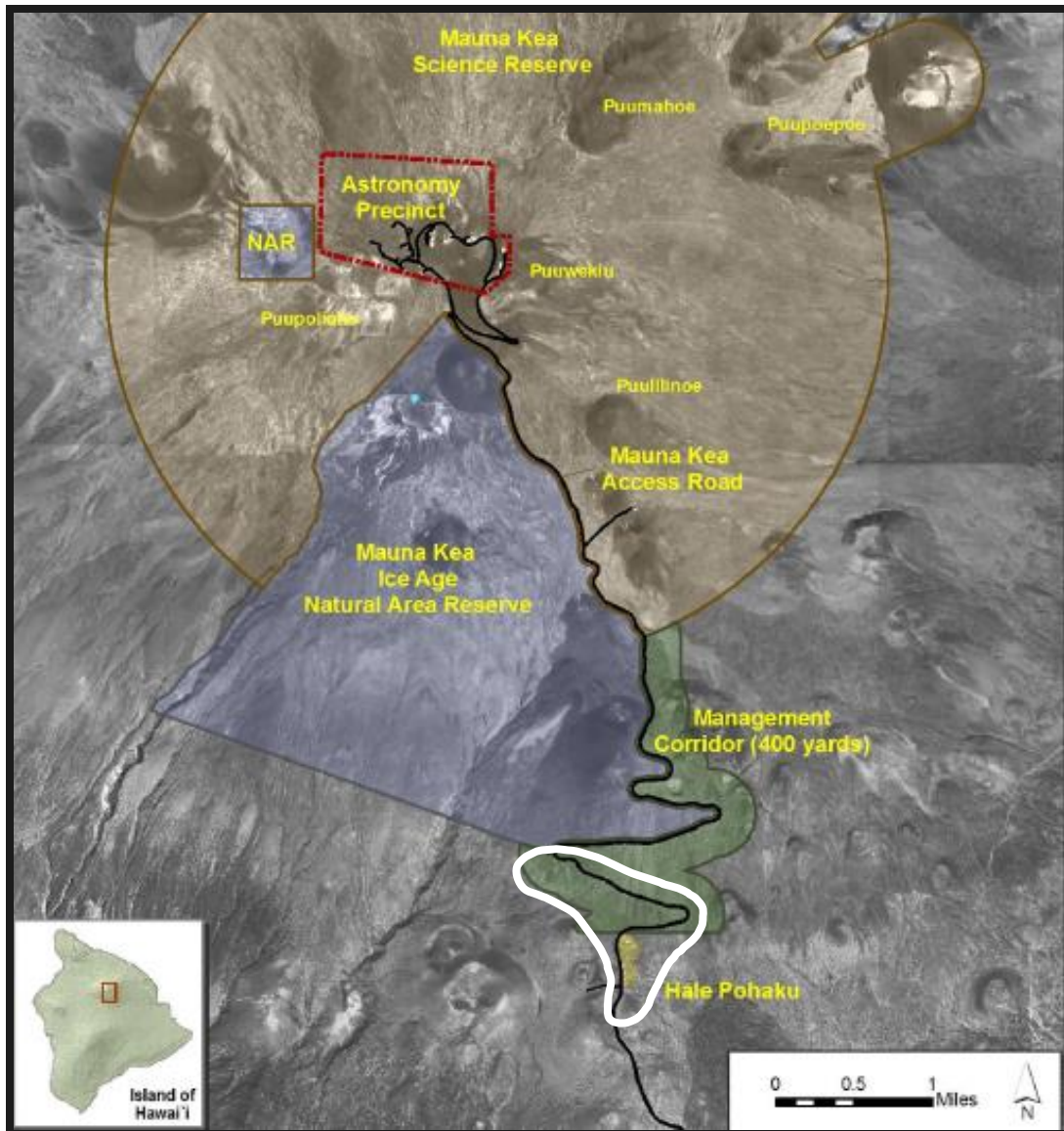


Figure 2. Map of the UH Management Areas in the subalpine and alpine regions of the Maunakea Volcano on Hawai'i Island. Study area outlined in white. Map derived from the Maunakea Comprehensive Management Plan (MKCMP 2009), and used with permission from the Office of Maunakea Management.

In 2011 a botanical baseline survey was conducted in the UH Management Areas on Maunakea to inventory the area's plants and guide natural resource management decisions to protect the native flora, conserve habitat for native arthropods, and minimize possible habitat for potentially harmful non-native arthropods by controlling non-native plants that may serve as habitat for destructive arthropod species (Gerrish 2013). Of the 73 recorded plant species in the UH Management Areas only 33 are native. However, native plants are typically more numerous and dominant than non-native plants, except for certain non-native grasses and herbs that cover the ground in some parts of the 2,800 - 3,000 m elevation Māmane Woodland (MKCMP 2009; Gerrish 2013). Since the Halepōhaku and Road Corridor management units encompass the Māmane Woodland habitat and a variety of native plant species, we focused this study on the arthropod diversity associated with three relatively common native plant species in this area.

The three plant species that we selected for this study are described in the Maunakea Comprehensive Management Plan (MKCMP 2009) and in the 2011 University of Hawai'i botanical baseline survey (Gerrish 2013). *G. cuneatum* (Hinahina) is an endemic shrub in rocky areas of the subalpine and alpine shrubland between 1,800 - 3,800 m elevation, *S. chrysophylla* (Māmane) is an endemic tree in the subalpine region between 1,800 - 2,900 m elevation, and *C. oahuense* (ʻĀweoweo) is an endemic plant in the subalpine region between 1,800 - 2,900 m elevation and it is considered either a shrub or tree. These plants are three of the five native plant species in the Halepōhaku region that have greater than 10% frequency along the 100 m transects in the 2011 botanical baseline survey (Gerrish 2013). Since many native insects require specific native plant hosts (Bernays & Graham 1988), we expected that sampling arthropods on and around these three plant species would likely yield native arthropod species. Non-native

arthropods are likely also on these plants because established adventive arthropods are often generalists (Krushelnicky et al. 2007).

Section 2.3 Methods and Materials

2.3.1 Study Design

Since this study is part of a large arthropod diversity project, previously collected arthropod diversity data were available for *C. oahuense*, *G. cuneatum*, and *S. chrysophylla*. These data, collected by Jesse Eiben and various student assistants in the UH Management Areas from 2011 - 2014, were used to create preliminary species accumulation curves to help determine the sample size necessary to be confident that the sampling for this study was complete, meaning additional sampling will not likely yield any additional arthropod species, and that the asymptotic estimate of arthropod species abundance and richness is representative of actual diversity. We used plant beat data to create these species accumulation curves because this sampling method had the largest sample size of all the methods used for *C. oahuense*, *G. cuneatum*, and *S. chrysophylla* between 2011 - 2014. We formatted these data into abundance matrices with sample information in the first row, arthropod species or morphospecies information in the first column, and abundance data in the cells (Table 1). The abundance matrices were loaded as text files into EstimateS version 9 (Colwell 2013) which is a free software package that computes a variety of biodiversity statistics based on either sample-based incidence data (occurrences), or individual-based abundance data (counts) (Colwell 2013). Following the methods of Leblanc et al. (2013) and the EstimateS User's Guide (Colwell 2013), we created species accumulation curves with 1,000 randomizations without replacement.

Table 1. Abundance matrix example

	A	B	C	D	E	F	G	H	I	J	K	L
1	taxa.id	c.1.b	c.2.b	c.3.b	c.4.b	c.5.b	c.6.b	c.6.l	c.6.p	c.6.s	c.7.b	c.8.b
2	cole2	0	0	0	0	0	0	0	0	1	1	0
3	hemi8	0	0	0	0	0	0	0	0	0	39	4
4	hemi12	0	0	0	0	0	0	0	0	0	0	0
5	hemi13	0	0	0	0	0	0	0	0	0	0	0
6	hemi14	0	0	0	0	0	0	0	0	0	0	0
7	hemi15	0	0	0	0	0	0	0	0	0	0	0
8	hemi16	0	0	0	0	0	0	0	0	0	0	1
9	hemi18	1	46	3	2	0	3	1	0	0	45	0
10	hemi21	0	0	0	0	0	0	0	0	0	0	0
11	hemi22	0	0	0	0	0	1	0	0	8	0	0
12	hemi23	0	0	0	0	0	0	0	0	0	0	0
13	hyme3	0	0	0	0	0	0	0	0	0	0	0
14	hyme5	0	0	0	0	0	0	0	0	1	0	0
15	hyme7	0	0	0	0	0	0	0	0	0	0	0
16	psoc3	0	0	0	0	0	0	0	0	1	0	0
17	psoc6	1	0	0	1	1	0	0	1	0	1	0

Although species accumulation curves can predict species richness with either incidence or abundance data, we opted to use abundance data so that our diversity estimates would weight rare and common species differently. Therefore, we used the Chao 1 diversity estimator for our initial species accumulation curves rather than the Chao 2 diversity estimator which would be more appropriate for incidence data (Meier & Dikow 2004; Colwell et al. 2012). The Chao 1 estimator ($\text{Chao 1} = S_{\text{obs}} + (a^2/2b)$) assumes that rare species can reveal information about the number of unobserved species, and it uses the observed number of species (S_{obs}) and the number of singletons (a) and doubletons (b) in a sample to estimate the actual number of species present (Colwell & Coddington 1994). Specifically, we used the Chao 1 mean, Chao1 95% Confidence Interval Upper Bound, and Chao1 95% Confidence Interval Lower Bound to create expected species accumulation curves to estimate the completeness of plant beat sampling data for *C. oahuense* 20 *G. cuneatum*, and 20 *S. chrysophylla* (Figure 3).

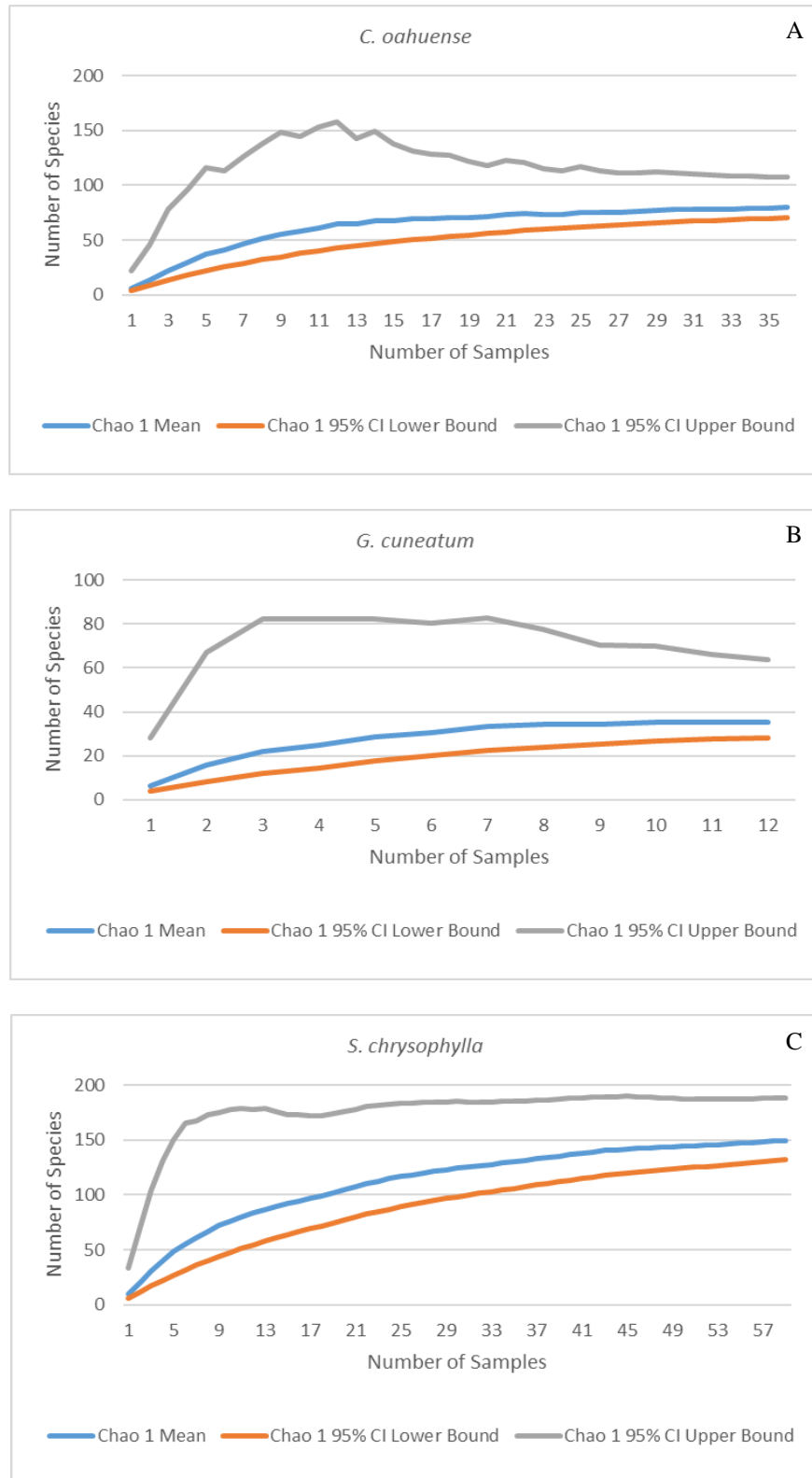


Figure 3. Preliminary expected species accumulation curves for (A) *C. oahuense*; (B) *G. cuneatum*; and (C) *S. chrysophylla* using plant beat data and Chao 1 and 95% CI upper and lower bounds.

We visually assessed the curves to determine the sample size at which the asymptote appeared to start approaching a plateau. Since 20 samples was the largest sample size required for the curves to approach an asymptotic plateau and the confidence intervals to approach their minimum spread from the mean, we sampled 20 *C. oahuense*, 20 *G. cuneatum*, and 20 *S. chrysophylla* in 2015 (July - November) to test whether this sample size would result in sampling completeness and improved arthropod abundance and richness estimates for all three of the plant species. Once the sample size was determined, we used a random number generator in Microsoft Excel (2016) to select 20 of each plant species from the botanical baseline survey spreadsheet file (Gerrish 2013). Only waypoints that were reported to have either *C. oahuense*, *G. cuneatum*, or *S. chrysophylla* present within a six meter or smaller radius of Gerrish's sample points were selected for sampling.

The sampling methods used for this study were plant beats, pitfall traps, yellow sticky cards, and leaf litter analysis. These methods were chosen because they are simple and cost effective, and they target a wide variety of arthropods. As sampling methods and effort were identical for *C. oahuense*, *G. cuneatum*, and *S. chrysophylla*, we pooled the data for each plant species into two sampling intensity categories: (1) Extensive and (2) Intensive. For extensive sampling, 15 individual plants of each plant species were randomly selected to be sampled with plant beats ($n = 15$ per plant species). For intensive sampling, we randomly selected five individual plants of each plant species to be sampled with plant beats, pitfall traps, sticky cards, and leaf litter analysis ($n = 20$ per plant species). After all the sample waypoints were randomly selected and designated for either extensive or intensive sampling, we loaded them onto a Garmin eTrex 20 Global Positioning System (GPS) handheld unit, and created a map of the waypoints with ArcGIS Version 10.2 (Esri 2013) (Figure 4).

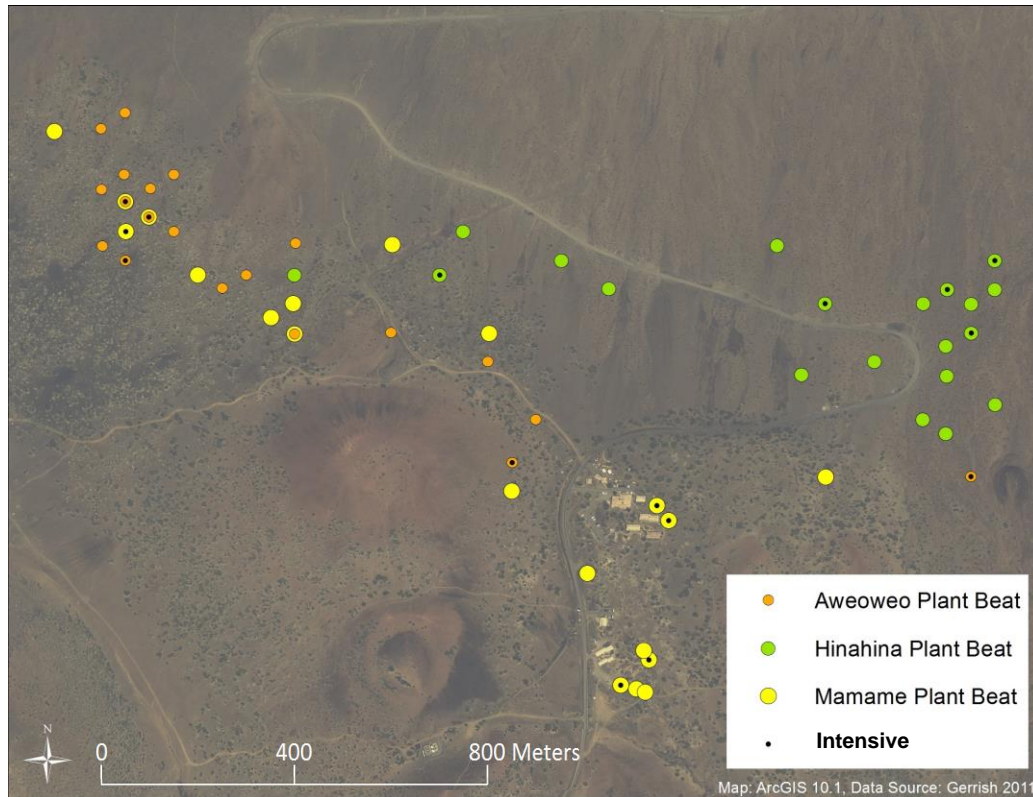


Figure 4. Map of sample points. Black dots indicate intensive sample points ($n = 5$ per plant species; methods = plant beat, pitfall, leaf litter, and sticky card). Points without black dots are extensive sample points ($n = 15$ per plant species; method = plant beat). Image source: 2014 WorldView 2 satellite data.

2.3.2 Arthropod Sampling

We sampled 34 plants in July, 16 in October, and 10 in November. A variety of factors affected the timing of our sampling efforts, including inclement weather and access limitations due to land manager imposed constraints on Maunakea in 2015. During arthropod sampling trips, we used a GPS unit to locate each of the preloaded waypoints. A hard copy of the map created to display the waypoints (Figure 4) was also used for a visual reference of the sample points, and to indicate what type of plant was at each point and what sample method(s) were to be used for that plant. Upon arrival at a preloaded waypoint, we recorded an additional waypoint at the exact location of the nearest plant that was to be sampled. Each new waypoint was given a unique

sample identification code (JUL15RPBMAM7, for example) that denotes the sample month (JUL), year (15), sample method (RPB: Random Plant Beat), and plant species and number (MAM7). The date, GPS coordinates, plant species, and the sample identification code assigned to each sampled plant was recorded on a field data sheet, and also on a paper sample label that was placed inside every sample container.

We sampled a total of 45 plants (15 *C. oahuense*, 15 *G. cuneatum*, and 15 *S. chrysophylla*) with plant beats by placing a net around five plant branches, and tapping the net several times to dislodge arthropods from the branches (Figure 5A). We then used a plastic vial attached to an aspirator to collect arthropods from the net (Figure 5B). As each arthropod was collected it was counted, preliminarily identified, and recorded on the field data sheet. This process was repeated five times for each plant beat. A sample label was inserted into the plastic vial once the five plant beats were complete. We used plant beats, pitfall traps, yellow sticky cards, and leaf litter analysis to sample 15 additional plants (five *C. oahuense*, five *G. cuneatum*, and five *S. chrysophylla*). To set a pitfall trap we used a hand trowel to dig a small hole underneath the plant being sampled, placed a 10-ounce plastic cup into the hole, filled the cup with approximately four ounces of soapy water, added a sample label to the cup, covered the cup with a cap rock, and retrieved the cup and its contents five - seven days after placement (Figure 5C). While digging the hole for the pitfall trap we also used the hand trowel to collect one scoop of leaf litter (approximately 200 cm³) from underneath the plant and place the litter in a sandwich-size sealable plastic bag with a sample label (Figure 5D). At the same time as the pitfall trap was set and the leaf litter was collected, a yellow sticky card with a sample label was attached with tape to the plant and left for five - seven days with the pitfall trap (Figure 5E). To retrieve the pitfall trap, we transferred the contents of the cup into a 30 mL Nalgene container with a screw cap. To

retrieve a sticky card, we removed the tape from the branch, placed the card in a clear plastic bag, and carefully pressed the plastic to both sides of the card so that the arthropod specimens were visible through the plastic. The time required to intensively sample one plant was typically about 15 minutes, but varied depending on the number of arthropods in the plant beat samples.



Figure 5. Photos of sampling methods. (A) Plant beat; (B) Plant beat with aspirator; (C) Pitfall trap; (D) Leaf litter; (E) Yellow sticky card.

2.3.3 Arthropod Processing, Curation, and Identification

All samples were stored in a freezer before being processed and curated for museum vouchering purposes and permit obligations. The reference collection of voucher specimens is stored in the University of Hawai‘i at Hilo College of Agriculture, Forestry, and Natural Resource Management’s Teaching and Research Arthropod Collection. To process plant beat samples, we removed the plastic vial from the freezer and allowed the contents to thaw before opening the vial. Once the contents were thawed, we placed them on a white piece of paper and used forceps to separate the specimens by species or morphospecies. Each species or

morphospecies, and the number of adults, nymphs, or larvae, were recorded on a morphospecies sorting data sheet. The information on the field data sheet was also transferred to the morphospecies data sheet. Nymphs, larvae, and other minute specimens were placed, with a sample label, in 3.7 mL glass vials filled with 70% Isopropyl alcohol. Adult specimens were pinned with insect pins, or glued to pinned paper points, and stored with paper labels in a Schmidt box or Cornell drawer. In samples where there were many adults of the same species, only the first five of the duplicates were pinned or glued to points, and the others were placed in an alcohol vial with a sample label. Only one morphospecies sorting data sheet was used for each plant beat sample, and separate glass alcohol vials, each with a sample label, were used for every species or morphospecies in the plant beat sample.

The same type of morphospecies sorting data sheet that was used for plant beats was also used for sorting and preliminarily identifying specimens in pitfall traps, leaf litter, and sticky cards, and the sampling method was indicated on the sheet. To extract the arthropods from either pitfall traps or leaf litter, we first removed a sample from the freezer and waited for it to thaw. Then we placed the contents in a clear plastic 143 mm diameter sorting dish underneath a microscope, added 70% Isopropyl alcohol until the contents were fully submerged, and used forceps to carefully separate the arthropods from the leaves and other material in the sample. As with plant beat samples, every specimen was counted, identified, and recorded on a morphospecies sorting data sheet, and only one morphospecies sorting data sheet was used for each pitfall or leaf litter sample. Separate glass vials filled with 70% Isopropyl alcohol and a sample label, were used for every distinct species or morphospecies in each sample. For sticky card samples, we used a hand lens or microscope to identify and count the arthropods on the

cards. We recorded these species and morphospecies on a morphospecies sorting data sheet, but left the specimens on the cards.

After all specimens were prepared, preliminarily identified, and recorded on morphospecies sorting data sheets, they were reexamined for formal identification and labeling. This process required the use of a microscope, taxonomic keys, textbooks, internet resources, and taxonomic specialists if they were available. We attempted to identify every specimen to the lowest possible taxonomic level, but some of the specimens were only able to be identified to genus, family, or order. Unidentified arthropods were assigned morphospecies designations that were checked and confirmed by at least two lab members. Specimens that we identified to species were recorded on species determination data sheets and given printed determining labels. All the information in the field data sheets, morphospecies sorting data sheets, and species determination data sheets were input in separate spreadsheets for record keeping and statistical analyses.

2.3.4 Data Analyses

Our original dataset includes the records of the 13,034 arthropod specimens that we collected during this study, regardless of level of identification. These original data will be available in 2017 on the OMKM website <http://www.malamamaunakea.org/library/>, and by request from the author, but they were not used in our statistical analyses. The final dataset that we used for analyses includes morphospecies from our original data if there was consensus among lab members that the morphospecies was a distinct taxon, otherwise debatable morphospecies were removed from our final dataset and our analyses. In their analyses of arthropods on Maui's Haleakala volcano, Krushelnycky et al. (2007) removed arthropods that

were not identified to species or morphospecies, and specifically Acari, parasitic Hymenoptera, and Pseudococcidae. Likewise, we removed from our final dataset Acari, Pseudococcidae, and minute Hymenoptera if they were singletons or doubletons since we could not confidently determine whether they were truly unique species, and these uncommonly collected and identified taxa would greatly influence biodiversity statistics. We also excluded singletons and doubletons that were collected on sticky cards because the generally poor condition of these specimens made it difficult or impossible to assign reliable morphospecies descriptions. Except for immature Lithobiomorpha specimens, we did not include in our final dataset morphospecies that consisted of only immature arthropods if they could not be identified beyond the level of order or family. Immature Collembola described as morphospecies 2 and morphospecies 3 were included due to their high abundance and presence in samples with adults identified as the same morphospecies. Of the original 13,034 individual arthropods collected during this study, 12,868 were included in our final dataset and used in our analyses.

We conducted all analyses with both the native and non-native arthropods in our final dataset, and we repeated the analyses with endemic arthropod data separately. Similar to the methods used by Leblanc et al. (2013) to compare the effectiveness of various trapping methods for detecting endemic Drosophilidae flies across a habitat disturbance gradient, we also used our final data and our endemic arthropod data to create species accumulation curves to determine whether our extensive and intensive sampling efforts were sufficient to detect arthropod diversity on Maunakea. As we did for our preliminary species accumulation curves, we analyzed these data in EstimateS with 1,000 randomizations without replacement. Instead of plotting the Chao 1 estimate output, as we did for our preliminary curves, we plotted these curves using the observed

species mean from the EstimateS output and then referred to the Chao 1 estimate to assess sampling completeness.

To compare arthropod diversity between plant species we used an ANOVA and Tukey post hoc analysis to detect and identify significant differences in the mean arthropod species richness and the mean Pielou's J measure of species evenness (Pielou 1966) associated with *C. oahuense*, *G. cuneatum*, and *S. chrysophylla*. To assess differences in arthropod community composition across plant species and levels of sampling intensity we utilized the vegan package (Oksanen et al. 2015) in R version 3.3.1 (R Development Core Team 2015), and followed the multivariate statistical methods described in Selmants et al. (2016). To visualize the differences in arthropod community composition across plant species, we used Bray-Curtis dissimilarity matrices to construct nonmetric multidimensional scaling (NMDS) ordinations. We also used Bray-Curtis dissimilarity matrices and the “adonis” function (Anderson 2001) for non-parametric permutational multivariate analysis of variance (PERMANOVA) to test whether variation in arthropod community composition was a function of plant species and sampling intensity.

Section 2.4 Results

2.4.1 Descriptive Statistics

Of the 13,034 arthropod individuals that we collected during this study, 7,365 were collected from *C. oahuense*, 316 were collected from *G. cuneatum*, and 5,353 were collected from *S. chrysophylla*. No Acari were identified beyond the order level, as was also the case for various minute Hymenoptera, Diptera, Lepidoptera, and immature specimens. Many of these specimens were included in our analyses as distinct morphospecies, but others were excluded from our final dataset as described above in section 2.3.4 Data Analyses. Our final arthropod

abundance was 12,868 individuals of which 7,278 individuals of 65 species were collected from *C. oahuense*, 280 individuals of 45 species were collected from *G. cuneatum*, and 5,310 individuals of 67 species were collected on *S. chrysophylla* (Table 2). In our final dataset, there were 2,888 arthropods in 25 endemic taxa, 914 arthropods in 26 non-native taxa, and 9,066 arthropods in 48 taxa of unknown origin (Table 3). Of the 9,066 arthropods of unknown origin, 8,684 were unidentified species of very small Collembola.

Table 2. Summary of arthropod diversity organized by plant species. C. oahuense (C), S. chrysophylla (S), and G. cuneatum (G). N = 105: n = 35 per plant species.

Orders	Abundance				Richness			
	Total	C	S	G	Total	C	S	G
Collembola	8,684	5,923	2,741	20	4	4	4	4
Hemiptera	3,486	1,057	2,358	71	23	21	16	12
Thysanoptera	199	104	44	51	4	4	1	1
Acari	122	56	47	19	6	5	6	5
Hymenoptera	111	30	24	57	17	11	10	8
Psocoptera	97	31	54	12	5	3	5	3
Diptera	70	16	14	40	16	4	9	6
Araneae	29	21	6	2	4	2	4	1
Coleoptera	17	11	3	3	8	7	3	1
Lithobiomorpha	16	11	5	0	1	1	1	0
Neuroptera	13	9	3	1	1	1	1	1
Lepidoptera	12	1	8	3	9	1	6	2
Dermoptera	12	8	3	1	1	1	1	1
Total	12,868	7,278	5,310	280	99	65	67	45

Table 3. Summary of arthropod diversity organized by arthropod origin. Endemic (E), Non-native (N), and Unknown (U). $N = 105$; $n = 35$ per plant species.

Orders	Abundance				Richness			
	Total	E	N	U	Total	E	N	U
Collembola	8,684	0	0	8,684	4	0	0	4
Hemiptera	3,486	2,804	592	90	23	12	8	3
Thysanoptera	199	0	190	9	4	0	2	2
Acari	122	0	0	122	6	0	0	6
Hymenoptera	111	11	14	86	17	4	2	11
Psocoptera	97	60	36	1	5	2	2	1
Diptera	70	4	39	27	16	2	4	10
Araneae	29	0	6	23	4	0	2	2
Coleoptera	17	3	10	4	8	1	3	4
Lithobiomorpha	16	0	0	16	1	0	0	1
Neuroptera	13	0	13	0	1	0	1	0
Lepidoptera	12	6	2	4	9	4	1	4
Dermaptera	12	0	12	0	1	0	1	0
Total	12,868	2,888	914	9,066	99	25	26	48

In Maunakea's subalpine region *C. oahuense* and *S. chrysophylla* are dominant native plants that are known to support high levels of arthropod richness (Krushelnycky & Gillespie 2008), and our results indicated that *G. cuneatum* was also host to comparable species richness. Figure 6 demonstrates the relatively high arthropod species richness associated with *G. cuneatum* despite its relatively small size and substantially lower arthropod abundance compared to *C. oahuense* and *S. chrysophylla*. Specifically, the 280 arthropod individuals collected from *G. cuneatum* are only 2% of the arthropod individuals collected during this study, but the 45 arthropod species represented by these 280 individuals is 45% of the overall arthropod species richness associated with all three plant species. The same is true for endemic arthropod abundance and richness: the 45 endemic arthropod individuals collected from *G. cuneatum* make up less than 2% of overall endemic arthropod abundance collected during this study, but the six endemic arthropod species represented by these 45 individuals is 24% of the 25 endemic arthropod species identified during this study. The 16 endemic arthropod species collected from

C. oahuense constitute 64% of the endemic arthropod species identified during this study, and the same is true for the 16 endemic arthropod species collected from *S. chrysophylla*. The 65 arthropod species collected from *C. oahuense* is 65% of the 99 arthropod species identified during this study, and the 67 total species collected from *S. chrysophylla* is 67% of these 99 species.

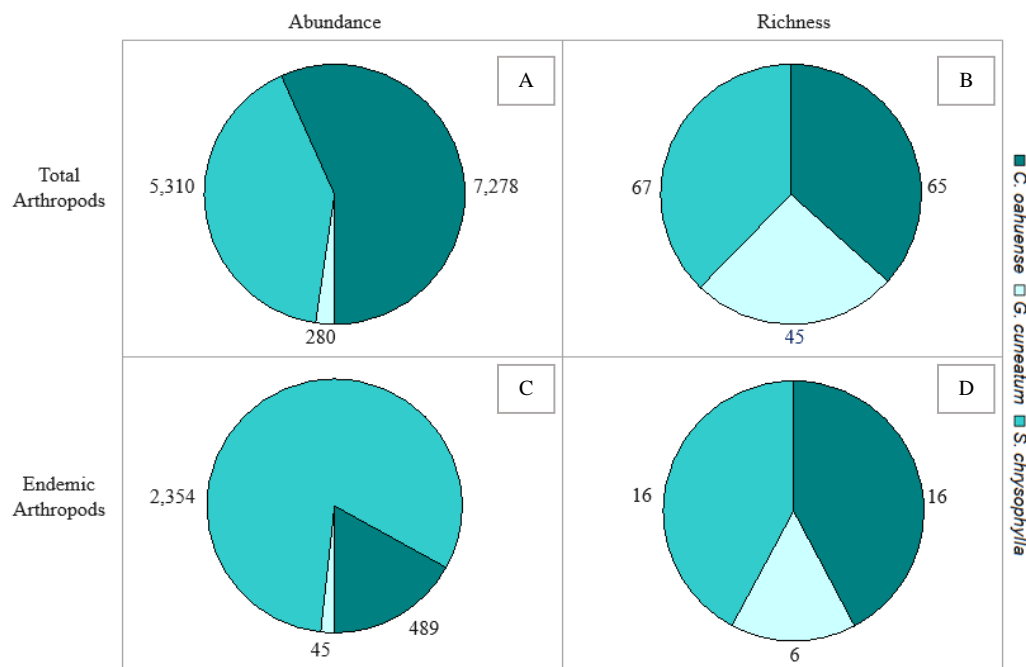


Figure 6. Summary of total and endemic arthropod abundance and species richness by plant species. $N = 105$; $n = 35$ per plant species. (A) Total abundance = 12,868; (B) Total richness = 99; (C) Endemic abundance = 2,888; (D) Endemic richness = 25.

2.4.2 Species Accumulation Curves

The species accumulation curves that we created with our data indicated that additional sampling is necessary to detect all arthropod species associated with *C. oahuense*, *G. cuneatum*, and *S. chrysophylla*. The curves representing total sampling effort for the three plant species combined (Figure 7) did not reach a plateau, and the empirical total arthropod species richness of 99 species was less than half of the Chao 1 mean estimated species richness (not shown in Figure

8) of 229 species with a 95% confidence interval lower bound of 145 and upper bound of 462.

The endemic species richness curve for total sampling effort for the three plant species combined (Figure 7) also did not reach a plateau, and the empirical endemic species richness was 25 species whereas the Chao 1 mean estimated species richness (not shown in Figure 7) was 90 species with a 95% confidence interval lower bound of 43 and upper bound of 197.

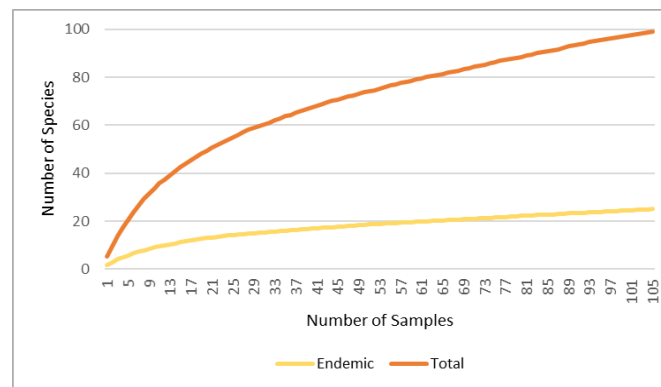


Figure 7. Species accumulation curves for total and endemic arthropod species with plant species and sampling methods combined ($n = 35$ per plant species).

As with the curves representing the total and endemic arthropods collected by total sampling effort and plant species combined, the curves representing these data separately for *C. oahuense*, *G. cuneatum*, and *S. chrysophylla* also failed to reach plateaus (Figure 8), and the empirical arthropod species richness was also lower than the Chao 1 mean estimated arthropod species richness (not shown in Figure 8). The results were similar when we created curves by separating extensive and intensive sampling intensities for each plant species, except for the curves representing endemic arthropods collected from *C. oahuense* and *G. cuneatum* with extensive sampling intensity (Figure 9). At 15 extensive samples, the *C. oahuense* empirical arthropod species richness of six species was equal to the Chao 1 mean estimated species richness of six species (not shown in Figure 9) with a 95% confidence interval lower bound of

six and upper bound of 7.48. The *G. cuneatum* empirical arthropod species richness equaled the Chao 1 mean estimated species richness of two species with 95% confidence interval upper and lower bounds of two at 12 samples. The Chao 1 data reported but not shown in this document, and additional diversity metrics and other data outputs from our EstimateS analyses, will be available in 2017 on the OMKM website <http://www.malamamaunakea.org/library/>, and by request from the author.

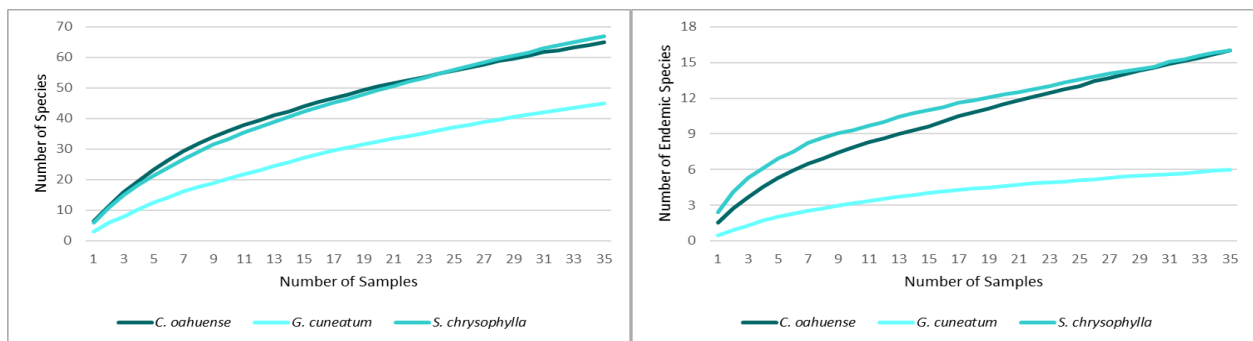


Figure 8. Species accumulation curves for total and endemic arthropod species with extensive and intensive sampling methods combined.

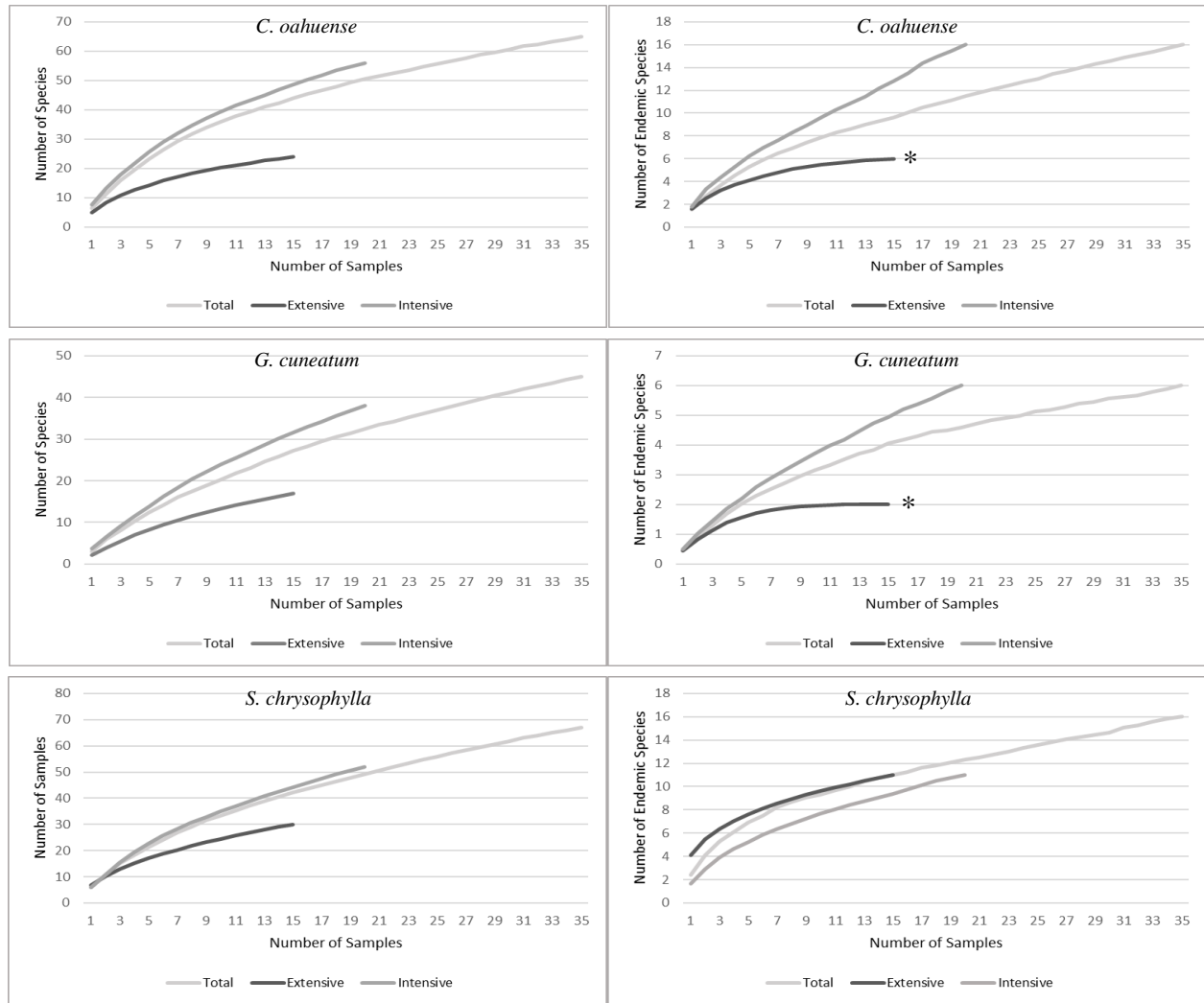


Figure 9. Species accumulation curves for total and endemic arthropod species collected on *C. oahuense*, *G. cuneatum*, and *S. chrysophylla* with extensive sampling, intensive sampling, and total sampling (extensive and intensive sampling combined). Curves that reached plateaus are indicated with an asterisk (*).

2.4.3 Arthropod Diversity Comparisons

Mean arthropod species richness for total sampling effort and total arthropod data (5.47 ± 0.287 se) varied significantly as a function of plant species ($F = 16.51$, $P < 0.001$), and an ANOVA showed that the significant difference was between *C. oahuense* and *G. cuneatum* ($P < 0.001$) and *S. chrysophylla* and *G. cuneatum* ($P < 0.001$), but not between *S. chrysophylla* and

C.oahuense ($P = 0.619$) (Figure 10). Mean endemic arthropod richness for total sampling effort (2.441 ± 0.182 se) also varied significantly as a function of plant species ($F = 17.46$, $P < 0.001$), and an ANOVA showed that the significant difference was between *S. chrysophylla* and *C.oahuense* ($P < 0.001$), and *S. chrysophylla* and *G. cuneatum* ($P < 0.001$), but not between *C. oahuense* and *G. cuneatum* ($P = 0.486$) (Figure 11). The mean Pielou's J arthropod evenness for total sampling effort and total arthropod data (0.595 ± 0.032 se) varied significantly as a function of plant species ($F = 10.79$, $P < 0.001$), and an ANOVA showed that the significant difference was between *S. chrysophylla* and *C.oahuense* ($P = 0.007$), and *S. chrysophylla* and *G. cuneatum* ($P < 0.001$), but not between *C. oahuense* and *G. cuneatum* ($P = 0.304$) (Figure 12). The mean Pielou's J endemic arthropod evenness (0.364 ± 0.044 se) did not vary significantly as a function of plant species ($F = 0.243$, $P = 0.785$) (Figure 13).

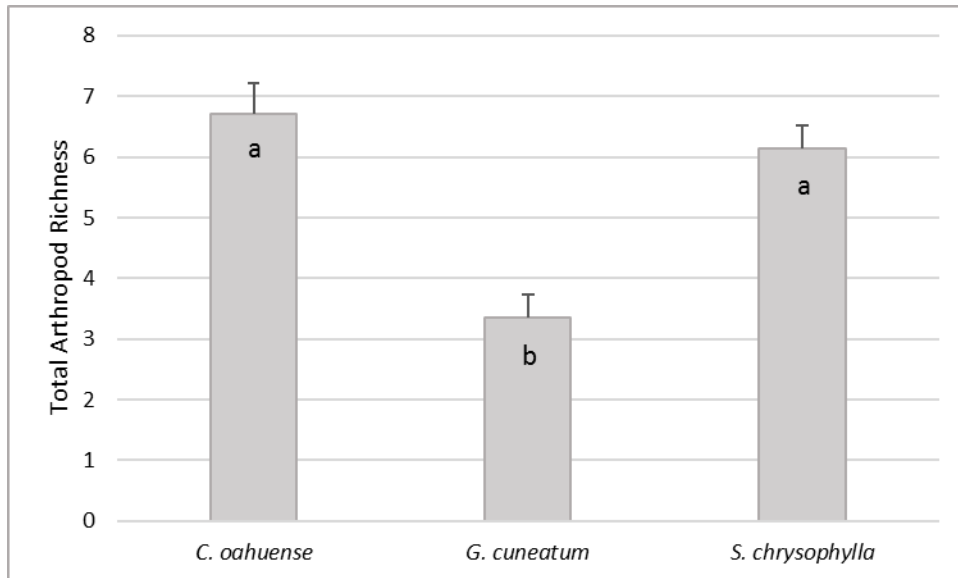


Figure 10. Comparison arthropod species richness between three native plant species in the subalpine region of the Maunakea Volcano on the Island of Hawai‘i. Mean arthropod species richness varied significantly among plant species, with *G. cuneatum* significantly lower in arthropod species richness than the other two plant species. Statistically significant differences determined by an ANOVA and Tukey post hoc analysis are indicated by lower case letters.

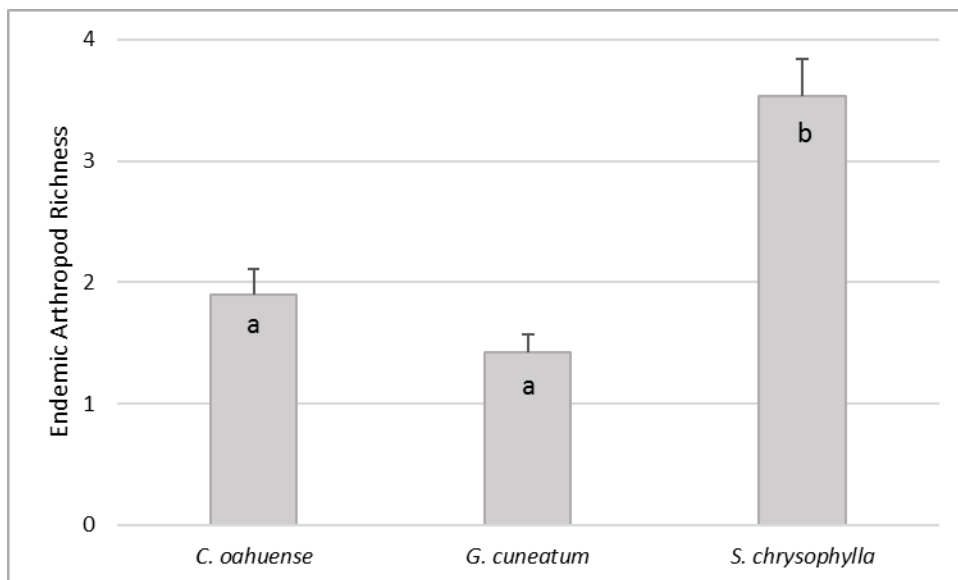


Figure 11. Comparison of endemic arthropod species richness between three native plant species in the subalpine region of the Maunakea Volcano on the Island of Hawai‘i. Mean endemic arthropod species richness varied significantly among plant species, with *S. chrysophylla* significantly higher in endemic arthropod species richness than the other two plant species. Statistically significant differences determined by an ANOVA and Tukey post hoc analysis are indicated by lower case letters.

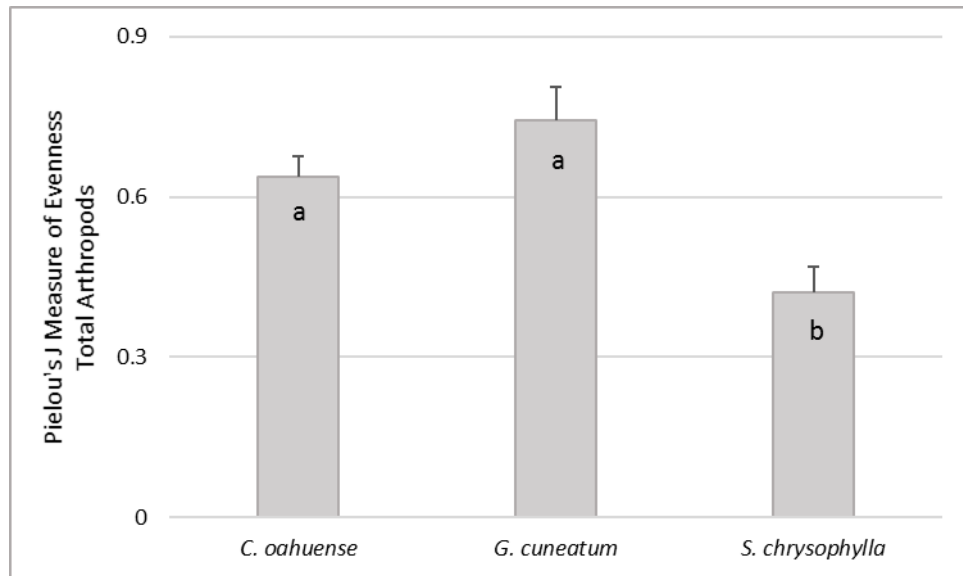


Figure 12. Comparison of arthropod evenness between three native plant species in the subalpine region of the Maunakea Volcano on the Island of Hawai'i. The mean Pielou's *J* arthropod evenness varied significantly among plant species, with *S. chrysophylla* significantly lower in endemic arthropod species evenness than the other two plant species. Statistically significant differences determined by an ANOVA and Tukey post hoc analysis are indicated by lower case letters.

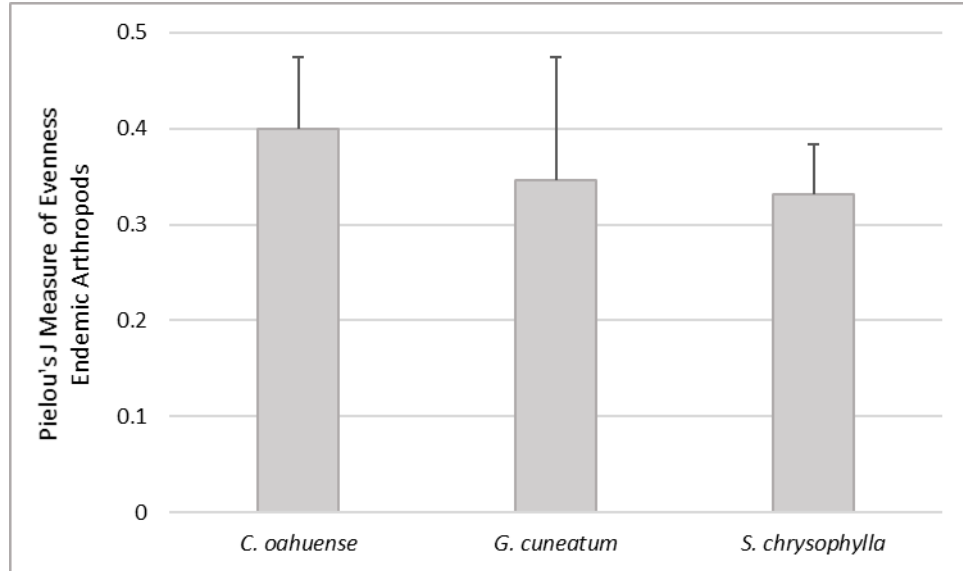


Figure 13. Comparison of endemic arthropod evenness between three native plant species in the subalpine region of the Maunakea Volcano on the Island of Hawai'i. The mean Pielou's *J* endemic arthropod evenness did not vary significantly among plant species. Lack of statistically significant differences determined by an ANOVA.

2.4.4 Multivariate Statistics

Total and endemic arthropod species richness patterns (Figure 6) indicate that there is some arthropod species overlap between *C. oahuense*, *G. cuneatum*, and *S. chrysophylla*. NMDS ordinations also indicate some overlap in total arthropod community composition between plant species when total sampling effort was combined (Figure 14). Despite this overlap, our PERMANOVA results showed that total arthropod community composition varied significantly as a function of plant species, although the effect was relatively weak ($R^2 = 0.147$, $P = 0.001$). There were insufficient endemic arthropod data for NMDS ordinations, but our PERMANOVA results showed that endemic arthropod community composition also varied significantly as a function of plant species ($R^2 = 0.290$, $P = 0.001$).

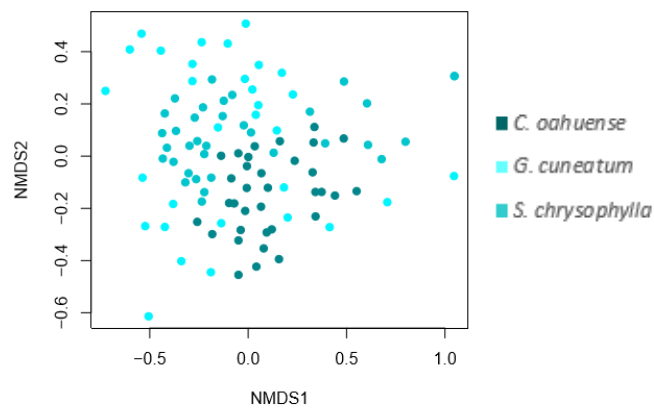


Figure 14. Nonmetric multidimensional scaling (NMDS) ordination of arthropod community composition across three native plant species in the subalpine region of the Maunakea Volcano on the Island of Hawai'i. Circles represent 35 total samples for each plant species ($n = 15$ extensive plant beat samples per plant species, and $n = 20$ intensive samples per plant species). NMDS stress = 0.15.

NMDS ordinations comparing arthropod communities collected by extensive and intensive sampling efforts for each plant species indicated that total arthropod community composition overlapped between sampling intensities for each plant species (Figure 15). Our

PERMANOVA results showed that arthropod community composition varied significantly as a function of sampling intensity for *C. oahuense* ($R^2 = 0.098$; $P = 0.001$) and *S. chrysophylla* ($R^2 = 0.191$; $P = 0.001$), but not for *G. cuneatum* ($R^2 = 0.039$; $P = 0.285$). There were insufficient endemic arthropod data for NMDS ordinations comparing sampling intensities for *C. oahuense* and *G. cuneatum*, but there were sufficient endemic arthropod data for *S. chrysophylla*, and it indicated overlap between endemic arthropod community composition collected by extensive and intensive sampling (Figure 16). Our PERMANOVA results showed that endemic arthropod diversity varied significantly as a function of sampling intensity for *S. chrysophylla* ($R^2 = 0.355$; $P = 0.001$), but not for *C. oahuense* ($R^2 = 0.048$; $P = 0.207$) or *G. cuneatum* ($R^2 = 0.054$; $P = 0.795$).

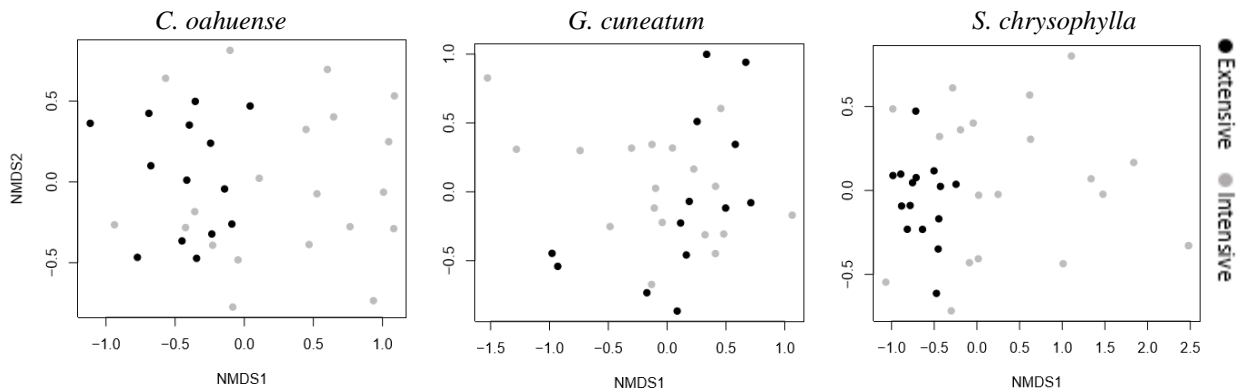


Figure 15. Nonmetric multidimensional scaling (NMDS) ordination of arthropod community composition in the subalpine region of the Maunakea Volcano on the Island of Hawai‘i across three native plant species and two levels of sampling effort intensity: (1) Extensive and (2) Intensive. Circles represent 35 total samples per plant species ($n = 15$ extensive plant beat samples; $n = 20$ intensive samples). *C. oahuense* NMDS stress = 0.22; *G. cuneatum* NMDS stress = 0.08; *S. chrysophylla* NMDS stress = 0.13.

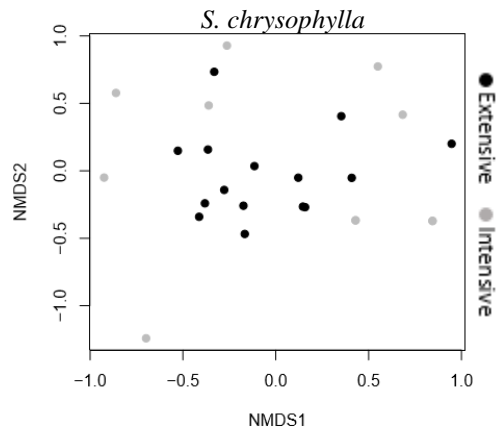


Figure 16. Nonmetric multidimensional scaling (NMDS) ordination of endemic arthropod community composition associated with *S. chrysophylla* in the subalpine region of the Maunakea Volcano on the Island of Hawai‘i across two levels of sampling effort intensity: (1) Extensive and (2) Intensive. Circles represent 35 total samples ($n = 15$ extensive plant beat samples; $n = 20$ intensive samples). NMDS stress = 0.19.

Section 2.5 Discussion

An important finding from this study was that neither Formicidae nor Vespidae were detected in any of our samples, nor were any observed while we were in the field. If social Hymenoptera were to become established in Maunakea’s subalpine region, their competition and predation pressures could reduce native arthropod diversity. Homogenization of arthropod communities would be considered a serious threat to Maunakea’s subalpine biodiversity, and is one reason why monitoring for social ants and wasps is listed as a priority in the Maunakea Comprehensive Management Plan (MKCMP 2009). Non-native arthropods occurred on all three plant species sampled during this study, indicating that generalist herbivores are non-native, but none of the non-native arthropods collected and identified in this study were considered to be particularly threatening to Maunakea’s subalpine biodiversity. For instance, there are no endemic or indigenous aphids in Hawai‘i (Nishida 2002; Messing et al. 2012), yet during this study we collected over 200 aphids on *C. oahuense*, *G. cuneatum*, and *S. chrysophylla*. Aphids were

inadvertently introduced to all the main Hawaiian Islands and are now known to feed on endemic Hawaiian plants as well as cause significant economic and agricultural damage (Messing et al. 2012). Fortunately, aphids were not particularly abundant in our samples, but these and other non-native arthropods should continue to be monitored so that potential arthropod community composition shifts can be detected.

Although most of our species accumulation curves indicate that our sampling effort did not completely detect all estimated arthropod species diversity associated with *C. oahuense*, *G. cuneatum*, and *S. chrysophylla*, the endemic arthropod diversity collected by extensive plant beat sampling was equal to the Chao 1 estimated endemic arthropod species diversity at 15 samples for *C. oahuense* and at 12 samples for *G. cuneatum*. After 15 extensive plant beat samples, the species accumulation curve for *S. chrysophylla* did not appear to approach an asymptotic plateau, and the number of observed endemic species was 11 which is nearly double the endemic arthropod species richness associated with *C. oahuense* (six species) and six times greater than the endemic species richness associated with *G. cuneatum* (two species). These disparities may be due to *S. chrysophylla*'s prevalence and relatively large size, compared to the other two plant species, which allow it to support more endemic arthropod diversity that requires additional sampling effort to fully detect.

Incomplete arthropod sampling and diversity estimations could be due to superficially high numbers of singletons and doubletons resulting from unidentifiable specimens being erroneously split into multiple morphospecies, and single non-resident arthropods that arrived through their dispersal events from other habitats and ecosystems, but are unable to survive and become established as residents. Additional definitive arthropod identifications could have decreased the numbers of singletons and doubletons in our data, thereby decreasing our Chao 1

diversity estimations since this metric uses singletons and doubletons to predict the presence of undetected species. While some unidentified and identified specimens may truly be rare, Bassett et al. (2004) suggest a variety of explanations for seemingly rare species in arthropod monitoring studies including sampling method limitations and insufficient sampling effort. Coddington et al. (2009) also suggest that undersampling rather than biological factors explain the high frequency of singletons in many arthropod surveys.

Another important factor in the high alpine ecosystem on Maunakea is likely the aeolian distribution of novel arthropod taxa that may have highly variable seasonal and annual detection (Howarth, 1987). The fact that our sampling effort did not result in sampling completeness for most plants and sampling intensities in our study may also be due to temporally inconsistent sampling between July and November 2015. Consistent sampling over time might have helped clarify some of the uncertainty in our morphospecies and immature specimen identifications by allowing us to compare arthropod diversity over time (Bassett et al. 2004). Additional samples collected between 2011 and 2014 are also available for future analysis (personal communication, Eiben 2016) such as the effects of time, elevation, or other abiotic factors of temporal changes on arthropod diversity, but these data and additional factors were not included in the scope of this study.

Our comparisons of arthropod diversity indicated that total arthropod species richness varied significantly as a function of plant species, and that *G. cuneatum* supports significantly fewer arthropod species than *C. oahuense* and *S. chrysophylla*, perhaps because of its relatively small size compared to the other two plant species. Our results also indicated that endemic arthropod species richness varied significantly as a function of plant species, and that the endemic arthropod species richness associated with *S. chrysophylla* was significantly greater

than the endemic arthropod species richness associated with *C. oahuense* and *G. cuneatum*. As previously mentioned, *S. chrysophylla* is the largest of the three plant species included in this study, and its size is likely a factor in the high species richness supported by this plant. Total arthropod evenness also varied as a function of plant species, with *S. chrysophylla* differing significantly from *C. oahuense* and *G. cuneatum*, but there was no significant difference in endemic arthropod evenness between any of the three plant species. The lack of significant differences in endemic arthropod evenness between plant species suggests that the endemic arthropods associated with each plant type are found consistently across samples even though, for example, certain species such as *Orthotylus sophoricola* are far more abundant and frequently encountered than *Nabis kahavalu* in *S. chrysophylla* samples. Endemic arthropod evenness also varied significantly as a function of plant species, and we determined that the difference was between *S. chrysophylla* and the other two plant species, but not between *C. oahuense* and *G. cuneatum*. We were unable to determine which arthropod taxa specifically influenced this difference, but the result suggests that non-native arthropods were found less consistently than the endemic arthropods associated with *S. chrysophylla*.

Although our arthropod diversity analyses indicated significant differences in arthropod species richness and evenness between certain plant species and not others, and NMDS ordinations also indicated some overlap in community composition between plant species, our PERMANOVA results showed that there are statistically significant differences in total and endemic arthropod community composition between *C. oahuense*, *G. cuneatum*, and *S. chrysophylla*. Because the arthropod communities varied between plant species, and there were instances of endemic species collected from one but not all plant species, there is a record of host-specific insects on Maunakea. Differences in arthropod community composition between

plant species also suggest that arthropod diversity in Maunakea's subalpine region is not homogenized, as might be the case if non-native arthropods were dominant in this area. Future comparisons of arthropod community composition between endemic plant species such as *C. oahuense*, *G. cuneatum*, and *S. chrysophylla* could reveal undesirable ecosystem changes that warrant natural resource management action. Ultimately, the effects of non-native species and shifts in arthropod community composition associated with these high elevation plants will depend on the novel competitive interactions that may replace host-specific endemic plant associations.

Section 2.6 Recommendations

Based on the results from this study we offer several recommendations to the OMKM and other land management entities for future arthropod inventory, research, and monitoring in Maunakea's subalpine region. For instance, simple sampling methods such as pitfall traps may decrease the amount of effort needed to sample arthropods (Morrison et al. 2012), and detect potentially harmful arthropod species such as ants that could threaten Maunakea's native flora and fauna. However, despite the functional simplicity of trapping methods such as pitfall traps, sticky cards, and leaf litter analysis, and their usefulness for detecting arthropod threats, we found that it is often more difficult to taxonomically identify many of the minute or highly degraded specimens collected by these methods as opposed to the plant beat method. For this reason, we recommend the continued use of morphospecies descriptions in future arthropod surveys on Maunakea because morphospecies are a convenient and effective way to offset taxonomic deficiencies (Oliver & Beattie 1996; Derraik et al. 2001, 2002; Bassett et al. 2004, 2012; Krushelnycky et al. 2007; Krushelnycky & Gillespie 2008; Cardoso et al. 2011; Morrison

et al. 2012), because there is an accessible reference collection of morphospecies derived from this study available in the University of Hawai‘i at Hilo College of Agriculture, Forestry, and Natural Resource Management’s Teaching and Research Arthropod Collection. We also recommend that molecular techniques such as mtDNA or nDNA barcoding for species identification be investigated as they could provide additional clarity to estimates of species richness (Moritz & Cicero 2004) especially for minute or damaged specimens as was evidenced by the identification of highly degraded fish specimens by Carvalho et al. (2015), and the identification of fish and Lepidoptera specimens of varying levels of DNA degradation by Hajibabaei et al. (2006).

Due to the October 2016 ruling by the United States Fish and Wildlife Service that seven species of Hawaiian *Hylaeus* bees are officially endangered (USFWS 2016), we recommend discontinuing the use of sticky cards as a sampling method for all future arthropod surveys in Maunakea’s subalpine region. *Hylaeus* bees are known pollinators of native plants (Magnacca 2007; MKCMP 2009; Hanna et al. 2013), and members of the *Hylaeus* genus were frequently observed during this study near *C. oahuense*, *G. cuneatum*, and *S. chrysophylla*, but none were collected in plant beats, pitfall traps, or leaf litter analysis. The only *Hylaeus* specimens that we collected were non-endangered species on sticky cards, and since this is a lethal collection method it should not be utilized in future arthropod surveys where *Hylaeus* bees are likely present, given the regulatory limitations imposed on collections of any individuals within the *Hylaeus* genus, even if non-endangered.

Since our species accumulation curves indicated that additional sampling is necessary to detect all arthropod diversity associated with *C. oahuense*, *G. cuneatum*, and *S. chrysophylla*, for an overall diversity inventory, we recommend increasing the sample size for future extensive

sampling to 20 plant beats per plant species with evaluation of species accumulation curves to determine whether sample size should be further increased. We also recommend increasing the sample size for intensive sampling to 10 plants per plant species, and excluding plant beats and sticky cards from intensive sampling efforts since plant beats are used for extensive sampling and sticky cards are likely to collect *Hylaeus* bees. Furthermore, we recommend the selection and use of indicator taxa in addition to morphospecies descriptions as another option for expediting and simplifying the assessment and monitoring of changes in arthropod diversity. Several papers suggest using a suite of indicator taxa for effective use of arthropods to effectively indicate a variety of biological and ecological factors (Kremen et al. 1993; Bassett et al. 2004; Morrison et al. 2012; Gerlach et al. 2013). Bassett et al. (2004) surveyed tropical arthropods to assess the effectiveness of using a variety of arthropod indicator taxa and taxonomic resolution to classify areas of varying anthropogenic disturbance. Their results indicated that the discriminatory power was low for datasets with arthropods identified to order, but increased when specimens were identified to family. The authors also determined that datasets that used morphospecies had variable discriminatory power, but were effective for many arthropod guilds. Based on these results, our study likely has good discriminatory power because we attempted to identify every specimen to the lowest taxonomic level, and we used morphospecies when taxonomic expertise was unavailable to identify specimens beyond order or family.

Indicator taxa may be particularly useful for future arthropod inventories, monitoring, and research on Maunakea. For instance, hand collection and release of native *Hylaeus* bees, and *Cydia* moth collection could be useful for indicating the status of pollination services and *L. bailleui* prey availability associated with *S. chrysophylla*. Furthermore, since ants and social wasps are apparently not established in our study area in Maunakea's subalpine region, the

detection of even one individual identified to only family level (Formicidae or Vespidae) would indicate a potentially serious threat that require immediate management action which may include baits with toxicants that are approved for social Hymenoptera. By using a variety of sampling methods to collect arthropods from the tops of foliage to the leaf litter and ground below the plants, our data includes many potential indicator taxa representing a wide variety of functional guilds, and Bassett et al. (2004) found that datasets that include indicator taxa regardless of inclusion or exclusion of rare morphospecies had the most discriminatory power. For our study, we were interested in overall arthropod diversity so we used as much of our data as possible to create species accumulation curves, but future surveys could be expedited with the use of indicator taxa such as the especially abundant and easily identifiable native species such as *Orthotylus sophoricola* on *S. chrysophylla*, and *Nysius terrestris* on *C. oahuense*. Although none of the arthropods collected on *G. cuneatum* were particularly abundant, and there were only two endemic species identified, the host specific leaf-hopper *Nesosydne geranii* could be an ideal indicator for that plant species.

In addition to incorporating indicator taxa into future arthropod surveys on Maunakea, we recommend that the plant species sampled should be expanded to include non-native plant species. Leblanc et al. (2013) found that many highly specialized endemic Hawaiian Drosophilidae were restricted to intact native forests, but some also seemed to persist in disturbed or mixed habitats which suggests that both native habitats as well as disturbed habitats should be monitored for native arthropods when planning conservation actions and pest management decisions. Native plants such as *S. chrysophylla* in the subalpine region on Maunakea should continue to be the main focus of arthropod surveys since these plants may prove to be especially important resources for native arthropods such as *L. bailleui* prey if

parasitism and predation pressures reduce *Cydia* and *Scotorythra* populations in lower elevation forests (Banko et al. 2002); however, the arthropod diversity associated with non-native plants such as the pervasive fireweed (*Senecio madagascariensis*) should also be assessed to possibly detect native arthropods, as well as potentially harmful invasive arthropods.

Section 2.7 Conclusion

In addition to the practical and applied utility of this study, there are larger scientific implications such as the documentation of endemic and non-native arthropods associated with native plant species in the relatively intact subalpine region on Maunakea. Furthermore, even though we were not able to identify every arthropod specimen that we collected during this study, they have all been curated and preserved for identification and research in the future.

Notwithstanding our taxonomic challenges, by collecting and identifying arthropods on *C. oahuense*, *G. cuneatum*, and *S. chrysophylla* we successfully documented endemic arthropods associated with these native plants, as well as confirmed that there were no particularly destructive ant or wasp species in any of our samples. Moreover, this study was the first to establish a comprehensive baseline arthropod inventory for *C. oahuense*, *G. cuneatum*, and *S. chrysophylla* in Maunakea's subalpine region. The OMKM has indicated that Hawaiian arthropods, specifically pollinators, are a high priority natural resource for research, inventory and monitoring on Maunakea (MKCMP 2009), and a baseline is a useful reference frame for evaluating the success or failure of conservation actions or natural resource management decisions (Bull et al. 2014). Besides establishing a baseline inventory, our arthropod species accumulation curves, diversity estimates, and community composition comparisons also provide

the OMKM with a functional framework for planning and implementing future arthropod surveys and conservation efforts on Maunakea.

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