FROM PHYLOGENETICS TO HOST PLANTS: MOLECULAR AND ECOLOGICAL INVESTIGATIONS INTO THE NATIVE URTICACEAE OF HAWAIʻI

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Abstract

The following study investigated the native Hawaiian Urticaceae in both an evolutionary and ecological context. First, the phylogenetic relationships of the native Urticaceae were determined using molecular DNA techniques. Second, the relationships between the native Urticaceae and an endemic Hawaiian specialist herbivore, *Vanessa tameamea* (Lepidoptera, Nymphalidae), were explored in order to assess variation among urticaceous species as host plants.

The family Urticaceae has undergone several taxonomic revisions in the past two decades as a result of molecular phylogenetic studies, although little phylogenetic attention has been paid to the Urticaceae taxa native to Hawai‘i despite four species being federally endangered and the presence of two endemic genera. Overall, results from the phylogenetic analysis using Bayesian inference presented here revealed that taxonomic revisions to five of the seven native-represented genera are necessary based on polyphyletic and paraphyletic relationships to other genera. Further DNA analysis is suggested to elucidate species-level relationships for the native species of *Pipturus* and several species of *Neraudia*. The analysis produced a well-supported, monophyletic Hawaiian *Urera/Touchardia* clade, and it can be inferred that a single colonization event, as opposed to the previously hypothesized two colonization events, led to the current three extant species in this clade.

Results from a no-choice bioassay experiment revealed that *V. tameamea* performed best on two native, but distantly related species, *Urera glabra* (tribe Urticeae) and *Pipturus albidus* (tribe Boehmerieae). Additionally, caterpillars from both O‘ahu populations recognized and readily ate the non-native *C. obtusifolia*, although caterpillars from Hawai‘i Island reared on this plant diet did not recognize *C. obtusifolia* as a food source and subsequently died within their

first instar. No significant correlations were found between putative defense or nutritive leaf traits and the metrics of performance. Thus, it remains unclear what factors underlie variation among plant species in suitability as host plants for *V. tameamea*. The bioassay experiment highlights the complex relationships between a herbivore and its host plants.

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Preface

The following thesis is a merger of what appears to be two very different fields of study: molecular phylogenetics and plant-herbivore interactions. Yet, understanding the evolutionary relationships of the native Urticaceae to each other and to other non-native species in the nettle family provided a much more interesting platform to test *Vanessa tameamea* (Kamehameha butterfly) performance across different larval plant diets. In addition, I was able to test the native specialist herbivore's recognition and acceptance of a non-native plant diet. Ultimately, the Kamehameha butterfly performed best on two distantly-related native species of Urticaceae and exhibited population-level differences in larval acceptance of the non-native *Cecropia obtusifolia* diet. My results highlight the complex relationships between herbivores and their host plants and the continued need to explore them.

Chapter 1

Phylogenetic analysis of Hawaiian Urticaceae using nuclear and chloroplast gene regions

Introduction

The Hawaiian Islands are home to fifteen recognized species in Urticaceae that are currently divided across seven genera: *Boehmeria*, *Hesperocnide*, *Neraudia*, *Pilea*, *Pipturus*, *Touchardia*, and *Urera* (Figure 1.1, Table 1.1, Wagner et al. 1999). Similar to many of the ca. 2600 species in Urticaceae, the native taxa have simple leaves often containing cystoliths; stipules that are usually present; flowers that are reduced in size, unisexual, and wind-pollinated resulting in the plants being monoecious, dioecious, or gynodioecious; staminate flowers with stamens inflexed at bud and equal to the number of sepals; pistillate flowers containing a single pistil and one-celled superior ovary; and fruit that is an achene (Friis 1993; Wagner et al. 1999; APG IV 2016; Christenhusz & Byng 2016; Stevens 2017). Consistent with many other native-represented families of the Hawaiian Archipelago, the native Urticaceae exhibit a high degree of endemism. Both *Neraudia* and the monotypic genus *Touchardia* are endemic genera to the Hawaiian Islands, and all but one of the fifteen species are endemic to Hawai‘i, with eight species being single island endemics. *Pilea peploides* is the lone indigenous exception (Wagner et al. 1999).

Several of the native urticaceous species are well known due to their economic value and/or cultural importance in Hawai‘i. *Touchardia latifolia* Gaud. (*olonā*) was historically harvested by Hawaiians to make cordage. Its fibers, anatomically recognized as laticifers and given the distinction of being the strongest known natural fibers in the world, produce a very durable and salt-resistant cordage that is well suited for fishing lines and nets (MacCaughey

1918; Funk 1979; Funk 1982; Abbott 1992; Krauss 1993; Loeffler & Morden 2003). Species of *Pipturus* and *Urera* were also used to make cordage. In addition, Hawaiians harvested *Pipturus* leaves to make a tea (Abbott 1992), and today *Pipturus albidus* (Hook. & Arnott) A. Gray is being commercially grown on multiple Hawaiian Islands for the same purpose. Lastly, *Neraudia*, *Pipturus* and *Boehmeria* (*grandis*) were harvested to make *kapa*, a type of cloth (Funk 1982; Abbott 1992).

The native Urticaceae are also ecologically important to Hawai‘i. The native taxa occupy dry to mesic to wet forest to subalpine habitats from less than 50m to over 2600m elevation (Wagner et al. 1999). Many of these species are known host plants to the archipelago's insect fauna including one of two native butterflies to Hawai‘i, *Vanessa tameamea*, the Kamehameha butterfly (See Chapter 2; Giffard 1922; Swezey 1924; Swezey 1954; Tabashnik et al. 1992). Additionally, the fleshy fruits of *Pipturus albidus* are known to be consumed by the Hawaiian Crow (*Corvus hawaiiensis*), or *‘alala*, but it is presumed that the fruits of many of these species were part of the diets of other native frugivorous birds (National Research Council 1992; Loeffler & Morden 2003; Culliney et al. 2012). Unfortunately, due to a suite of threats from alien plant competition, depredation by feral pigs and sheep, introduced pathogens (e.g. *māmaki* rust), and climate change, native Urticaceae are facing serious threats and some species have declined in numbers and/or are found in extremely restricted ranges (World Conservation Monitoring Centre 1998a, b; Fortini et al. 2013; Keir et al. 2015; Weisenberger et al. 2015; [HDOA] 2016; Keir et al. 2016; Yoshioka et al. 2017). Additionally, four of these species have been placed on the USFWS endangered species list: *Neraudia angulata* R. Cowan, *N. ovata* Gaud., *N. sericea* Gaud., and *Urera kaalae* Wawra ([USFWS] 1991, 1994, 1996).

In spite of the fact that many Hawaiian Urticaceae are threatened, their phylogenetic relationships remain poorly known (Wagner et al. 1999). Some Hawaiian-represented genera have been shown via molecular studies to be paraphyletic or polyphyletic to other genera. For example, three phylogenetic studies have shown that the genus *Hesperocnide* is paraphyletic with respect to *Urtica* (Wu et al. 2013; Kim et al. 2015; Grosse-Veldmann et al. 2016). In addition, *Hesperocnide sandwicensis* has been called into question as a unique species (Grosse-Veldmann et al. 2016). Wu et al. (2013) have shown that the genus *Boehmeria* is polyphyletic with respect to other genera within the tribe Boehmerieae, though at least one *Boehmeria* species has been moved to the genus *Pouzolzia* (Wilmot-Dear et al. 2009). Furthermore, distantly related genera within Urticaceae as a whole show remarkable character homoplasy that have been difficult for placement of species in monophyletic genera (Wu et al. 2015). For example, *Touchardia* has been placed in the tribe Boehmerieae, though phylogenetic data support its inclusion in Urticeae, a tribe of species distantly-related to species in Boehmerieae (Friis 1993; Wu et al. 2013).

Multiple studies over the past fifteen years have included phylogenetic analyses of Urticaceae (Sytsma et al. 2002; Hadiah et al. 2003; Monro 2006; Hadiah et al. 2008; Jestrow et al. 2012; Wu et al. 2013; Kim et al. 2015; Grosse-Veldmann et al. 2016; Treiber et al. 2016). Several of these studies have included representative species of the Hawaiian taxa (Table 1.1). However, one third of the Hawaiian species have never been placed in a phylogenetic context, including all federally endangered species. Phylogenetic studies have played a key role in plant conservation by yielding important information for natural resource managers that can enable them to make informed decisions regarding the conservation of native plant taxa (Buerki et al. 2010; Namoff et al. 2010; Morden et al. 2015). In order to implement successful management

initiatives for the conservation of native taxa, especially with regard to endangered species, it is essential to understand whether the current taxonomic nomenclature at the genus and species levels represent monophyletic genera and genetically distinct species. With the addition of genera-rich phylogenetic studies of Urticaceae (e.g., Sytsma et al. 2002; Wu et al. 2013; Kim et al. 2015; Grosse-Veldmann et al. 2016), it is now possible to incorporate all of the Hawaiian taxa into both a broad evolutionary framework as well as a narrower scope in order to obtain evolutionary relationships at the genus and species levels.

The following study incorporated nuclear and chloroplast sequences of the 15 native Urticaceae taxa into the greater phylogenetic framework of the family established by previous authors in order to determine whether the current taxonomic nomenclature reflects the evolutionary monophylies of the native taxa, especially with regard to rare/endangered taxa. Several of the same nuclear (ITS) and chloroplast gene sequences (*rbcL, trnL-trnF* spacer, and *rpl14-rps8-infA-rpl36* spacer) as were used by Wu et al. (2013) were applied here. Ultimately, placing all urticaceous species native to Hawai‘i in a comprehensive phylogenetic tree produces evidence that will support or refute current taxonomic nomenclature for the native Urticaceae taxa of Hawai‘i and assist with management initiatives for these taxa.

Materials and Methods

Materials and *DNA extractions*

A total of 47 individual plants were examined (Table 1.2). Plants were sampled from recent field collections that were preserved in silica gel, collected from herbarium specimens obtained from National Tropical Botanical Garden (PTBG) or Bishop Museum (BISH), or were obtained from previously extracted and preserved DNA accessions in the Hawaiian Plant DNA

Library (HPDL) (Morden et al. 1996; Randell & Morden 1999). All plant DNA was given a HPDL identification number after extraction.

For plants sampled in this study, DNA was extracted using the CTAB method by Doyle (1987) with some modifications (Morden et al. 1996) or a modified extraction protocol using the Qiagen DNeasy® Plant Mini Kit (Qiagen, Santa Clarita, California) for several herbarium specimen samples (Costa & Roberts 2014). The concentration and quality of DNA were determined using Nano Drop Spectrophotometer (ND-1000, v 3.8.1, Thermo Scientific, Waltham, Massachusetts). All DNA samples were diluted to 10-15ng/µl and stored at -20°C until used.

GenBank sequences

GenBank was gleaned for relevant Urticaceae species' sequences. In particular, sequences from Monro (2006), Hadiah et al. (2008), Liao et al. (2009), Wu et al. (2013), Henning et al. (2014), Kim et al. (2015), and Grosse-Veldmann et al. (2016) were used the most (Appendix A1, Table A1.1). The majority of species sequenced in Wu et al. (2013) were used for the phylogenetic analysis in order to place the native Hawaiian Urticaceae into a broad evolutionary framework. Other sequences that were most often selected for phylogenetic analysis were from species of most relevance to the Hawaiian taxa (e.g., from the following clades: *Urtica/Hesperocnide*, *Urera/Obetia/Poikilospermum*, *Boehmeria*, *Pipturus/Nothocnide*, *Neraudia*, and *Pilea*). In most cases, only species' accessions in GenBank represented by two or more of the four gene regions of interest (see below) were chosen for this study. In several instances, species' accessions were included in the analysis that were only represented by one gene region because of their relevance to the Hawaiian taxa (e.g., *Nothocnide repanda*). Several

relevant species and their respective sequences were left out of the final analysis if it became apparent during preliminary data analysis that species were identified incorrectly. When possible, voucher specimens were checked to verify species identifications.

Species Sequence Divergence

One nuclear DNA (ITS) and three chloroplast DNA gene regions (*rbcL*, *trnL-trnF* spacer, and *rpl14-rps8-infA-rpl36* spacer) were tested for sequence variation. These regions were used in the most comprehensive phylogenetic analysis of Urticaceae to date (Wu et al. 2013). The gene regions are used for providing overall generic structure relations within the family (*rbcL*) or are considered faster-evolving loci (ITS and chloroplast spacers) that are better at determining genus level resolutions that are of primary interest for this study. Samples were PCR amplified in 25 µl volumes under the following conditions: 25 ng of DNA, ca. 0.2 mM each of dATP, dCTP, dGTP, dTTP, 1X *Taq* Polymerase buffer (10mM Tris-HCL [pH 9.0 at 25°C], 50mM KCL, and 0.1% Triton X-100 [Promega]), 1.5mM MgCl2, 0.50 mg BSA, 0.2mM forward and reverse primers (White et al. 1990; Taberlet et al. 1991; Fay et al. 1997; Shaw et al. 2007), and ca. 1 unit of *Taq* DNA Polymerase (Promega, Madison, Wisconsin, USA). PCR amplifications were performed on a MJ Research PTC-200 DNA thermal cycler (MJ Research, Waltham, Massachusetts) using one of two sets of reaction conditions. ITS PCR amplifications were subjected to an initial 95°C for 2 minutes, denaturation at 93°C for 1 minute, annealing at 51°C for 1 minute and extension for 2 minutes each at 72°C for 30 cycles. For all chloroplast PCR amplifications, DNA underwent the same amplification protocol except that annealing occurred at 55°C for 1 minute instead of 51°C. Negative control reactions were run without DNA for all PCR amplifications to ensure reaction components were uncontaminated. PCR amplified

products were loaded on 1.0% agarose gel, stained with EtBr and visualized with an ultraviolet light source. Size of amplification product was estimated using the 100 kb ladder (Promega). Final gel products were viewed using Gel Doc XR (BIO-RAD, Hercules, California, USA) and digitally recorded on Quantity One software (BIO-RAD, v.4.5.1). The PCR products were cleaned using Exo-Sap-It (Affymetrix, Thermo Scientific) according to the manufacturer's instructions. Double stranded PCR products were bi-directionally sequenced using amplification and internal primers as needed at the ASGPB Sequencing Facility (http://cgpbr.hawaii.edu/) of the University of Hawai'i using BigDye Terminator chemistry (Applied Biosystems, Foster City, California) and visualized on an ABI 3730XL capillary-based DNA sequencer (Applied Biosystems).

Sequence Data Analysis

Sequence results were edited and concatenated using Sequencher® v.5.0 (Gene Codes Corporation, Ann Arbor, MI USA http://www.genecodes.com) and aligned using MEGA v7.0.20-mac (Kumar et al. 2016) by Muscle function with default parameters. Single region and combined (ITS and 3 chloroplast gene regions) trees were produced using Bayesian inference in the following manner. Aligned sequences for each gene region, or in the case of the combined tree, aligned sequences were combined for all regions into one MEGA file, and exported as nexus files from MEGA to Mesquite v3.31-mac (Maddison & Maddison 2017), and reformatted as new nexus files for the online CIPRES Science Gateway portal (Miller et al. 2010). Phylogenetic analysis was performed by Bayesian inference using MrBayes XSEDE 3.2.6 via the CIPRES Science Gateway (Ronquist & Huelsenbeck 2003). Bayesian inference was run using a GTR substitution model with gamma-distributed rate variation across sites and a

proportion of invariable sites. Four Markov chain Monte Carlo simulations were run simultaneously and sampled every 1000 generations for a total of 1 million generations with a 25% burn-in (i.e., first 250,000 sample trees were discarded). Fig Tree v1.4.3 (http://tree.bio.ed.ac.uk/, (Rambaut 2016) was used to visualize the Tree Annotator output files.

Results

Forty-seven individual specimens representing the 15 native Hawaiian species and two non-native species (*Urtica urens* and *Hesperocnide tenella*) were successfully sequenced. Urticaceae species were sequenced across one nuclear and 3 chloroplast gene regions. For some individuals, all four gene regions were not able to be sequenced, but all sequences of interest were obtained for the majority of individual specimens. Using Bayesian inference (BI), the 15 native Urticaceae to Hawai‘i were incorporated into a phylogenetic tree that encompassed 44 of the ca. 53 genera in the family (Figure 1.2, Christenhusz & Byng 2016). Three species from Cannabaceae (*Cannabis sativa*, *Humulus scandens*, and *Celtis kunmingensis*) and two accessions of *Fatuoa villosa* (Moraceae) were used for outgroup comparison. Single gene region trees using BI for each of the four gene regions of interest in this study were examined prior to combining sequences for the final analysis (Appendix, Figures A1.1-A1.4).

Hesperocnide/Urtica (Tribe Urticeae)

Results from the combined BI phylogenetic tree shows that *Hesperocnide* is paraphyletic with respect to *Urtica* (Figures 1.3). *H. sandwicensis* is nested within the *H. tenella* clade for the combined BI tree and all chloroplast single region consensus trees. The ITS BI tree shows distinct clades for *H. sandwicensis* and *H. tenella* (i.e., distinct species, Figure A1). Removal of

H. tenella sequences from Grosse-Veldmann et al. (2016) also results in combined tree showing distinct species separation between *H. sandwicensis* and *H. tenella* (data not shown).

Urera/Touchardia (Tribe Urticeae)

Results from the combined BI phylogenetic tree show that *Touchardia latifolia*, *Urera glabra*, and *U. kaalae* form a distinct clade that is sister to the clade the contains *Obetia*, *Poikilospermum*, and other non-Hawaiian *Urera* from the Americas, Asia, Pacific and Africa (Figures 1.3). The single-region BI ITS tree shows that the Hawaiian *Urera*/*Touchardia* clade is sister to the *Poikilospermum* clade but with low support (Figure A1.1).

Pilea peploides (Tribe Elatostemateae)

In congruence with other phylogenetic studies, *Pilea peploides* is found in the monophyletic *Pilea* clade (Monro 2006, Wu et al. 2013) based on BI combined data set and individual gene region BI analysis (Figures 1.4, A1.1-A1.4). *Sarcopilea domingensis*, the single species in the genus *Sarcopilea* that was shown to be paraphyletic to *Pilea* by Jestrow et al. (2012) and Wu et al. (2013), has undergone a name change based on morphological and phylogenetic review and was designated in this study as *Pilea fairchildiana* (Jestrow et al. 2012). This name change was reflected in Figure 1.4. Both *Pilea peploides* specimens from Hawai‘i formed a monophyletic clade with a *P. peploides* var. *major* specimen from Taiwan, supporting the species' current indigenous status. *Pilea peploides* is more closely related to *P. lapestris* and *P. cavaleriei* subsp. *cavaleriei* than other *Pilea* species used in this study. Monro (2006) previously demonstrated the close relationship between these three species of *Pilea*.

Neraudia/Pipturus (Tribe Boehmerieae)

Based on results from the combined gene-region tree using BI, *Neraudia* forms a monophyletic clade that is sister to a *Pouzolzia* clade and the paraphyletic *Nothocnide*/*Pipturus* clade (Figure 1.5). *Neraudia kauaiensis* forms a clade that is sister to the remainder of the *Neraudia* clade. *Neraudia angulata*, an O‘ahu endemic, forms its own branch within the clade sister to *N. kauaiensis*, but species relationships between the other three species of *Neraudia* are not resolved.

The Hawaiian *Pipturus* forms a distinct clade within the paraphyletic *Nothocnide*/*Pipturus* clade based on the combined tree using BI, although the relationships between species remain unresolved (Figure 1.5). *Pipturus arborescens*, *P. argenteus*, *Nothocnide mollisma*, and *N. repanda* branch within the same clade that includes the Hawaiian *Pipturus* subclade.

Boehmeria grandis (Tribe Boehmerieae)

Based on results from the combined BI gene-region tree*, B. grandis* is placed in the well-supported *Boehmeria* clade that contains *Boehmeria* species native to Southeast Asia (Figure 1.6). There is some divergence between *B. grandis* individuals from O‘ahu and Kaua‘i. *B. platphylla*, a species that was thought to be closely related to *B. grandis* by Wagner et al. (1999) was found to be more distantly related (based on a single ITS region) to *B. grandis* than other species of *Boehmeria.*

Discussion

The Hawaiian Urticaceae represent a morphologically and genetically diverse group of species. All 15 currently recognized species of Urticaceae that are native to Hawai‘i were sequenced and placed in a large, comprehensive phylogenetic tree via the inclusion of available GenBank sequences of Urticaceae species.

Results for the genus *Hesperocnide* concur with previous phylogenetic studies that demonstrate *Hesperocnide* is paraphyletic with respect to *Urtica* (Wu et al. 2013; Kim et al. 2015; Grosse-Veldmann et al. 2016). There are very few morphological distinctions between the two genera. The most striking differences between these two genera based on genus descriptions is with regard to stipule size and connation of the pistillate calyx (Wagner et al. 1999). Stipules are prominent in *Urtica* whereas stipules are minute in *Hesperocnide* (Wagner et al. 1999). In *Hesperocnide*, the pistillate calyx is connate (Wagner et al. 1999). In *Urtica*, the pistillate calyx is described as being four-lobed with the lobes being "nearly distinct," which suggests that the lobes are also actually connate (Wagner et al. 1999). Thus, it would be relatively easy to dissolve *Hesperocnide* as a genus and subsume its species into *Urtica* based on such slight morphological differences. Indeed, a previous synonym for *H. sandwicensis* (Wedd.) is *Urtica sandwicensis* Wedd. (Wagner et al. 1999).

The phylogenetic placement of *Hesperocnide sandwicensis* nested within the *H. tenella* clade based on the combined sequence results supports the prediction by Grosse-Veldmann et al. (2016) that *H. sandwicensis* is not a distinct species based on the molecular species concept. Yet, the ITS single region BI tree and interestingly, removal of *Hesperocnide tenella* accessions that lack all four gene sequences in the analysis (i.e., *H. tenella* 331, 2026, and 2586) show clear genetic separation between the two species (data not shown), and therefore supports the distinction of two separate *Hesperocnide* species. Wagner et al. (1999) describe *H. sandwicensis*

and *H. tenella* as "clearly distinct" species based solely on vegetative characteristics. They describe *H. tenella* as having "less coarsely, but more deeply divided leaves and in general is a more delicate plant [compared to *H. sandwicensis*]." Plants in general can exhibit extensive plasticity in their vegetative characteristics depending on location. Based on the results from this study, there appears to be species resolution among the two species of *Hesperocnide* based on the DNA sequencing. Ideally, a key for this ditypic genus would include both vegetative and reproductive characters. Interestingly, it was also posited by Hillebrand (1888) and referenced in Wagner et al. (1999) that *H. sandwicensis* may represent a recent colonization possibly brought over by a cattle introduction in the late 1700s. If this is found to be true based on further analysis (e.g., molecular clock dating), then *H. sandwicensis* could represent a native species that arose post-human contact in the Hawaiian Islands and further complicate the native species definition for island archipelagoes. Further and more detailed molecular analysis of populations of *H. tenella* across its native range should be made to verify or refute this possibility.

In the combined gene-region BI tree, the Hawaiian *Urera/Touchardia* clade was highly supported as sister to the clade containing *Urera/Obetia/Poikilospermum* species from Asia, Africa, and South America. Thus, based on this analysis, it can be inferred that a single colonization event led to the speciation of two *Urera* species (*U. glabra* and *U. kaalae*) and *Touchardia latifolia*. Wagner et al. (1999) and others have previously hypothesized that the Hawaiian *Urera/Touchardia* species represent two different colonization events, because of the morphological differences between *U. kaalae* to that of *U. glabra* and *T. latifolia*. For example, *U. kaalae* is monoecious or dioecious, whereas *U. glabra* and *T. latifolia* are strictly dioecious. In addition, *U. glabra* has dichotomous paniculate cymes and lanceolate to ovate leaves (or derivations of these leaf types), whereas *U. kaalae* has cordate leaves and trichotomous

paniculate cymes. The placement of the Hawaiian *Urera/Touchardia* clade in this paper's analysis does not agree with Wu et al. (2013)'s phylogenetic analysis. Their results placed *Urera glabra* and *Touchardia latifolia* in a clade sister to the South American *Urera*, although they did not sequence *U. kaalae*. The different results may be due to the addition of more *Urera/Obetia/Poikilospermum* sequences in this paper's analysis. Although the placement of the Hawaiian *Urera/Touchardia* within the tribe Urticeae is not consistent between this study and Wu et al. (2013), there is very little genetic dissimilarity between *U. glabra*, *U. kaalae*, and *T. latifolia*, and therefore the results from the BI combined tree highly support that a single colonization event resulted in the following three extant species.

Results from the combined BI tree support the current indigenous status of *Pilea peploides* based on the inclusion of single *trnL-trnF* sequence from a *P. peploides var. major* specimen from Taiwan. The genus *Pilea* contains over 600 species (Burger 1977; Monro 2006), and thus without including a large number of *Pilea* species in the phylogenetic analysis, it is difficult to pinpoint the closest extant relative to *P. peploides*. Sequences from species that were made available on GenBank from Monro (2006) and that were shown in that paper to be the most closely related to *P. peploides* (including the previously mentioned *P. peploides var. major*) were included in this study's phylogenetic analysis. *P. lapestris* is the most genetically similar species to *P. peploides* in the combined gene BI tree. This species is native to Indonesia (Monro 2004).

The genus *Pipturus* was found to be paraphyletic with respect to *Nothocnide*. One of the main differences between species placed in *Nothocnide* versus *Pipturus* is habit. *Pipturus* species are shrubs to small trees whereas species in the genus *Nothocnide* are lianas (Chew 1969). Based on the paraphyly of *Nothocnide* and *Pipturus*, it is suggested that the genus *Nothocnide* is

dissolved as it is a congeneric for *Pipturus* (as originally detailed by H.A. Weddell in his 1856-1857 monograph of the family Urticaeae) and the four known species in the genus *Nothocnide* are moved into the genus *Pipturus* (Chew 1969).

No species level resolution for the *Pipturus* species native to Hawai‘i was obtained in this study. Although Hawaiian species of *Pipturus* separate into their own subclade within the paraphyletic *Pipturus/Nothocnide* clade, the non-Hawaiian *Pipturus* and *Nothocnide* branch equally from the main clade. The number of species of *Pipturus* that are native to Hawai‘i has fluctuated greatly over the years. A morphological study on the Hawaiian *Pipturus* led Nicharat and Gillett (1970) to conclude that only two species of *Pipturus* were warranted for the archipelago. The same authors concluded that many of the species denoted by C. Skottsberg and others represent intraspecific variation and/or hybrid introgression. Further DNA sequencing that includes additional nuclear regions may be able to tease apart species that may or may not be consistent with the current species delimitations for the native taxa of this genus. If genetic divergence is not seen across the native *Pipturus* taxa, it should be questioned whether the current taxonomic delimitations of the Hawaiian species of *Pipturus* are valid at the species level. Three of the four native *Pipturus* species are single island endemics, and thus from a conservation perspective and in order to maintain genetic diversity of the native *Pipturus*, it would be worthwhile to conduct a population genetics study on *Pipturus* in order to understand island-level divergence for the native *Pipturus*.

Unlike the genus *Pipturus*, the endemic genus *Neraudia* was found to be highly supported as monophyletic. The genus *Neraudia* is very closely related to the genus *Pipturus* with the *Neraudia* clade being sister to a larger clade that contains several *Pouzolzia* species and the paraphyletic *Nothocnide*/*Pipturus* clade. Similar to the phylogenetic results of the Hawaiian

Pipturus, very little species resolution was found for the native *Neraudia*. The combined gene region BI analysis shows that the *N. kauaiensis* clade is sister to the remaining *Neraudia* clade, inferring that the first colonization of the main Hawaiian Islands occurred to Kaua‘i. Additionally, *N. angulata*, an O‘ahu endemic and federally listed endangered species, groups in a separate subclade that branches from the main clade that contains the other three *Neraudia* species. *N. ovata*, a Hawai‘i Island endemic, and *N. sericea*, endemic to Maui Nui, are both endangered species. It is important for the conservation of the native *Neraudia* that further genetic sequencing at the population level be conducted in order to tease apart and understand species delimitations for the endemic *Neraudia*.

Boehmeria grandis is nested within a *Boehmeria* clade that contains other *Boehmeria* species from Southeast Asia. The genus *Boehmeria*, along with other genera in the tribe Boehmerieae, has undergone many taxonomic revisions in the past few decades by C.M. Wilmot-Dear, I. Friis and others but still remains a polyphyletic genus (see (Wilmot-Dear 1988; Wilmot-Dear & Friis 2004; Wilmot-Dear et al. 2009; Wilmot-Dear & Friis 2013). For the purpose of this study, the only *Boehmeria* speices that was assigned a new name (with respect to species names used by authors in previous phylogenetic studies) was *Boehmeria rugulosa* that was designated as *Pouzolzia rugulosa* based on Wilmot-Dear et al. (2009). In this phylogenetic study, *B. grandis* was most closely related to a *B. spicata* specimen from China. *B. spicata* is native to China, Korea and Japan (e.Floras.org). The original type species for the genus is *Boehmeria ramiflora*, a New World species, that is found in Mesoamerica to South America. New World and Old world species of *Boehmeria* separate into phylogenetically distinct clades and augment the current polyphyly of the genus. Further genetic sequencing of the polyphyletic *Boehmeria* and closely related genera (i.e., those genera nested within the polyphyly) are

necessary to delimit monophyletic genera and tease apart differences in morphology for taxonomic purposes. It is the hope of this study, that the inclusion of *B. grandis* within the *Boehmeria* phylogeny will assist with future taxonomic revisions relating to this genus.

Conclusion

The Urticaceae taxa native to Hawai‘i are genetically diverse and represent 6 different colonization events to the Hawaiian Islands. Based on the results from this phylogenetic study, revisions to the current Urticaceae taxonomy need to be made to five of the seven genera that are represented by native species to Hawai‘i (*Boehmeria*, *Hesperocnide*, *Pipturus*, *Touchardia*, and *Urera*). Thus, this study highlights the importance of including taxa endemic to oceanic archipelagoes in order to resolve generic level relationships to better represent evolutionary monophylies within the family Urticaceae.

Acknowledgments

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Tables

Table 1.1. List of species of Urticaceae that are native to Hawai‘i. Information about the native species includes: endemism, endangered species status, tribal placement, common name, habit, distribution and previously published phylogenetic studies that included the species. Tribal placement is based on consensus from Friis 1993, Hadiah et al. 2009, Wilmot-Dear & Friis 2013, and/or Wu et al. 2013. *Touchardia* is placed in Urticeae based on Wu et al. (2013). Species, common name, habit, and distribution information are from Wagner et al. (1999). Key: [a] endemic genus, [b] USFWS federally-endangered

Genus	Species	Tribe	Common name	Habit	Distribution	Previous studies
Boehmeria	*Boehmeria* grandis (Hook. & Arnott) A. Heller	Boehmerieae	‘ākōlea, false nettle	Shrub	All main HI islands, except Ni‘ihau and Kaho‘olawe	(Sytsma et al. 2002; Hadiah et al. 2008)
Hesperocnide	*Hesperocnide sandwicensis* (Wedd.) Wedd.	Urticeae	NA	Herb, annual	Hawai‘i	(Kim et al. 2015)
Neraudia[a]	*Neraudia angulata*[b] R. Cowan	Boehmerieae	NA	Shrub	O‘ahu	NA
	Neraudia kauaiensis (Hillebr.) R. Cowan		NA	Shrub	Kaua‘i	(Wu et al. 2013)
	Neraudia melastomifolia Gaud.		ma‘aloa, ma‘oloa, ‘oloa	Shrub, small tree	Kaua‘i, O‘ahu, West Maui, Moloka‘i	(Wu et al. 2013)
	Neraudia ovata[b] Gaud.		NA	Shrub	Hawai‘i	NA
	Neraudia sericea[b] Gaud.		NA	Shrub	Moloka‘i, Lāna‘i, Maui, formerly Kaho‘olawe	NA
Pilea	*Pilea peploides* (Gaud.) Hook. & Arnott	Elatostemateae	NA	Herb, short-lived perennial	All main HI islands, except Ni‘ihau and Kaho‘olawe	(Monro 2006; Hadiah et al. 2008)
Pipturus	*Pipturus albidus* (Hook. & Arnott) A. Gray	Boehmerieae	māmaki, māmake, Waimea	Shrub, small tree	All main HI islands, except Ni‘ihau and Kaho‘olawe	(Howarth et al. 2007)
	Pipturus forbesii Kraj.		māmaki, māmake	Shrub	East Maui	NA

	Pipturus kauaiensis A. Heller		māmaki, māmake	Shrub	Kauaʻi	(Wu et al. 2013)
	Pipturus ruber A. Heller		māmaki, māmake, Waimea	Shrub	Kauaʻi	(Wu et al. 2013)
Touchardia[a]	*Touchardia latifolia* Gaud.	Urticeae	Olonā	Shrub	All main HI islands, except Niʻihau and Kahoʻolawe	(Wu et al. 2013)
Urera	*Urera glabra* (Hook. & Arnott) Wedd.	Urticeae	ōpuhe, hōpue, hona	Shrub, small tree	All main HI islands, except Niʻihau and Kahoʻolawe	(Sytsma et al. 2002; Wu et al. 2013)
	Urera kaalae[b] Wawra		Ōpuhe	Shrub, small tree	Oʻahu (Waianae Mts. only)	NA

Table 1.2. New specimens sequenced for phylogenetic study of Hawaiian Urticaceae. Table includes voucher number and herbarium location, geographic location of plant collection, Hawai‘i Plant DNA Library (HPDL) identification number, and name used for phylogenetic tree. Asterisks in the column for Voucher No. signify that no voucher specimen was collected at the time that the plant specimen sequenced. See appendix (Table A1.1) for list of sequences obtained for each specimen.

Genus	Species	Voucher No. (Herbarium Location)	Location	HPDL Identification No.
Boehmeria	*B. grandis*	W. Loeffler, s.n. (HAW)	O‘ahu	1120
	B. grandis	W.P. Haines 005 (HAW)	Kaua‘i	10015
Hesperocnide	*H. sandwicensis*	Morden 1332 (HAW)	Hawai‘i	566
	H. sandwicensis	Morden 1421 (HAW)	Hawai‘i	918
	H. tenella	T. Sagar, SMM-MCSP1 (HAW)	Santa Monica Mountains, California	8089
Neraudia	*N. angulata*	*	O‘ahu	2670
	N. angulata	*	O‘ahu	2682
	N. angulata	*	O‘ahu	2953
	N. kauaiensis	Perlman 22408 (BISH)	Kaua‘i	10018
	N. kauaiensis	Wood 8793 (BISH)	Kaua‘i	10019
	N. melastomifolia	Perlman 17,093 (PTBG)	O‘ahu	2696
	N. melastomifolia	Morden, s.n. (HAW)	O‘ahu	2769
	N. ovata	Morden 1411 (HAW)	Hawai‘i	542
	N. ovata	L.M. Castle, s.n. (PTA)	Hawai‘i	5907
	N. sericea	Perlman 22293 (PTBG)	Moloka‘i	9572
	N. sericea	Oppenheimer H71301 (BISH)	West Maui	10021
Pipturus	*P. albidus*	Wood 13854 (BISH)	Kaua‘i	10022
	P. albidus	Wood 14944 (BISH)	Kaua‘i	10023
	P. albidus	W.P. Haines 001A, 001B (HAW)	East Maui	9076
	P. albidus	W.P. Haines 002 (HAW)	East Maui	9077
	P. albidus	W.P. Haines 003 (HAW)	East Maui	9079
	P. albidus	Imada 2002-19 (BISH)	East Maui	10024

	P. albidus	Morden 1105 (HAW)	Hawai‘i	124
	P. albidus	W. Loeffler, s.n. (HAW)	O‘ahu	1122
	P. albidus	V. Caraway 17 (HAW)	O‘ahu	1307
	P. albidus	Morden 1532 (HAW)	O‘ahu	1670
	P. albidus	Morden 1584 (HAW)	Hawai‘i	2156
	P. albidus	Morden 1802 (HAW)	O‘ahu	4421
	P. albidus	J.L. Birch 086 (HAW)	Hawai‘i	5437
	P. albidus	Morden 2211 (HAW)	O‘ahu	5688
	P. forbesii	Wood 15049 (PTBG)	East Maui	9571
	P. forbesii	Oppenheimer H91415 (PTBG)	East Maui	10025
	P. forbesii	Wood 6646 (BISH)	East Maui	10026
	P. kauaiensis	Lorence 10368 (BISH)	Kaua‘i	10027
	P. ruber	Wood 15551 (BISH)	Kaua‘i	10030
	P. ruber	Tangalin 2498 (BISH)	Kaua‘i	10029
	P. ruber	W.P. Haines 006 (HAW)	Kaua‘i	10016
	sp. (hybrid)	W.P. Haines 007 (HAW)	O‘ahu	10017
Pilea	*P. peploides*	Morden 1627 (HAW)	West Maui	1627
	P. peploides	Morden 1939 (HAW)	O‘ahu	1939
Touchardia	*T. latifolia*	W. Loeffler 46 (HAW)	O‘ahu	1114
Urera	*U. glabra*	Perlman 20517 (BISH)	Hawai‘i	10031
	U. glabra	Morden 1534 (HAW)	O‘ahu	1672
	U. kaalae	Morden 1533 (HAW)	O‘ahu	1671
	U. kaalae	*	O‘ahu	2086
	U. kaalae	*	O‘ahu	4687
	U. urens	Morden 1339 (HAW)	Hawai‘i	582

Figures

Figure 1.1. Photos of Urticaceae species that are native to Hawai‘i. From top left: male *Touchardia latifolia;* infructescence of female *T. latifolia* containing achenes enclosed by orange, fleshy calyx; *Boehmeria grandis* (monoecious); female *Urera glabra* with inflorescence; fruit on female *Pipturus albidus*, achenes embedded in fleshy receptacle; male *Pipturus albidus*. All photos were taken of plants in the Ko'olau Mountain range (O‘ahu).

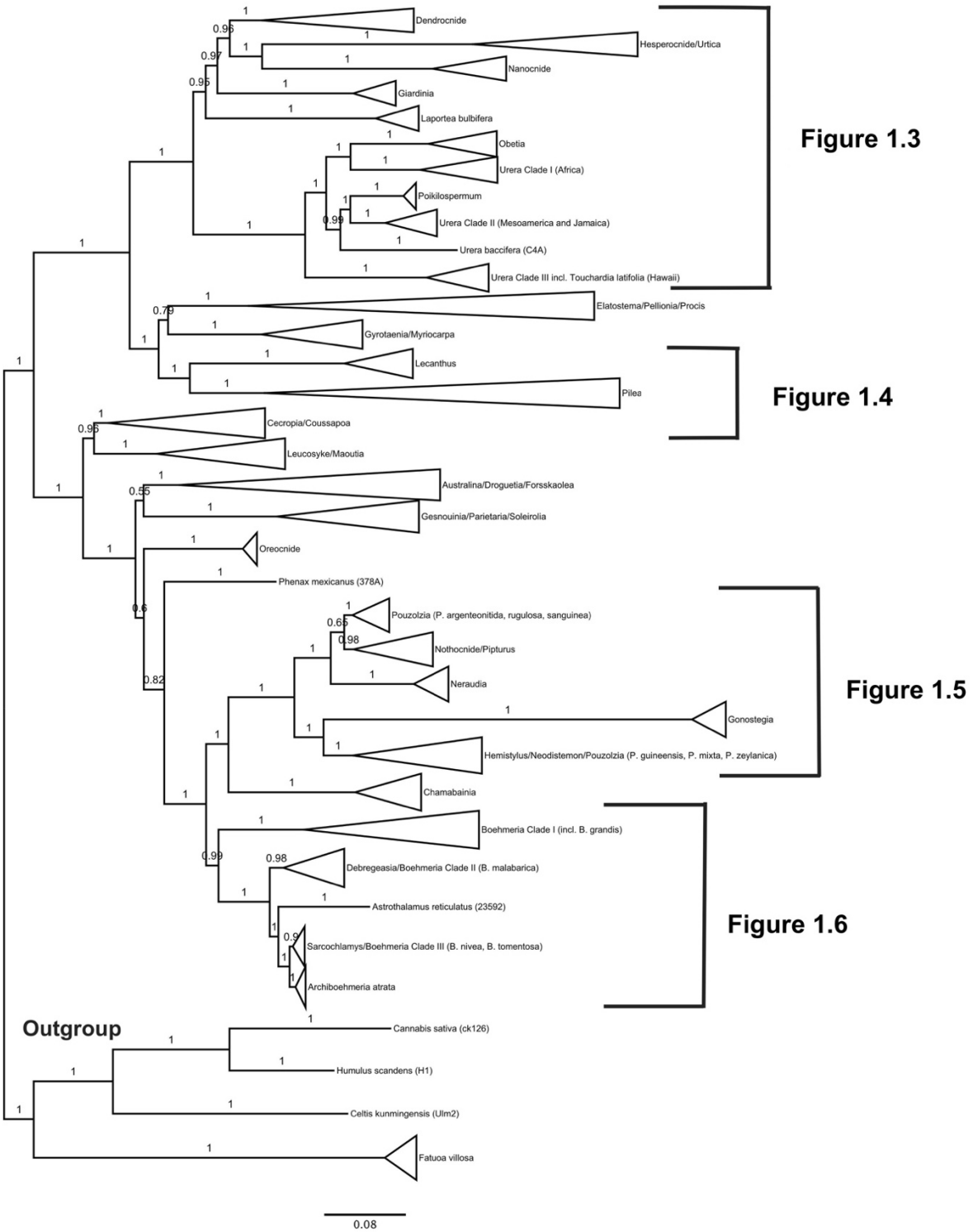

Figure 1.2. Combined four-gene region phylogenetic tree of Urticaceae based on nuclear (ITS) and chloroplast (*trnL-trnF* spacer*, rbcL,* and *rpl14-rps8-infA-rpl36* spacer) gene regions using Bayesian inference (1,000,000 iterations, 25% burn-in) for 257 accessions. Branch labels represent posterior probabilities. Wedge height represents two or more accessions present and does not represent the number of accessions associated with it.

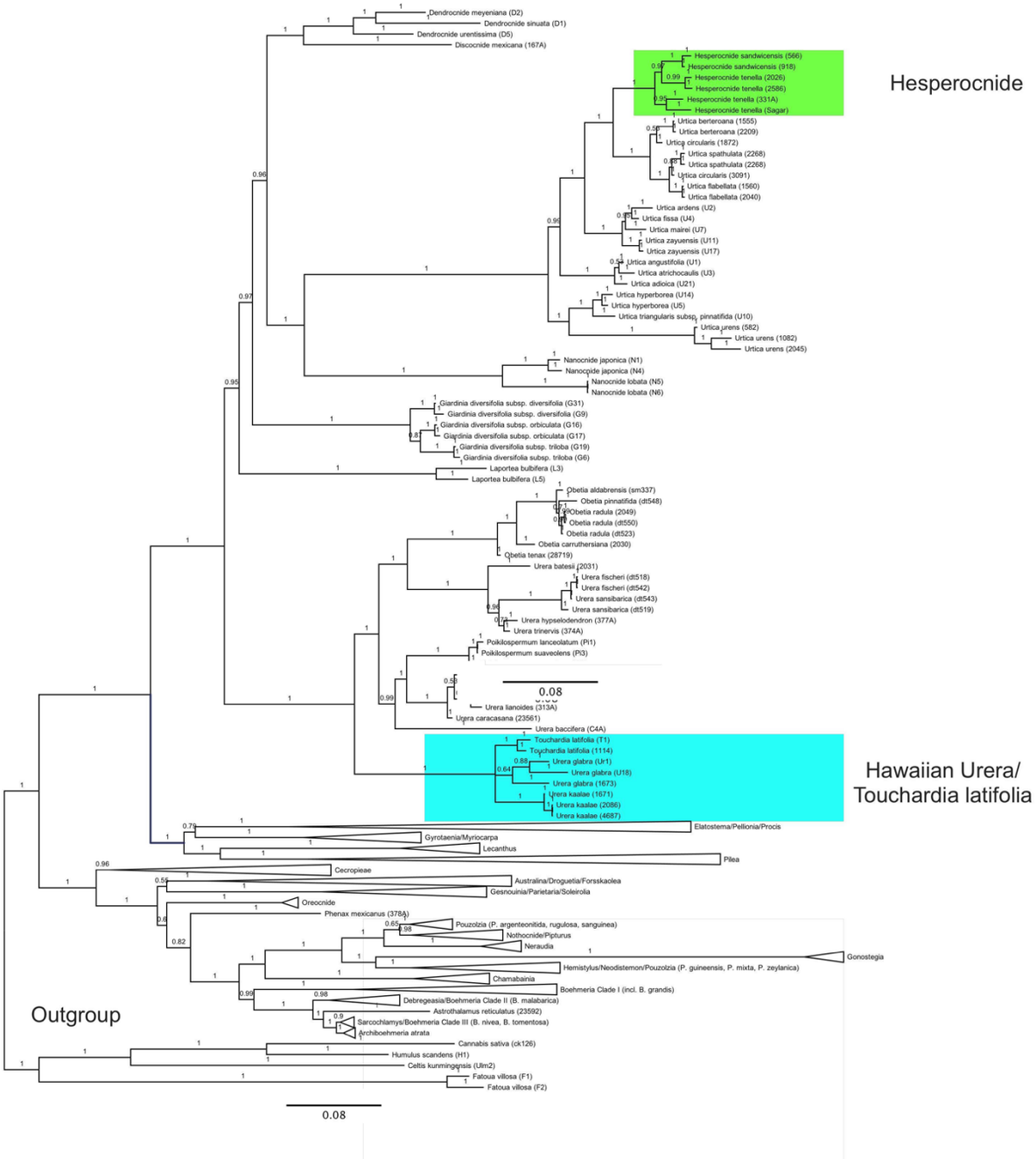

Figure 1.3. Combined four-gene region phylogenetic tree of Urticaceae based on nuclear (ITS) and chloroplast (*trnL-trnF* spacer*, rbcL,* and *rpl14-rps8-infA-rpl36* spacer) gene regions using Bayesian inference (1,000,000 iterations, 25% burn-in) for 257 accessions. Tree highlights Hawaiian species in tribe Urticeae. Branch labels represent posterior probabilities. Wedge height represents two or more accessions present and does not represent the number of accessions associated with it.

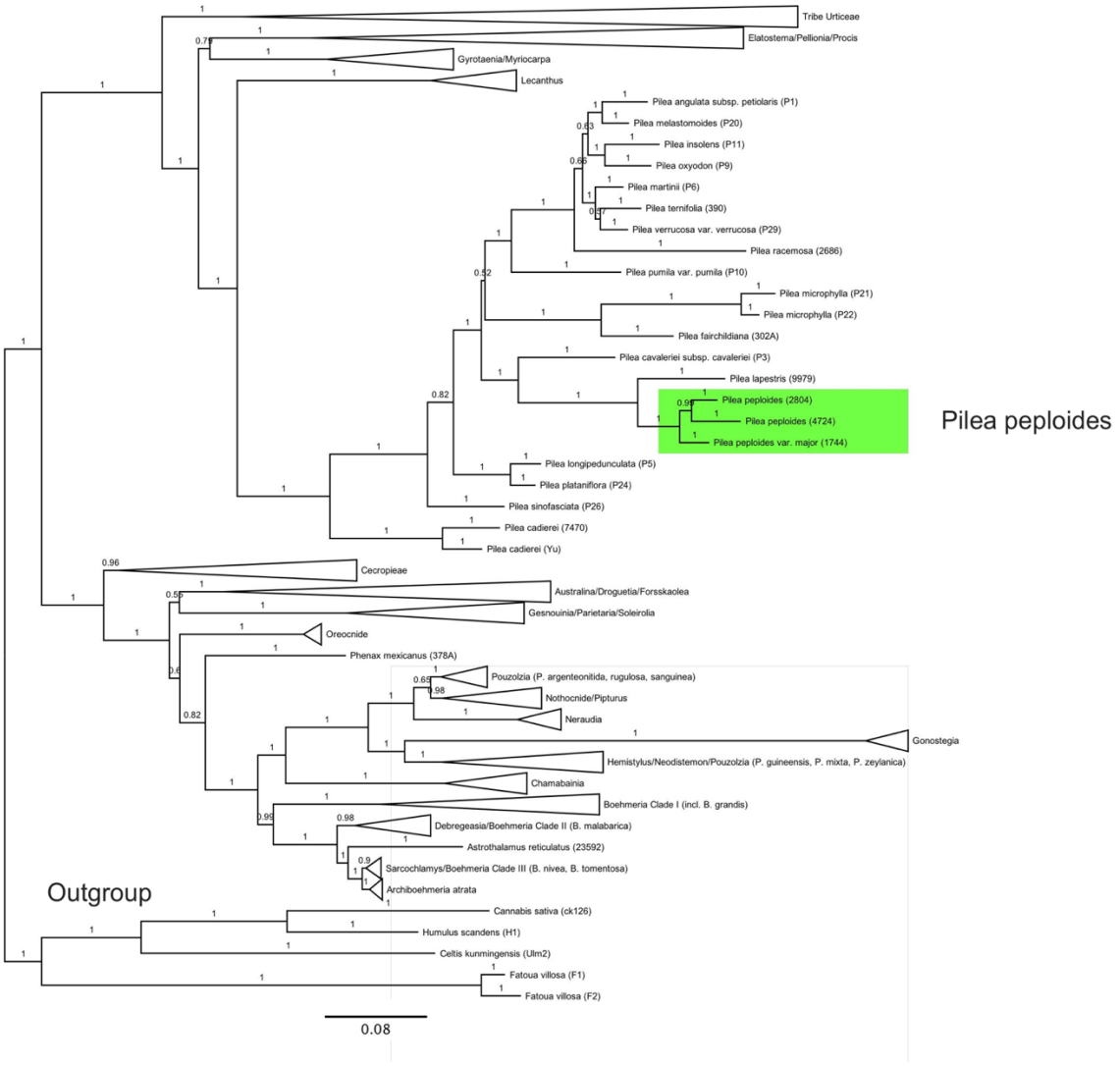

Figure 1.4. Combined four-gene region phylogenetic tree of Urticaceae based on nuclear (ITS) and chloroplast (*trnL-trnF* spacer*, rbcL,* and *rpl14-rps8-infA-rpl36* spacer) gene regions using Bayesian inference (1,000,000 iterations, 25% burn-in) for 257 accessions. Tree highlights Hawaiian species *Pilea peploides*. Branch labels represent posterior probabilities. Wedge height represents two or more accessions present and does not represent the number of accessions associated with it.

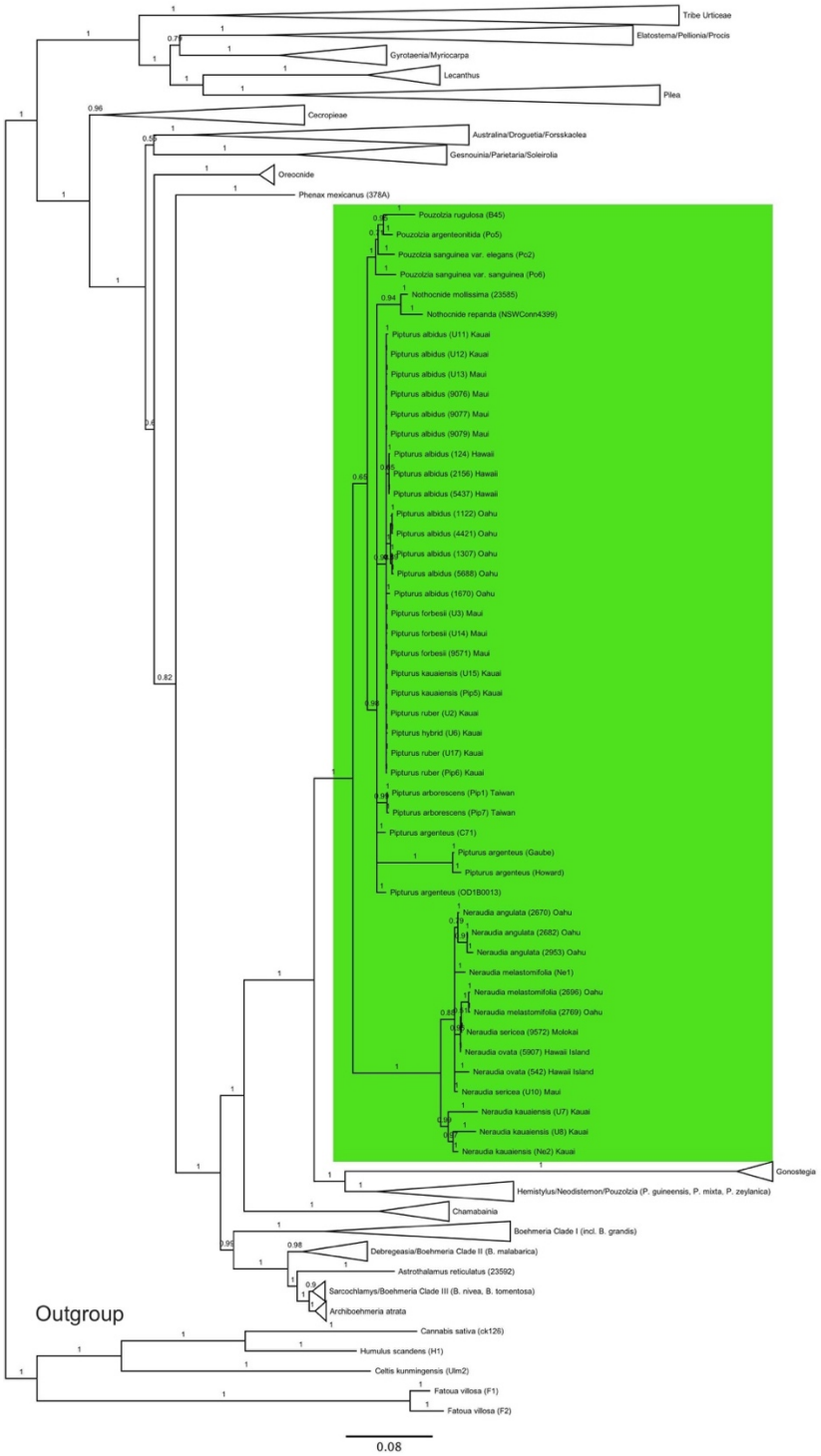

Figure 1.5. Combined four-gene region phylogenetic tree of Urticaceae based on nuclear (ITS) and chloroplast (*trnL-trnF* spacer*, rbcL,* and *rpl14-rps8-infA-rpl36* spacer) gene regions using Bayesian inference (1,000,000 iterations, 25% burn-in) for 257 accessions. Tree highlights Hawaiian species in the genera *Pipturus* and *Neraudia*. Branch labels represent posterior probabilities. Wedge height represents two or more accessions present and does not represent the number of accessions associated with it.

Figure 1.6. Combined four-gene region phylogenetic tree of Urticaceae based on nuclear (ITS) and chloroplast (*trnL-trnF* spacer*, rbcL,* and *rpl14-rps8-infA-rpl36* spacer) gene regions using Bayesian inference (1,000,000 iterations, 25% burn-in) for 257 accessions. Tree highlights Hawaiian species *Boehmeria grandis*. Branch labels represent posterior probabilities. Wedge height represents two or more accessions present and does not represent the number of accessions associated with it.

Appendix A1

Table A1.1. GenBank identification numbers and associated phylogenetic studies for sequences used in phylogenetic study of Urticaceae, N= 257. Grey cells indicate sequences used for phylogentic analysis. No GenBank identification numbers have been given to the sequences of the 47 new specimens sequenced in this study. *Name changed in this study to *Pouzolzia rugulosa* from *Boehmeria rugulosa* based on Wilmot-Dear et al. (2009). ** Name changed in this study to *Pilea fairchildiana* from *Sarcopilea domingensis* based on Jestrow et al. (2012).

Species	Accession	Abbreviation for tree	ITS	*trnL-trnF*	*rbcL*	*rpl14-rps8-infA-rpl36*
Archiboehmeria atrata	A1	Archiboehmeria_atrata_A1	KF137798	KF138269	KF138106	KF138434
Archiboehmeria atrata	A2	Archiboehmeria_atrata_A2	KF137799	KF138270	KF138107	KF138434
Astrothalamus reticulatus	23592	Astrothalamus_reticulatus_23592	KF137800	KF138271	KF123108	
Australina flaccida	23601	Australina_ flaccida_23601	KF137801	KF138272	KF138109	KF138436
Boehmeria blinii var. *blini*	Li009	Boehmeria_blinii_var_blinii_Li009	FJ750373	FJ750399		
Boehmeria clidemioides var. *clidemioides*	B2	Boehmeria_clidemioides_B2	KF137802	KF138274	KF138111	KF138438
Boehmeria clidemioides var. *clidemioides*	B15	Boehmeria_clidemioides_B15	KF137803	KF138273	KF138110	KF138437
Boehmeria clidemioides var. *diffusa*	B16	Boehmeria_clidemioides_var_diffusa_B16	KF137804	KF138275	KF138112	KF138439
Boehmeria densiflora	B52	Boehmeria_densiflora_B52	KF137805	KF138276	KF138113	
Boehmeria densiflora	B53	Boehmeria_densiflora_B53	KF137806	KF138277	KF138114	
Boehmeria densiglomerata	Li0024	Boehmeria_densiglomerata_Li0024	FJ750378	FJ750417		
Boehmeria formosana	Li0018	Boehmeria_formosana_Li0018	FJ750379	FJ750426		
Boehmeria glomerulifera	B5	Boehmeria_glomerulifera_B5	KF137807	KF138278	KF138115	KF138440

Boehmeria gracilis	Li0025	Boehmeria_gracilis_Li0025	FJ750361	FJ750409		
Boehmeria grandis	10015	Boehmeria_grandis_10015				
Boehmeria grandis	1120	Boehmeria_grandis_1120				
Boehmeria leiophylla	Li003	Boehmeria_leiophylla_Li003	FJ750353	FJ750403		
Boehmeria longispica	B20	Boehmeria_longispica_B20	KF137809	KF138280	KF138117	KF138441
Boehmeria macrophylla var. *macrophylla*	B24	Boehmeria_macrophylla_B24	KF137810	KF138281	KF138118	KF138442
Boehmeria macrophylla var. *macrophylla*	B28	Boehmeria_macrophylla_B28	KF137811	KF138282	KF138119	KF138443
Boehmeria macrophylla var. *rotundifolia*	B21	Boehmeria_macrophylla_var_rotundifolia_B21	KF137812	KF138283	KF138120	KF138444
Boehmeria macrophylla var. *rotundifolia*	B26	Boehmeria_macrophylla_var_rotundifolia_B26	KF137813	KF138284	KF138121	KF138445
Boehmeria malabarica	Li002	Boehmeria_malabarica_Li002	FJ750387	FJ750439		
Boehmeria malabarica	Li00121	Boehmeria_malabarica_Li00121	FJ750388	FJ750402		
Boehmeria nivea var. nipononivea	B32	Boehmeria_nivea_var_nipononivea_B32	KF137814	KF138285	KF138122	KF138446
Boehmeria nivea var. *nivea*	B6	Boehmeria_nivea_B6	KF137815	KF138286	KF138123	KF138447
Boehmeria pannosa	P188	Boehmeria_pannosa_P188	JF980316	JN102155		
Boehmeria penduliflora	B33	Boehmeria_penduliflora_B33	KF137816	KF138287	KF138124	KF138448
Boehmeria pilosiuscula	Li0017	Boehmeria_pilosiuscula_Li0017	FJ750372	FJ750422		
Boehmeria platyphylla	BpYu	Boehmeria_platyphylla_BpYu	KF835876			
Boehmeria polystachya	Li0029	Boehmeria_polystachya_Li0029	FJ750376	FJ750421		

Boehmeria pseudotricuspis	Li0012	Boehmeria_pseudotricuspis_Li0012	FJ750375	FJ750400		
*Pouzolzia rugulosa**	B45	Pouzolzia_rugulosa_B45	KF137817	KF138288	KF138125	KF138449
Boehmeria siamensis	PS1032MT01	Boehmeria_siamensis_PS1032MT01	FJ980384		GQ436555	
Boehmeria silvestrii	Li0026	Boehmeria_silvestrii_Li0026	FJ750380	FJ750411		
Boehmeria spicata	B9	Boehmeria_spicata_B9	KF137819	KF138290	KF138127	KF138451
Boehmeria strigosifolia	Li0020	Boehmeria_strigosifolia_Li0020	FJ750358	FJ750419		
Boehmeria strigosifolia	Li0021	Boehmeria_strigosifolia_Li0021	FJ750383	FJ750418		
Boehmeria tomentosa	B38	Boehmeria_tomentosa_B38	KF137820	KF138291	KF138128	KF138452
Boehmeria tricuspis	B39	Boehmeria_tricuspis_B39	KF137821	KF138292	KF138129	
Boehmeria umbrosa	B12	Boehmeria_umbrosa_B12	KF137822	KF138293	KF138130	KF138453
Boehmeria umbrosa	B40	Boehmeria_umbrosa_B40	KF137823	KF138294	KF138131	KF138454
Boehmeria zollingeriana var. *blinii*	B1	Boehmeria_zollingeriana_var_blinii_B1	KF137824	KF138295	KF138132	
Cannabis sativa	Kim	Cannabis_sativa_Kim	KM586391	KM586563	KM586477	
Cecropia ficifolia	23606	Cecropia_ficifolia_23606	KF137825	KF138296	KF138133	
Cecropia obtusifolia	162A	Cecropia_obtusifolia_162A		KF138297	KF138134	KF138455
Celtis kunmingensis	ULM2	Celtis_kunmingensis_ULM2	KF137826	KF138298	KF138135	KF138456
Chamabainia cuspidata	C1	Chamabainia_cuspidata_C1	KF137827	KF138299	KF138136	KF138457
Chamabainia cuspidata	C2	Chamabainia_cuspidata_C2	KF137828	KF138300	KF138137	KF138458

Coussapoa parvifolia	386A	Coussapoa_parvifolia_386A		KF138301		KF138459
Debregeasia elliptica	De19	Debregeasia_elliptica_De19	KF137829	KF138302	KF138138	KF138460
Debregeasia elliptica	De7	Debregeasia_elliptica_De7	KF137830	KF138303	KF138139	KF138461
Debregeasia longifolia	De10	Debregeasia_longifolia_De10	KF137831	KF138304	KF138140	KF138462
Debregeasia longifolia	De9	Debregeasia_longifolia_De9	KF137832	KF138305	KF138141	KF138463
Debregeasia orientalis	De13	Debregeasia_orientalis_De13	KF137833	KF138306	KF138142	KF138464
Debregeasia orientalis	De15	Debregeasia_orientalis_De15	KF137834	KF138307	KF138143	KF138465
Debregeasia saeneb	De17	Debregeasia_saeneb_De17	KF137835	KF138308	KF138144	KF138466
Debregeasia squamata	De5	Debregeasia_squamata_De5	KF137837	KF138310	KF138146	KF138468
Dendrocnide meyeniana	D2	Dendrocnide_meyeniana_D2	KF137838	KF138311	KF138147	KF138469
Dendrocnide sinuata	D1	Dendrocnide_sinuata_D1	KF137839	KF138312	KF138148	KF138470
Dendrocnide urentissima	D5	Dendrocnide_urentissima_D5	KF137841	KF138314	KF138150	KF138472
Didymodoxa caffra	23599	Didymodoxa_caffra_23599		KF138315	KF138151	KF138473
Discocnide mexicana	167A	Discocnide_mexicana_167A	KF137842	KF138316	KF138152	KF138474
Droguetia ambigua	28892	Droguetia_ambigua_28892	KF137843	KF138317	KF138153	KF138475
Droguetia iner subsp. urticoides	Dr1	Droguetia_iner_subsp_urticoides_Dr1	KF137844	KF138318	KF138154	KF138476
Droguetia iner subsp. *urticoides*	Dr4	Droguetia_iner_subsp_urticoides_Dr4	KF137845	KF138319	KF138155	KF138477
Elatostema albopilosum	E1	Elatostema_albopilosum_E1	KF137846	KF138320	KF138156	KF138478

Elatostema atropurpureum	E2	Elatostema_atropurpureum_E2	KF137847	KF138321	KF138157	KF138479
Elatostema cuspidatum var. *cuspidatum*	E4	Elatostema_cuspidatum_E4	KF137848	KF138322	KF138158	KF138480
Elatostema cyrtandrifolium var. *cyrtandrifolium*	E3	Elatostema_cyrtandrifolium_E3	KF137849	KF138323	KF138159	KF138481
Elatostema densistriolatum	E9	Elatostema_densistriolatum_E9	KF137850	KF138324	KF138160	KF138482
Elatostema longibracteatum	E6	Elatostema_longibracteatum_E6	KF137851	KF138325	KF138161	KF138483
Elatostema parvum var. *parvum*	E7	Elatostema_parvum_E7	KF137852	KF138326	KF138162	KF138484
Elatostema petelotii	E8	Elatostema_petelotii_E8	KF137853	KF138327	KF138163	KF138485
Elatostema stewardii	E10	Elatostema_stewardii_E10	KF137855	KF138329	KF138165	KF138487
Elatostema subtrichotomum var. *subtrichotomum*	E11	Elatostema_subtrichotomum_E11	KF137856	KF138330	KF138166	KF138488
Elatostema tenuicaudatum var. tenuicaudatum	E12	Elatostema_tenuicaudatum_E12	KF137857		KF138167	KF138489
Fatoua villosa	F1	Fatoua_villosa_F1	KF137858	KF138331	KF138168	KF138490
Fatoua villosa	F2	Fatoua_villosa_F2	KF137859	KF138332	KF138169	KF138491
Forsskaolea angustifolia	6515	Forsskaolea_angustifolia_6515	KF137860	KF138333	KF138171	KF138492
Forsskaolea angustifolia	16132	Forsskaolea_angustifolia_16132	KF137861	KF138334	KF138170	KF138493
Gesnouinia arborea	177A	Gesnouinia_arborea_177A	KF137862	KF138335	KF138172	KF138494
Giardinia diversifolia subsp. *diversifolia*	G31	Giardinia_diversifolia_G31		KF138336	KF138173	KF138495
Giardinia diversifolia subsp. *diversifolia*	G9	Giardinia_diversifolia_G9	KF137863	KF138337	KF138174	KF138496
Giardinia diversifolia subsp. *suborbiculata*	G16	Giardinia_diversifolia_subsp_suborbiculata_G16		KF138338	KF138175	KF138497

Giardinia diversifolia subsp. *Suborbiculata*	G17	Giardinia_diversifolia_subsp_suborbiculata_G17		KF138339	KF138176	KF138498
Giardinia diversifolia subsp. *triloba*	G19	Giardinia_diversifolia_subsp_triloba_G19	KF137864	KF138340	KF138177	KF138499
Giardinia diversifolia subsp. *triloba*	G6	Giardinia_diversifolia_subsp_triloba_G6		KF138341	KF138178	KF138500
Gonostegia hirta	Go3	Gonostegia_hirta_Go3	KF137865	KF138342	KF138179	
Gonostegia parvifolia	Go1	Gonostegia_parvifolia_Go1	KF137866	KF138343	KF138180	KF138501
Gonostegia parvifolia	Go4	Gonostegia_parvifolia_Go4	KF137867	KF138344	KF138181	KF138502
Gyrotaenia microcarpa	473A	Gyrotaenia_microcarpa_473A		KF138345		
Hemistylus macrostachya	23597	Hemistylus_macrostachya_23597	KF137868	KF138346	KF138182	KF138503
Hesperocnide sandwicensis	566	Hesperocnide_sandwicensis_566				
Hesperocnide sandwicensis	918	Hesperocnide_sandwicensis_918				
Hesperocnide tenella	331A	Hesperocnide_tenella_331A				KF138504
Hesperocnide tenella	8089	Hesperocnide_tenella_8089				
Hesperocnide tenella	2026	Hesperocnide_tenella_2026	KF558907	KF559027		
Hesperocnide tenella	2586	Hesperocnide_tenella_2586	KF558930	KF559050		
Humulus scandense	H1	Humulus_scandense_H1	KF137869	KF138347	KF138183	KF138505
Laportea bulbifera	L3	Laportea_bulbifera_L3		KF138348	KF138184	KF138506
Laportea bulbifera	L5	Laportea_bulbifera_L5	KF137870	KF138349	KF138185	KF138507
Lecanthus penduncularis	Le1	Lecanthus_penduncularis_Le1	KF137871	KF138350	KF138186	KF138508

Lecanthus penduncularis	Le3	Lecanthus_penduncularis_Le3	KF137872	KF138351	KF138187	KF138509
Lecanthus petelotii var. *corniculata*	Le2	Lecanthus_petelotii_var_corniculata_Le2	KF137873	KF138352	KF138188	KF138510
Lecanthus petelotii var. *corniculata*	Le4	Lecanthus_petelotii_var_corniculata_Le4	KF137874	KF138353	KF138189	KF138511
Leucosyke quadrinervia	Leu3	Leucosyke_quadrinervia_Leu3	KF137875	KF138354	KF138190	
Leucosyke quadrinervia	Leu4	Leucosyke_quadrinervia_Leu4	KF137876	KF138355	KF138191	
Maoutia setosa	M2	Maoutia_setosa_M2		KF138356	KF138192	
Myriocarpa cordata	C2A	Myriocarpa_cordata_C2A	KF137877	KF138357	KF138193	KF138512
Myriocarpa obovata	370A	Myriocarpa_obovata_370A	KF137878	KF138358		KF138513
Nanocnide japonica	N1	Nanocnide_japonica_N1	KF137879	KF138359	KF138194	KF138514
Nanocnide japonica	N4	Nanocnide_japonica_N4	KF137880	KF138360	KF138195	KF138515
Nanocnide lobata	N5	Nanocnide_lobata_N5	KF137881	KF138361	KF138196	KF138516
Nanocnide lobata	N6	Nanocnide_lobata_N6	KF137882	KF138362	KF138197	KF138517
Neodistemon indicum	279A	Neodistemon_indicum_279A		KF138363	KF138198	
Neraudia angulata	2670	Neraudia_angulata_2670				
Neraudia angulata	2682	Neraudia_angulata_2682				
Neraudia angulata	2953	Neraudia_angulata_2953				
Neraudia kauaiensis	10018	Neraudia_kauaiensis_10018				
Neraudia kauaiensis	10019	Neraudia_kauaiensis_10019				

Neraudia kauaiensis	Ne2	Neraudia_kauaiensis_Ne2	KF137883	KF138364	KF138199	
Neraudia melastomifolia	Ne1	Neraudia_melastomifolia_Ne1	KF137884	KF138365	KF138200	KF138518
Neraudia melastomifolia	2696	Neraudia_melastomifolia_2696				
Neraudia melastomifolia	2769	Neraudia_melastomifolia_2769				
Neraudia ovata	542	Neraudia_ovata_542				
Neraudia ovata	5907	Neraudia_ovata_5907				
Neraudia sericea	10021	Neraudia_sericea_10021				
Neraudia sericea	9572	Neraudia_sericea_9572				
Nothocnide mollissima	23585	Nothocnide_mollissima_23585	KF137885	KF138366	KF138201	
Nothocnide repanda	NSWConn4399	Nothocnide_repanda_NSWConn4399		FJ432253		
Obetia aldabrensis	sm337	Obetia_aldabrensis_sm337	KM586460	KM586632	KM586546	
Obetia carruthersiana	2030	Obetia_carruthersiana_2030	KF971187	KF971220		
Obetia pinnatifida	dt548	Obetia_pinnatifida_dt548	KM586449	KM586621	KM586535	
Obetia radula	2049	Obetia_radula_2049	KX271352	KX271433		
Obetia radula	dt523	Obetia_radula_dt523	KM586431	KM586603	KM586517	
Obetia radula	dt550	Obetia_radula_dt550	KM586451	KM586623	KM586537	
Obetia tenax	28719	Obetia_tenax_28719	KF137886	KF138367	KF138202	KF138520

Oreocnide frutescens subsp. *frutescens*	O2	Oreocnide_frutescens_O2	KF137887	KF138368	KF138203	KF138521
Oreocnide frutescens subsp. *frutescens*	O8	Oreocnide_frutescens_O8	KF137888	KF138369	KF138204	KF138522
Oreocnide frutescens subsp. *occidentalis*	O12	Oreocnide_frutescens_subsp_occidentalis_O12		KF138370	KF138205	KF138523
Parietaria judaica	11077	Parietaria_judaica_11077		KF138371	KF138206	KF138524
Parietaria micrantha	Pa1	Parietaria_micrantha_Pa1		KF138372	KF138207	KF138525
Pellionia macrophylla	Pe1	Pellionia_macrophylla_Pe1	KF137889	KF138373	KF138208	KF138526
Pellionia paucidentata var. paucidentata	Pe2	Pellionia_paucidentata_Pe2	KF137890	KF138374	KF138209	KF138527
Pellionia radicans	Pe3	Pellionia_radicans_Pe3	KF137891	KF138375	KF138210	KF138528
Pellionia repens	Pe4	Pellionia_repens_Pe4	KF137892	KF138376	KF138211	KF138529
Pellionia tsoongii	Pe5	Pellionia_tsoongii_Pe5	KF137893	KF138377	KF138212	KF138530
Phenax mexicanus	378A	Phenax_mexicanus_378A		KF138378		
Pilea angulata subsp. *petiplaris*	P1	Pilea_angulata_subsp_petiplaris_P1	KF137894	KF138379	KF138213	KF138531
Pilea cadierei	RBGE19697470	Pilea_cadierei_7470	DQ175608.1	DQ179359.1		
Pilea cadierei	N/A	Pilea_cadierei_Yu	KF835854	KF835853		
Pilea cavaleriei	P3	Pilea_cavaleriei_subsp_cavalerei_P3	KF137895	KF138380	KF138214	KF138532
Pilea insolens	P11	Pilea_insolens_P11	KF137896	KF138381	KF138215	KF138533
Pilea lapestris	Johns9979	Pilea_lapestris_9979	DQ175598	DQ179341		
Pilea longipedunculata	P5	Pilea_longipedunculata_P5	KF137897	KF138382	KF138216	KF138534

Pilea martinii	P6	Pilea_martinii_P6	KF137898	KF138383	KF138217	KF138535
Pilea melastomoides	P20	Pilea_melastomoides_P20	KF137899	KF138384	KF138218	KF138536
Pilea microphylla	P21	Pilea_microphylla_P21	KF137900	KF138385	KF138219	KF138537
Pilea microphylla	P22	Pilea_microphylla_P22	KF137901	KF138386	KF138220	KF138538
Pilea oxyodon	P9	Pilea_oxyodon_P9	KF137902	KF138387	KF138221	KF138539
Pilea peploides	2804	Pilea_peploides_2804				
Pilea peploides	4724	Pilea_peploides_4724				
Pilea peploides	Tanaka_1744	Pilea_peploides_var_major_1744		DQ179342		
Pilea plantaniflora	P24	Pilea_plantaniflora_P24	KF137903		KF138222	KF138540
Pilea pumila	P10	Pilea_pumila_P10	KF137904	KF138388	KF138223	KF138541
Pilea racemosa	Ho et al. 2686	Pilea_racemosa_2686	DQ175602	DQ179347		
Pilea sinofasciata	P26	Pilea_sinofasciata_P26	KF137905	KF138389	KF138224	KF138542
Pilea ternifolia	L.H.S. Williams 390	Pilea_ternifolia_390	DQ175597	DQ179346		
Pilea verrucossa	P29	Pilea_verrucossa_P29	KF137907	KF138391	KF138226	KF138544
Pipturus albidus	10022	Pipturus_albidus_10022				
Pipturus albidus	10023	Pipturus_albidus_10023				
Pipturus albidus	10024	Pipturus_albidus_10024				
Pipturus albidus	9076	Pipturus_albidus_9076				

Pipturus albidus	9077	Pipturus_albidus_9077				
Pipturus albidus	9079	Pipturus_albidus_9079				
Pipturus albidus	124	Pipturus_albidus_124				
Pipturus albidus	1122	Pipturus_albidus_1122				
Pipturus albidus	1307	Pipturus_albidus_1307				
Pipturus albidus	1670	Pipturus_albidus_1670				
Pipturus albidus	2156	Pipturus_albidus_2156				
Pipturus albidus	4421	Pipturus_albidus_4421				
Pipturus albidus	5437	Pipturus_albidus_5437				
Pipturus albidus	5688	Pipturus_albidus_5688				
Pipturus arborescens	Pip1	Pipturus_arborescens_Pip1	KF137908	KF138392	KF138227	KF138545
Pipturus arborescens	Pip7	Pipturus_arborescens_Pip7	KF137909	KF138393	KF138228	
Pipturus argenteus	C71	Pipturus_argenteus_C71			KF496559	
Pipturus argenteus	Gaube	Pipturus_argenteus_Gaube	HQ110082			
Pipturus argenteus	Howard	Pipturus_argenteus_Howard			KU564846	
Pipturus argenteus	OD1B0013	Pipturus_argenteus_OD1B0013			JF738411	
Pipturus forbesii	10026	Pipturus_forbesii_10026				
Pipturus forbesii	10025	Pipturus_forbesii_10025				

Pipturus forbesii	9571	Pipturus_forbesii_9571				
Pipturus kauaiensis	10027	Pipturus_kauaiensis_10027				
Pipturus kauaiensis	Pip5	Pipturus_kauaiensis_Pip5	KF137910	KF138394	KF138229	KF138546
Pipturus ruber	10016	Pipturus_ruber_10016				
Pipturus sp.	10017	Pipturus_hybrid_10017				
Pipturus ruber	10030	Pipturus_ruber_10030				
Pipturus ruber	Pip6	Pipturus_ruber_Pip6	KF137911	KF138395	KF138230	KF138547
Poikilospermum lanceolatum	Pi1	Poikilospermum_lanceolatum_Pi1	KF137912	KF138396	KF138231	KF138548
Poikilospermum suaveolens	Pi2	Poikilospermum_suaveolens_Pi2	KF137913	KF138397	KF138232	KF138549
Poikilospermum suaveolens	Pi3	Poikilospermum_suaveolens_Pi3	KF137914	KF138398	KF138233	KF138550
Pouzolzia argenteonitida	Po5	Pouzolzia_argenteonitida_Po5	KF137915	KF138399	KF138234	KF138551
Pouzolzia guineensis	282A	Pouzolzia_guineensis_282A		KF138400	KF138235	KF138552
Pouzolzia mixta	288A	Pouzolzia_mixta_288A	KF137916	KF138401	KF138236	KF138553
Pouzolzia sanguinea var. *elegans*	Po2	Pouzolzia_sanguinea_var_elegans_Po2	KF137917	KF138402	KF138237	KF138554
Pouzolzia sanguinea var. *sanguinea*	Po6	Pouzolzia_sanguinea_Po6	KF137918	KF138403	KF138238	KF138555
Pouzolzia zeylanica var. *zeylanica*	Po4	Pouzolzia_zeylanica_Po4	KF137920	KF138405	KF138240	KF138557
Pouzolzia zeylanica var. *zeylanica*	Po7	Pouzolzia_zeylanica_Po7	KF137921	KF138406	KF138241	KF138558
Procris wightiana	Pr1	Procris_wightiana_Pr1	KF137922	KF138407	KF138242	KF138559

Procris wightiana	Pr2	Procris_wightiana_Pr2	KF137923	KF138408	KF138243	KF138560
Sarcochlamys pulcherrima	S1	Sarcochlamys_pulcherrima_S1	KF137924	KF138409	KF138244	KF138561
*Pilea fairchildiana***	302A	Pilea_fairchildiana_302A	KF137925	KF138410	KF138245	KF138562
Soleirolia soleirolii	312A	Soleirolia_soleirolii_312A	KF137926	KF138411	KF138246	KF138563
Touchardia latifolia	T1	Touchardia_latifolia_T1	KF137927	KF138412	KF138247	KF138564
Touchardia latifolia	1114	Touchardia_latifolia_1114				
Urera alceifolia	C11A	Urera_alceifolia_C11A		KF138413	KF138248	KF138565
Urera baccifera	C4A	Urera_baccifera_C4A	KF137928	KF138414	KF138249	KF138566
Urera batesii	2031	Urera_batesii_2031	KF971186	KF971219		
Urera caracasana	23561	Urera_caracasana_23561	KF137929	KF138415	KF138250	KF138567
Urera elata	sm351	Urera_elata_sm351	KM586470	KM586642	KM586556	
Urera elata	sm352	Urera_elata_sm352	KM586471	KM586643	KM586557	
Urera fischeri	dt518	Urera_fischeri_dt518	KM586427	KM586599	KM586513	
Urera fischeri	dt542	Urera_fischeri_dt542	KM586443	KM586615	KM586529	
Urera glabra	Ur1	Urera_glabra_Ur1	KF137930	KF138416	KF138251	KF138568
Urera glabra	10031	Urera_glabra_10031				
Urera glabra	1673	Urera_glabra_1673				
Urera hypselodendron	377A	Urera_hypselodendron_377A		KF138417	KF138252	KF138569

Urera kaalae	1671	Urera_kaalae_1671				
Urera kaalae	2086	Urera_kaalae_2086				
Urera kaalae	4687	Urera_kaalae_4687				
Urera lianoides	313A	Urera_lianoides_313A		KF138418	KF138253	KF138570
Urera sansibarica	dt519	Urera_sansibarica_dt519	KM586428	KM586600	KM586514	
Urera sansibarica	dt543	Urera_sansibarica_dt543	KM586444	KM586616	KM586530	
Urera trinervis	374A	Urera_trinervis_374A	KF137932	KF138420	KF138255	KF138572
Urtica angustifolia	U1	Urtica_angustifolia_U1	KF137933	KF138421	KF138256	KF138573
Urtica ardens	U2	Urtica_ardens_U2	KF137934	KF138422	KF138257	KF138574
Urtica atrichocaulis	U3	Urtica_atrichocaulis_U3	KF137935	KF138423	KF138258	KF138575
Urtica bertoroana	1555	Urtica_bertoroana_1555	KX271384	KX271460		
Urtica bertoroana	2209	Urtica_bertoroana_2209	KX271383	KX271459		
Urtica circularis	1872	Urtica_circularis_1872	KX271386	KX271462		
Urtica circularis	3091	Urtica_circularis_3091	KF971200	KF971233		
Urtica dioica	U21	Urtica_dioica_U21	KF137936	KF138424	KF138259	KF138576
Urtica fissa	U4	Urtica_fissa_U4	KF137937	KF138425	KF138260	KF138577
Urtica flabellata	1560	Urtica_flabellata_1560	KF971199	KF971232		
Urtica flabellata	2040	Urtica_flabellata_2040	KF558908	KF559028		

Urtica hyperborea	U14	Urtica_hyperborea_U14	KF137938	KF138426	KF138261	KF138578
Urtica hyperborea	U5	Urtica_hyperborea_U5	KF137939	KF138427	KF138262	KF138579
Urtica mairei	U7	Urtica_mairei_U7	KF137940	KF138428	KF138263	KF138580
Urtica masafuerae	1879	Urtica_masafuerae_1879	KX271380			
Urtica spathulata	2268	Urtica_spathulata_2268	KX271385	KX271461		
Urtica trangularis subsp. *pinnatifida*	U10	Urtica_trangularis_subsp_pinnatifida_U10	KF137943	KF138431	KF138266	KF138583
Urtica urens	582	Urtica_urens_582				
Urtica urens	1082	Urtica_urens_1082	KF558889	KF559010		
Urtica urens	2045	Urtica_urens_2045	KX271359	KX271440		
Urtica zayuensis	U11	Urtica_zayuensis_U11	KF137944	KF138432	KF138267	KF138584
Urtica zayuensis	U17	Urtica_zayuensis_U17	KF137945	KF138433	KF138268	KF138585

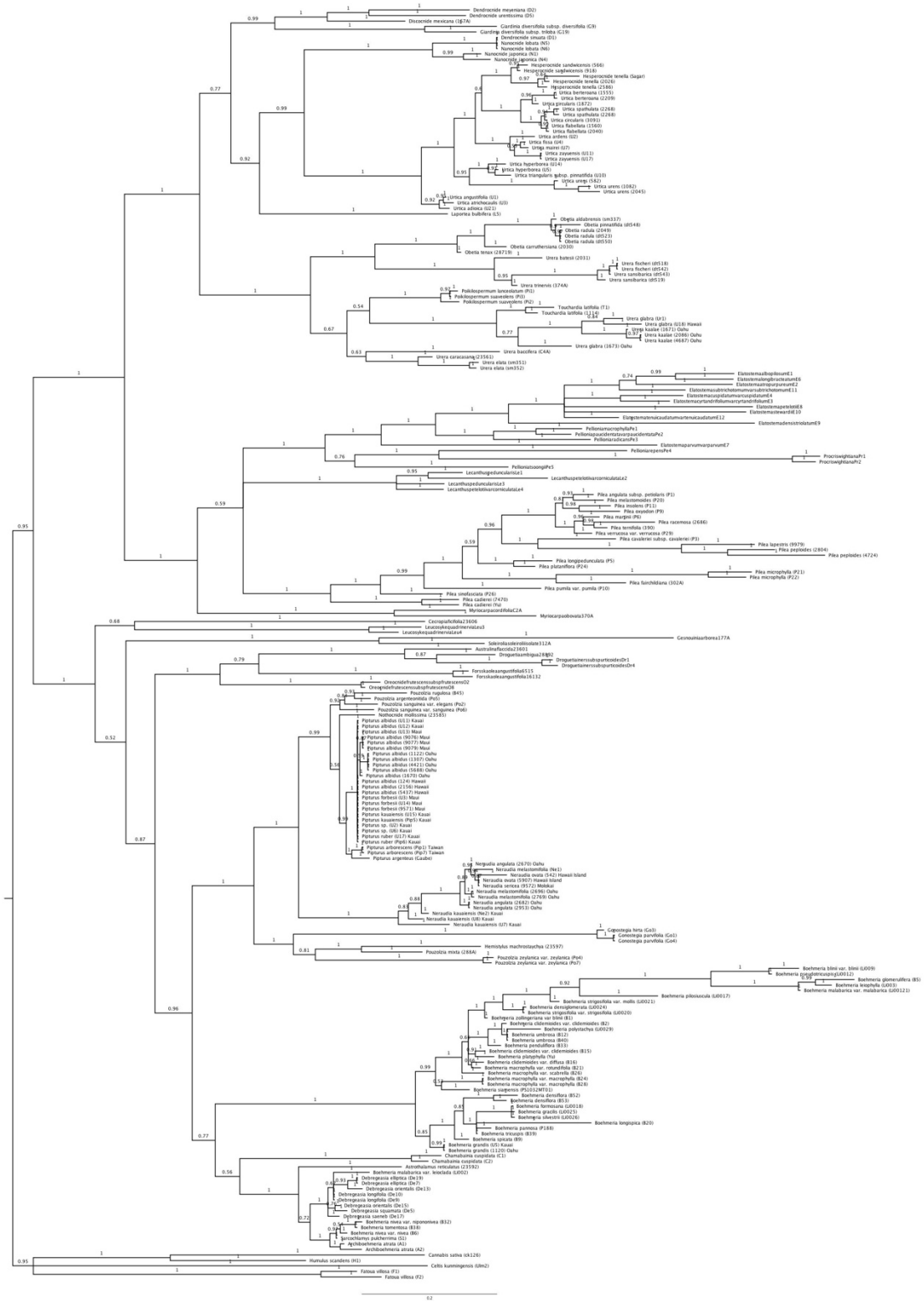

Figure A1.1 Phylogenetic tree of Urticaceae using Bayesian Inference (1,000,000 iterations, 25% burn-in) from single nuclear ITS gene region. Branch labels represent posterior probabilities. Number of accessions=232.

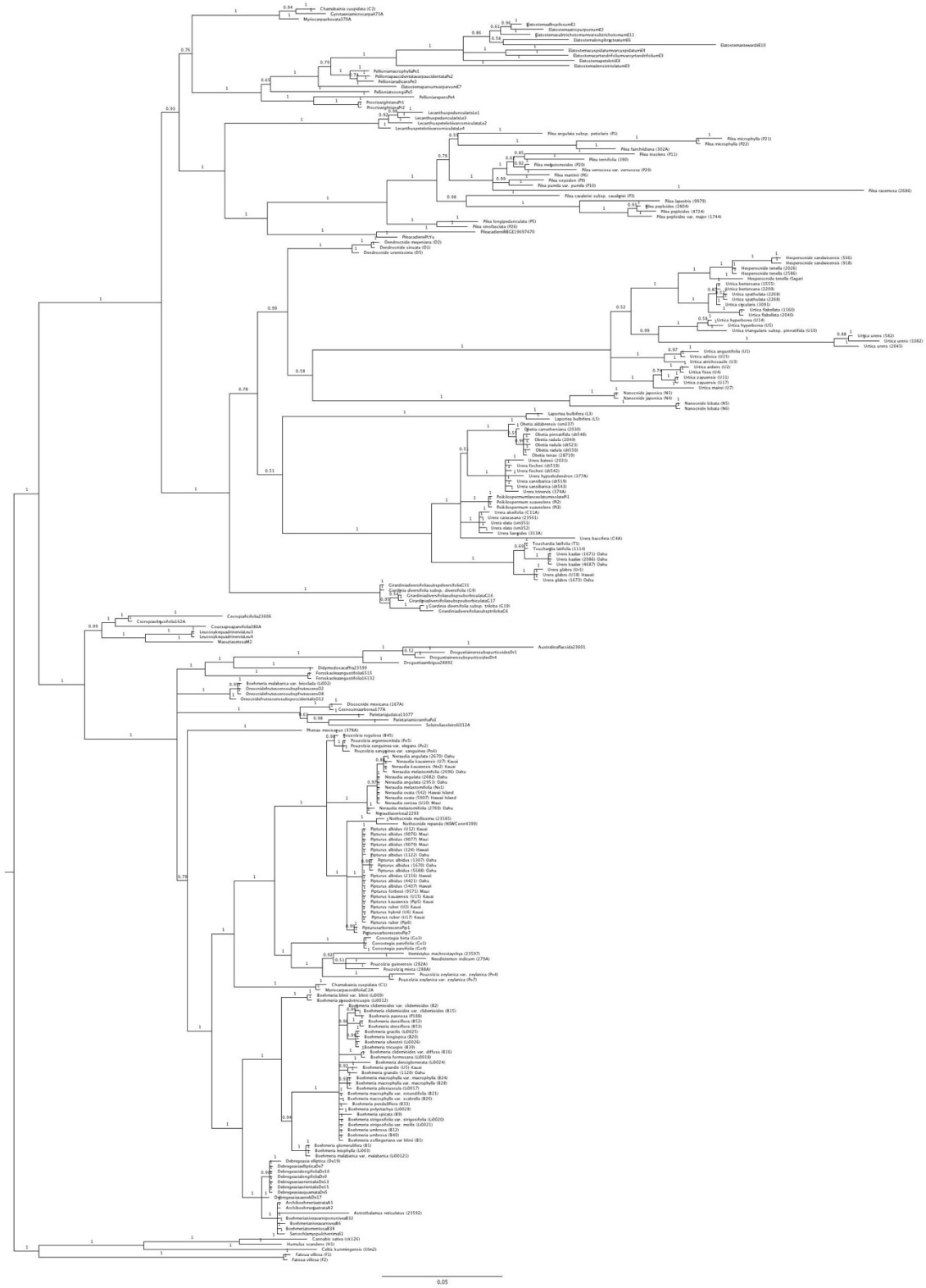

Figure A1.2. Phylogenetic tree of Urticaceae using Bayesian Inference (1,000,000 iterations, 25% burn-in) from single chloroplast *trnL-trnF* gene region. Branch labels represent posterior probabilities. Number of accessions=242.

Figure A1.3. Phylogenetic tree of Urticaceae using Bayesian Inference (1,000,000 iterations, 25% burn-in) from single chloroplast *rbcL* gene region. Branch labels represent posterior probabilities. Number of accessions=208.

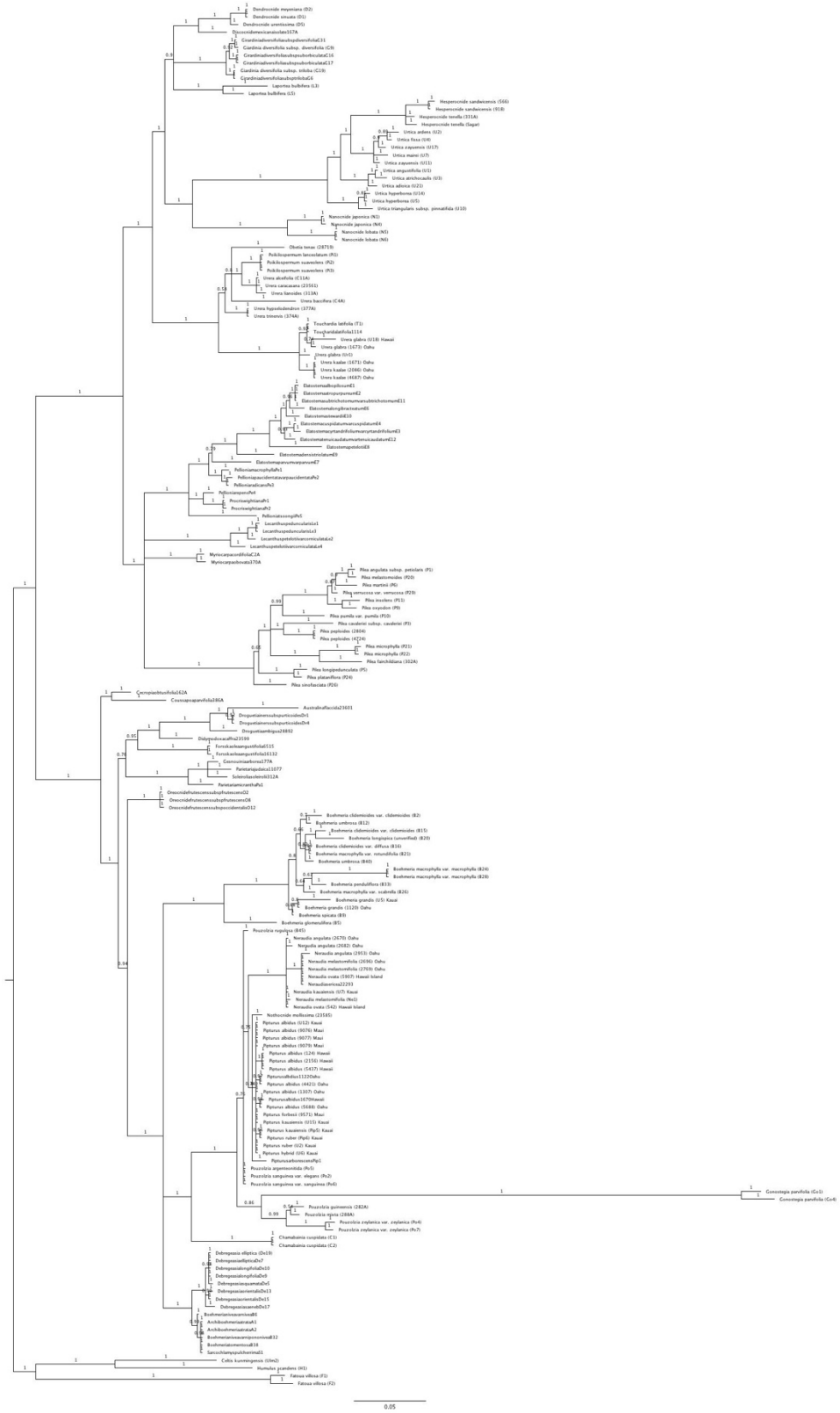

Figure A1.4. Phylogenetic tree of Urticaceae using Bayesian Inference (1,000,000 iterations, 25% burn-in) from single chloroplast *rpll4–rps8–infA–rpl36* gene region. Branch labels represent posterior probabilities. Number of accessions=180.

Chapter 2

No-choice bioassay reveals variation in performance and plant recognition of the Hawai‘i endemic butterfly, *Vanessa tameamea* Esch. (Nymphalidae) on native and novel non-native Urticacean hostplants

Introduction

Hawaiian flora and fauna exhibit high levels of species endemism and suffer from disproportionately high rates of plant and animal extinctions compared to continental taxa (Dobson et al. 1997; Kier et al. 2009). Current and historical declines in biodiversity in oceanic islands are strongly linked to the introduction of non-native animals, direct and indirect resource competition from introduced flora, and habitat loss (Savidge 1987; Brooks et al. 2002; Blackburn et al. 2004; Gurevitch & Padilla 2004; Fordham & Brook 2010; Powell et al. 2013). The loss of native flora may result in the extinction of fauna that depend on specific host plants to complete their life cycle, leading to the co-extinction of both plant and animal taxa (Dunn 2005). For example, Koh et al. (2004) demonstrated that the extinction of native butterflies in Singapore was directly correlated with the extinction of host plants. Such co-extinctions can lead to secondary extinctions and potentially trigger extinction cascades within an ecosystem (Colwell et al. 2012). Therefore, endemic insects threatened by habitat loss should be assessed in terms of their host specialization in order to identify taxa at risk of co-extinction in the case of host plant declines. Insect performance assays on novel plant diets may elucidate the likelihood that endemic insects may persist even in the case of host plant extinction events due to their ability to utilize additional plant species as host plants.

In Hawai‘i, dozens of native insect and plant species have gone extinct in the past 200 years, including over 30 Lepidoptera and over 90 plant taxa (Sax & Gaines 2008; Bishop Museum 2017). The high extinction rates of native Hawaiian Lepidoptera and flora are alarming and highlight the need for research that investigates plant-insect interactions. For example, information on larval performance across a variety of plant diets sheds light on how host plant selection impacts butterfly fitness, which in turn may impact the level of conservation and protection needed for both the lepidopteran species and their host plants. However, few studies have assessed larval performance of Hawaiian Lepidoptera across different plant diets or attempted to determine the plant traits underlying variation in herbivore preference and performance (Rubinoff & San Jose 2010; Barton & Haines 2013).

Vanessa tameamea Eschscholtz (1821), the Kamehameha butterfly and state insect of Hawai‘i, is one of only two butterflies native to the Archipelago (Williams 1928; Riotte & Uchida 1978(79)). *V. tameamea* is classified as an oligophagous specialist herbivore in its larval stages as the caterpillar has been found to feed solely on species from several genera in Urticaceae, the nettle family (Williams 1928; Swezey 1954; Leeper 1975; Riotte & Uchida 1978(79); Tabashnik et al. 1992; Ali & Agrawal 2012). Its populations have been declining in Hawaii in recent decades based on historical records and current species distribution data (Williams 1928; Tabashnik et al. 1992; Haines et al. 2017). Although *V. tameamea* is still present on all of the main Hawaiian Islands with the exception of Kaho‘olawe, it is generally observed in restricted areas of montane and/or riparian native forests (Williams 1928; Gorelick & Wielgus 1968; Tabashnik et al. 1992).

The Hawaiian Archipelago is home to 15 currently recognized native species in Urticaceae, representing 7 genera: *Boehmeria* Jacq., *Hesperocnide* Torr., *Neraudia* Gaud., *Pilea*

Lindl. nom. cons., *Pipturus* Wedd., *Touchardia* Gaud., and *Urera* Gaud. (Wagner et al. 1999). The seven genera belong to the following three tribes: Boehmerieae (i.e., *Boehmeria*, *Neraudia*, and *Pipturus*), Elatostemateae (i.e., *Pilea*), and Urticeae (*Hesperocnide*, *Touchardia*, and *Urera*) (Conn and Hadiah 2009, Wu *et al.* 2013). All of the native taxa are endemic to the archipelago with the exception of the indigenous *Pilea peploides* (Gaud.) Hook. & Arnott, and four of these species are federally listed endangered species ([USFWS] 1991, 1994, 1996; Wagner et al. 1999). There are also several non-native urticaceous species that have become recently established in Hawai‘i, including but not limited to *Boehmeria nivea* (L.) Gaud., *Laportea aestuans* (L.) Chew, several *Pilea* species including *P. microphylla* (L.) Liebm., *P. nummulariifolia* (Sw.) Weddell, and *P. cadierei* Gagnep. & Guill., *Cecropia obtusifolia* Bertol., and *Urtica urens* L. (Wagner et al. 1999; Staples & Herbst 2005; Arakaki & Lao 2012).

Little is known about *V. tameamea* performance and preference on the different urticaceous species with the exception of anecdotal observations (Williams 1928; Gorelick & Wielgus 1968; Tabashnik et al. 1992). *V. tameamea* caterpillars have been observed feeding on native species of *Pipturus*, *Urera*, *Neraudia, Touchardia latifolia,* and *Boehmeria grandis* (Swezey 1954). There are no records of *V. tameamea* caterpillars feeding or ovipositing on non-native urticaceous species in the wild, with the possible exception of *B. nivea* (Gorelick & Wielgus 1968), and attempts to feed *V. tameamea* non-native plants (e.g., in the genus *Pilea*) in the laboratory have proved unsuccessful (William Haines unpublished data). From these observations, it appears that the caterpillar's host plants are restricted to species confined to two tribes within Urticaceae: Urticeae and Boehmerieae. It remains unclear whether this is because species in other tribes cannot support *V. tameamea* development, or whether butterflies do not recognize the plants for oviposition.

To provide a more comprehensive assessment of *V. tameamea* performance on Hawaiian Urticaceae, a no-choice bioassay experiment was conducted in order to test the performance of *V. tameamea* (representing populations from three different geographic regions) on four native and one non-native urticaceous species in Hawai‘i: *Boehmeria grandis* (Hook. & Arnott) A. Heller, *Pipturus albidus* (Hook. & Arnott) A. Gray, *Touchardia latifolia* Gaud., *Urera glabra* (Hook. & Arnott) Wedd., and *Cecropia obtusifolia*. The four native species in the study are in the tribes Urticeae (i.e. *T. latifolia* and *U. glabra*) or Boehmerieae (i.e. *B. grandis* and *P. albidus*). *C. obtusifolia* is a non-native, invasive forest species in the tribe Cecropieae (Conn & Hadiah 2009; [HPWRA] 2012; Treiber et al. 2016). *P. albidus* is a common native species found in mesic to wet forest, whereas *B. grandis*, *T. latifolia* and *U. glabra* are less common native forest species (Wagner *et al.* 1999). In addition to quantifying herbivore performance across the species diets, leaf trait data of the focal species were examined in order to identify key traits that mediate these interactions, including both nutritive (leaf nitrogen and phosphorus content) as well as putative defense traits (leaf toughness, calcium).

The main objectives of this study were three-fold: (1) to determine whether the plant species varied in their suitability as hostplants for *V. tameamea* based on a variety of larval and adult performance metrics; (2) to relate the plant leaf traits to *V. tameamea* performance to identify traits underlying variation in hostplant suitability, and (3) to test whether the endemic *V. tameamea* can successfully develop on the non-native *Cecropia obtusifolia*, thereby assessing at the larval stage the potential for an endemic insect to utilize a plant outside of its known host plant range. It was predicted that *V. tameamea* performance will vary across all plant five diets and that first instar caterpillars would not recognize *C. obtusifolia* as a food source because it falls outside of the two Urticaceae tribes that include confirmed host plants. Based on a multitude

of published studies, it was predicted that butterfly performance would be negatively correlated with putative physical defense traits, including leaf toughness by calculating leaf mass per area (LMA) and leaf thickness, and leaf calcium, which was used as a potential proxy for biomineralization in the form of calcium-based cystoliths that are common in species of Urticaceae (Awmack & Leather 2002; Hanley et al. 2007; Poorter et al. 2009; Morehouse & Rutowski 2010; He et al. 2014). Conversely, butterfly performance was predicted to be positively correlated with metrics of leaf nutritional value, including phosphorus concentration, a key macronutrient, and chlorophyll concentration, an indicator of leaf nitrogen, another macronutrient, and inversely related to C:N ratio (i.e. low nitrogen per carbon ratio) (Perkins et al. 2004; Van den Berg & Perkins 2004; Huberty & Denno 2006; Visanuvimol & Bertram 2010; Pellissier et al. 2014; Cease et al. 2016; Liman et al. 2017). This study is the first rigorous assessment of host plant suitability of a Hawaiian endemic butterfly, providing critical information for the conservation of this iconic insect and shedding light on the potential for the species to persist despite declines in its coevolved host plants.

Methods

Study system

To examine host plant preferences and performance of *V. tameamea*, a no-choice laboratory bioassay was performed at the University of Hawai‘i at Mānoa (UHM, Honolulu, Hawai‘i) in fall of 2015. Caterpillars were obtained from two UHM-reared *V. tameamea* colonies developed from founder butterflies collected on two separate mountain ranges on O‘ahu (i.e., Ko‘olau and Waianae) hereafter referred to as the “Koolau” and “Waianae” populations (no *okina*). The Ko‘olau colony was founded from a single wild female collected in Kahana Valley

in April 2014 and was never augmented with additional wild individuals. The Waianae colony was founded from several individuals collected at Waianae-Kaʻala Trail in January 2015, and periodically augmented with wild individuals from Palikea Trail. All colonies were maintained on *Pipturus albidus* leaves. The third population of caterpillars used in this experiment came from the F2 generation of caterpillars collected in July 2015 at Puʻu Huluhulu Kīpuka on Saddle Road, Hawaiʻi Island, hereafter referred to as the Saddle Road population.

To characterize suitability of the urticaceous species as host plants, caterpillars were reared on leaves from one of five plant species throughout development: *B. grandis*, *P. albidus*, *T. latifolia*, *U. glabra*, or *C. obtusifolia***.** During the bioassay experiment, fresh leaves from sapling and reproductively mature plants growing in natural conditions in the Koʻolau Mountains were collected every three to four days, washed with tap water and stored in a lab refrigerator until used.

Bioassay

The bioassay was performed from October to November 2015 in a controlled laboratory setting. Naïve caterpillars were reared on a single species diet with 15 replicate caterpillars per population per diet, giving a total sample size of N=225 (3 diets x 3 populations x 15 replicates).

Caterpillars were reared on benches with a 12L:12D light cycle that represents roughly the mean day length in Hawaiʻi (Carlquist 1980). Mean temperature and relative humidity were recorded hourly throughout the experiment using two data loggers (Onset HOBO U23 Pro v2 External Temperature/Relative Humidity data logger, Bourne, MA). For the first three instars, caterpillars were reared in lidded 2 oz. clear plastic ramekin containers with a moistened disc of filter paper. During this time, the ramekins were cleaned every other day by replacing the filter

paper and removing frass. New plant material was placed in the ramekins every other day. At the fourth instar, caterpillars were transferred to larger 12 oz. clear, lidded containers with a moistened disc of filter paper. The lids of these containers had two small, center slits in order to allow for ventilation. At this time, the holding containers were cleaned and new leaf material was replaced daily. The caterpillars remained in the larger, plastic containers through eclosion. The arrangement of holding containers was randomized daily in order to minimize any effect of microclimate variations on growth.

Caterpillars were observed daily to record development and mortality. Recorded data included dates of: hatching, molting between each instar, pre-pupation (behavioral positioning prior to pupation), pupation, and eclosion. Pupation was indicated by the presence of a hardened chrysalis, and eclosion was marked by the emergence of the adult butterfly from the chrysalis.

In order to account for the variety of ways in which host plants can affect performance of an insect herbivore throughout its life cycle (Kariyat & Portman 2016), the following performance metrics were recorded during this study: mortality (at each life stage), larval duration (i.e., time from egg hatch to pupation in days), pupal duration (i.e., time from pupation to eclosion in days, pupal mass, and adult mass. Adult butterfly mass was recorded 8 to 12 hours after the butterfly had eclosed in order to minimize variance in adult weights due to hemolymph loss and expulsion of the meconium. All butterfly mass data were obtained using a Mettler Toledo AB204-S balance (Greifensee, Switzerland) and measured to the nearest .01mg.

Plant Traits

To investigate traits potentially underlying variation in *V. tameamea* performance across the five plant diets, leaf trait data were collected from mature individuals of each of the five plant

species over the course of two days in October 2016. Two leaves were collected from 8 to 9 wild individuals of each species, and individual plants included many of the same plants in which leaves were harvested for the bioassay. Before harvesting the two leaves in the field, chlorophyll and leaf thickness were measured. Chlorophyll was measured using a chlorophyll meter (SPAD-502 plus, Konica Minolta, Inc., Japan), and leaf thickness was measured using a 0.001 cm precision micrometer (Model 54-850/860, Fowler High Precision, Newton, Massachusetts). Leaves were harvested and immediately stored on ice until returned to the lab, at which time photos were taken to calculate leaf area using ImageJ (version 1.50i, Wayne Rasband, National Institutes of Health, USA). After photos were taken, the two leaves were kept frozen until they were dried at 60°C until constant mass was obtained. The two leaves were weighed to obtain dry mass and then ground together and sent to the University of Hawai‘i at Hilo Analytical Laboratory (Hilo, Hawai‘i) for carbon (C), nitrogen (N), phosphorous (P), and calcium (Ca) nutrient analysis. Nutrient analysis was conducted according to Hue et al. (2000). Briefly, for carbon and nitrogen analysis, 5-6 mg of ground samples were weighed and packaged into tin capsules and then analyzed using a Costech 4010 Elemental Combustion System (ECS) elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA). For phosphorus and calcium analysis, ca. 0.25 g of sample was weighed and then combusted at 500°C for five hours. Once cooled, 10 mL of 1N HCl was added to each sample and left for 30 minutes. Samples were transferred to centrifuge tubes and analyzed using a Varian Vista MPX ICP-OES spectrometer (Varian Inc., Palo Alto, CA). The quality control for all nutrient analyses was NIST® SRM ® 1547.

Data analysis

All analyses were conducted and figures produced in R v3.3.3 (R Development Core Team 2017) using RStudio v.1.1.383 (RStudio Team 2017). Aikake's Information Criterion (AIC) was used to select the best model for each of the following response variables: survival, adult mass, pupal mass, larval duration (i.e., time from egg hatch to pupation in days), and pupal duration (i.e., time from pupation to eclosion in days). The package 'MuMIn' in R was used for each model comparison (Barton 2017). For each response variable, plant diet, sex, and butterfly population, and all interactions were included as the full model, and AIC scores were calculated for all subsets of this full model. All predictor variables were fixed factors. For these analyses, with the exception of survival, data were only used for individuals that survived through eclosion (N=157). Butterfly sex was determined post-eclosion based on sexually-dimorphic wing color patterns (Figure 2.1). Adult mass met linear model assumptions for normality of residuals and homogeneity of variance based on Shapiro-Wilk's and Fligner-Killeen tests, respectively. In order to meet the same assumptions for pupal mass, three outliers were excluded from the data set (N=154) for pupal mass general linear models. The outliers were selected based on quantile-quantile (qq) norm plots as well as other diagnostic plots. An ANOVA was conducted on the best model (lowest AIC) for each of the mass response variables. A Tukey honest significance test was conducted to assess post-hoc pairwise comparisons for main effects and interactions after an ANOVA was performed. Butterfly survival was analyzed using a generalized linear model with a binomial distribution (link = "logit"). Survival was based on N=224, because one caterpillar was lost in the study. Butterflies that survived through eclosion were given the designation of "1," whereas those individuals that died before eclosion occurred were given the designation of "0". There were six instances in which butterflies eclosed with severe deformities that prevented them from taking flight. These individuals were also assigned an identity of "0"

for the logistic regression analysis and excluded from other data analyses. Larval duration and duration of pupation in response to sex, diet, and butterfly population, were analyzed using a generalized linear model (glm) with a Poisson distribution. Tukey post-hoc pairwise comparisons with a Holm correction (Holm 1979) for larval and pupal duration were conducted using the package 'multcomp' in R (Hothorn et al. 2017).

To determine which leaf traits covary, correlations between the following response variables were calculated: C:N ratio, chlorophyll content, leaf P, leaf Ca, leaf thickness, and leaf mass per area. Additionally, a principal component analysis (PCA) based on the six response variables was performed. Each variable was log transformed or scaled prior to conducting the PCA. A generalized linear mixed model (GLMM) on each response variable was performed that included species as a fixed factor and site as the random factor using the package 'lme4' (Bates et al. 2014). Site referred to one of two locations in the Ko'olau Mountains (Oahu) where leaves were harvested for caterpillars during the bioassay. Model comparisons based on AIC values were conducted in the same manner as linear models for performance response variables. Leaf C:N ratio and leaf P were log-transformed to achieve normality. All other response variables met the normality assumption. Tukey post-hoc pairwise comparisons with a Holm correction were performed on significant leaf trait response variables.

In order to directly assess links between hostplant leaf traits and herbivore performance, regression analyses were conducted on mean pupal mass, adult mass, and median larval duration and the mean of each of the plant response variables for leaf traits by plant species. Additionally, a regression analysis was conducted using the PCA1 axis values, which were positively related to leaf defense traits and negatively related to leaf nutrition, and PCA2.

Results

Survival

Vanessa tameamea survival was generally high (Figure 2.2), and did not vary significantly across diets (Wald χ^2=6.37, residual d.f. 4, P=0.17), or among populations (Wald χ^2=0.15, d.f. 2, P=0.93). The crossover interaction of these two variables was also tested but not found to be significant (Diet × Population, Wald χ^2=5.55, d.f. 8, P=0.70). Among the native host plant species, survival was highest on *U. glabra* (87-93% among populations) and lowest on *B. grandis* (53-87% among populations, Figure 2.2). First instar larvae from the Saddle Road population did not recognize the non-native *C. obtusifolia* as a food source and consequently died within their first instar, whereas both populations from Oahu readily ate *C. obtusifolia* and had survival rates similar to the native host plants (Figure 2.2).

Butterfly Performance

Butterfly performance, as assessed by body mass, varied significantly across diets, populations, and sex. With regard to pupal mass, there were significant main effects of diet (F_4=16.79, P<<0.001), sex (F_1=6.46, P=0.012), and population (F_2=6.02, P=0.0031), as well as a significant interaction of Population × Sex (F_2=6.35, P=0.023). There was a marginally significant interaction of Population × Diet on pupal mass (F_7=1.96, P=0.064). For adult mass, there were significant main effects of diet (F_4=16.96, P<<0.001) and sex (F_7=8.98, P=0.0032), and a marginally significant interaction between Population × Sex (F_2=3.039, P=0.051). Adult and pupal mass varied significantly among populations and between sexes (Figure 2.3). Females were 1.04 times larger than males at the pupal stage and 1.07 times larger at the adult stage. Both pupal and adult mass were significantly lower for caterpillars fed *T. latifolia* and *C. obtusifolia*

compared to the pupal and adult masses of larvae fed the other plant diets (Figure 2.4). Larvae that were reared on *B. grandis* had pupal masses 1.16 times greater and adult masses 1.27 times greater than those larvae reared on *T. latifolia* leaves (Figure 2.4).

Diet strongly affected the duration of the larval stage, leading to a highly significant effect of diet on larval duration. Based on model comparisons, the best predictive model for larval duration included the main effects of diet and population. Post-hoc comparisons revealed caterpillars fed *P. albidus* (median larval duration=20.0 days) pupated significantly faster than those fed *B. grandis*, *T. latifolia*, and *U. glabra*. *B. grandis* diets resulted in the longest larval duration (median=28.0 days; Figure 2.5). Caterpillars reared on *C. obtusifolia* (median larval duration=23.5 days) had a larval duration that was intermediate to the range for caterpillars reared on native host plants. Post-hoc comparisons with a Holm correction revealed no significant differences in larval duration among populations. In contrast to the highly significant effect of diet on larval duration, there was no detectable effect of diet, sex or population on the pupal duration (median=12 days) based on model comparisons, and follow-up univariate glm analyses suggested that the duration of this ontogenetic stage is highly conserved with regard to the three predictor variables used in this study.

Plant Trait Analysis

Correlation analysis of the leaf traits revealed that many of the leaf traits are significantly correlated (Figure A2.1). Principal component analysis for the six leaf trait response variables revealed that 43.5% of the variance could be explained by PC axis 1 (PC1) and 25.3% by PC axis 2 (PC2). When the two axes are graphed (Figure 2.6), the native species grouped closely together in trait space whereas *C. obtusifolia* was separate. Higher PC1 axis values indicate

overall higher leaf defenses and less nutritious leaves. The majority of the variance (54.9%) for PC1 was explained by leaf thickness and 68% of the variance for PC2 was explained by leaf chlorophyll (Table 2.1).

Univariate analyses on leaf trait response variables by plant species did not reveal host plants that were clearly best for herbivore development with respect to both defense and nutrition traits (Figure 2.7). For examples, *U. glabra* had a significantly higher P concentration compared to three of the other species, although the C:N ratio of *U. glabra* was not significantly lower than *T. latifolia* and *C. obtusifolia*—diets that resulted in significantly lower pupal and adult *V. tameamea* masses. *B. grandis* had significantly higher C:N ratio and significantly lower chlorophyll concentration in its leaves compared to the other plant species, indicating lower leaf nitrogen compared to the other species (Figure 2.7).

Regression analyses failed to reveal significant relationships between any of the leaf traits and corresponding herbivore performance on the diets, most likely due to the limited sample size (N=5 species; Table 2.2). Two regression analyses that were marginally significant ($P<0.1$) showed an unexpected inverse relationship between leaf chlorophyll concentration and pupal and adult mass, suggesting that greener, more nitrogenous leaves were negatively related to performance (Figure 2.8).

Discussion

Vanessa tameamea butterfly performance varied significantly across unknown and confirmed urticaceous host plant diets. The results from this bioassay highlight how host plants may influence different aspects of herbivore performance, emphasizing the need to examine multiple fitness metrics in a bioassay study (Kariyat & Portman 2016). Overall, butterflies

performed best on *Urera glabra* and *Pipturus albidus*. Butterflies reared on these diets outperformed or equally performed well (in comparison to other diets) across three of five performance metrics. Individuals reared on *U. glabra* exhibited high survival and high pupal and adult masses. Individuals reared on *P. albidus* exhibited high pupal and adult masses and reached pupation significantly faster than individuals reared on other native plant diets. In comparison, individuals reared on *B. grandis* had high pupal and adult masses, but reached pupation significantly slower than individuals reared on all of the other diets. Individuals reared on *C. obtusifolia* did not pupate significantly faster than individuals fed *P. albidus*, but they did have significantly lower pupal and adult masses than *P. albidus*. With regard to larval duration, the large difference in length of larval phase (i.e., between butterflies reared on *B. grandis* versus *P. albidus*) is biologically significant as the risk of parasitism and predation may increase with prolonged exposure at this ontogenetic stage (Benrey & Denno 1997).

Additional variation in performance metrics was detected among populations of *V. tameamea* and between sexes. Males from the Saddle Road population had significantly lower pupal and adult masses compared to Saddle Road females. In general, females were heavier than males among the O‘ahu populations, but there were no significant differences in pupal or adult mass between sexes for these populations. In the order Lepidoptera, sexual size dimorphism favoring larger females is not uncommon (Lederhouse et al. 1982; Rutowski 1997).

Of particular interest, both O‘ahu populations recognized the non-native *C. obtusifolia* as a viable food source, whereas the Saddle Road population from Hawai‘i Island did not. *Cecropia obtusifolia* is in the tribe Cecropieae and is phylogenetically distinct from the other plant species used in this study. *C. obtusifolia* also grouped in a separate trait space than the native plant species in the PCA plot, signifying that it is not only evolutionarily distinct but also functionally

distinct from the four native plant species based on the leaf traits used in this study. Until recently, the species was placed in a separate family (Cecropiaceae) until this family was found to be monophyletic to Urticaceae (Conn & Hadiah 2009; Wu et al. 2013; Treiber et al. 2016). In Hawai‘i, *C. obtusifolia* has become naturalized, and is considered invasive. It is a fast-growing tree species that can grow to heights greater than 15 m (Wagner et al. 1999; [CTAHR] 2003; Daehler 2009). The differences in larval recognition and acceptance of *C. obtusifolia* as a larval diet among the different populations could be due to genetic drift (Massonnet & Weisser 2004). Because *C. obtusifolia* is found on both O‘ahu and Hawai‘i Island, it is difficult to understand why there would be selective pressure for O‘ahu butterflies and not Hawai‘i Island butterflies to recognize the invasive species as a viable food source as larvae. Future studies should investigate adult female *V. tameamea* recognition of *C. obtusifolia* as a host plant by quantifying female oviposition preferences among *C. obtusifolia* and known native host plants.

Despite including a range of putative defensive and nutrition-based leaf traits, no significant relationships between leaf traits and herbivore performance were detected. This could be due to the small sample size of the regression analyses (N=5), or may indicate that the most bioactive traits were ignored. For example, examination of secondary chemistry of the diets could reveal chemical defenses for those species associated with poor butterfly performance. Interestingly, the two marginally significant negative correlations of pupal and adult mass to leaf chlorophyll were in the opposite direction to initial predictions, revealing that herbivore performance was on average higher on species with lower leaf chlorophyll content. Given that nitrogen is a key nutrient for herbivores and chlorophyll is an important source of nitrogen, this result is surprising. None of the other traits, nor the composite PCA vector, were significantly related to any metric of herbivore performance. Leaf traits can be highly variable across regions

(Hulshof & Swenson 2010; Kichenin et al. 2013). Because all of the leaves for the bioassay and for the subsequent leaf trait analyses were collected from the Ko‘olau mountain range on Oahu, results from the bioassay and from the trait analyses may be subject to plant population bias. Additional leaf trait analyses conducted on other populations of the five plant species may reveal significant differences in these parameters among populations.

Insect herbivore performance on its host plants depends on a wide variety of factors, including not only the leaf functional traits investigated in this study. Differences in performance can be explained by other factors such as the protein to carbohydrate ratio of the plant diet and also secondary compounds (Lee 2007; Roeder & Behmer 2014). A class of phenolic compounds known as ellagitannins have been implicated as Lepidoptera-targeted defense compounds (Barbehenn et al. 2006; Moilanen & Salminen 2008). Lepidoptera have high pH (alkaline) midguts (Berenbaum 1980; Dow 1992). Ellagitannins are plant compounds that at high pH ranges become oxidized and thus damaging to tissue (Moilanen & Salminen 2008). Future analysis of ellagitannins of the five plant species used in the bioassay would determine whether butterfly performance is negatively correlated to bioactive ellagitannin concentration.

Conclusion

The variation in *V. tameamea* butterfly performance across native diets suggests that not all native and known host plants for the insect provide equally suitable diet. The ability of the endemic butterfly to utilize and pupate on a non-native, invasive plant species that is distantly related to and groups in a separate trait space (i.e. functionally distinct) from recognized native host plants suggests that the butterfly’s ability to switch hostplants, or at least expand its host plant range, in the wild is feasible and lends reason to believe that population declines are not

due to host plant declines. At the very least, all plants diets used in this experiment, if recognized, allowed for complete development of the insect from caterpillar to butterfly. Interestingly, *Vanessa tameamea* performed best on *Urera glabra* and *Pipturus albidus*. These two species are phylogenetically disjunct and are placed in two different tribes. The following bioassay highlights not only population-level differences in larval recognition of (potential) host plants, but also that evolutionarily distant host plants can provide equally suitable diets for an insect endemic to an oceanic archipelago.

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Tables

Table 2.1. PCA loadings for six leaf trait response variables for the five plant species used in the *Vanessa tameamea* bioassay. PC1 and PC2 jointly accounted for 68.8% of variation among leaf traits.

Response variable	PC1	PC2
% total variation explained	43.5%	25.3%
C:N ratio	0.474	-0.032
Chlorophyll concentration	-0.206	0.688
LMA	0.428	0.274
Leaf thickness	0.549	0.036
Leaf phosphorous	-0.408	-0.418
Leaf calcium	0.287	-0.524

Table 2.2. Results from regression analysis that compared butterfly performance to leaf traits of plant species used in *Vanessa tameamea* bioassay. All F-statistics are based on 1 and 3 degrees of freedom. P-values with (+) indicated p-value<0.1. Meaningful regression analyses (i.e. positive adjusted R^2) are highlighted in bold. PCA 1 is a combined leaf trait variable in which higher values indicate plant species that are less nutritious and exhibit greater leaf trait defenses.

Response variable	Regression statistic	Mean pupal weight	Mean adult weight	Mean larval duration	Median larval duration
C:N ratio	Multiple R^2	0.1946	0.271	0.2316	0.2371
	Adjusted R^2	0.07383	0.02794	-0.0246	-0.01726
	F-statistic	0.725	1.115	0.904	0.9321
	P-value	0.4571	0.3685	0.4119	0.4055
Chlorophyll	Multiple R^2	**0.6643**	**0.6754**	0.1006	0.08104
	Adjusted R^2	**0.5523**	**0.5672**	-0.1992	-0.2253
	F-statistic	**5.935**	**6.242**	0.3355	0.2646
	P-value	**0.09281**+	**0.08784**+	0.6031	0.6425
LMA	Multiple R^2	0.05417	0.1099	0.006919	0.002495
	Adjusted R^2	-0.2611	-0.1868	-0.3241	-0.33
	F-statistic	0.1718	0.3703	0.0209	0.007505
	P-value	0.7064	0.5858	0.8942	0.9364
Leaf thickness	Multiple R^2	**0.2619**	0.2082	0.002076	0.00514
	Adjusted R^2	**0.01585**	-0.05573	-0.3306	-0.3265
	F-statistic	**1.064**	0.7889	0.006242	0.0155
	P-value	**0.3781**	0.4399	0.942	0.9088
Mean leaf P	Multiple R^2	0.02148	0.01416	0.02537	0.03123
	Adjusted R^2	-0.3047	-0.3145	-0.2995	-0.2917
	F-statistic	0.06584	0.04308	0.07809	0.09672
	P-value	0.8141	0.8489	0.7981	0.7762
Mean leaf C	Multiple R^2	0.1564	0.09509	0.03002	0.01452
	Adjusted R^2	-0.1248	-0.2065	-0.2933	-0.314
	F-statistic	0.5561	0.3153	0.09286	0.04419
	P-value	0.5099	0.6137	0.7805	0.847
PCA 1	Multiple R^2	0.1979	0.09509	0.06964	0.05544
	Adjusted R^2	-0.06952	-0.2065	-0.2405	-0.2594
	F-statistic	0.74	0.3153	0.2246	0.1761
	P-value	0.4529	0.6137	0.6679	0.703

Figures

Figure 2.1. *Vanessa tameamea* (from bottom left, clockwise): adult butterflies exhibit sexually dimorphic wing color patterns; larval *V. tameamea* (showing fifth instar caterpillars) can exhibit non-sex related color differences; hardened chrysalis of *V. tameamea*. Photos courtesy of Will Haines and Kari Bogner.

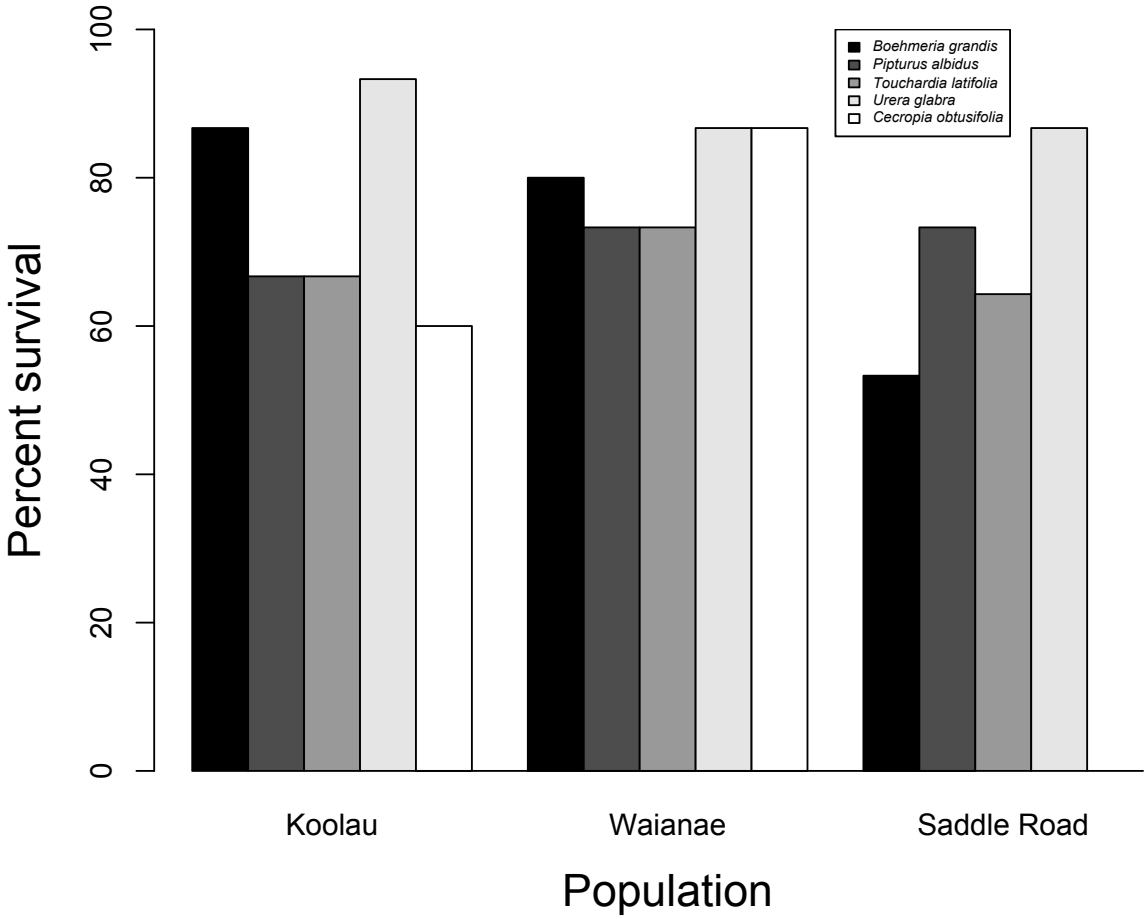

Figure 2.2. Percent survival for N=224 *Vanessa tameamea* butterflies for bioassay experiment. Survival bars are grouped by butterfly population. Butterflies that eclosed with deformities that prevented them from taking flight were classified as dead. All Saddle Road larvae that were fed *C. obtusifolia* died within the first instar.

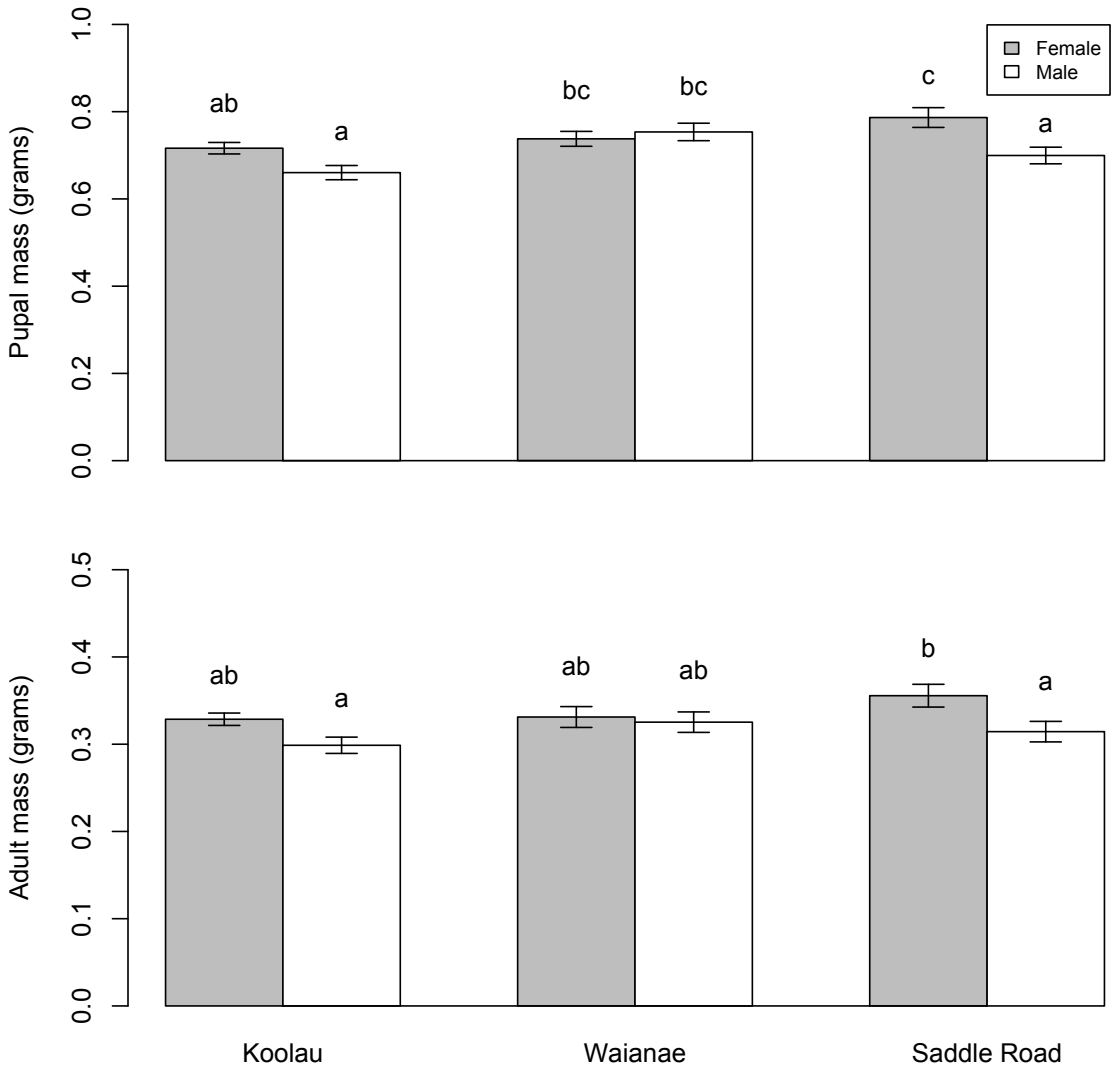

Figure 2.3. The interaction of sex and population on *Vanessa tameamea* pupal mass and adult mass. Tukey post-hoc pairwise comparisons (adjusted $P<0.05$) are made for each mass variable. Error bars represent standard error.

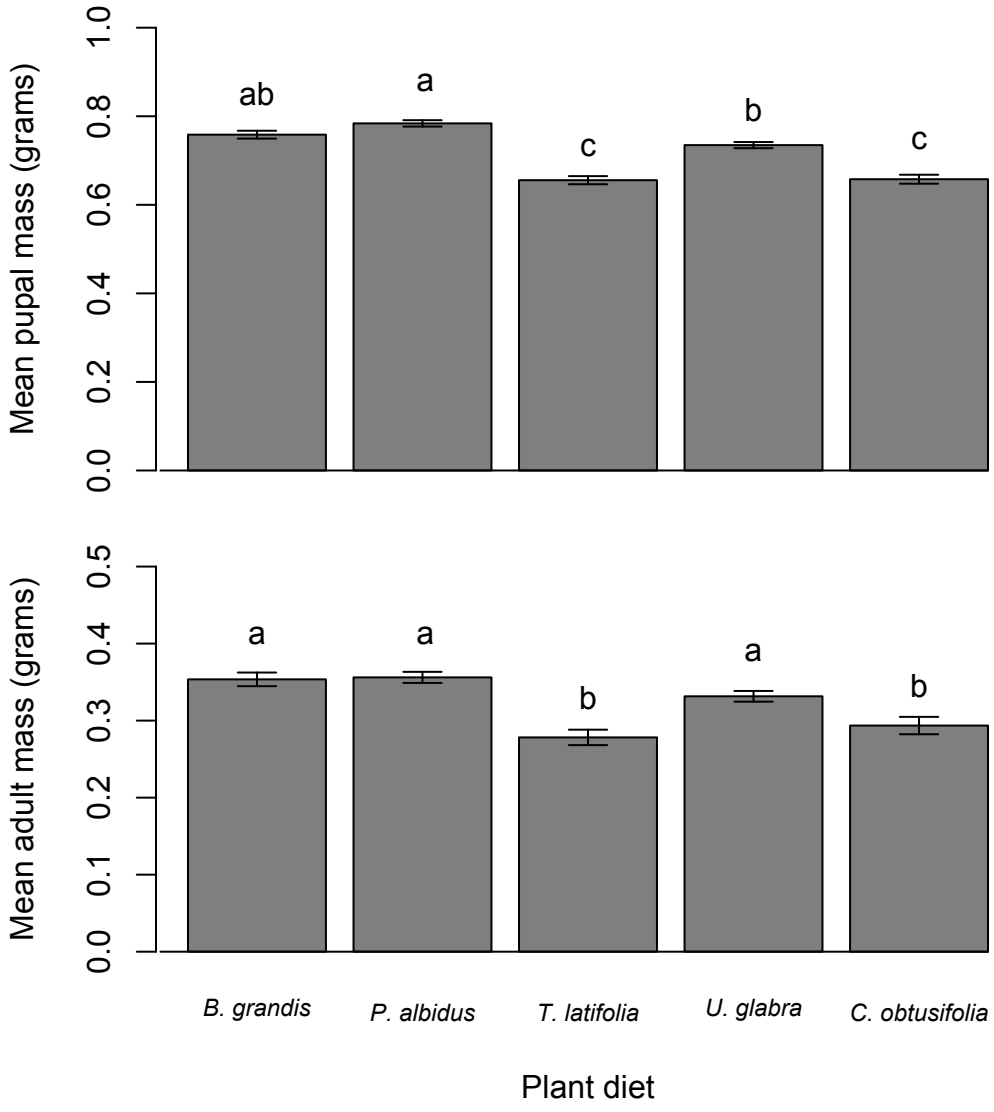

Figure 2.4. Main effect of plant diet on *Vanessa tameamea* pupal and adult mass. Post hoc pairwise comparisons were conducted for each mass performance metric. Error bars represent standard error.

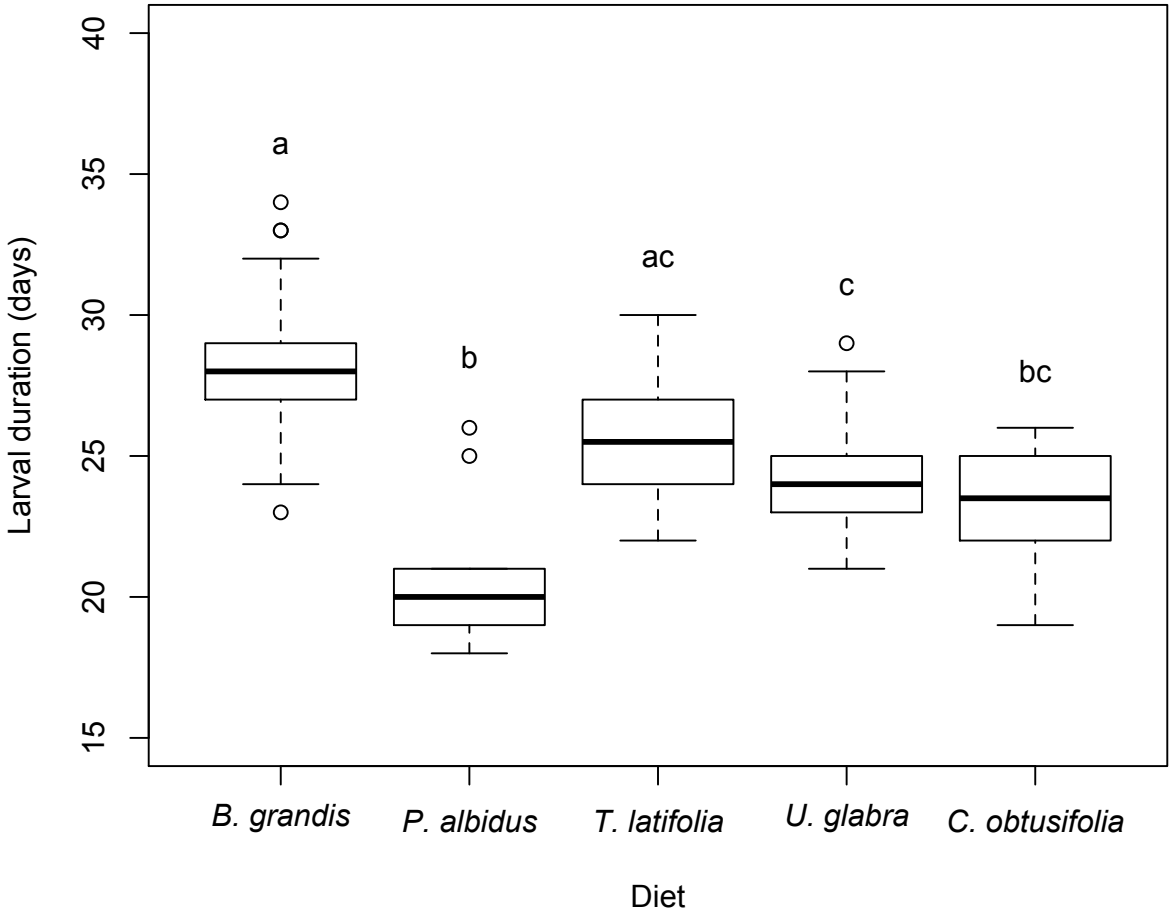

Figure 2.5. Box-plot for the main effect of plant diet by larval duration of *Vanessa tameamea*.

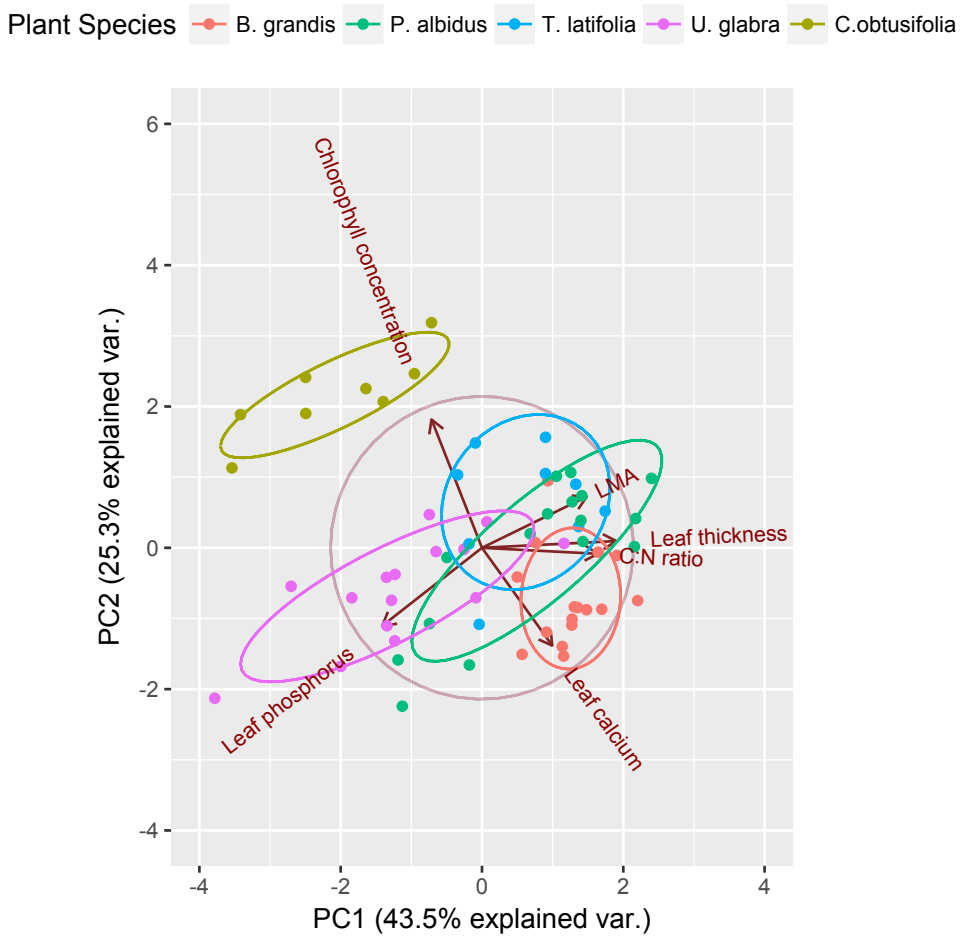

Figure 2.6. Scatterplot of PCA results for leaf trait variables grouped by one of five species of Urticaceae used in *Vanessa tameamea* bioassay study. PC1 and PC2 axes (combined) explained 68.8% variation among leaf traits. Table 2.1 has loadings for PC1 and PC2 axes.

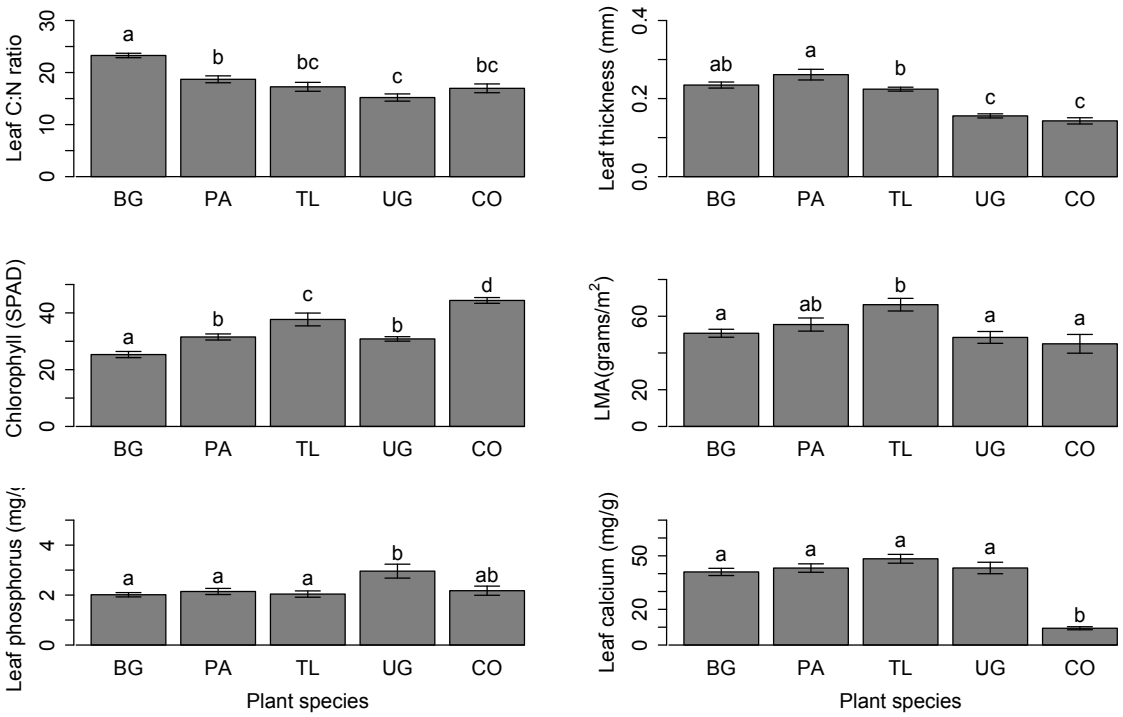

Figure 2.7. Main effect of leaf trait response variables across plant species. Tukey post-hoc pairwise comparisons with a Holm correction are restricted to each figure. Error bars represent standard error. (BG=*Boehmeria grandis*, PA=*Pipturus albidus*, TL=*Touchardia latifolia*, UG=*Urera glabra*, CO=*Cecropia obtusifolia*).

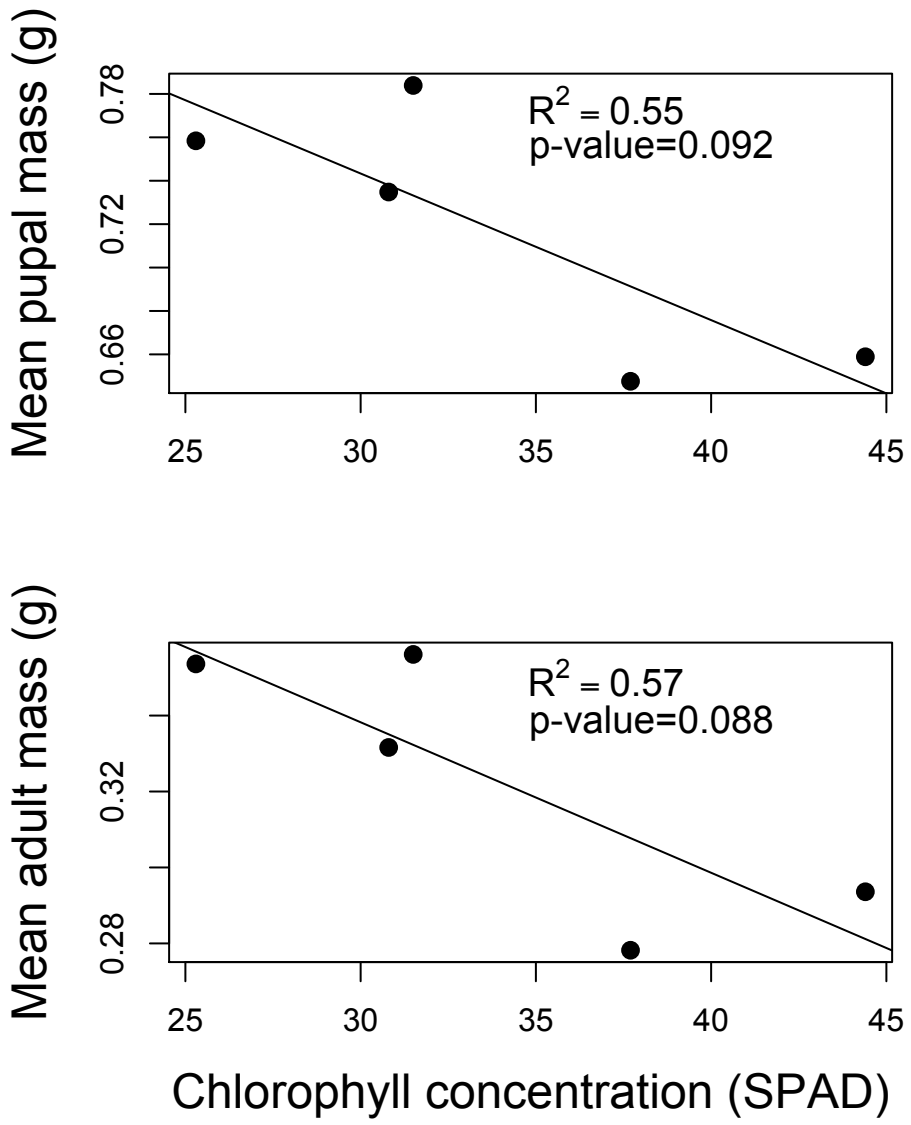

Figure 2.8. Scatterplots with fitted regression lines for *Vanessa tameamea* butterfly performance (pupal and adult mass) by chlorophyll concentration across five plant diets. Analyses were marginally significant ($P<0.1$). Adjusted R^2 values are reported.

Appendix A2

Figure A2.1. Experimental layout for *Vanessa tameamea* bioassay.

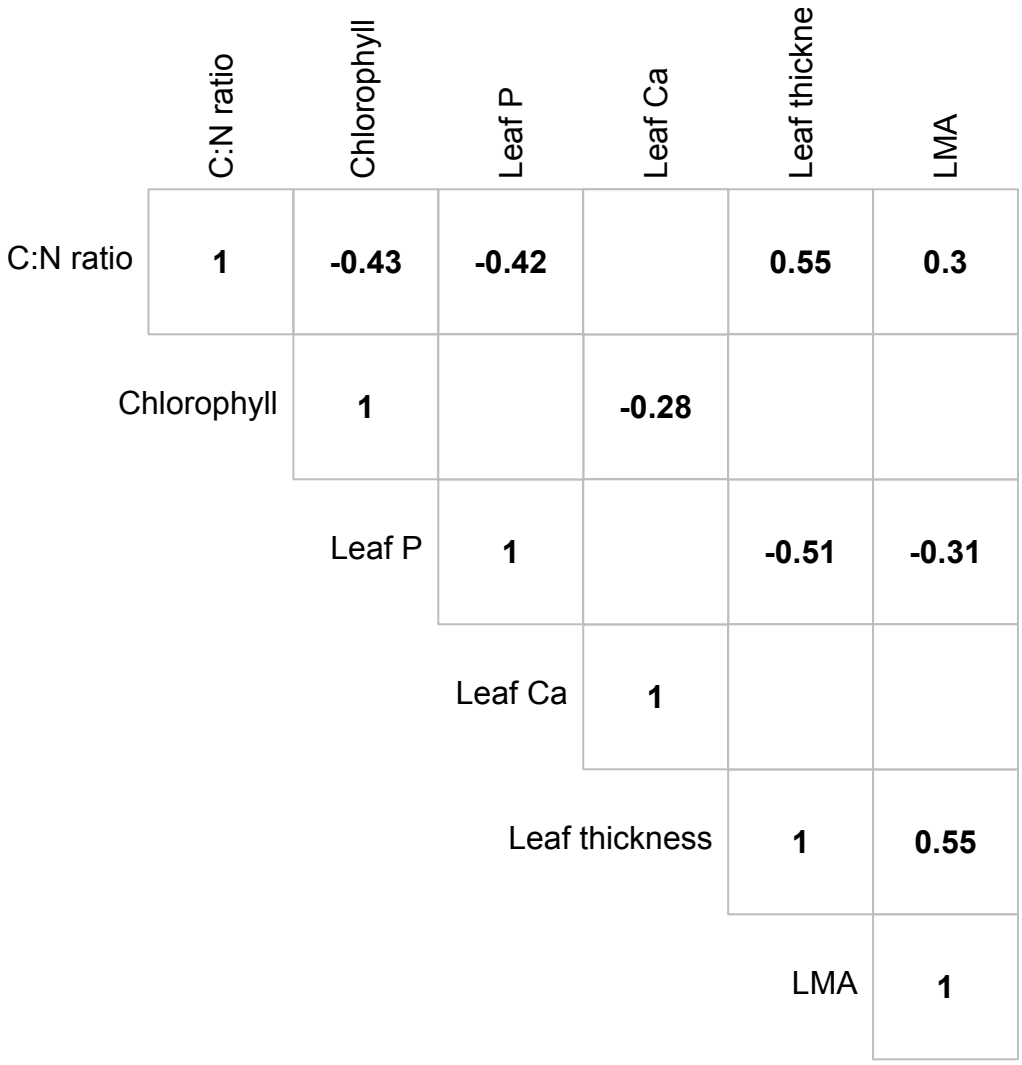

Figure A2.2. Correlogram displaying results from correlation matrix of leaf trait response variables. Significant correlations between response variables are listed in squares (α=0.05, Spearman's test). Empty squares represent non-significant correlations.

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