# GENETIC RELATIONSHIPS AND ANCESTRAL CHARACTER STATE RECONSTRUCTIONS OF *PSYCHOTRIA* L. SECT. *STRAUSSIA* (A. GRAY) FOSBERG (RUBIACEAE)

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# CHAPTER 1. LITERATURE REVIEW AND THESIS PROPOSAL

## INTRODUCTION

Species are the most fundamental unit of biological systems, recognized as a (genetically) distinct organism unique unto itself. However, there are many different species concepts (i.e., ecological, morphological, phylogenetic, reproductive etc.) (Mayr, 1982; Mayden, 1997; De Queiroz, 2005) and as a consequence, no universal criterion for delimiting species exist (Morrison et al., 2009). Morphological similarities have traditionally been used as the major criteria in describing species. However, these can be misleading as morphological traits can be influenced by environmental factors leading to problems such as convergence where unrelated organisms that look similar are classified together. With advancements in molecular techniques and the integration of phylogenetics and population genetics, it is possible to uncover novel relationships among taxa that were thought to be closely related due to morphological similarities and potentially reveal cryptic species when morphological differences are not apparent (Harbaugh et al., 2010; McGlaughlin and Friar, 2011). This is particularly important in understanding species relationships and their delimitation where separate entities are often treated as one. A better understanding of the kinds of differences observed in these closely related taxa and the patterns and processes that drive speciation can contribute much to studies in systematics, evolution, biogeography, ecology, and conservation biology (Duminil et al., 2011).

The Hawaiian archipelago is one of the most isolated land masses in the world but is the site of one of the highest rates of endemism among angiosperms (Price and Wagner, 2004; Keeley and Funk, 2011) despite its isolation and small size. Hawaiian plant radiations also typically exhibit extreme morphological diversity raising questions as to whether to treat

populations with relatively small morphological differences as distinct species or to recognize a large polymorphic taxon with inherent variation (Sohmer, 1977; Harbaugh et al., 2010). This is a particular problem with cryptic species because speciation may not always be accompanied by clear morphological differentiation (Kenfack, 2011). Similarly, a lack of clear morphological differences can occur among distantly related congeners can display morphological similarities through convergence  making it difficult to understand evolutionary relationships (Howarth et al., 1997; Morden et al., 2003). Given these difficulties in correctly recognizing genetic relationships with morphology it is important to investigate these relationships through molecular analyses, especially in morphologically complex Hawaiian plant groups (Dunbar-Co et al., 2008; Baldwin and Friar, 2010; Harbaugh et al., 2010; McGlaughlin and Friar, 2011; Morden and Ching-Harbin, 2013; Appelhans et al., 2014; Morden et al., 2015).

Previous taxonomic treatments and revisions of Hawaiian *Psychotria* L. (Rubiaceae) were based solely on morphology, and there seemed to be disagreements on how many taxa should be recognized and their relationships amongst each other (Rock, 1913; Fosberg, 1964; Sohmer, 1977; Wagner et al., 1990). To date, only two molecular studies on Hawaiian *Psychotria* have been done to assess species relationships and their biogeographical patterns (Nepokroeff et al., 2003; Zhang, 2016). These studies show support of Hawaiian *Psychotria* is a monophyletic group arising from a single colonization event that occurred about 8.73 Ma (Zhang, 2016) and are separated into two clades, corresponding to taxa in sect. *Straussia* (A. Gray) Fosberg and sect. *Pelagomapouria* Fosb., respectively. The phylogenetic relationships among some of the members in sect. *Straussia* are not fully resolved due to lack of genetic variation and do not support current species circumscriptions; this is especially true for *P. kaduana*, *P. mauiensis*, and *P. hawaiiensis* (Nepokroeff et al., 2003; Zhang, 2016). The lack of

resolution and non-monophyletic relationships among some taxa in sect. *Straussia* from the previous studies suggest that future work should explore the utility of other highly variable molecular markers to elucidate species relationships and delimit boundaries.

Several PCR-based dominant marker systems have been developed since the earlier studies of *Psychotria* that can be used for investigating genetic relationships at lower taxonomic levels (Robarts and Wolfe, 2014). These widely used marker systems include random amplified polymorphic DNA (RAPD), sequence related amplified polymorphisms (SRAP), inter-simple sequence repeat (ISSR), and amplified fragment length polymorphism (AFLP). Among these, SRAP and ISSR markers especially have the potential to resolve relationships of closely related species (Wolfe et al., 1998; Martín and Sánchez-Yélamo, 2000; Archibald et al., 2006; Robarts and Wolfe, 2014; Liao et al., 2016). SRAP is simple DNA-based method that is inexpensive and effective for producing genome-wide fragments with high reproducibility and versatility (Li and Quiros, 2001). SRAP markers consist of primers 17 or 18 nucleotides in length that are used to amplify open reading frames. The most common approach to scoring SRAP bands has been by their presence/absence (typically scored as 0 or 1) via electrophoresis and gel visualization. Similar to other dominant markers, limitations of SRAP markers have not yet been described, as this marker system is relatively new and their use is still in its early stages. (Robarts and Wolfe, 2014).

ISSR is a PCR-based technique that involves amplification of a DNA segment between two inversely oriented identical microsatellite repeat regions (Reddy et al. 2002). The longer primers (16–25 mer) used in this method permit higher annealing temperatures leading to higher stringency and improves its reproducibility (Reddy et al., 2002). Like other dominant marker systems, it does have the disadvantage of the possible non-homology of similar sized fragments

(Kumar et al., 2009). However, a new two-step PCR-based method called multiplexed ISSR genotyping by sequencing (MIG-seq) developed by Suyama and Matsuki (2015) overcomes this problem. This technique utilizes reduced representation libraries in which DNA fragments between a selected size range from multiple individuals are pooled together. This allows *de novo* single-nucleotide polymorphisms (SNPs) discovery and genotyping using next-generation sequencing. Using multiplexed ISSR primers, thousands of genome-wide regions can be amplified from a wide variety of genomes without prior genetic information. Furthermore, this method can be utilized in assessing relationships among individuals, populations, closely related species, like those in Hawaiian *Psychotria* and hybrids (Suyama and Matsuki, 2015; Tamaki et al., 2016; Takahashi, 2016; Binh et al., 2018).

**Family Rubiaceae**

Rubiaceae (Coffee family) is one of the largest angiosperm families with more than 13,000 species in ca. 600 genera distributed worldwide with the majority of the species diversity found within the tropics (Davis et. al, 2009). Most members of Rubiaceae are traditionally characterized by leaves that are simple, opposite or whorled, and entire, well developed interpetiolar stipules, and flowers with an inferior ovary (Davis et. al, 2009). Some well-known plants in Rubiaceae include the economically important *Coffea arabica* L. (coffee), medicinal herbs such as *Cinchona* L. which is used to treat malaria, and ornamentals such as *Gardenia* Ellis, *Ixora* L., *Pentas* Benth., and *Mussaenda* L. (Plechakova et. al, 2009).

**Genus *Psychotria***

*Psychotria* L. is the largest genus within the Rubiaceae family with an estimated 1600-1800 species recognized (Davis et al., 2009; Paul et al., 2009). Members of *Psychotria* are distributed pantropically and are typically found as mesic to wet forest understory plants

(Nepokroeff et al., 1999). Taxa are typically shrubs or trees with white heterostylous flowers and are characterized by caducuous stipules, pyrenes without preformed germination slits (except in Pacific members), and usually with a reddish seed coat pigment (Andersson, 2002; Davis et al., 2001; Robbrecht and Manen, 2006). Several phylogenetic studies on *Psychotria* that species relationships within the genus were paraphyletic. Two opposing approaches have been advocated to solve this problem, either for the genus to be delimited in either the very narrowly, or conversely, very broadly (Andersson, 2002; Nepokroeff et al., 1999; Robbrecht and Manen, 2006). Interestingly, Andersson (2002) recognized the genus *Straussia* a name typically applied to *Psychotria* species from Hawai‘i as a separate genus that would include other *Psychotria* members from the western Pacific and Indian Ocean. However, there was no evidence at the time to support that *Straussia* as delimited by Andersson (2002) represented a monophyletic group. However, if it were to be recognized as a distinct genus, its correct name would be *Grumelia* Gaertn. (Lorence and Wagner 2005). Additionally, a number of *Psychotria* species have been transferred to *Margaritopsis* C. Wright due to the phylogenetic placement of these taxa nested within this genus (Barrabé et al. 2012; Razafimandimbison et al., 2014), but were more recently transferred to the genus *Eumachia* DC which had priority (Taylor et al., 2017). Lastly, molecular studies have shown *Psychotria* to be highly paraphyletic and has resulted in separation of *Psychotria* and its relatives into two tribes: Psychotrieae and Palicoureeae with both groups currently undergoing further study, with corresponding nomenclatural changes forthcoming (Barrabé et al., 2012; Barrabé et al., 2014; Razafimandimbison et al., 2014; Lorence et al., 2017).

The first molecular study focused on Hawaiian *Psychotria* was that of Nepokroeff (2003). Using a combination of nuclear ITS and ETS regions, the phylogeny revealed that all

Hawaiian *Psychotria* are from a single colonization event and that the Hawaiian taxa are most closely related to a group of species in subg. *Psychotria* supporting earlier hypotheses based on morphology (Fosberg, 1964; Sohmer, 1977; Sohmer,1978). Kaua‘i was found to be the island most likely colonized by the ancestor of all Hawaiian *Psychotria* and subsequent colonizations occurred from older to younger islands with species of sect. *Straussia* being derived from those in sect. *Pelagomapouria*. Phylogenetic relationships among species of sect. *Pelagomapouria* appear to be well resolved, whereas the relationships among members of sect. *Straussia* are not, especially for *P. kaduana*, *P. mauiensis*, and *P. hawaiiensis* which were paraphyletic.

The following studies applied the data from Nepokroeff et al. (2003) into model-based inference methods to explore the processes that drive evolution of geographic range size using Hawaiian *Psychotria* as their study system. Using the dispersal-extinction-cladogenesis (DEC) model Ree and Smith (2008) found evidence that suggested dispersal was the main cause of speciation in this group. Matzke (2014) modified the DEC model and created the DEC+J model that incorporates founder-event speciation and then compared the two models. Comparison of both models showed that the DEC+J model better reflected the data suggesting that the biogeography of Hawaiian *Psychotria* is best explained by a series of founder events within an island and among islands (Matzke, 2014).

The most recent phylogenetic work done on Hawaiian *Psychotria* was by Zhang (2016). It built upon the work of Nepokroeff et al. (2003) by sampling from several individuals of each species and included more loci to construct a phylogeny. This study showed that a single colonization event about 8.73 Ma gave rise to Hawaiian *Psychotria* with their closest relatives being species from Papua New Guinea. Hawaiian *Psychotria* are separated into two clades, corresponding to the two sections, *Pelagomapouria* and *Straussia*. Taxa in sect. *Pelagomapouria*

each species form well supported monophyletic groups. Also, within sect. *Straussia*, *P. wawrae*, *P. mariniana*, *P. greenwelliae*, *P. hathewayi*, and *P. fauriei* were species that each formed their own monophyletic clade but there were some instances of hybridization or species misidentification (e.g. placement of one individual of *P. fauriei* in the *P. kaduana* clade) (Zhang, 2016). However, *P. kaduana*, *P. mauiensis*, and *P. hawaiiensis* were polyphyletic and continue to be the most problematic with regards to resolving their relationships. The authors also built a chloroplast haplotype network that showed that the younger lineages from sect. *Straussia* possessed a considerable number of similar haplotypes, suggesting that there is a lack of sufficient genetic variation to distinguish among these taxa.

**Taxonomic history of Hawaiian *Psychotria* sect. *Straussia***

When Linnaeus (1759) first described *Psychotria*, he designated *P. asiatica* as the type species. Additionally, while no specimens were cited but in Linnaeus's description he makes a reference to Browne (1756). However, this taxon was from Jamaica in the genus *Psychotrophum*. Suggesting that Linnaeus may have made his description of *P. asiatiaca* based on features of two different species (Petit, 1964; Davis et al., 2001). Petit (1964) designated a lectotype for *P. asiatica* that was accepted as a valid lecotypification by Jarvis et al. (1993). With all the controversy surrounding *P. asiatica*, Davis et al. (2001) resolved this issue by providing a description and identifying specimens of the type species that define the genus *Psychotria*.

The first specimens of *Psychotria* recorded from Hawai‘i (authorities listed in Table 1.1) were collected on the island of O‘ahu by Chamisso and Schlechtendal (1829) and were originally placed in the genus *Coffea* (as *C. kaduana* and *C. mariniana*). Almost 30 years later, Asa Gray (1858) recognized that these taxa are not related to *Coffea*, although Nuttall believed that these species belonged to a new genus *Apionema* and labeled his specimens with three species:

Table 1.1. Current Classification of Hawaiian *Psychotria* by Wagner et al. (1990)

| Current Names | Synonyms |
|---|---|
| *Psychotria fauriei* (H. Lév.) Fosberg | |
| | *Straussia fauriei* H. Lév. |
| *Psychotria grandiflora* H. Mann | |
| | *Straussia grandiflora* Caum |
| *Psychotria greenwelliae* Fosberg | |
| | *Psychotria psychotrioides* (A. Heller) Fosberg |
| | *Straussia psychotrioides* A. Heller |
| *Psychotria hathewayi* Fosberg var. *brevipetiolata* Fosberg | |
| | *Straussia sessilis* O. Deg. & Hosaka |
| *Psychotria hathewayi* Fosberg var. *hathewayi* | |
| | *Psychotria hathewayi* Fosberg |
| | *Psychotria waianensis* Fosberg |
| *Psychotria hawaiiensis* (A. Gray) Fosberg var. *hawaiiensis* | |
| | *Psychotria hawaiiensis* (A. Gray) Fosberg |
| | *Straussia hawaiensis* A. Gray |
| *Psychotria hawaiiensis* (A. Gray) Fosberg var. *hillebrandii* (Rock) Fosberg | |
| | *Straussia hillebrandii* Rock |
| *Psychotria hawaiiensis* (A. Gray) Fosberg var. *scoriacea* (Rock) Fosberg | |
| | *Psychotria hawaiiensis* var. *glomerata* (Rock) Fosberg |
| | *Straussia glomerata* Rock |
| | *Straussia oncocarpa* Hillebr. var. *scoriacea* Rock |
| *Psychotria hexandra* H. Mann var. *hexandra* | |
| | *Psychotria hexandra* var. *hirta* Wawra |
| | *Psychotria hexandra* var. *kealiae* Fosberg |
| | *Psychotria hexandra* f. *waialuana* Fosberg |
| | *Psychotria hirta* (Wawra) A. Heller |
| | *Psychotria hirtula* Skottsb. |
| *Psychotria hexandra* H. Mann var. *oahuensis* O. Deg. & Fosberg | |
| | *Psychotria hexandra* f. *forbesii* Fosberg |
| | *Psychotria hexandra* f. *hosakana* Fosberg |
| | *Psychotria hexandra* var. *hosakana* Fosberg |
| | *Psychotria hexandra* subsp. *oahuensis* O. Deg. & Fosberg |
| | *Psychotria hexandra* var. *rockii* Fosberg |
| | *Psychotria hexandra* var. *st.-johnii* Fosberg |
| *Psychotria hobdyi* Sohmer | |
| | *Psychotria rosacea* H. St. John |
| *Psychotria kaduana* (Cham. & Schltdl.) Fosberg | |
| | *Coffea chamissonis* Hook. & Arn. |
| | *Coffea kaduana* Cham. & Schltdl. |
| | *Psychotria kaduana* var. *longissima* (Rock) Fosberg |
| | *Psychotria kaduana* var. *pubiflora* (A. Heller) Fosberg |
| | *Psychotria leptocarpa* (Hillebr.) Fosberg |
| | *Psychotria longissima* (Rock) H. St. John |
| | *Straussia kaduana (* Cham. & Schltdl.) A. Gray |
| | *Straussia kaduana* var. *coriacea* Hillebr. |
| | *Straussia leptocarpa* Hillebr. |
| | *Straussia longissima* Rock |
| | *Straussia pubiflora* A. Heller |
| *Psychotria mariniana* (Cham. & Schltdl.) Fosberg | |
| | *Coffea mariniana* Cham. & Schltdl. |
| | *Psychotria hawaiiensis* var. *glabrithyrsa* Fosberg |
| | *Straussia mariniana* (Cham. & Schltdl.) A. Gray |
| *Psychotria mauiensis* Fosberg | |
| | *Psychotria hawaiiensis* var. *molokaiensis* (Rock) Fosberg |
| | *Psychotria hawaiiensis* var. *rotundifolia* (Skottsb.) Fosberg |
| | *Psychotria mauiensis* var. *subcordata* (Rock) H. St. John |
| | *Straussia hillebrandii* var. *molokaiensis* Rock |
| | *Straussia hillebrandii* var. *rotundifolia* Skottsb. |
| | *Straussia oncocarpa* Hillebr. |
| | *Straussia oncocarpa* var. *subcordata* Rock |
| *Psychotria wawrae* Sohmer | |
| | *Straussia kaduana* var. *grandifolia* Wawra |

*obovata*, *penduliflora* and *sulcate*, he never published a description of them, so De Candolle's sectional name of *Straussia* was given priority. Thus, Gray (1858) described the new genus, *Straussia*, in which he placed the two species described by the previous authors and described a third species, *S. hawaiiensis*. Two additional new species were described in the genus *Straussia* by Hillebrand (1888) as a part of the *Flora of the Hawaiian Islands* (*S. oncocarpa*, and *S. leptocarpa*). Heller (1897) followed with the description of *S. psychotrioides* and *S. pubiflora*.

In 1911, H. Léviellé described another new species, *S. fauriei*. Shortly after, Joseph Rock (1913) described two new species, *S. longissima* and *S. hillebrandii* including var. *molokaiensis* and two new varieties of *S. oncocarpa* (var. *subcordata* and var. *scoriacea*). Additionally, Degener and Hosaka (1940) described *S. sessilis*, followed by Skottsberg (1944) who described a new variety, *S. hillebrandii* var. *rotundifolia*

The first comprehensive treatment classifying all species of Hawaiian *Psychotria* was done by Fosberg (1964). Fosberg reduced the genus *Straussia* to a section of *Psychotria* and described another section, *Pelagomapouria*. Section *Pelagomapouria* included two species *P. grandiflora* and *P. hexandra*. Section *Straussia* included seven species previously described (*P. mariniana*, *P. kaduana* with var. *longissima* and var. *pubiflora*, *P. leptocarpa*, *P. mauiensis*, *P. fauriei*, *P. psychotrioides*, and *P. hawaiiensis* that included var. *hawaiiensis*, var. *hillebrandii*, var. *scoriacea*, var. *rotundifolia*, var. *glomerata*, var. *glabrithrysa* and var. *molokaiensis*) and an additional three species which he described (*P. hathewayi* with var. *hathewayi* and var. *brevipetiolata*, *P. waianensis*, and *P. greenwelliae*). However, his treatment of sect. *Straussia* was unfinished due to the difficulty in classifying members in this section (Sohmer, 1977).

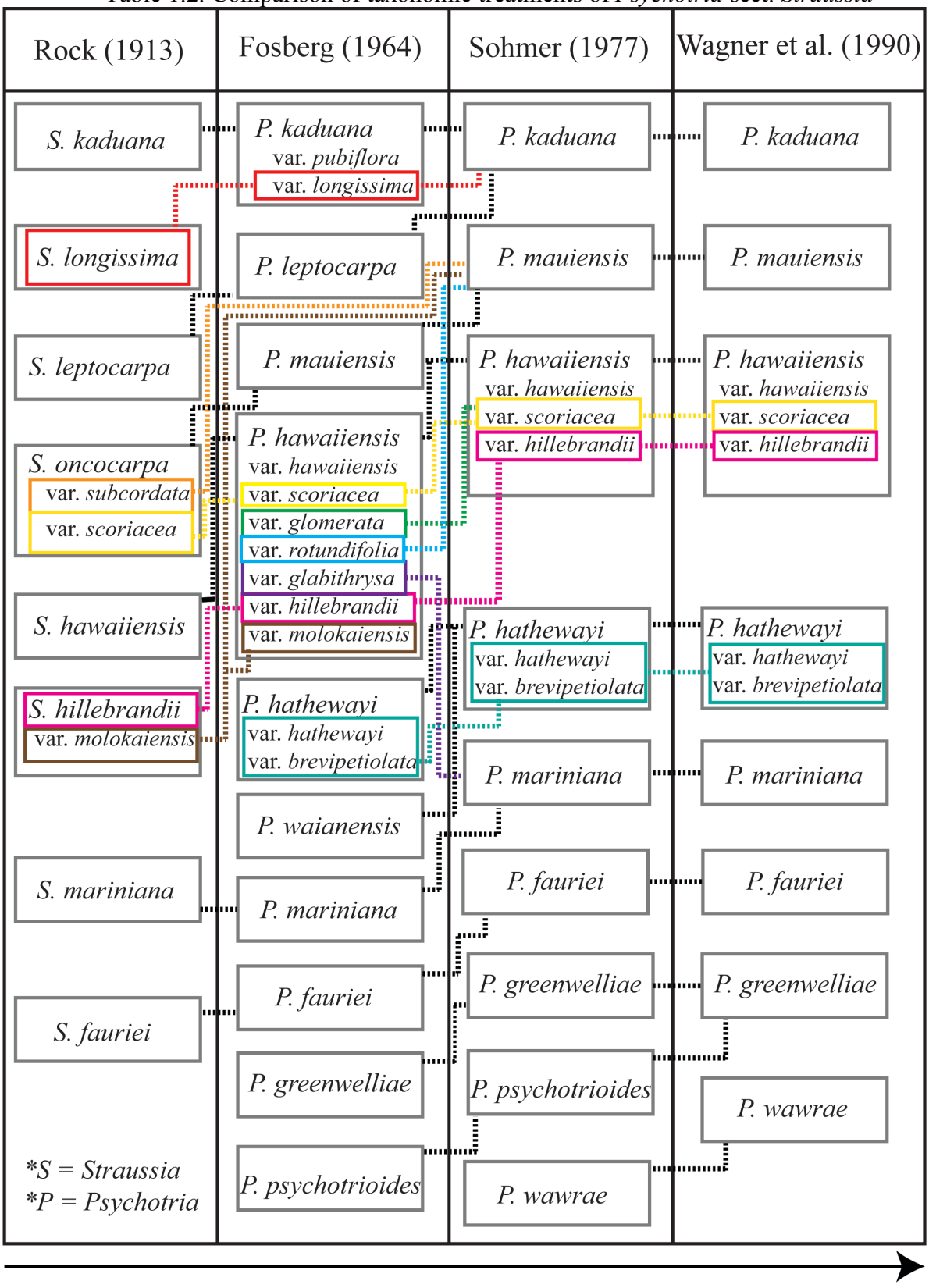
Sohmer (1977) more recently revised the Hawaiian species of *Psychotria*. He reduced to synonymy some species, subspecies, varieties, and forms that Fosberg (1964) had distinguished

stating they represented artificial divisions that are connected by intergrading forms (Table 1.2). A year later, St. John (1978) stated that his taxonomic treatment does not have to agree with that of Sohmer (1977), so he resurrected *P. longissima* to specific status and elevated *subcordata* to varietal rank under *P. mauiensis*. The most recent treatment of Hawaiian *Psychotria* is that of Wagner et al. (1990) as a part of *The Manual of Flowering Plants of Hawai'i*, in which the taxonomic treatment of Sohmer (1977) was largely maintained. The only taxonomic change within sect. *Straussia* was that *P. psychotrioides* was recognized as an illegitimate name and reduced to synonymy under the new name *P. greenwelliae*. There are presently 11 recognized *Psychotria* species endemic to Hawai'i, three in sect. *Pelagomapouria* and eight in sect. *Straussia* (Table 1.1).

**Traditional Uses and Ecological Importance**

In Hawaiian, *Psychotria* spp. are called Kōpiko. The wood from certain species belonging to this genus was made into anvils which were then used to make kapa and was also good for fuel (Malo, 1951). Kōpiko are an integral part of the Hawaiian forest flora (Gagne and Cuddihy, 1990). The shade tolerance of some *Psychotria* species could be useful in restoration efforts in invaded Hawaiian forests (Mascaro, 2011; Schulten, et al. 2014). Hawaiian *Psychotria* are host plants for a couple of insect genera such as *Nesophrosyne,* (Bennet and O'Grady 2012) and *Orthotylus* (Polhemus, 2011). Some species possess conspicuous domatia which is a common character in Rubiaceae (Robbrecht, 1988). These domatia are typically inhabited by mites and are thought to benefit the plant as predators and/or fungivores (Pemberton and Turner, 1989). In return, the domatia are thought to shelter mites from larger predators and fluctuations 1989). In return, the domatia are thought to shelter mites from larger predators and fluctuations in relative humidity (Grostal and O'Dowd 1994; Norton et al., 2001).

Table 1.2. Comparison of taxonomic treatments of *Psychotria* sect. *Straussia*



**Purpose of this study and hypotheses**

The phylogeny of Nepokroeff et al. (2003) revealed that the taxonomic relationships within members of sect. *Straussia* are inconsistent with the way current species are delineated, and that morphological characters that have been traditionally used to circumscribe species may misrepresent species diversity, specifically within members of sect. *Straussia* (Figure 1.1). A population-level study will help discern relationships by comparing genetic markers in the nuclear and chloroplast genomes. The main purpose of this study is two-fold. First, phylogenies constructed based on these regions will be used to test the taxonomic validity of current classifications, specifically: Are they congruent with species established using morphological data? And are there genetically distinct taxa that warrant species recognition? Second, this research will assess the degree of genetic variation within and among representative populations of all species of *Psychotria* sect. *Straussia*. Based on knowledge of the previous taxonomic treatments and phylogeny of Hawaiian *Psychotria*, five hypotheses are proposed:

**Hypothesis one**: Members within sect. *Straussia* have similar morphological character states due to convergent evolution.

Based on herbarium specimens and personal field observations, vast morphological differentiation within the species, especially *P. hawaiiensis*, *P. kaduana* and *P. mauiensis*, causes me to believe that some valid taxa were erroneously submerged into species of the current taxonomic treatment. This will be determined by phylogenetic and population analyses.

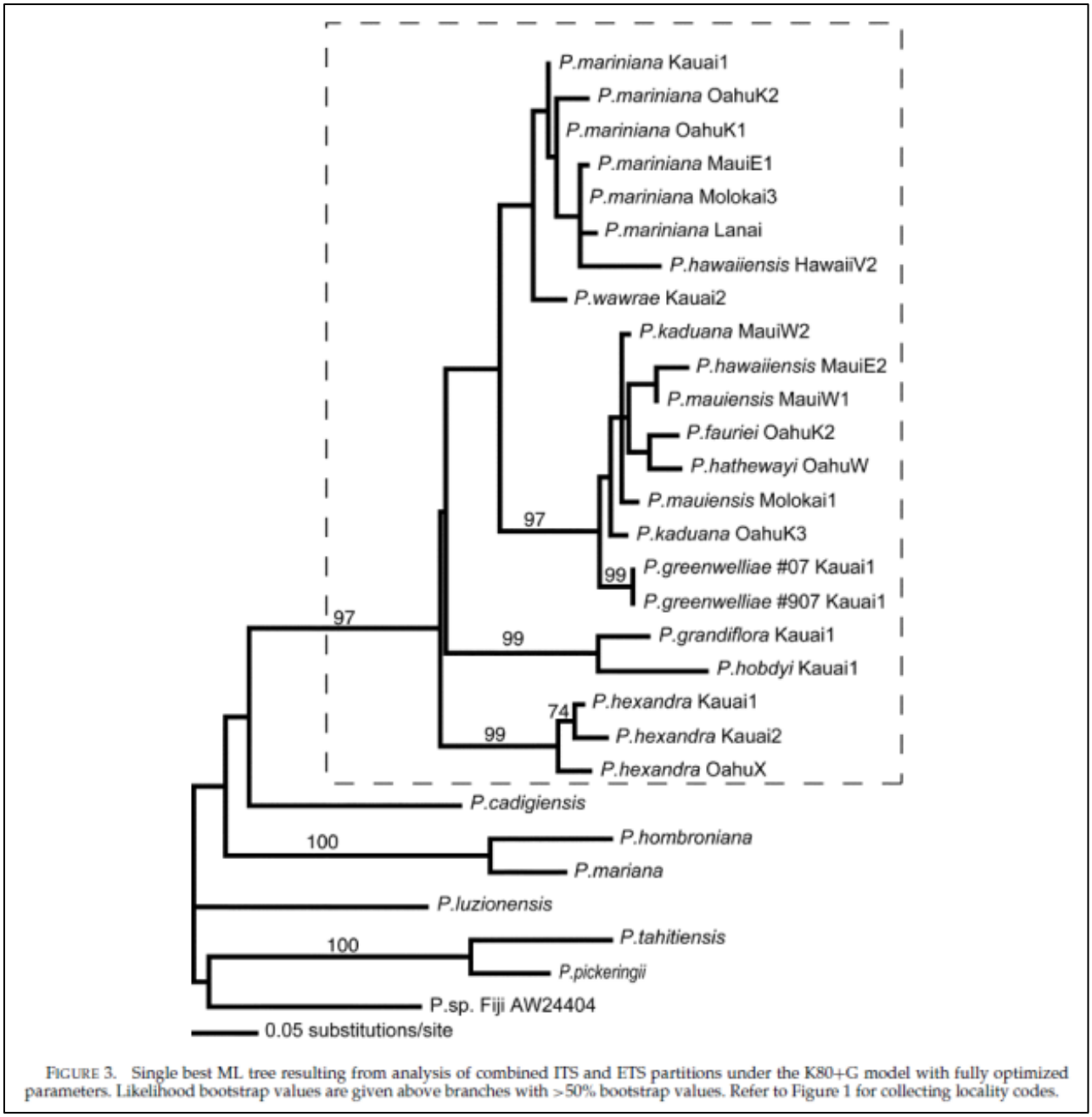



FIGURE 3. Single best ML tree resulting from analysis of combined ITS and ETS partitions under the K80+G model with fully optimized parameters. Likelihood bootstrap values are given above branches with >50% bootstrap values. Refer to Figure 1 for collecting locality codes.

Figure 1.1. Single best maximum likelihood tree resulting from analysis of combined ITS and ETS regions (from Nepokroeff et. al, 2003).

**Hypothesis two**: Plants referred to as *P. hawaiiensis* var. *hillebrandii* and *P. hawaiiensis* var. *scoriacea* are genetically distinct from *P. hawaiiensis* var. *hawaiiensis*.

*Psychotria hawaiiensis* var. *hawaiiensis* is characterized by having usually glabrous leaf blades (Sohmer, 1977; Wagner et al. 1990) whereas *P. hawaiiensis* var. *hillebrandii* originally described as *Straussia hillebrandii* by Rock (1913) was considered a distinct species from *Straussia hawaiiensis* due to its rounded leaf bases, pubescence on the undersides of leaves, lack of domatia, and rusty pubescent inflorescence axes (Rock 1913). Also, Figure 1.2 shows that *P. hawaiiensis* var. *hillebrandii* (labeled "MauiE2") and *P. hawaiiensis* var. *hawaiiensis* (labeled "HawaiiV2") are in two separate clades.

*Psychotria hawaiiensis* var. *scoriacea* was first described by Rock from the lava fields of southern part of Hawai‘i Island as a variety of *Straussia oncocarpa* (now *P. mauiensis*). It is characterized by its elliptic to orbicular leaves and the inflorescence axes with a whitish-yellowish brown pubescence (Sohmer, 1977; Wagner et al., 1990). It was not represented in previous molecular analyses of (Nepokroeff et. al., 2003; Zhang, 2016).

**Hypothesis three**: Plants previously referred to as *Straussia longissima* by Rock (1913) are genetically distinct from *Psychotria kaduana.*

*Straussia longissima* was first described by Rock (1913) in the back of Nu‘uanu Valley, O‘ahu and appears to be present only on the island of O‘ahu. *Psychotria kaduana* has leaves on short petioles or even sessile with leaf bases that are rounded, shortly acuminate or cuneate, and an inflorescence between 4-12.5 cm long, whereas *S. longissima* has subsessile and prominently nerved leaves with a cuneate leaf base, and an inflorescence up to 25 cm long (Rock 1913) . Fosberg (1964) recognized this species as a variety of *P. kaduana,* and it was eventually reduced

to that species by Sohmer (1977). Although St. John (1978) continued to recognize this taxon as a distinct species, but it was again reduced to synonymy to *P. kaduana* in the current treatment by Wagner et al. (1990).

**Hypothesis four**: *Psychotria mauiensis* populations on different islands are genetically distinct (Kauaʻi, Maui, Molokaʻi, and Hawaiʻi).

Hillebrand (1888) first described *Sraussia oncocarpa* from an individual in Ulupalakua, Maui. Fosberg (1964) later transferred *S. oncocarpa* to *Psychotria* with the new name of *P. mauiensis* due to *P. oncocarpa* K. Schum. having priority (Table 1.1). Hillebrand (1888) described a *Straussia oncocarpa* var. β from the island of Kauaʻi that is characterized by its obovate-oblong to rounded leaves, short peduncle, and large corolla lobes. Rock (1913) described *S. oncocarpa* var. *subcordata* and *S. hillebrandii* var. *molokaiensis* from the island of Molokaʻi, both later reduced into synonymy under *P. mauiensis* by Sohmer (1977). Also, Skottsberg (1944) described *S. hillebrandii* var. *rotundifolia* from Hawaiʻi Island and this, too, was also reduced into synonymy under *P. mauiensis* by Sohmer (1977). Individuals of these previously described varieties occur on separate islands. The geographic separation and the possible lack of seed dispersers would suggest that populations on different islands would show some evidence of genetic drift coupled with differential selection. Ree and Smith (2008) suggested that dispersal to a different island may potentially result in speciation event. Only individuals from Maui (labeled "MauiW1") and Molokaʻi (labeled "Molokai1") were examined by Nepokroeff et al. (2003) (Figure 1.1) and they were not monophyletic.

**Hypothesis five**: There are low levels of genetic differentiation among species of *Psychotria* sect. *Straussia*

Low genetic differentiation is common in closely related insular oceanic species that evolved through adaptive radiation (Knope et al. 2012, Lindqvist et al. 2003; Okada et al. 1997). A molecular study of Hawaiian *Psychotria* done by Nepokroeff (2003) (Figure 1.1) revealed poorly resolved basal lineages, especially among members of sect. *Straussia* that may be the result of rapid radiation following a colonization event. In addition, work done by Zhang (2016) showed the some species of within sect. *Straussia* were polyphyletic.

**Future Directions**

Given that previous molecularly based studies of *Psychotria* sect. *Straussia* were unable resolve species boundaries this study aims to use new techniques and a combination of phylogenetics and population genetics to delimit species boundaries and elucidate relationships. Morphological characters will then be used to determine if convergent evolution has occurred and investigate if there are other characters that may aid in elucidating relationships. The combined sequence and population data using SRAPs and SNPs will be used to test the validity of the current taxonomic treatments, and especially to assess whether recognition of separate species is warranted.

# CHAPTER 2. GENETIC RELATIONSHIPS AND ANCESTRAL CHARACTER STATE RECONSTRUCTIONS OF *PSYCHOTRIA* L. SECT. *STRAUSSIA* (A. GRAY) FOSBERG (RUBIACEAE)


## ABSTRACT

Taxonomic classifications based on morphology and molecular studies of members in *Psychotria* sect. *Straussia* have been problematic leaving a number of species identities' in doubt. Also, previous phylogenetic studies showed that *Psychotria hawaiiensis* may represents multiple taxa currently circumscribed under a single species. To resolve the identity of the taxa currently included in this species two nuclear and two chloroplast markers were used and analyzed using Maximum Likelihood and Bayesian Inference. Additionally, two morphological characters were examined in BayesTraits to elucidate relationships among taxa. The molecular analyses reveal that *P. hawaiiensis* is polyphyletic and varieties *hillebrandii* and *scoriacea* are not closely related to variety *hawaiiensis* and they may represent distinct species. Variation in domatia size and leaf venation are consistent with this interpretation of *P. hawaiiensis* and its lack of monophyly, but future work should investigate their taxonomic value within this sect. *Straussia*. Nomenclatural changes that may be needed in the future include reducing *P. hawaiiensis* var. *hawaiiensis* to varietal rank of *P. mariniana*. In addition, recognizing *P. hawaiiensis* var. *hillebrandii* and var. *scoriacea* as separate distinct species. Also, many of the recognized taxa are not well differentiated suggesting that other methods such as next-generation sequencing (NGS) technique should be pursed to disentangle the relationships of these taxa.


## INTRODUCTION

Species are the most fundamental unit of biological systems, recognized as a (genetically) distinct organism unique unto itself. However, there are many different species concepts (i.e., ecological, morphological, phylogenetic, reproductive etc.) (Mayr, 1982; Mayden, 1997; De Queiroz, 2005) and as a consequence, no universal criterion for delimiting species exists (Morrison et al., 2009). Morphological similarities have traditionally been used as the major criteria in describing species. However, these can be misleading as morphological traits can be influenced by environmental factors leading to problems such as convergence where unrelated organisms that look similar are classified together. With advancements in molecular techniques and the integration of phylogenetics and population genetics, it is possible to uncover novel relationships among taxa that were thought to be closely related due to morphological similarities and potentially reveal cryptic species when morphological differences are not apparent (Harbaugh et al., 2010; McGlaughlin and Friar, 2011). This is particularly important in understanding species relationships and their delimitation where separate entities are often treated as one. A better understanding of the kinds of differences observed in these closely related taxa and the patterns and processes that drive speciation can contribute much to studies in systematics, evolution, biogeography, ecology, and conservation biology (Duminil et al., 2011).

The Hawaiian archipelago is one of the most isolated land masses in the world but is the site of one of the highest rates of endemism among angiosperms (Price and Wagner, 2004; Keeley and Funk, 2011) despite its isolation and small size. Hawaiian plant radiations also typically exhibit extreme morphological diversity raising questions as to whether treat populations with relatively small morphological differences as distinct species or to recognize a large polymorphic taxon with inherent variation (Sohmer, 1977; Harbaugh et al., 2010). This is a particular problem with cryptic species because speciation may not always be accompanied by

clear morphological differentiation (Kenfack, 2011). Similarly, a lack of clear morphological differences can occur among distantly related congeners can display morphological similarities through convergence  making it difficult to understand evolutionary relationships (Howarth et al., 1997; Morden et al., 2003). Given these difficulties in correctly recognizing genetic relationships with morphology it is important to investigate these relationships through molecular analyses, especially in morphologically complex Hawaiian plant groups (Dunbar-Co et al., 2008; Baldwin and Friar, 2010; Harbaugh et al., 2010; McGlaughlin and Friar, 2011; Morden and Ching-Harbin, 2013; Appelhans et al., 2014; Morden et al., 2015).

*Psychotria* L. is the largest genus of Rubiaceae with an estimated 1600-1800 species recognized (Paul et al., 2009; Davis et al. 2009). Members of *Psychotria* are distributed pantropically and are typically found as mesic to wet forest understory plants (Nepokroeff et al. 1999). *Psychotria* taxa are typically shrubs or trees with white heterostylous flowers and are characterized by caducuous stipules, absence of preformed germination slits (except in Pacific members), and usually with a reddish seed coat pigment (Andersson 2002; Davis et al. 2001; Robbrecht and Manen 2006). *Psychotria* species in Hawaii are typically referred to as Kōpiko and are important components of the Hawaiian forests and ecologically important (Gagne and Cuddihy, 1990; Mascaro, 2011; Polhemus, 2011; Bennet and O'Grady, 2012; Schulten et al., 2014). Hawaiian *Psychotria* are separated into two sections: *Pelagomapouria* Fosb. and *Straussia* (A. Gray) Fosberg. Section *Pelagomapouria* consists of 3 species and 2 varieties that are characterized by corolla tubes more than 6 mm. long, anthers dorsifixed, pyrenes triangular in cross section and without invagination of the seed coat. Section *Straussia* consist of 8 species and 5 varieties that are characterized by corolla tubes smaller than 3 mm long, anthers basifixed, and pyrenes that are semi-circular in cross section and with a T-shaped seed coat invagination on

the inner or adaxial surface (Sohmer, 1978; Wagner et al., 1990). Furthermore, proper identification of members in sect. *Straussia* can be difficult due to lack of unique morphological characters which has made the taxonomy of this section rather challenging (Sohmer, 1977; Sohmer, 1978; Nepokroeff et al., 2003).

Previous taxonomic treatments and revisions of Hawaii *Psychotria* were based solely on morphology and there seemed to be disagreements on how many taxa should be recognized and their relationships amongst each other (Table 2.1). Additionally, delimitation of members of sect. *Straussia* has proven to be problematic owing to highly variable morphological character states that often overlapping among species (Table 2.2), and in which boundaries are not always clearly defined; to complicate the situation further, species can grow in sympatrically and hybridization may be occurring (Fosberg 1964; Sohmer 1977). Conversely, speciation is not always accompanied by clear morphological differentiation and it is important to look at genetic relationship because morphological characters used to circumscribe species may be homoplasious (Kenfack 2011).

To date, only two molecular studies on Hawaiian *Psychotria* has been done to assess species relationships and biogeographical patterns (Neporkoeff et al. 2003; Zhang 2016). These studies show support of Hawaiian *Psychotria* arising from a single colonization event that occurred about 8.73 Ma (Zhang, 2016) and are separated into two clades, corresponding to taxa in sect. *Straussia* and sect. *Pelagomapouria*, respectively. The phylogenetic relationships among the members in sect. *Pelagomapouria* showed each taxon conforming to well supported monophyletic groups, whereas the relationships among some of the members in sect. *Straussia* are not fully resolved due to lack of genetic variation and do not support current species circumscriptions; this is especially true for *P. kaduana* (Cham & Schltdl.) Fosberg, *P. mauiensis*

Table 2.1. Comparison of taxonomic treatments of *Psychotria* sect. *Straussia*

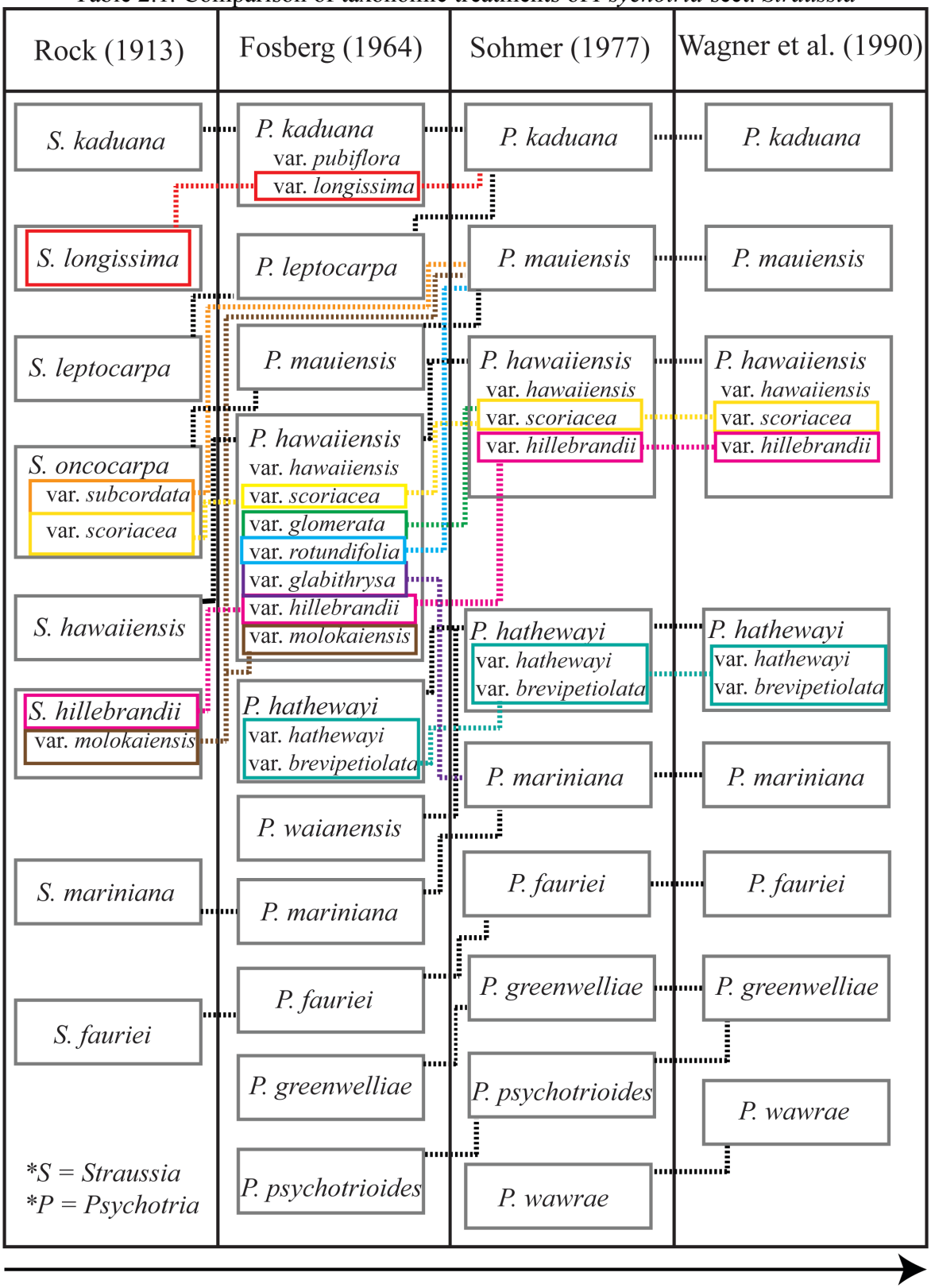


Table 2.2. Distribution, habitat and morphological characteristics of members of *Psychotria* sect. *Straussia*

| | ***Psychotria fauriei*** | ***Psychotria greenwelliae*** | ***Psychotria hathewayi* var. *brevipetiolata*** | ***Psychotria hathewayi* var. *hathewayi*** | ***Psychotria hawaiiensis* var. *hawaiiensis*** |
|---|---|---|---|---|---|
| Distribution | O | K | O | O | Mo, M, H |
| Elevation (m) | (450-)520-860 | 610-1,280 | 360-940 | 360-940 | 50-1,590 |
| Habitat | wet forest | mesic to occasionally wet forest | mesic to occasionally dry forest | mesic to occasionally dry forest | mesic to wet forest |
| Plant height (m) | 3—5 | up to 5 | up to 8 | up to 8 | up to 12 |
| Petiole length (cm) | up to 0.3 | 0.2-2.5 | 0-0.5(-0.8) | 0.4-1.2 | 0.8-4.7 |
| Leaves | stiffly coriaceous, rugose | coriaceous | chartaceous | chartaceous | chartaceous to coriaceous |
| Leaf shape | obovate, obovate-oblong to nearly elliptic-ovate | obovate, oblanceolate-oblong, or elliptic-oblong | oblong-oblanceolate, oblong-elliptic to rotund | obovate to elliptic-oblong to subrotund | mostly obovate |
| Leaf apex | obtuse, rounded, or subtruncate | acute to obtuse or sometimes rounded | rounded | rounded to obtuse | obtuse to rounded, sometimes with a short abrupt point |
| Leaf base | obtuse, rounded, or subcordate | attenuate to narrowly or boradly cuneate | obtuse, rounded, or cordate | acute, obtuse, or subcordate | acuminate, acute, obture, or nearly truncate |
| Leaf length (cm) | 2.5-10 | 2.2-14 | 1.5-12.6 | 1.1-9.5 | 2.2-20.5 |
| Leaf width (cm) | 1.8-5.5 | 1.5-7 | 1.8-7.3 | 2-5.5 | 4.5-9 |
| Abaxial leaf surface | glabrous or puberulent | glabrous or occasionally hirtellous | glabrous or puberulent | glabrous to hirtellous | usually glabrous |
| Tertiary veins | conspicuous | conspicuous | conspicuous | conspicuous | inconspucous |
| Domatia | absent or very small and inconspicuous | absent or small | absent or very small | absent or very small and inconspicuous | conspicuous |
| peduncle length (cm) | 0-5 | N/A | 0-0.5(-0.8) | (0.5-)1-5 | 1.6-8 |
| Inflorescence axes | reddish pubescence, sometimes glabrous | glabrous | pubescent | pubescent | pubescent |
| Fruit shape | pyriform-globose | globose-pyriform | ellipsoid-pyriform | ellipsoid-pyriform | ovoid or obpyriform |
| Fruit length (mm) | 9-11 | 10-13 | 12-18 | 12-18 | 6-10 |

Table 2.2. (Continued) Distribution, habitat and morphological characteristics of members of *Psychotria* sect. *Straussia*

| | ***Psychotria hawaiiensis* var. *hillebrandii*** | ***Psychotria hawaiiensis* var. *scoriacea*** | ***Psychotria kaduana*** | ***Psychotria mariniana*** | ***Psychotria mauiensis*** | ***Psychotria wawrae*** |
|---|---|---|---|---|---|---|
| Distribution | Mo, M, H | M, H | K, O, Mo, M, L | K, O, Mo, M, L | K, Mo, M, L, H | K |
| Elevation (m) | 150-1,530 | 460-1,370 | (15-)180-1,220 | (60-)180-1,220 | 215-1,470 | 120-850 |
| Habitat | wet to moderatly wet forest | dry forest | mesic valleys, mesic forest, and wet forest | mesic to wet forest | mesic to wet forest | mesic forest |
| Plant height (m) | up to 12 | up to 10 | 2-4(-8) | up to 20 | 4 to 12 | up to 5 |
| Petiole length (cm) | 0.5-3 | 0.5-2.5 | up to 2.5 | 0.5-3.3 | 0.5-2 | 0-2 |
| Leaves | chartaceous to coriaceous | coriaceous | chartaceous or coriaceous | coriaceous | membranous to coriaceous | coriaceous |
| Leaf shape | obovate to obovate-oblong, rarely suborbicular | obovate to oblong to subrotund | obovate to oblanceolate or elliptic | oblacneolate to obovate | broadly obovate to oblanceolate, elliptic, or suborbicular | oblacneolate to obovate |
| Leaf apex | obtuse to rounded, sometimes with a short abrupt point | obtuse to rounded, sometimes with a short abrupt point | rounded, obtuse, or acute, often with an abrupt, short, but wide point | acute to obtuse, or rounded, usually with an obtuse, abrupt point | obtuse or rounded | rounded or obtuse and usually with a thick, stout point |
| Leaf base | acuminate, acute, obtuse, or nearly truncate | acuminate, acute, obture, or nearly truncate | cuneate or acuminate, occasionally attenuate, or rarely rounded | usually acuminate or attenuate | acute, rounded, or subtruncate | attenuate to obtuse or subcordate |
| Leaf length (cm) | 3-12.6 | 2—11 | 2.5-14.5 | 5—13 | 1.8-13 | (8.5-)15-29.2 |
| Leaf width (cm) | 5-7.5 | 2.5-7.5 | 0.9-6 | 1.5-6 | 1-9.3 | 4-10.1 |
| Abaxial leaf surface | reddish or rusty pubescent | glabrous or hirtellous | glabrous, sometimes puberulent | glabrous | glabrous or pubescent | usually glabrous |
| Tertiary veins | conspicuous | conspicuous | conspicuous | inconspicuos | conspicuous | inconspucous |
| Domatia | absent or very small and inconspicuous | conspicuous | small and inconspicuous | very conspicous | absent or inconspicuous | absent or extremely small and inconspicuous |
| peduncle length (cm) | 6-10 | N/A | (0.2-)1.6-6.5(-15.5) | 1.2-6.2 | 1-7.4 | 1.8-10 |
| Inflorescence axes | rusty pubescent | whitish or yellowish brown pubescent | glabrous or puberulent | glabrous | glabrous or pubescent | glabrous |
| Fruit shape | ovoid or obpyriform | ovoid or obpyriform | pyriform or ellipsoid | pyriform to oblong-globose | oblong-pyriform | pyriform |
| Fruit length (mm) | 6-8 | 6-7 | 5-15 | 10-12 | 9-15 | 7-15 |

Fosberg, and *P. hawaiiensis* (A. Gray) Fosberg (Nepokroeff et al. 2003; Zhang 2016). Perhaps the characters used to distinguish *P. hawaiiensis* from its congeners may be convergent and as a result current species circumscriptions may be based on elements from two or more species. The lack of resolution and non-monophyletic relationships among taxa in sect. *Straussia* shown in previous studies suggest that future work should explore the utility of other molecular approaches to resolve relationships and delimit species boundaries.

Dominant markers such as sequence related amplified polymorphisms (SRAP) inter-simple sequence repeat (ISSR) are highly variable markers that have the potential to resolve relationships of closely related species (Wolfe et al., 1998; Martín and Sánchez-Yélamo, 2000; Archibald et al., 2006; Robarts and Wolfe, 2014; Liao et al., 2016). Additionally, A new method called multiplexed ISSR genotyping by sequencing (MIG-seq) was recently developed by Suyama and Matsuki (2015) for constructing highly reduced representation libraries which involves de novo single nucleotide polymorphisms (SNP) discovery and their genotyping using next-generation sequencing (NGS). Other commonly used NGS methods typically use restriction enzymes to produce reduced representation libraries which require high quality DNA, whereas MIG-seq is a PCR-based method that can accommodate a wide range of DNA qualities and quantities to run analyses. Additionally, this method can be utilized in assessing relationships and genetic differentiation among individuals, populations, closely related species and hybrids (Suyama and Matsuki 2015; Tamaki et al. 2016; Takahashi 2016; Binh et al., 2018).

This study aims to use a combination of phylogenetics and population genetics to delimit species boundaries, elucidate relationships and assess genetic differentiation among taxa in sect. *Straussia* using molecular markers. Here, sequence analysis of nuclear and chloroplast DNA regions were used to reconstruct phylogenies to investigate species relationship. Morphological

characters were then overlaid onto the phylogenies to determine if convergent evolution had occurred in the morphological characters previously used to delimit *P. hawaiiensis* and to investigate the potential usefulness of other characters in elucidating relationships. Population genetic markers based on sequence related amplified polymorphisms (SRAP) and multiplexed ISSR genotyping by sequencing (MIG-seq) methods were used to assess the level of variation within and among populations throughout the Hawaiian Islands to estimate genetic differentiation, examine genetic structure and gene flow. Combined population and sequence data were used to assess current taxonomic treatments and whether nomenclatural changes is warranted.

## METHODS

### *Taxon sampling —*

Leaf tissues was sampled from all recognized taxa in sect. *Straussia* and preserved in silica gel (Table 2.3). Identification of collections were verified using keys provided by Rock (1913), Fosberg (1964), Sohmer (1977), and Wagner et al. (1990). A total of 49 sampled were analyzed for phylogenetic inference, including at least two samples from each taxon, in order to account for population differences. Moreover, a total of 121 were analyzed for SRAP analyses with up to three samples per population (Table 2.4). Furthermore, a total of 177 samples were analyzed for MIG-seq analyses, with up to 10 samples used per population (Table 2.5). The taxon most difficult to identify and collect in the field was *P. mauiensis* due to its morphological similarities with *P. kaduana* and it is found in areas that are difficult to access. Also, individuals of *P. hawaiiensis* var. *scoriacea* were represented from one population. However, this population

Table 2.3. Taxa used for phylogenetic analyses with collection locality, Hawaii Plant DNA Library accession, and voucher ID

| Taxon | Location (Island) | HPDL | Voucher ID |
|---|---|---|---|
| P. fauriei | Kuliouou (O) | 9322 | JKS74 |
| P. fauriei | Maunawili (O) | 9334 | JKS77 |
| P. fauriei | Moanalua (O) | 9410 | JKS53 |
| P. fauriei | Poamoho (O) | 9309 | JKS56 |
| P. greenwelliae | Awaawapuhi (K) | 9012 | David H. Lorence 10464 (PTBG 068480) |
| P. greenwelliae | Kaluapuhi (K) | 8943 | Michael Kiehn MK-890804-2/1 (BISH 580936) |
| P. hathewayi var. brevipetiolata | Makaha (O) | 9563 | KMW4299 |
| P. hathewayi var. brevipetiolata | Pahole (O) | 9567 | KMW281 |
| P. hathewayi var. brevipetiolata | Palikea (O) | 8777 | JKS36 |
| P. hathewayi var. hathewayi | Kahanahaiki (O) | 9397 | JKS73 |
| P. hathewayi var. hathewayi | Palikea (O) | 8762 | JKS30 |
| P. hawaiiensis var. hawaiiensis | Kalopa (H) | 9096 | JKS111 |
| P. hawaiiensis var. hawaiiensis | Keauohana (H) | 9209 | JKS107 |
| P. hawaiiensis var. hawaiiensis | S. Kona (H) | 9188 | JKS104 |
| P. hawaiiensis var. hillebrandii | Kalopa (H) | 9086 | JKS110 |
| P. hawaiiensis var. hillebrandii | Kapilau (M) | 9568 | H121502 |
| P. hawaiiensis var. hillebrandii | Kaumana (H) | 9140 | JKS109 |
| P. hawaiiensis var. hillebrandii | Kipahulu (M) | 9745 | JKS118 |
| P. hawaiiensis var. hillebrandii | Kipuka (H) | 9230 | S.H. Sohmer 6317 (BISH 436773) |
| P. hawaiiensis var. hillebrandii | S. Kona (H) | 9146 | JKS106 |
| P. hawaiiensis var. hillebrandii | Waiakea (H) | 9261 | JKS112 |
| P. hawaiiensis var. scoriacea | Manuka (H) | 9128 | JKS117 |
| P. hawaiiensis var. scoriacea | Manuka (H) | 9131 | JKS117 |
| P. hawaiiensis var. scoriacea | Manuka (H) | 9136 | JKS117 |
| P. hexandra | Awaawapuhi (K) | 9743 | David H. Lorence 10466 (PTBG 068482) |
| P. kaduana | Hawaii Loa (O) | 9293 | JKS41 |
| P. kaduana | Iao (M) | 9056 | JKS66 |
| P. kaduana | Kuliouou (O) | 8784 | JKS28 |

| | | | |
|---|---|---|---|
| P. kaduana | Limahuli (K) | 8921 | Natalia Tangalin 3284 (PTBG 072995) |
| P. kaduana | Makaua (O) | 9314 | JKS94 |
| P. kaduana | Manoa Cliff (O) | 8727 | JKS19 |
| P. kaduana | Maunawili (O) | 9331 | JKS90 |
| P. kaduana | Moanalua (O) | 8372 | JKS2 |
| P. kaduana | Poamoho (O) | 9380 | JKS61 |
| P. kaduana | Waikamoi (M) | 9048 | JKS116 |
| P. kaduana | Wiliwilinui (O) | 9344 | JKS45 |
| P. mariniana | Alakai (K) | 9027 | Michael Kiehn MK-900907-1/29 (PTBG 008542) |
| P. mariniana | Hanakapiai (K) | 8832 | Steve Perlman 16898 (PTBG 033887) |
| P. mariniana | Hauula (O) | 9282 | JKS70 |
| P. mariniana | Kamakou (MO) | 8791 | JKS16 |
| P. mariniana | Lanipo (O) | 8742 | JKS23 |
| P. mariniana | Makaleha (K) | 8908 | David Lorence 6384(PTBG 003141) |
| P. mariniana | Palikea (O) | 9414 | JKS38 |
| P. mariniana | Waihee (M) | 9039 | R.W. Hobdy 3044 (BISH 572301) |
| P. mariniana x hathewayi | Makaha (O) | 9565 | JKS102 |
| P. mauiensis | Auwahi (M) | 9045 | JKS69 |
| P. mauiensis | Kamakou (MO) | 8802 | JKS17 |
| P. wawrae | Makaleha (K) | 8902 | Natalia Tanglin 3256 (PTBG 72842) |
| P. wawrae | Powerline (K) | 8885 | David H. Lorence 8845 (PTBG 034377) |

Table 2.4. Taxa, collection locality, Hawaii Plant DNA Library accession number used for SRAP analysis

| HPDL ID | Species | Location |
|---|---|---|
| 9322 | fauriei | Kuliouou Summit, Oahu |
| 9323 | fauriei | Kuliouou Summit, Oahu |
| 9334 | fauriei | Maunawili, Oahu |
| 9337 | fauriei | Maunawili, Oahu |
| 9340 | fauriei | Maunawili, Oahu |
| 9405 | fauriei | Moanalua Summit, Oahu |
| 9410 | fauriei | Moanalua Summit, Oahu |
| 9411 | fauriei | Moanalua Summit, Oahu |
| 9308 | fauriei | Poamoho Trail, Oahu |
| 9309 | fauriei | Poamoho Trail, Oahu |
| 9310 | fauriei | Poamoho Trail, Oahu |
| 9011 | greenwelliae | Awaawapuhi, Kauai |
| 9012 | greenwelliae | Awaawapuhi, Kauai |
| 9018 | greenwelliae | Awaawapuhi, Kauai |
| 8941 | greenwelliae | Kaluapuhi, Kauai |
| 8943 | greenwelliae | Kaluapuhi, Kauai |
| 8945 | greenwelliae | Kaluapuhi, Kauai |
| 9385 | hathewayi | Kahanahaiki, Oahu |
| 9397 | hathewayi | Kahanahaiki, Oahu |
| 9400 | hathewayi | Kahanahaiki, Oahu |
| 9563 | hathewayi var. brevipetiolata | Makaha, Oahu |
| 8761 | hathewayi var. hathewayi | Palikea, Oahu |
| 8767 | hathewayi var. hathewayi | Palikea, Oahu |
| 8777 | hathewayi var. hathewayi | Palikea, Oahu |
| 9567 | hathewayi x kaduana | Pahole NAR, Oahu |
| 9101 | hawaiiensis var. hawaiiensis | Hamakua FR, Hawaii |
| 9108 | hawaiiensis var. hawaiiensis | Hamakua FR, Hawaii |
| 9111 | hawaiiensis var. hawaiiensis | Hamakua FR, Hawaii |
| 9209 | hawaiiensis var. hawaiiensis | Keauohana FR, Hawaii |
| 9213 | hawaiiensis var. hawaiiensis | Keauohana FR, Hawaii |
| 9215 | hawaiiensis var. hawaiiensis | Keauohana FR, Hawaii |
| 9188 | hawaiiensis var. hawaiiensis | South Kona FR, Hawaii |
| *** | hawaiiensis var. hawaiiensis | South Kona FR, Hawaii |
| 9205 | hawaiiensis var. hawaiiensis | South Kona FR, Hawaii |
| 9086 | hawaiiensis var. hillebrandii | Hamakua FR, Hawaii |

| | | |
|---|---|---|
| 9092 | hawaiiensis var. hillebrandii | Hamakua FR, Hawaii |
| 9096 | hawaiiensis var. hillebrandii | Hamakua FR, Hawaii |
| 9137 | hawaiiensis var. hillebrandii | Hilo FR, Hawaii |
| 9140 | hawaiiensis var. hillebrandii | Hilo FR, Hawaii |
| 9142 | hawaiiensis var. hillebrandii | Hilo FR, Hawaii |
| 9568 | hawaiiensis var. hillebrandii | Kapilau ridge, Maui |
| 9569 | hawaiiensis var. hillebrandii | Kapilau ridge, Maui |
| 9230 | hawaiiensis var. hillebrandii | Kipuka Puaulu, Hawaii |
| 9236 | hawaiiensis var. hillebrandii | Kipuka Puaulu, Hawaii |
| 9246 | hawaiiensis var. hillebrandii | Kipuka Puaulu, Hawaii |
| 9745 | hawaiiensis var. hillebrandii | Lower Kipahulu Valley, Maui |
| 8756 | hawaiiensis var. hillebrandii | South Kona FR, Hawaii |
| 9146 | hawaiiensis var. hillebrandii | South Kona FR, Hawaii |
| 9261 | hawaiiensis var. hillebrandii | Waiakeakua FR, Hawaii |
| 9262 | hawaiiensis var. hillebrandii | Waiakeakua FR, Hawaii |
| 9271 | hawaiiensis var. hillebrandii | Waiakeakua FR, Hawaii |
| 9128 | hawaiiensis var. scoriacea | Manuka NAR, Hawaii |
| 9131 | hawaiiensis var. scoriacea | Manuka NAR, Hawaii |
| 9136 | hawaiiensis var. scoriacea | Manuka NAR, Hawaii |
| 9285 | kaduana | Hawaii Loa Ridge Trail, Oahu |
| 9290 | kaduana | Hawaii Loa Ridge Trail, Oahu |
| 9293 | kaduana | Hawaii Loa Ridge Trail, Oahu |
| 9056 | kaduana | Iao valley, Maui |
| 9062 | kaduana | Iao valley, Maui |
| 9072 | kaduana | Iao valley, Maui |
| 8782 | kaduana | Kuliouou, Oahu |
| 8784 | kaduana | Kuliouou, Oahu |
| 8919 | kaduana | Limahuli, Kauai |
| 8921 | kaduana | Limahuli, Kauai |
| 8922 | kaduana | Limahuli, Kauai |
| 9313 | kaduana | makaua Valley, Oahu |
| 9314 | kaduana | makaua Valley, Oahu |
| 9318 | kaduana | makaua Valley, Oahu |
| 8727 | kaduana | Manoa Cliff Trail, Oahu |
| 8728 | kaduana | Manoa Cliff Trail, Oahu |
| 8730 | kaduana | Manoa Cliff Trail, Oahu |
| 9326 | kaduana | Maunawili, Oahu |
| 9331 | kaduana | Maunawili, Oahu |
| 9332 | kaduana | Maunawili, Oahu |

| | | |
|---|---|---|
| 8372 | kaduana | Moanalua, Oahu |
| 8379 | kaduana | Moanalua, Oahu |
| 8382 | kaduana | Moanalua, Oahu |
| 9378 | kaduana | Poamoho Trail, Oahu |
| 9380 | kaduana | Poamoho Trail, Oahu |
| 9381 | kaduana | Poamoho Trail, Oahu |
| 9046 | kaduana | Waikamoi Preserve, Maui |
| 9048 | kaduana | Waikamoi Preserve, Maui |
| 9053 | kaduana | Waikamoi Preserve, Maui |
| 9350 | kaduana | Wailupe Valley, Oahu |
| 9344 | kaduana | Wiliwilinui Trail, Oahu |
| 9346 | kaduana | Wiliwilinui Trail, Oahu |
| 8825 | mariniana | Hanakapia'i Valley, Kauai |
| 8829 | mariniana | Hanakapia'i Valley, Kauai |
| 8832 | mariniana | Hanakapia'i Valley, Kauai |
| 9279 | mariniana | Hauula Uka Loop Trail, Oahu |
| 9282 | mariniana | Hauula Uka Loop Trail, Oahu |
| 9283 | mariniana | Hauula Uka Loop Trail, Oahu |
| 8788 | mariniana | Kamakou Preserve, Molokai |
| 8789 | mariniana | Kamakou Preserve, Molokai |
| 8791 | mariniana | Kamakou Preserve, Molokai |
| 9565 | mariniana | Makaha, Oahu |
| 8904 | mariniana | Makaleha Mts., Kauai |
| 8908 | mariniana | Makaleha Mts., Kauai |
| 8909 | mariniana | Makaleha Mts., Kauai |
| 8742 | mariniana | Maumae Trail, Oahu |
| 8745 | mariniana | Maumae Trail, Oahu |
| 8750 | mariniana | Maumae Trail, Oahu |
| 9414 | mariniana | Palikea, Oahu |
| 9415 | mariniana | Palikea, Oahu |
| 9420 | mariniana | Palikea, Oahu |
| 9022 | mariniana | Pihea Trail, Kauai |
| 9027 | mariniana | Pihea Trail, Kauai |
| 9029 | mariniana | Pihea Trail, Kauai |
| 9032 | mariniana | Waihe'e Ridge Trail, Maui |
| 9039 | mariniana | Waihe'e Ridge Trail, Maui |
| 9040 | mariniana | Waihe'e Ridge Trail, Maui |
| 9045 | mauiensis | Auwahi, Maui |
| 8795 | mauiensis | Kamakou Preserve, Molokai |

| | | |
|---|---|---|
| 8804 | mauiensis | Kamakou Preserve, Molokai |
| 8813 | mauiensis | Kamakou Preserve, Molokai |
| 8892 | wawrae | Makaleha, Kauai |
| 8895 | wawrae | Makaleha, Kauai |
| 8902 | wawrae | Makaleha, Kauai |
| 8881 | wawrae | Powerline Trail, Kauai |
| 8882 | wawrae | Powerline Trail, Kauai |
| 8885 | wawrae | Powerline Trail, Kauai |

Table 2.5. Taxon samples of sect. *Straussia* used for MIG-seq analysis, showing Hawaii Plant DNA Library ID (HPDL), and the location/island the sample was originally collected from

| HPDL ID | Genus | Species | Locality |
|---|---|---|---|
| 9322 | Psychotria | fauriei | Kuliouou Summit, Oahu |
| 9323 | Psychotria | fauriei | Kuliouou Summit, Oahu |
| 9334 | Psychotria | fauriei | Maunawili, Oahu |
| 9336 | Psychotria | fauriei | Maunawili, Oahu |
| 9337 | Psychotria | fauriei | Maunawili, Oahu |
| 9339 | Psychotria | fauriei | Maunawili, Oahu |
| 9340 | Psychotria | fauriei | Maunawili, Oahu |
| 9405 | Psychotria | fauriei | Moanalua Summit, Oahu |
| 9407 | Psychotria | fauriei | Moanalua Summit, Oahu |
| 9410 | Psychotria | fauriei | Moanalua Summit, Oahu |
| 9411 | Psychotria | fauriei | Moanalua Summit, Oahu |
| 9412 | Psychotria | fauriei | Moanalua Summit, Oahu |
| 9307 | Psychotria | fauriei | Poamoho Trail, Oahu |
| 9308 | Psychotria | fauriei | Poamoho Trail, Oahu |
| 9309 | Psychotria | fauriei | Poamoho Trail, Oahu |
| 9310 | Psychotria | fauriei | Poamoho Trail, Oahu |
| 9311 | Psychotria | fauriei | Poamoho Trail, Oahu |
| 9382 | Psychotria | hathewayi | Kahanahaiki, Oahu |
| 9385 | Psychotria | hathewayi | Kahanahaiki, Oahu |
| 9396 | Psychotria | hathewayi | Kahanahaiki, Oahu |
| 9397 | Psychotria | hathewayi | Kahanahaiki, Oahu |
| 9400 | Psychotria | hathewayi | Kahanahaiki, Oahu |
| 8759 | Psychotria | hathewayi | Palikea, Oahu |
| 8761 | Psychotria | hathewayi | Palikea, Oahu |
| 8767 | Psychotria | hathewayi | Palikea, Oahu |
| 8777 | Psychotria | hathewayi | Palikea, Oahu |
| 8780 | Psychotria | hathewayi | Palikea, Oahu |
| 9563 | Psychotria | hathewayi var. brevipetiolata | Makaha, Oahu |
| 9097 | Psychotria | hawaiiensis var. hawaiiensis | Hamakua FR, Hawaii |
| 9100 | Psychotria | hawaiiensis var. hawaiiensis | Hamakua FR, Hawaii |
| 9101 | Psychotria | hawaiiensis var. hawaiiensis | Hamakua FR, Hawaii |
| 9104 | Psychotria | hawaiiensis var. hawaiiensis | Hamakua FR, Hawaii |
| 9107 | Psychotria | hawaiiensis var. hawaiiensis | Hamakua FR, Hawaii |
| 9108 | Psychotria | hawaiiensis var. hawaiiensis | Hamakua FR, Hawaii |
| 9111 | Psychotria | hawaiiensis var. hawaiiensis | Hamakua FR, Hawaii |
| 9207 | Psychotria | hawaiiensis var. hawaiiensis | Keauohana FR, Hawaii |

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|---|---|---|---|
| 9208 | Psychotria | hawaiiensis var. hawaiiensis | Keauohana FR, Hawaii |
| 9209 | Psychotria | hawaiiensis var. hawaiiensis | Keauohana FR, Hawaii |
| 9210 | Psychotria | hawaiiensis var. hawaiiensis | Keauohana FR, Hawaii |
| 9212 | Psychotria | hawaiiensis var. hawaiiensis | Keauohana FR, Hawaii |
| 9213 | Psychotria | hawaiiensis var. hawaiiensis | Keauohana FR, Hawaii |
| 9214 | Psychotria | hawaiiensis var. hawaiiensis | Keauohana FR, Hawaii |
| 9215 | Psychotria | hawaiiensis var. hawaiiensis | Keauohana FR, Hawaii |
| 9185 | Psychotria | hawaiiensis var. hawaiiensis | South Kona FR, Hawaii |
| 9186 | Psychotria | hawaiiensis var. hawaiiensis | South Kona FR, Hawaii |
| 9188 | Psychotria | hawaiiensis var. hawaiiensis | South Kona FR, Hawaii |
| 9195 | Psychotria | hawaiiensis var. hawaiiensis | South Kona FR, Hawaii |
| 9197 | Psychotria | hawaiiensis var. hawaiiensis | South Kona FR, Hawaii |
| *** | Psychotria | hawaiiensis var. hawaiiensis | South Kona FR, Hawaii |
| 9201 | Psychotria | hawaiiensis var. hawaiiensis | South Kona FR, Hawaii |
| 9205 | Psychotria | hawaiiensis var. hawaiiensis | South Kona FR, Hawaii |
| 9086 | Psychotria | hawaiiensis var. hillebrandii | Hamakua FR, Hawaii |
| 9087 | Psychotria | hawaiiensis var. hillebrandii | Hamakua FR, Hawaii |
| 9088 | Psychotria | hawaiiensis var. hillebrandii | Hamakua FR, Hawaii |
| 9092 | Psychotria | hawaiiensis var. hillebrandii | Hamakua FR, Hawaii |
| 9093 | Psychotria | hawaiiensis var. hillebrandii | Hamakua FR, Hawaii |
| 9095 | Psychotria | hawaiiensis var. hillebrandii | Hamakua FR, Hawaii |
| 9096 | Psychotria | hawaiiensis var. hillebrandii | Hamakua FR, Hawaii |
| 9137 | Psychotria | hawaiiensis var. hillebrandii | Hilo FR, Hawaii |
| 9138 | Psychotria | hawaiiensis var. hillebrandii | Hilo FR, Hawaii |
| 9139 | Psychotria | hawaiiensis var. hillebrandii | Hilo FR, Hawaii |
| 9140 | Psychotria | hawaiiensis var. hillebrandii | Hilo FR, Hawaii |
| 9141 | Psychotria | hawaiiensis var. hillebrandii | Hilo FR, Hawaii |
| 9142 | Psychotria | hawaiiensis var. hillebrandii | Hilo FR, Hawaii |
| 9143 | Psychotria | hawaiiensis var. hillebrandii | Hilo FR, Hawaii |
| 9144 | Psychotria | hawaiiensis var. hillebrandii | Hilo FR, Hawaii |
| 9568 | Psychotria | hawaiiensis var. hillebrandii | Kapilau ridge, Maui |
| 9569 | Psychotria | hawaiiensis var. hillebrandii | Kapilau ridge, Maui |
| 9222 | Psychotria | hawaiiensis var. hillebrandii | Kipuka Puaulu, Hawaii |
| 9230 | Psychotria | hawaiiensis var. hillebrandii | Kipuka Puaulu, Hawaii |
| 9231 | Psychotria | hawaiiensis var. hillebrandii | Kipuka Puaulu, Hawaii |
| 9235 | Psychotria | hawaiiensis var. hillebrandii | Kipuka Puaulu, Hawaii |
| 9236 | Psychotria | hawaiiensis var. hillebrandii | Kipuka Puaulu, Hawaii |
| 9237 | Psychotria | hawaiiensis var. hillebrandii | Kipuka Puaulu, Hawaii |
| 9245 | Psychotria | hawaiiensis var. hillebrandii | Kipuka Puaulu, Hawaii |

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|---|---|---|---|
| 9246 | Psychotria | hawaiiensis var. hillebrandii | Kipuka Puaulu, Hawaii |
| 9745 | Psychotria | hawaiiensis var. hillebrandii | Lower Kipahulu Valley, Maui |
| 8756 | Psychotria | hawaiiensis var. hillebrandii | South Kona FR, Hawaii |
| 9146 | Psychotria | hawaiiensis var. hillebrandii | South Kona FR, Hawaii |
| 9261 | Psychotria | hawaiiensis var. hillebrandii | Waiakeakua FR, Hawaii |
| 9262 | Psychotria | hawaiiensis var. hillebrandii | Waiakeakua FR, Hawaii |
| 9271 | Psychotria | hawaiiensis var. hillebrandii | Waiakeakua FR, Hawaii |
| 9114 | Psychotria | hawaiiensis var. scoriacea | Manuka NAR, Hawaii |
| 9115 | Psychotria | hawaiiensis var. scoriacea | Manuka NAR, Hawaii |
| 9117 | Psychotria | hawaiiensis var. scoriacea | Manuka NAR, Hawaii |
| 9125 | Psychotria | hawaiiensis var. scoriacea | Manuka NAR, Hawaii |
| 9128 | Psychotria | hawaiiensis var. scoriacea | Manuka NAR, Hawaii |
| 9129 | Psychotria | hawaiiensis var. scoriacea | Manuka NAR, Hawaii |
| 9131 | Psychotria | hawaiiensis var. scoriacea | Manuka NAR, Hawaii |
| 9134 | Psychotria | hawaiiensis var. scoriacea | Manuka NAR, Hawaii |
| 9135 | Psychotria | hawaiiensis var. scoriacea | Manuka NAR, Hawaii |
| 9136 | Psychotria | hawaiiensis var. scoriacea | Manuka NAR, Hawaii |
| 9284 | Psychotria | kaduana | Hawaii Loa Ridge Trail, Oahu |
| 9285 | Psychotria | kaduana | Hawaii Loa Ridge Trail, Oahu |
| 9290 | Psychotria | kaduana | Hawaii Loa Ridge Trail, Oahu |
| 9293 | Psychotria | kaduana | Hawaii Loa Ridge Trail, Oahu |
| 9304 | Psychotria | kaduana | Hawaii Loa Ridge Trail, Oahu |
| 9054 | Psychotria | kaduana | Iao valley, Maui |
| 9056 | Psychotria | kaduana | Iao valley, Maui |
| 9062 | Psychotria | kaduana | Iao valley, Maui |
| 9070 | Psychotria | kaduana | Iao valley, Maui |
| 9072 | Psychotria | kaduana | Iao valley, Maui |
| 8782 | Psychotria | kaduana | Kuliouou, Oahu |
| 8784 | Psychotria | kaduana | Kuliouou, Oahu |
| 8919 | Psychotria | kaduana | Limahuli, Kauai |
| 8920 | Psychotria | kaduana | Limahuli, Kauai |
| 8921 | Psychotria | kaduana | Limahuli, Kauai |
| 8922 | Psychotria | kaduana | Limahuli, Kauai |
| 8927 | Psychotria | kaduana | Limahuli, Kauai |
| 9312 | Psychotria | kaduana | makaua Valley, Oahu |
| 9313 | Psychotria | kaduana | makaua Valley, Oahu |
| 9314 | Psychotria | kaduana | makaua Valley, Oahu |
| 9318 | Psychotria | kaduana | makaua Valley, Oahu |
| 9320 | Psychotria | kaduana | makaua Valley, Oahu |

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|---|---|---|---|
| 8727 | Psychotria | kaduana | Manoa Cliff Trail, Oahu |
| 8728 | Psychotria | kaduana | Manoa Cliff Trail, Oahu |
| 8729 | Psychotria | kaduana | Manoa Cliff Trail, Oahu |
| 8730 | Psychotria | kaduana | Manoa Cliff Trail, Oahu |
| 8731 | Psychotria | kaduana | Manoa Cliff Trail, Oahu |
| 9326 | Psychotria | kaduana | Maunawili, Oahu |
| 9328 | Psychotria | kaduana | Maunawili, Oahu |
| 9331 | Psychotria | kaduana | Maunawili, Oahu |
| 9332 | Psychotria | kaduana | Maunawili, Oahu |
| 9333 | Psychotria | kaduana | Maunawili, Oahu |
| 8372 | Psychotoria | kaduana | Moanalua, Oahu |
| 8375 | Psychotoria | kaduana | Moanalua, Oahu |
| 8379 | Psychotoria | kaduana | Moanalua, Oahu |
| 8382 | Psychotoria | kaduana | Moanalua, Oahu |
| 8384 | Psychotoria | kaduana | Moanalua, Oahu |
| 9370 | Psychotria | kaduana | Poamoho Trail, Oahu |
| 9373 | Psychotria | kaduana | Poamoho Trail, Oahu |
| 9378 | Psychotria | kaduana | Poamoho Trail, Oahu |
| 9380 | Psychotria | kaduana | Poamoho Trail, Oahu |
| 9381 | Psychotria | kaduana | Poamoho Trail, Oahu |
| 9046 | Psychotria | kaduana | Waikamoi Preserve, Maui |
| 9047 | Psychotria | kaduana | Waikamoi Preserve, Maui |
| 9048 | Psychotria | kaduana | Waikamoi Preserve, Maui |
| 9052 | Psychotria | kaduana | Waikamoi Preserve, Maui |
| 9053 | Psychotria | kaduana | Waikamoi Preserve, Maui |
| 9349 | Psychotria | kaduana | Wailupe Valley, Oahu |
| 9350 | Psychotria | kaduana | Wailupe Valley, Oahu |
| 9352 | Psychotria | kaduana | Wailupe Valley, Oahu |
| 9343 | Psychotria | kaduana | Wiliwilinui Trail/Waianae Nui, Oahu |
| 9344 | Psychotria | kaduana | Wiliwilinui Trail/Waianae Nui, Oahu |
| 9346 | Psychotria | kaduana | Wiliwilinui Trail/Waianae Nui, Oahu |
| 9567 | Psychotria | kaduana x hathewayi | Pahole NAR, Oahu |
| 9565 | Psychotria | mariana x hathewayi | Makaha, Oahu |
| 8825 | Psychotria | mariniana | Hanakapia'i Valley, Kauai |
| 8826 | Psychotria | mariniana | Hanakapia'i Valley, Kauai |
| 8829 | Psychotria | mariniana | Hanakapia'i Valley, Kauai |
| 8832 | Psychotria | mariniana | Hanakapia'i Valley, Kauai |
| 9278 | Psychotria | mariniana | Hauula Uka Loop Trail, Oahu |
| 9279 | Psychotria | mariniana | Hauula Uka Loop Trail, Oahu |

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|---|---|---|---|
| 9282 | Psychotria | mariniana | Hauula Uka Loop Trail, Oahu |
| 9283 | Psychotria | mariniana | Hauula Uka Loop Trail, Oahu |
| 8788 | Psychotoria | mariniana | Kamakou Preserve, Molokai |
| 8789 | Psychotoria | mariniana | Kamakou Preserve, Molokai |
| 8790 | Psychotoria | mariniana | Kamakou Preserve, Molokai |
| 8791 | Psychotoria | mariniana | Kamakou Preserve, Molokai |
| 8904 | Psychotria | mariniana | Makaleha Mts., Kauai |
| 8908 | Psychotria | mariniana | Makaleha Mts., Kauai |
| 8909 | Psychotria | mariniana | Makaleha Mts., Kauai |
| 8752 | Psychotria | mariniana | Maumae (Lanipo) Trail, Oahu |
| 8742 | Psychotria | mariniana | Maumae Trail, Oahu |
| 8745 | Psychotria | mariniana | Maumae Trail, Oahu |
| 8750 | Psychotria | mariniana | Maumae Trail, Oahu |
| 9413 | Psychotria | mariniana | Palikea, Oahu |
| 9414 | Psychotria | mariniana | Palikea, Oahu |
| 9415 | Psychotria | mariniana | Palikea, Oahu |
| 9420 | Psychotria | mariniana | Palikea, Oahu |
| 9022 | Psychotria | mariniana | Pihea Trail, Kauai |
| 9024 | Psychotria | mariniana | Pihea Trail, Kauai |
| 9025 | Psychotria | mariniana | Pihea Trail, Kauai |
| 9027 | Psychotria | mariniana | Pihea Trail, Kauai |
| 9029 | Psychotria | mariniana | Pihea Trail, Kauai |
| 9030 | Psychotria | mariniana | Pihea Trail, Kauai |
| 9032 | Psychotria | mariniana | Waihe'e Ridge Trail, Maui |
| 9034 | Psychotria | mariniana | Waihe'e Ridge Trail, Maui |
| 9036 | Psychotria | mariniana | Waihe'e Ridge Trail, Maui |
| 9039 | Psychotria | mariniana | Waihe'e Ridge Trail, Maui |
| 9040 | Psychotria | mariniana | Waihe'e Ridge Trail, Maui |
| 9045 | Psychotria | mauiensis | Auwahi, Maui |
| 8795 | Psychotria | mauiensis | Kamakou Preserve, Molokai |
| 8802 | Psychotria | mauiensis | Kamakou Preserve, Molokai |
| 8803 | Psychotria | mauiensis | Kamakou Preserve, Molokai |
| 8804 | Psychotria | mauiensis | Kamakou Preserve, Molokai |
| 8806 | Psychotria | mauiensis | Kamakou Preserve, Molokai |
| 8812 | Psychotria | mauiensis | Kamakou Preserve, Molokai |
| 8813 | Psychotria | mauiensis | Kamakou Preserve, Molokai |
| 8814 | Psychotria | mauiensis | Kamakou Preserve, Molokai |
| 8815 | Psychotria | mauiensis | Kamakou Preserve, Molokai |
| 8819 | Psychotria | mauiensis | Kamakou Preserve, Molokai |

collected from this taxon's type locality. Lastly, one voucher specimen per population was collected and will be deposited into the Bernice Pauahi Bishop Museum Herbarium (BISH).

***DNA extraction, sequencing, and alignments —***

Total genomic DNA of each sample was extracted using a modified CTAB (cetyl trimethylammonium bromide) protocol (Morden et al., 1996), purified with phenol and chloroform extractions and ethanol precipitation, and accessioned into the Hawaiian Plant DNA Library (HPDL; Morden et al., 1996). Polymerase Chain Reaction (PCR) reactions were performed in 25 μl reaction mixtures containing 1 x GoTaq Flexi PCR buffer, 15 mM $MgCl_2$, 0.1% BSA, 0.2 mM each dNTP, 0.2 mM each amplification primer, and 1 U GoTaq polymerase (Promega, Madison, Wisconsin). Samples were used to amplify two nuclear gene regions, internal transcribed spacer (ITS) and external transcribed spacer (ETS) and two chloroplast intergenic spacers (*rpl32-trnL* and *trnH-psbA*). The primers used to amplify each region examined for this study can be found in Table 2.6 and marker characteristics after PCR amplification in Table 2.7 . For the nuclear markers, the internal transcribed spacer (ITS) and external transcribed spacer (ETS) regions were amplified using the following reaction conditions: an initial denaturation cycle of 95ºC for 2 min, followed by 30 cycles of 93ºC for 1 min; 55ºC for 1 min; 72ºC for 2 min; and a final extension at 72ºC for 3 minutes. For the chloroplast DNA, the *trnH-psbA* region was amplified using an initial denaturation cycle of 80ºC for 5 min, followed by 35 cycles of 94ºC for 30 seconds; 57ºC for 30 seconds; 72ºC for 1 min; and a final extension at 72ºC for 10 minutes; *rpl32-trnL* region was amplified using an initial template denaturation at 80ºC for 5 min followed by 30 cycles of denaturation at 95ºC for 1 min, primer annealing at 50ºC for 1 min, followed by a ramp of 0.38C/s to 65ºC, and primer extension at 65ºC for 4 min; followed by a final extension step of 5 min at 65ºC. All PCR products were

Table 2.6. List of sequence primers used with references in this study used for phylogenetic analyses

| Gene Region | Genome origin, type of DNA | Primer Name, Sequence (5'-3') and reference |
|---|---|---|
| Internal Transcribed Spacer (ITS) | Nuclear, spacer | ITS 5: GGAAGTAAAAGTCGTAACAAGG ITS 4: TCCTCCGCTTATTGATATGC (White et al., 1990) |
| External Transcribed Spacer (ETS) | Nuclear, spacer | ETS 18S: GAGCCATTCGCAGTTTCACAG (Wright et al., 2001) jkETS 9: CGTWMAGGYGYATGAGTGGT (Mitchell, Heenan & Patterson, 2009) |
| *rpl32-trnL* | Chloroplast, spacer | rpl32-f: GCGTATTCGTAAAAATATTTGGAA trnL-r: TTCCTAAGAGCAGCGTGTCTACC (Dong et al., 2012) |
| *trnH-psbA* | Chloroplast, spacer | trnH-f: CGCGCATGGTGGATTCACAAATC psbA-r: TGCATGGTTCCTTGGTAACTTC (Dong et al., 2012) |

Table 2.7. Characteristics of nuclear and chloroplast markers used for inferring phylogenies

| Markers | Aligned sequence length (bp) | # of conserved sites | # of variable sites (parsimony-uninformative and parsimony-informative) | # of parsimony-informative sites |
|---|---|---|---|---|
| ITS | 642 | 400 | 228 | 109 |
| ETS | 499 | 338 | 150 | 79 |
| *rpl32-trnL* | 782 | 725 | 43 | 12 |
| *trnH-psbA* | 465 | 391 | 70 | 49 |

visualized on 1% agarose gel to check for amplification and that the primers were not contaminated. PCR products were then cleaned using exoSAP-IT (Affymetrix, Cleveland, Ohio) following the exoSAP-IT PCR product cleanup protocol: incubation for 37ºC for 15 min followed by 80ºC for 15 min. Samples were bi-directionally sequenced using each amplification primer at the University of Hawaii's ASGPB Sequencing Facility (http://cgpbr.hawaii.edu/) using BigDye Terminator chemistry (Applied Biosystems, Foster City, California) and visualized on an ABI 3730XL capillary-based DNA sequencer (Applied Biosystems). In addition, *Psychotria* sequences were downloaded from GenBank for phylogenetic inference (Table 2.8).

All sequences were assembled and edited in Geneious v10.2.2 (Biomatters, Auckland, NZ) and aligned using the MAFFT plugin (Katoh et al., 2002). All gene regions were first aligned individually and then concatenated to be subsequently analyzed as a nuclear, chloroplast, and combined data sets. The program PartitionFinder 2.1.1 (Lanfear et al., 2016; Lanfear et al., 2012) was used to determine partitioning scheme and the most appropriate model of nucleotide substitution for each gene region and the concatenated alignment using the corrected Akaike information criterion (AICc).

***Phylogenetic analyses —***

To infer relationships among taxa within *Psychotria* sect. *Straussia*, one sample from each representative of population was used, except for *P. hawaiiensis* var. *scoriacea* where three samples were used since this is the first study in which this variety was included in any molecular analyses and it was only sampled from one location. Maximum Likelihood (ML) and Bayesian Inference (BI) analyses of nuclear, chloroplast, and concatenated alignments were preformed using CIPRES Science Gateway (Miller et al., 2010). ML analyses performed in RAxML 8.2.9 (Stamatakis, 2014), ITS, ETS, *rpl32-trnL*, and *trnH-psbA* regions were treated as

Table 2.8 *Psychotria* species from GenBank used for phylogenetic analyses, listing the GenBank accession number and the reference in which the sequence was originally published

| Taxon | Location | Reference | ITS | ETS | *trnH-psbA* |
|---|---|---|---|---|---|
| Psychotria fauriei | Oahu | Nepokroeff et al. 2003 | AY350663 | AY350692 | |
| Psychotria grandiflora | Kauai | Nepokroeff et al. 2003 | AY350670 | AY350699 | |
| Psychotria greenwelliae | Kauai | Nepokroeff et al. 2003 | AY350665 | AY350694 | |
| Psychotria greenwelliae | Kauai | Nepokroeff et al. 2003 | AY350666 | AY350695 | |
| Psychotria hathewayi | Oahu | Nepokroeff et al. 2003 | AY350664 | AY350693 | |
| Psychotria hawaiiensis | Hawaii | Nepokroeff et al. 2003 | AY350659 | AY350688 | |
| Psychotria hawaiiensis | Maui | Nepokroeff et al. 2003 | AY350660 | AY350689 | |
| Psychotria hawaiiensis | Hawaii | Barrabé et al. 2014 | KF675941 | KF675840 | KF676296 |
| Psychotria hexandra | Kauai | Nepokroeff et al. 2003 | AY350667 | AY350697 | |
| Psychotria hexandra | Kauai | Nepokroeff et al. 2003 | AY350668 | AY350696 | |
| Psychotria hexandra var. oahuensis | Oahu | Nepokroeff et al. 2003 | AY350669 | AY350698 | |
| Psychotria hobdyi | Kauai | Nepokroeff et al. 2003 | AY350671 | AY350700 | |
| Psychotria hombroniana | Kosrae | Nepokroeff et al. 2003 | AY350676 | AY350705 | |
| Psychotria kaduana | Maui | Nepokroeff et al. 2003 | AY350657 | AY360686 | |
| Psychotria kaduana | Oahu | Nepokroeff et al. 2003 | AY350658 | AY350687 | |
| Psychotria luzoniensis | Philippines | Nepokroeff et al. 2003 | AY350674 | AY350703 | |
| Psychotria mariana | Tinian Islands | Nepokroeff et al. 2003 | AY350677 | AY350706 | |
| Psychotria mariniana | Kauai | Nepokroeff et al. 2003 | AY350651 | AY350680 | |
| Psychotria mariniana | Oahu | Nepokroeff et al. 2003 | AY350652 | AY350681 | |
| Psychotria mariniana | Oahu | Nepokroeff et al. 2003 | AY350653 | AY350682 | |
| Psychotria mariniana | Maui | Nepokroeff et al. 2003 | AY350654 | AY350683 | |
| Psychotria mariniana | Molokai | Nepokroeff et al. 2003 | AY350655 | AY350684 | |
| Psychotria mariniana | Lanai | Nepokroeff et al. 2003 | AY350656 | AY350685 | |
| Psychotria mauiensis | Maui | Nepokroeff et al. 2003 | AY350661 | AY350690 | |
| Psychotria mauiensis | Molokai | Nepokroeff et al. 2003 | AY350662 | AY350691 | |
| Psychotria pickeringii | Fiji | Nepokroeff et al. 2003 | AY350679 | AY350708 | |
| Psychotria rubra | Hong Kong | Li et al. 2012 | | | JN407051 |
| Psychotria rubra | Hong Kong | Li et al. 2012 | | | JN407052 |
| Psychotria rubra | Hong Kong | Li et al. 2012 | | | JN407053 |
| Psychotria rubra | Hong Kong | Li et al. 2012 | | | JN407054 |
| Psychotria sp. (Whistler) | Fiji | Nepokroeff et al. 2003 | AY350678 | AY350707 | |
| Psychotria tahitiensis | Tahiti | Nepokroeff et al. 2003 | AY350675 | AY350704 | |
| Psychotria wawrae | Kauai | Nepokroeff et al. 2003 | AY350672 | AY350701 | |

separate partitions and the models selected for each partition were TrNef+I+G, TVM+G, K81UF+I+G, and GTR+I+G, respectively based on the results from PartitionFinder. RAxML was set to halt boostrapping automatically using the Majority Rule Criterion (Pattengale et al., 2010). For the BI analyses performed in MrBayes 3.2.6 (Ronquist et al., 2012), the GTR+I+G model was selected as the best model of nucleotide substitution for the combined dataset and was specified in the Bayes run by setting the number of substitution types to "mixed." The analysis was run for 10 million generations and trees were sampled every 1000 generations with the initial 25% of the trees were discarded as burn-in. The MCMC outputs were examined using Tracer version 1.6 (Rambaut et al., 2014) to confirm convergence among MCMC runs and to assess effective sample size scores (ESS) for all parameters. All ESS values were greater than 200.

The statistical significance of conflict among individual nuclear and chloroplast regions and between chloroplast and nuclear datasets was estimated by the ILD test (Farris et al., 1994), implemented as the partition homogeneity test in PAUP v4.0a159 (Swofford, 2002). Five hundred replicates were performed using a random addition heuristic search. No incongruence was observed between plastid and nuclear datasets, so gene regions were concatenated into a single alignment and used for phylogenetic analyses.

*Ancestral character state reconstructions —*

For ancestral state reconstructions, two morphological characters were used to determine their taxonomic value: leaf venation (0) tertiary veins present (1) tertiary veins absent; domatia on abaxial leaf surface (2) conspicuous (3) inconspicuous or absent. The two morphological characters' states were assigned to each taxon based on information from personal observations and previous taxonomic treatments (Rock, 1913; Fosberg, 1964; Sohmer, 1977; and Wagner et

al., 1990). Ancestral state reconstruction was carried out using maximum likelihood reconstruction in BayesTraits v2.02 (Pagel and Meade, 2013) using the 'MultiState' option on 1,000 trees from the Bayesian analysis and the 'AddMRCA' (most recent common ancestor) command was used to reconstruct each node. To illustrate final values of probabilities for ancestral reconstruction of character evolution, values were represented on a 50% majority rule consensus Bayesian tree using pie charts at each node using Interactive Tree Of Life (iTOL) v3 (Letunic and Bork, 2016).

***SRAP analyses —***

There were 168 SRAP primer combinations that were initially screened in nine samples, representing one sample of each species in *Psychotria* sect. *Straussia*. After initial primer screening of 168 primer combinations, 20 were selected to analyze all samples (Table 2.9). SRAP markers were amplified in 25 µl volumes under the following conditions: ca. 0.2 mM each of dATP, dCTP, dGTP, dTTP, 1X Taq DNA polymerase buffer, 1.5 mM $MgCl_2$, 0.25 mg BSA, 1 µl of each primer, and ca. 1 unit of Taq DNA Polymerase (Promega). PCR was performed in a DNA thermocycler (MJ Research) using the following reaction conditions: initial denaturation at 94ºC for 5 min, followed by five cycles of denaturation for 1 min at 94ºC, annealing at 35ºC for 1 min, and elongation at 72ºC for 1 min, and then 35 cycles of denaturation for 1 min at 94ºC and annealing at 50ºC for 1 min, ending with an elongation step at 72ºC for 5 min. Samples were then stored in a refrigerator at 4ºC until use. PCR amplified products were mixed with loading dye and separated on 2% agarose gels with size of amplification products estimated using a 100 kb ladder (Promega) and gels visualized with a UV light source. Negative control reactions were run without DNA for all PCR amplifications to ensure that reaction components were

Table 2.9. Primers used for sequence-related amplified polymorphism (SRAP)

| **Forward Primers** | | **Reverse Primers** | |
|---|---|---|---|
| Name | Sequence (5'–3') | Name | Sequence (5'–3') |
| Me1 | TGAGTCCAAACCGGATA | Em2 | GACTGCGTACGAATTTGC |
| Me3 | TGAGTCCAAACCGGAAT | Em3 | GACTGCGTACGAATTGAC |
| Me4 | TGAGTCCAAACCGGACC | Em4 | GACTGCGTACGAATTTGA |
| Me5 | TGAGTCCAAACCGGAAG | Em5 | GACTGCGTACGAATTAAC |
| Me6 | TGAGTCCAAACCGGTAA | Em6 | GACTGCGTACGAATTGCA |
| Me7 | TGAGTCCAAACCGGTCC | Em7 | GACTGCGTACGAATTGAG |
| Me8 | TGAGTCCAAACCGGTGC | Em8 | GACTGCGTACGAATTGCC |
| Me9 | TGAGTCCAAACCGGACA | Em9 | GACTGCGTACGAATTTCA |
| Me10 | TGAGTCCAAACCGGACG | Em10 | GACTGCGTACGAATTCAA |
| Me11 | TGAGTCCAAACCGGACT | | |
| Me12 | TGAGTCCAAACCGGAGG | | |
| Me13 | TGAGTCCAAACCGGAAA | | |
| Me14 | TGAGTCCAAACCGGAAC | | |

uncontaminated. Amplified bands were scored as either 0 (marker absent) or 1 (marker present), and a data matrix was constructed.

Genetic similarity indices were estimated using both Gower (1971) and Nei and Li (1979) similarity coefficients for populations using MVSP Plus ver. 3.1 (Kovach 2007). Relationships within and among populations and the species were projected from the similarity matrixes using principal coordinate analysis (PCO) with MVSP Plus ver. 3.1 (Kovach 2007) using Gower similarity (Gower 1971).

***MIG-seq analyses —***

A total of 192 samples representing eight taxa were used with up to 10 samples per population was used (Table 2.3). Taxa included in this analysis were from the *mariniana* group (*P. mariniana* and *P. hawaiiensis* var. *hawaiiensis*) and kaduana group (*P. kaduana*, *P. fauriei*, *P. mauiensis*, *P. hathewayi*, and *P hawaiiensis* var. *hillebrandii* and var. *scoriacea*). *Psychotria wawrae* and *P. greenwelliae*, were the only two species from sect. *Straussia* that were not included in this analysis due to their forming well supported monophyletic groups in previous phylogenetic study (Zhang, 2016).

Multiplexed ISSR genotyping by sequencing (MIG-seq) was used for single nucleotide polymorphism (SNP) detection (Suyama and Matsuki 2015). Preparation of the MIG-seq library was performed under standard conditions according to Suyama and Matsuki (2015). Repeat motifs and anchor sequences of the tailed first PCR primers used in this study were as follows: $(ACT)_4TG$, $(CTA)_4TG$, $(TTG)_4AC$,$(GTT)_4CC$, $(GTT)_4TC$, $(GTG)_4AC$, $(GT)_6TC$, and $(TG)_6AC$. To the diluted products of the first round of PCR, the necessary sequences for the Illumina sequencing and indices for identifying samples were added in the second PCR. The products of the second PCR were pooled in equimolar concentrations, and fragments in the size range 300–

800 bp were isolated. After the measurement of the final concentration of the library, approximately 8.5 pM of the library was used for sequencing on an Illumina MiSeq Sequencer (Illumina, San Diego, California), using a MiSeq Reagent Kit v3 (150 cycle, Illumina). Removing the primer regions and quality filtering were conducted according to Suyama and Matsuki (2015).

Prior to SNP calling, 15 individuals had fewer than 50,000 reads and these individuals were removed from further genetic analyses. SNPs were called using Stacks v1.47 (Catchen et al., 2011). First, using the 'ustacks' option, a set of identical reads was bundled together in a 'stack', and several of these stacks were merged to form putative loci with the settings: maximum distance between stacks (M) = 2, enable the deleveraging algorithm (d), and the removal algorithm (r). The minimum depth of coverage required to create a stack (m) was set as 20. Second, a catalog was created for all possible loci and alleles with the 'cstacks' option. The parameter 'number of allowed mismatches between samples (n)' was set as four. All stacks created by 'ustacks' were then matched against the catalog produced by 'cstacks', using the 'sstacks' option. Additionally, the 'write_single_snp' option was also used to select only the first SNP of each locus. If a locus could not be detected in a sample, the genotype in the sample was treated as missing data.

Genetic population structure using SNPs were assessed using STRUCTURE v2.3.4 (Pritchard et al., 2000). Burn-in and run lengths of 100,000 replicates, with 10 iterations per *K* were used for all analyses. As gene flow is expected among populations, the admixture model using correlated allele frequencies was implemented. Species designation was used *a priori* for the analysis including all sampled individuals that was incorporated into the STRUCTURE algorithm (Hubisz et al., 2009). Species designation or island origin was used *a priori* for

subsequent analyses examining subsets of the sampled individuals. The number of inferred populations or clusters, *K*, ranged from one to three more than the actual number of populations included in the analysis The optimal number of genetic populations or clusters (*K* value) was determined from the greatest Δ*K* value, an ad hoc statistic based on the rate of change in the log probability of data between successive *K* values (Evanno et al. 2005) using STRUCTURE Harvester v0.6 (Earl and Bridgett, 2012). Multimodality across the 10 replicate iterations of the STRUCTURE analysis was addressed by permuting 1,000 times using the greedy algorithm and averaging across membership coefficients in CLUMPP v1.1.2 (Jakobsson and Rosenberg, 2007); the results were graphically displayed using Distruct v1.1 (Rosenberg, 2004).

Several analyses were conducted in GenoDive v2.0b27 (Meirmans and Van Tienderen, 2004). First, to investigate the genetic structure further, and to test how the genetic diversity might be structured, analyses of molecular variance (AMOVA) were conducted (Excoffier, 1992; Michalakis and Excoffier, 1996). For each analysis, the standard AMOVA settings were used, with 1,000 permutations. Standard AMOVA analyses were used to test for the significance of grouping of the data by island and taxa. Secondly, several principal coordinate (PCO) analyses was performed on a matrix of covariance values calculated from population allele frequencies to investigate relationships among species. Lastly, an assessment of the correlation between geographic distance and pairwise genetic distance ($F_{ST}$) were analyzed by the Mantel test with 1,000 permutations.

## RESULTS

***Phylogenetic analyses —***

The nuclear phylogeny using ML and BI methods produced similar topologies. Species of sect. *Straussia* are separated into two groups hereafter referred to as the *marinana* and *kaduana* clades (named after a species that typifies each clade, respectively). The *mariniana* clade consists of *P. wawrae*, *P. mariniana*, and *P. hawaiiensis* var. *hawaiiensis*, whereas the *kaduana* clade consists of *P. greenwelliae*, *P. fauriei*, *P. kaduana*, *P. mauiensis*, *P. hathewayi*, and *P. hawaiiensis* var. *hillebrandii* and var. *scoriacea*. The separation of these clades are strongly supported in the ML analysis with 98% boostrap support (BS) and in the BI analysis with 100% posterior probability (PP) (Figure 2.1). In the *mariniana* clade, *P. wawrae* and *P. hawaiiensis* var. *hawaiiensis* each have a paraphyletic relationship with *P. mariniana*. One *P. mariniana* individual maintained an intermediate position between the *mariniana* and *kaduana* clades and another is nested within a clade with *P. hathewayi* that is weakly supported in the ML analysis (57% BS) but strongly supported BI (90% PP) analyses and they could be of hybrid origin (Figure 2.1A and 2.1B). In the *kaduana* clade, only one subclade is found in both the ML (59% BS) and BI (100% PP) analyses, consisting of the only *P. kaduana* individual from Kaua‘i and *P. greenwelliae*. Additionally, the analyses show *P. hawaiiensis* var. *hillebrandii* and var. *scoriacea* grouping with *P. mauiensis* that are weakly supported in the ML analysis (29% BS; Fig. 2.1A). However, the two varieties showed a moderately supported relationship in the BI analysis (78% PP; Figure 2.1B). The two individuals of var. hillebrandii collected on Maui show relationships with *P. kaduana* and *P. fauriei* in the ML analysis. Interestingly, *P. fauriei*, *P. hathewayi*, *P. kaduana*, *P. mauiensis*, and one of the *P. hawaiiensis* samples from Nepokoreff et al. (2003) group together in both analyses that are separate from other members of each of their respective species.

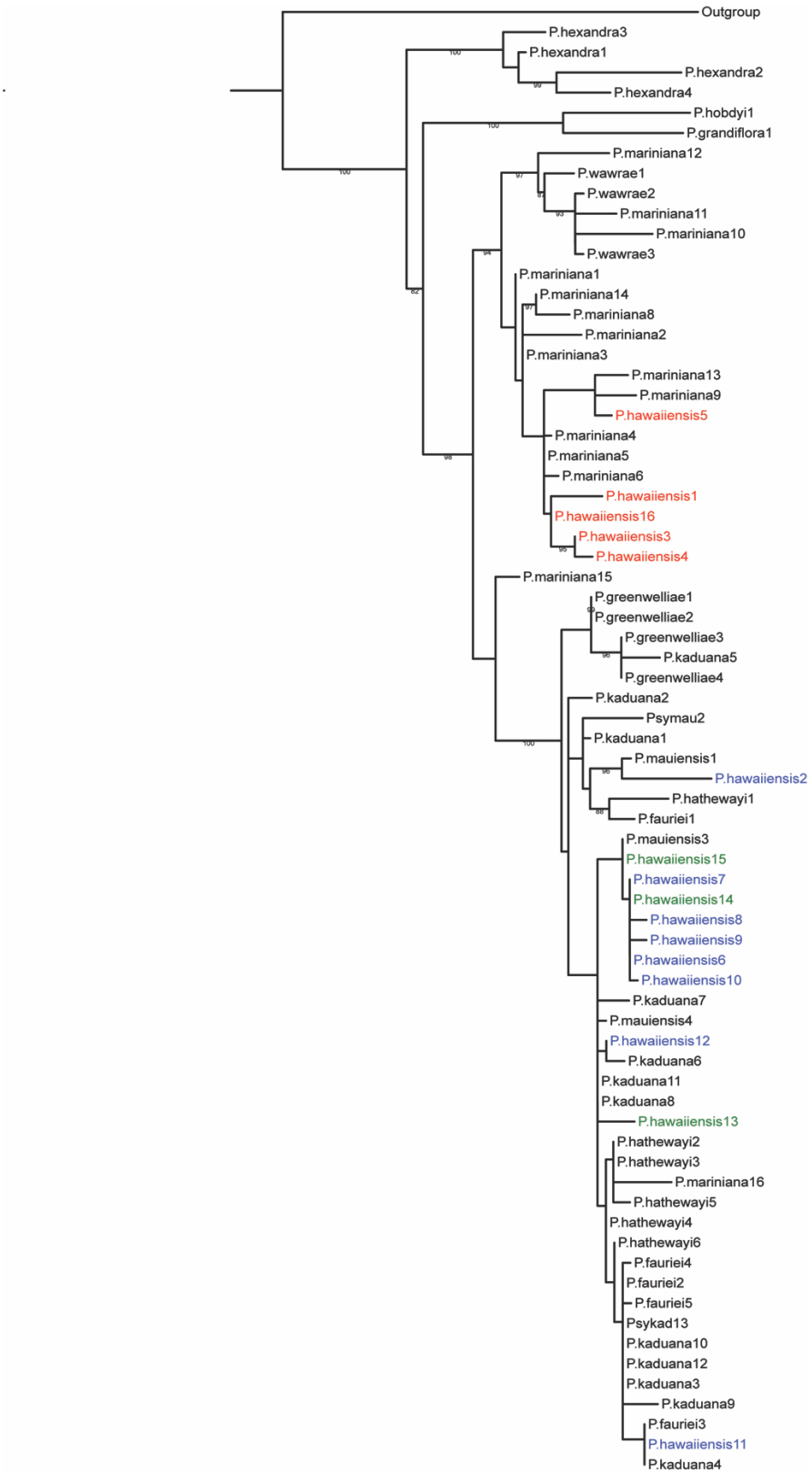


Figure 2.1A. Maximum likelihood analysis of nuclear data set (ITS and ETS). The values below branches correspond to boostrap values (>75&%). The red taxa represent *P. hawaiiensis* var. *hawaiiensis*; blue taxa represent *P. hawaiiensis* var. *hillebrandii* and green taxa represent *P. hawaiiensis* var. *scoriacea*.

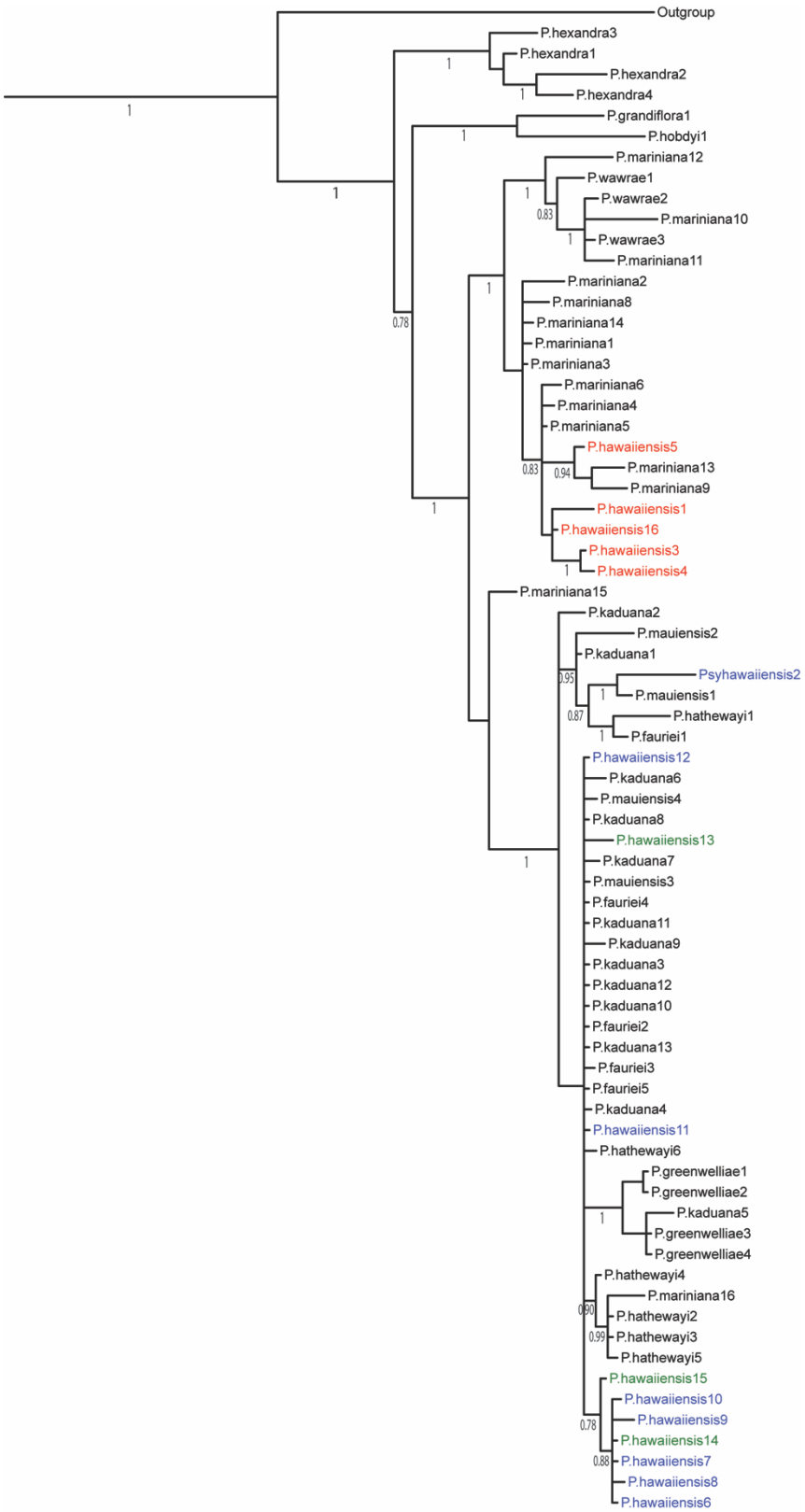


Figure 2.1B. 50% consensus tree of Bayesian Inference analysis of nuclear data set (ITS and ETS). The values below branches correspond to posterior probabilities (>0.75). The red taxa represent *P. hawaiiensis* var. *hawaiiensis*; blue taxa represent *P. hawaiiensis* var. *hillebrandii* and green taxa represent *P. hawaiiensis* var. *scoriacea*.

The chloroplast phylogeny showed a similar pattern for the *marinana* clade that is weakly supported in the ML analysis (56% BS) but strongly supported in the BI analyses (100% PP). Additionally, a couple *P. mariniana* samples group with taxa in the *kaduana* clade. Taxa of the *kaduana* clade form a polytomy in the BI analysis with only two subclades consisting of *P. fauriei* (92% PP) and *P. hawaiiensis* var. *scoriacea* (88% PP). But, in the ML analysis there are many weakly supported relationships among these taxa (Figure 2.2A). Lastly, one *P. mariniana* sample groups with the other *P. mariniana* individuals in the chloroplast phylogeny, but in the nuclear phylogeny it groups with *P. hathewayi* and could be of hybrid origin (Figure 2.1B and Figure 2.2B, respectively).

Regions within the same genome are not incongruent according to the ILD test ($p = 0.134$, between plastid datasets, and $p = 0.241$, between nuclear datasets), also plastid and nuclear datasets were not incongruent ($p = 0.062$). The combined dataset showed similar topologies and support at each node as the nuclear phylogenies. The biggest disparity among taxa sampled in the combined analyses is the polyphyletic relationships among *P. hawaiiensis* samples (Figure 2.3A and Figure 2.3B) which is congruent with the previous phylogenetic studies (Nepokroeff et al., 2003: Zhang, 2016). Variety *hawaiiensis* is grouped in the *mariniana* clade, whereas var. *hillebrandii* and var. *scoriacea* are grouped in the *kaduana* clade. In both analyses, variety *hawaiiensis* is nested within and shows a paraphyletic relationship with *P. mariniana*. In the ML analyses several variety *hillebrandii* samples form a weakly supported monophyletic group (33% BS), additional samples show a weak relationship with *P. mauiensis* (38% BS), and one sample forms a polytomy with *P. kaduana* and *P. fauriei* (Figure 2.3A). In the BI analysis, var. *hillebrandii* samples from Hawai‘i Island from a weakly supported

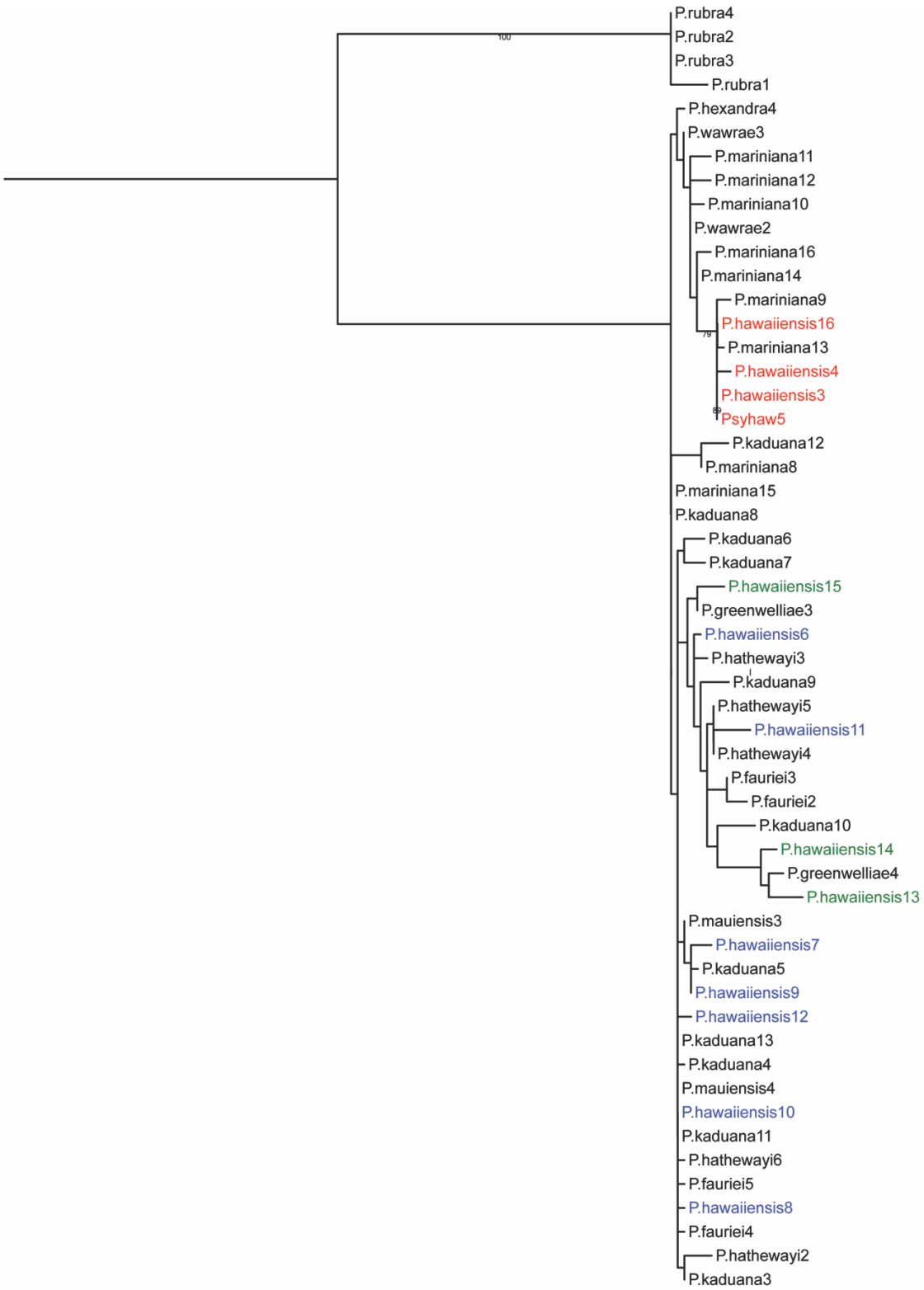


Figure 2.2A. Maximum likelihood analysis of chloroplast data set (*rpl32-trnL* and *trnH-psbA*). The values below branches correspond to boostrap values (>75%). The red taxa represent *P. hawaiiensis* var. *hawaiiensis*; blue taxa represent *P. hawaiiensis* var. *hillebrandii* and green taxa represents *P. hawaiiensis* var. *scoriacea*.

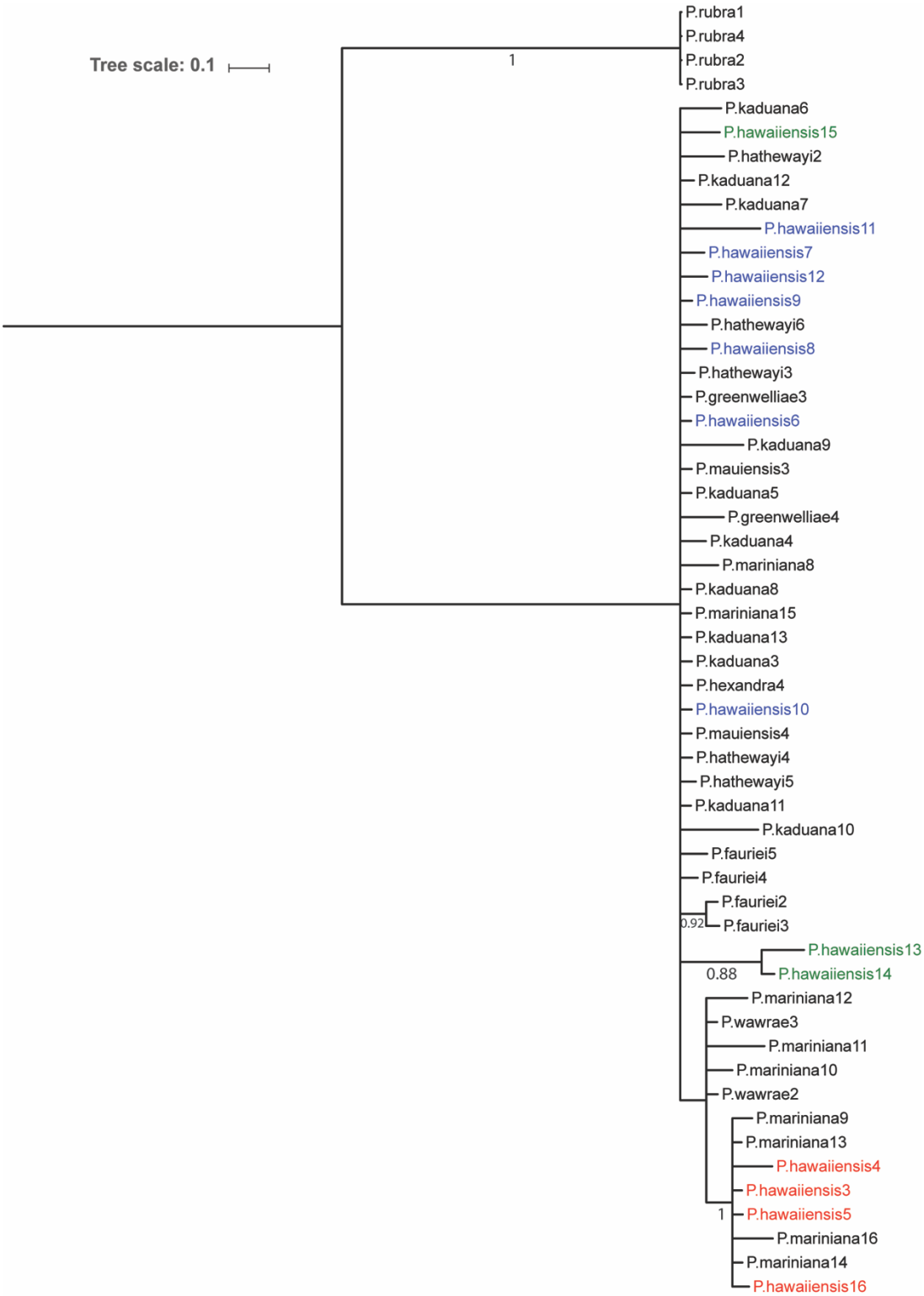


Figure 2.2B. 50% consensus tree of Bayesian Inference analysis of chloroplast data set (*rpl32-trnL* and *trnH-psbA*). The values below branches correspond to posterior probabilities (>0.75). The red taxa represent *P. hawaiiensis* var. *hawaiiensis*; blue taxa represent *P. hawaiiensis* var. *hillebrandii* and green taxa represents *P. hawaiiensis* var. *scoriacea*.


Figure 2.3A. Maximum likelihood analysis of concatenated data set. The values above branches correspond to boostrap values (>75%). The red taxa represent *P. hawaiiensis* var. *hawaiiensis*; blue taxa represent *P. hawaiiensis* var. *hillebrandii* and green taxa represents *P. hawaiiensis* var. *scoriacea*.

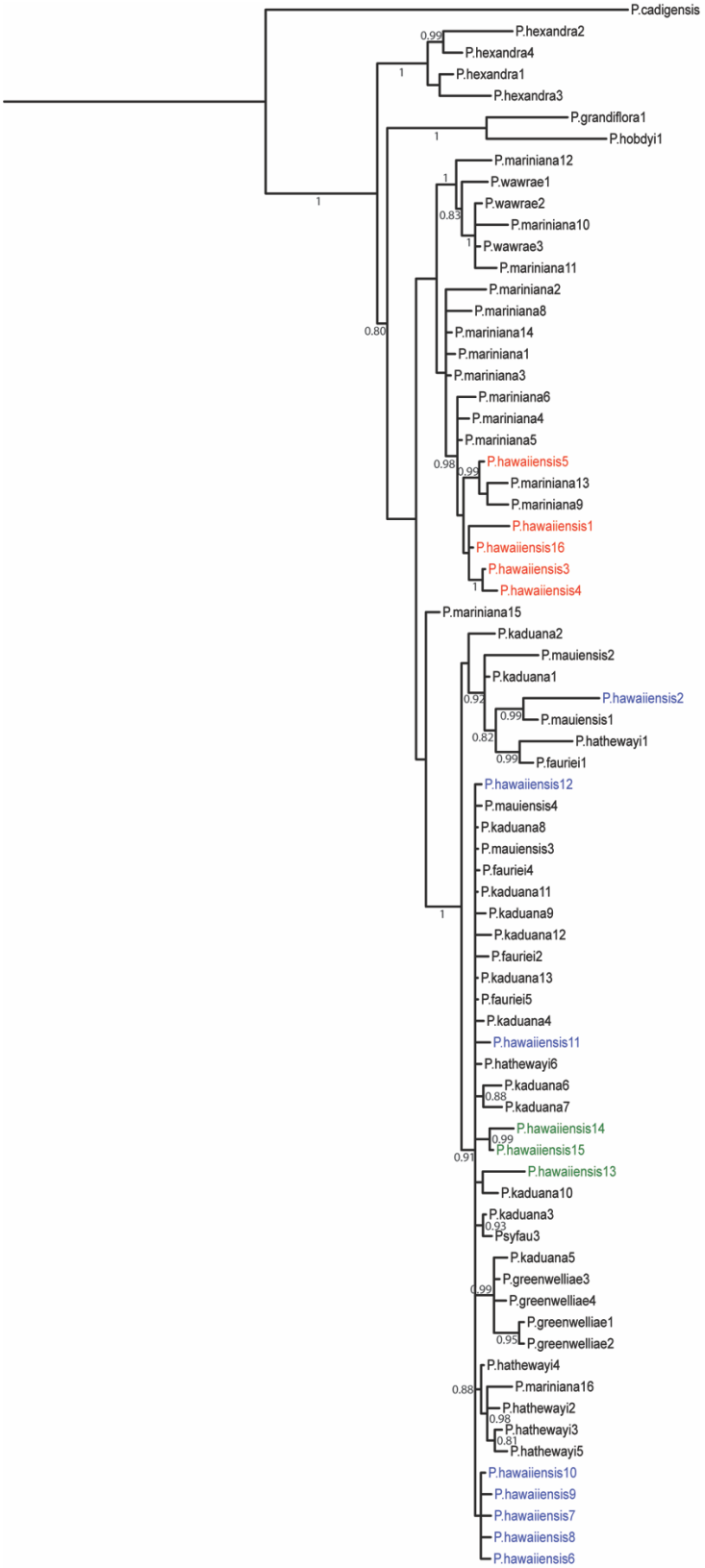


Figure 2.3B. 50% consensus tree of Bayesian Inference analysis of the concatenated data set. The values at nodes correspond to posterior probabilities >0.75. The red taxa represent *P. hawaiiensis* var. *hawaiiensis*; blue taxa represent *P. hawaiiensis* var. *hillebrandii* and green taxa represent *P. hawaiiensis* var. *scoriacea*.

monophyletic group (63%); the two samples from Maui form a polytomy with other members in the *kaduana* clade. Additionally, in the ML analysis, two *P. hawaiiensis* var. *scoriacea* samples group together (73% BS) with the third sample forming a polytomy with *P. kaduana* and *P. mauiensis*. In the BI analyses two var. *scoriacea* samples group together (99% PP)with the third forming a weakly supported relationship with *P. kaduana* (69% PP) (Fig. 2.3B).

***Ancestral character state reconstructions —***

Ancestral state reconstruction examining leaf reticulate tertiary venation and domatia size was made. Presence of a reticulate tertiary leaf venation is either visible or obscure, whereas domatia is either conspicuous or inconspicuous to absent. Plants of the *mariniana* clade are characterized by the lack of visible tertiary leaf venation, whereas the *kaduana* clade is characterized by having visible reticulate leaf venation (Fig. 2.4A). Furthermore the *mariniana* clade is characterized by having conspicuous domatia, except for *P. wawrae* in which the domatia are inconspicuous or absent, whereas the *kaduana* clade is characterized by having inconspicuous domatia, the exception being *P. hawaiiensis* var. *scoriacea* which has conspicuous domatia (Fig. 2.4B).

***SRAP analyses —***

Of the 121 individuals representing all recognized species within sect. *Straussia*, 597 clearly defined genetic markers were amplified using 20 combinations of 13 forward and 9 reverse primers (Table 2.9). The total number of markers ranged from 19 to 36 per primer combnination (average 29.85). Levels of polymorphism in each species were comparably low, with an average of 136 (22.80%) polymorphic loci across sect. *Straussia* (Table 2.10). Additionally, levels of estimated heterozygosity varied among species with *P. wawrae* the lowest at 0.027 and *P. kaduana* with the highest at 0.127 (Table 2.10). There were 50 bands each that

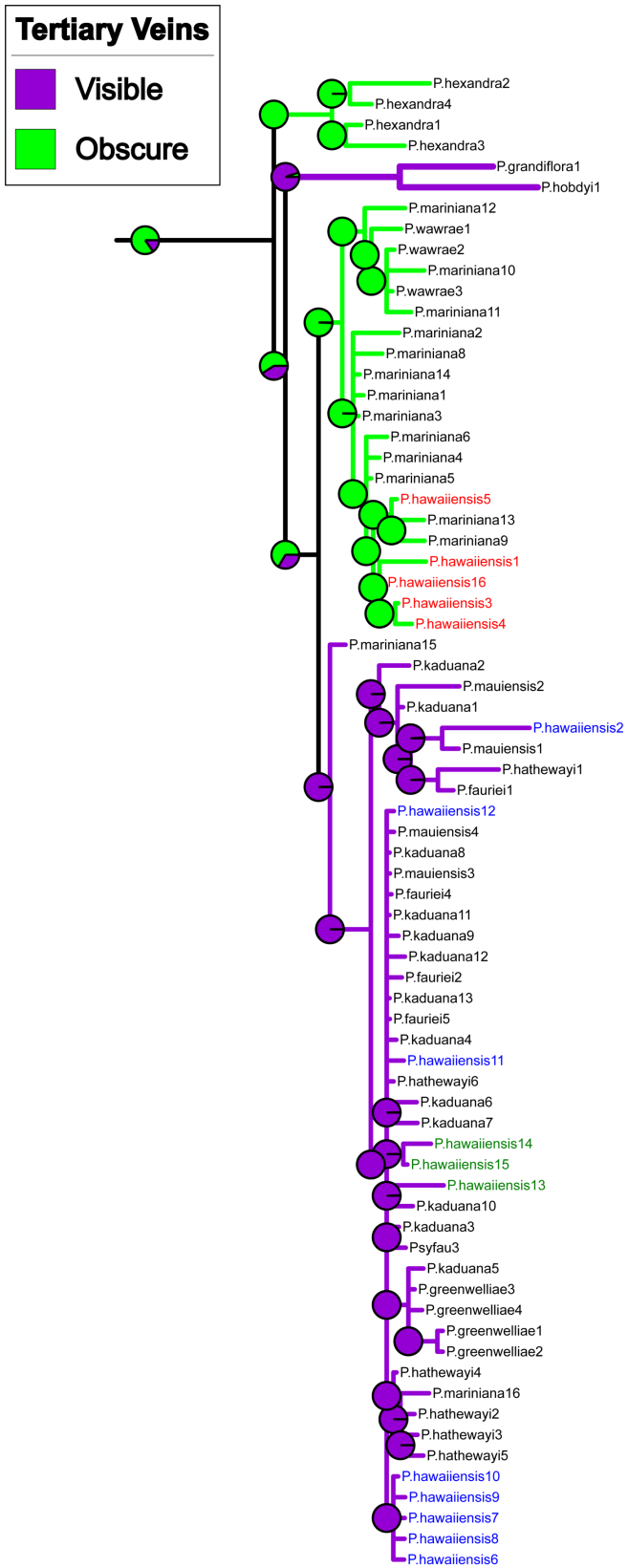


Figure 2.4A. Ancestral state reconstructions of leaf tertiary venation (green = obscure, purple = visible) using BayesTraits mapped onto consensus tree from Bayesian Inference of the concatenated data set. The red taxa represent *P. hawaiiensis* var. *hawaiiensis*; blue taxa represent *P. hawaiiensis* var. *hillebrandii* and green taxa represents *P. hawaiiensis* var. *scoriacea*.

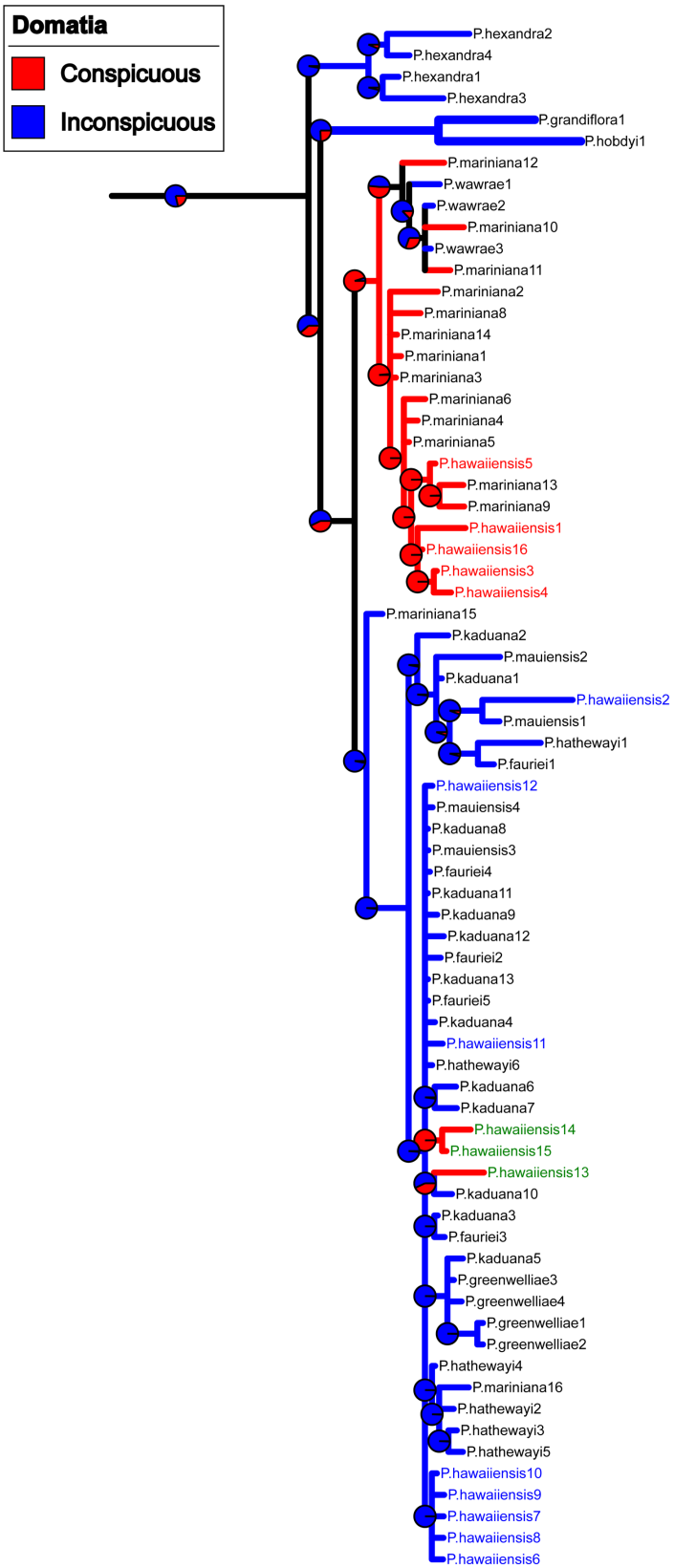


Figure 2.4B. Ancestral state reconstructions of leaf domatia (red= conspicuous, blue = inconspicuous) using BayesTraits mapped onto consensus tree from Bayesian Inference of the concatenated data set. The red taxa represent *P. hawaiiensis* var. *hawaiiensis*; blue taxa represent *P. hawaiiensis* var. *hillebrandii* and green taxa represents *P. hawaiiensis* var. *scoriacea*.

Table 2.10. Sample size per species, total number of bands amplified, number of unique bands, percent polymorphisms, and expected heterozygosity using SRAP data

| Species | No. Samples | No. Bands | No. Private Bands | Percent Polymorphisms (%) | He |
|---|---|---|---|---|---|
| hawaiiensis var. hillebrandii | 17 | 193 | 16 | 23.95% | 0.074 |
| mauiensis | 4 | 162 | 2 | 10.22% | 0.039 |
| wawrae | 6 | 133 | 6 | 7.71% | 0.027 |
| fauriei | 11 | 201 | 19 | 18.93% | 0.065 |
| hawaiiensis var. scoriacea | 3 | 157 | 2 | 7.20% | 0.028 |
| mariniana | 25 | 314 | 50 | 45.56% | 0.108 |
| kaduana | 32 | 337 | 50 | 49.75% | 0.127 |
| greenwelliae | 6 | 183 | 3 | 18.93% | 0.069 |
| hathewayi | 8 | 176 | 5 | 18.09% | 0.066 |
| hawaiiensis var. hawaiiensis | 9 | 191 | 16 | 27.64% | 0.104 |

were diagnostic of *P. mariniana* and *P. kaduana* (i.e. present only in each species), the most of all species examined while each of the other species varied in the number of diagnostic markers that were polymorphic within species (Table 2.10).

Populations were compared for genetic similarities based on the genetic identity (I) of Nei and Li (1979) where a value of 1.0 indicates complete genetic identity (Table 2.11). Genetic similarity showed that species within the *marinana* and *kaduana* clades inferred from the phylogenies were generally more similar to plants within these respective clades (Table 2.11). The similarity between the populations of *P. hawaiiensis* var. *hawaiiensis*, var. *hillebrandii*, and var. *scoriacea* is higher with *P. mauiensis*, *P. fauriei*, and *P. mariniana* (0.960, 0.943, and 0.901, respectively) than is the similarity between the populations of the same species.

PCO was performed with three samples of each populations examined in sect. *Straussia*. This PCO plot resulted in three distinct groupings: group 1) with plants of *P. mariniana*, *P.wawrae*, *P. hawaiiensis var. hawaiiensis*; group 2) with plants of *P. kaduana*, *P. greenwelliae*, and *P. hathewayi*; group 3) with plants of *P. fauriei*, *P. hawaiiensis* varieties *hillebrandii* and *scoriacea*, and *P. mauiensis* (Figure 2.5). The first PCO axis accounts for the distinction of *mariniana* clade from the *kaduana* clades consistent with the phylogenetic analyses. Two observations were evident from examining the second axis: (1) individuals of *P. mariniana* and *P. hawaiiensis* var. *hawaiiensis* were genetically differentiated from *P. wawrae*, and (2) individuals of *P. kaduana*, *P. greenwelliae*, and *P. hathewayi* were genetically differentiated from *P. fauriei*, *P. mauiensis P. hawaiiensis* vars. *hillebrandii* and *scoriacea*. Overall, individuals of the same species generally group together, except several individuals of *P. mariniana* are positioned closer to the *kaduana*/*greenwelliae*/*hathewayi* cluster. Also, the three varieties of *P. hawaiiensis* did not conform to the current taxonomic circumscription. Variety

Table 2.11. Genetic similarity values for all species within sect. *Straussia* (varieties are of *P. hawaiiensis*) using SRAP data based on Nei and Li (1979) coefficient

| var.hillebrandii | mauiensis | wawrae | fauriei | var. scoriacea | mariniana | kaduana | greenwelliae | hathewayi | var. hawaiiensis | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1.000 | | | | | | | | | | var. hillebrandii |
| 0.960 | 1.000 | | | | | | | | | mauiensis |
| 0.825 | 0.787 | 1.000 | | | | | | | | wawrae |
| 0.861 | 0.832 | 0.855 | 1.000 | | | | | | | fauriei |
| 0.849 | 0.819 | 0.848 | 0.943 | 1.000 | | | | | | var. scoriacea |
| 0.844 | 0.815 | 0.909 | 0.834 | 0.826 | 1.000 | | | | | mariniana |
| 0.897 | 0.877 | 0.845 | 0.863 | 0.845 | 0.889 | 1.000 | | | | kaduana |
| 0.878 | 0.861 | 0.816 | 0.821 | 0.803 | 0.852 | 0.929 | 1.000 | | | greenwelliae |
| 0.890 | 0.863 | 0.822 | 0.836 | 0.816 | 0.851 | 0.917 | 0.905 | 1.000 | | hathewayi |
| 0.860 | 0.825 | 0.859 | 0.820 | 0.806 | 0.901 | 0.878 | 0.867 | 0.882 | 1.000 | var. hawaiiensis |

*hawaiiensis* groups with *P. marinana* and *P. wawrae* whereas var. *hillebrandii* and *scoriacea* group closely to each other than they are to var. *hawaiiensis* and show close affinities with *P. fauriei*, and *P. mauiensis*, respectively. Genetic variation among populations of *P. fauriei*, *P. mauiensis* and *P. hawaiiensis* vars. *hillebrandii* and *scoriacea* was not clearly distinguishable (Figure 2.5A) although clustering of populations was evident. Therefore, a separate PCO analysis with only these species was conducted (Figure 2.5B). Individuals aligned into three distinct clusters. The first axis distinguishes *P. fauriei* and *P. hawaiiensis* var. *scoriacea* from *P. mauiensis* and P. hawaiiensis var. *hillebrandii*. Two observations were evident from examining the second axis: (1) *P. hawaiiensis* var. *scoriacea* were genetically well differentiated from *P. fauriei*, and (2) most individuals of *P. hawaiiensis* var. *hillebrandii* were genetically differentiated from *P. mauiensis*.

***MIG-seq analyses —***

*Principal Coordinate Analyses (PCO) —*

The PCO analyses including all samples showed two clusters and reveal that *P. mariniana* and *P. hawaiiensis* var. *hawaiiensis* of the *mariniana* group were genetically distinct from the *kaduana* group (*P. kaduana*, *P. fauriei*, *P. mauiensis*, *P. hathewayi*, and *P hawaiiensis* var. *hillebrandii* and var. *scoriacea*; Figure 2.6). *Psychotria mariniana* appears to be diverging among island populations and *P. hawaiiensis* var. *hawaiiensis* also is forming a distinct cluster (Figure 2.7). Additionally, there was very little genetic differentiation among members of the *kaduana* group and taxa appear to be clustering by island (Figure 2.8A and Figure 2.8B). Since there were no clear groupings, several more PCO analyses were conducted to investigate taxa by islands. On O‘ahu individuals of *P. kaduana*, *P. fauriei* and *P. hathewayi* cluster together with no distinct groupings observed (Figure 2.9). On Maui Nui and Hawai‘i , there were three clusters

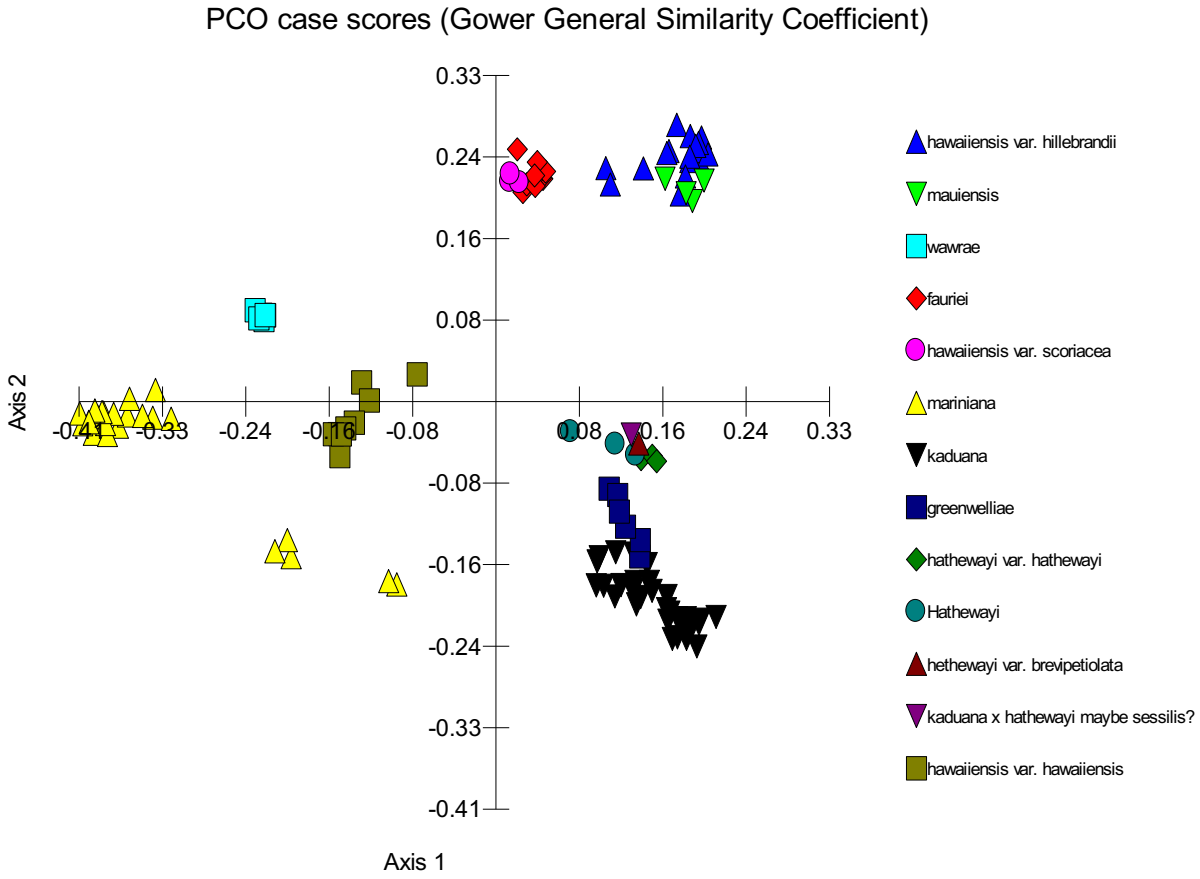


Figure 2.5A. PCO analysis using SRAP data of individuals of sect. *Straussia* species based on Gower general similarity coefficient. Axes 1 and 2 represent 19% and 13% of the variation, respectively.

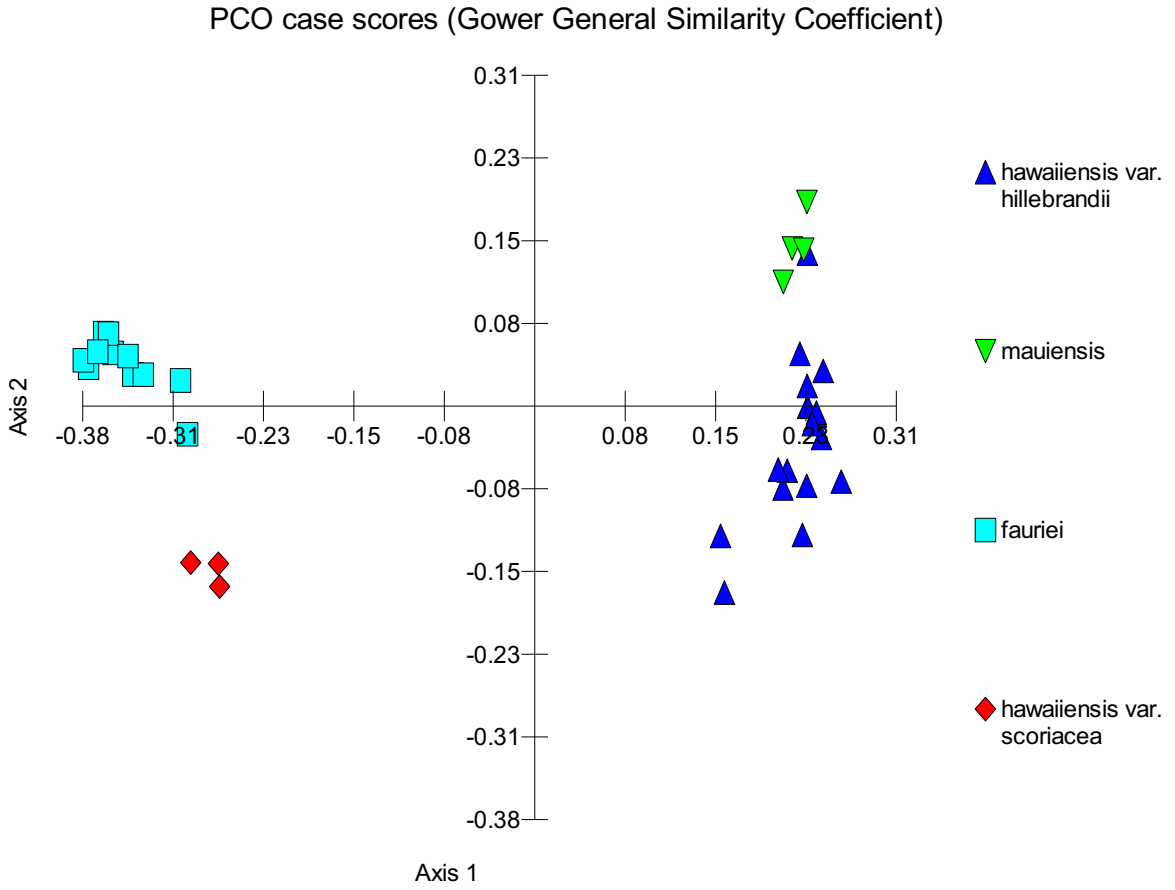


Figure 2.5B. PCO analysis using SRAP data of individuals of *P. fauriei*, *P.mauiensis*, and *P. hawaiiensis* varieties *hillebrandii* and *scoriacea* based on Gower general similarity coefficient.

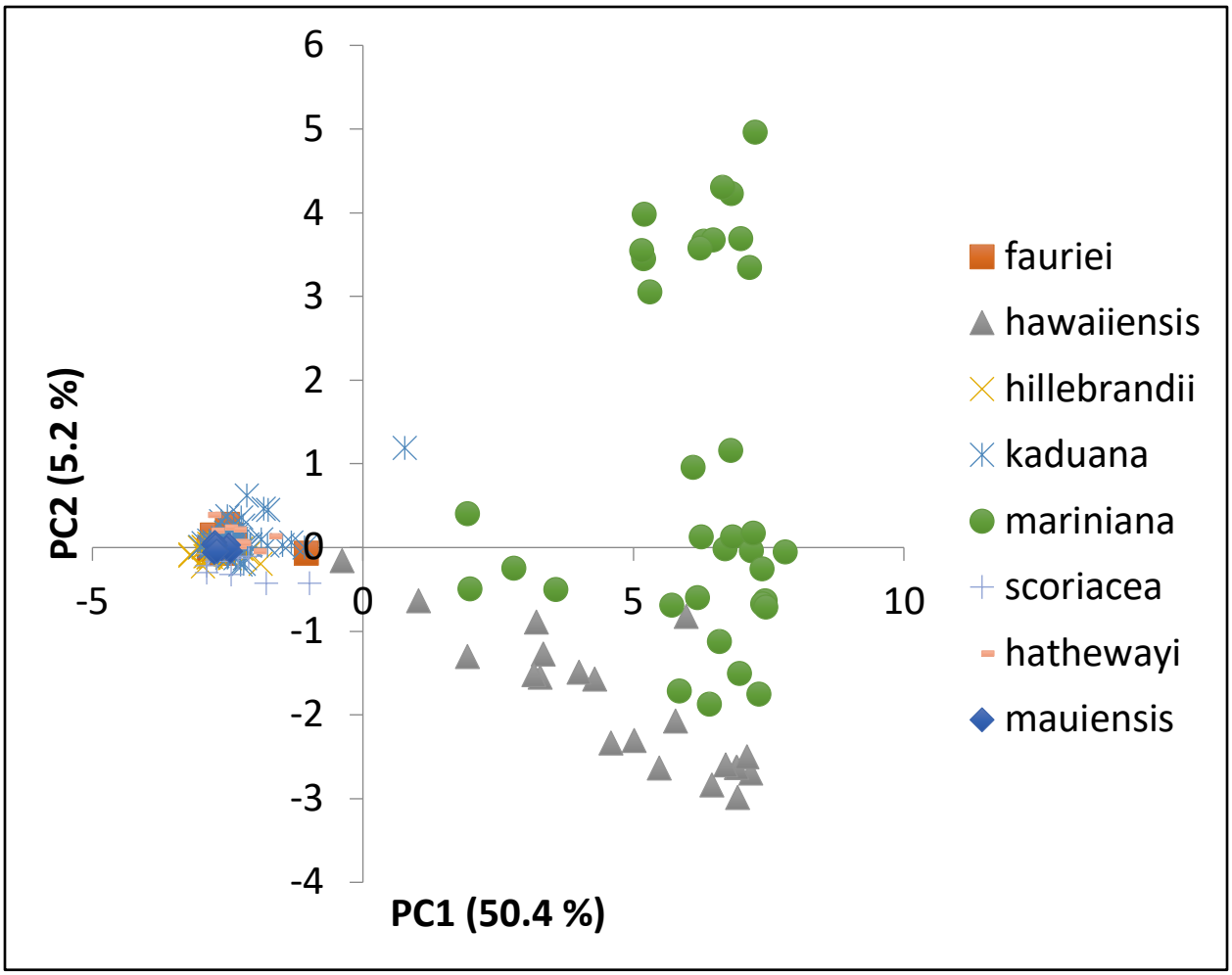


Figure 2.6. Principal coordinate analysis using MIG-seq data of taxa in the *mariniana* and *kaduana* groups using a matrix of covariance values calculated from population allele frequencies.

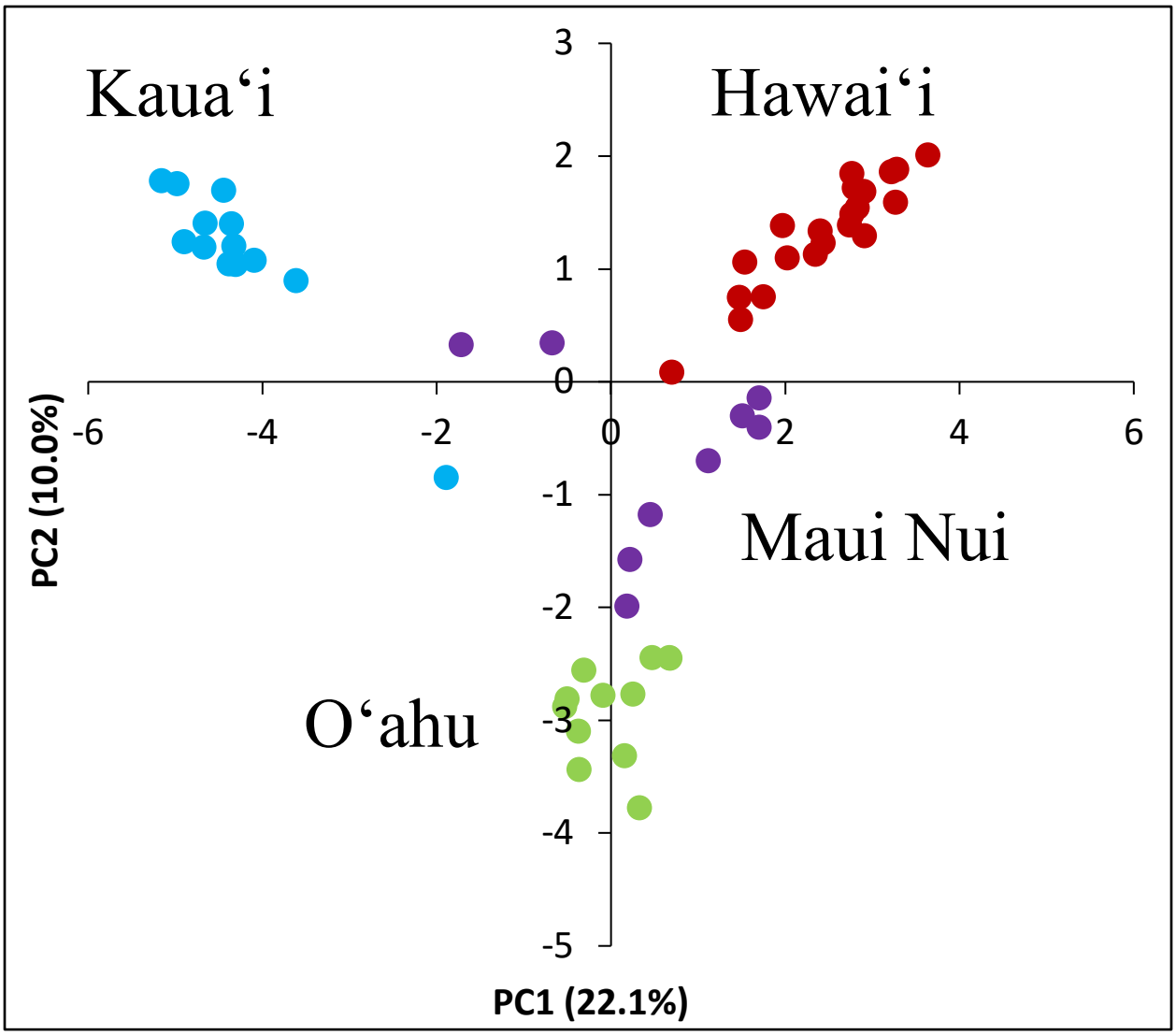

Figure 2.7. Principal coordinate analysis using MIG-seq data of *P. mariniana* and *P. hawaiiensis* var. *hawaiiensis* by island locality (Blue = Kaua‘i, Green = O‘ahu, Purple = Maui Nui, Red = Hawai‘i) using a matrix of covariance values calculated from population allele frequencies.

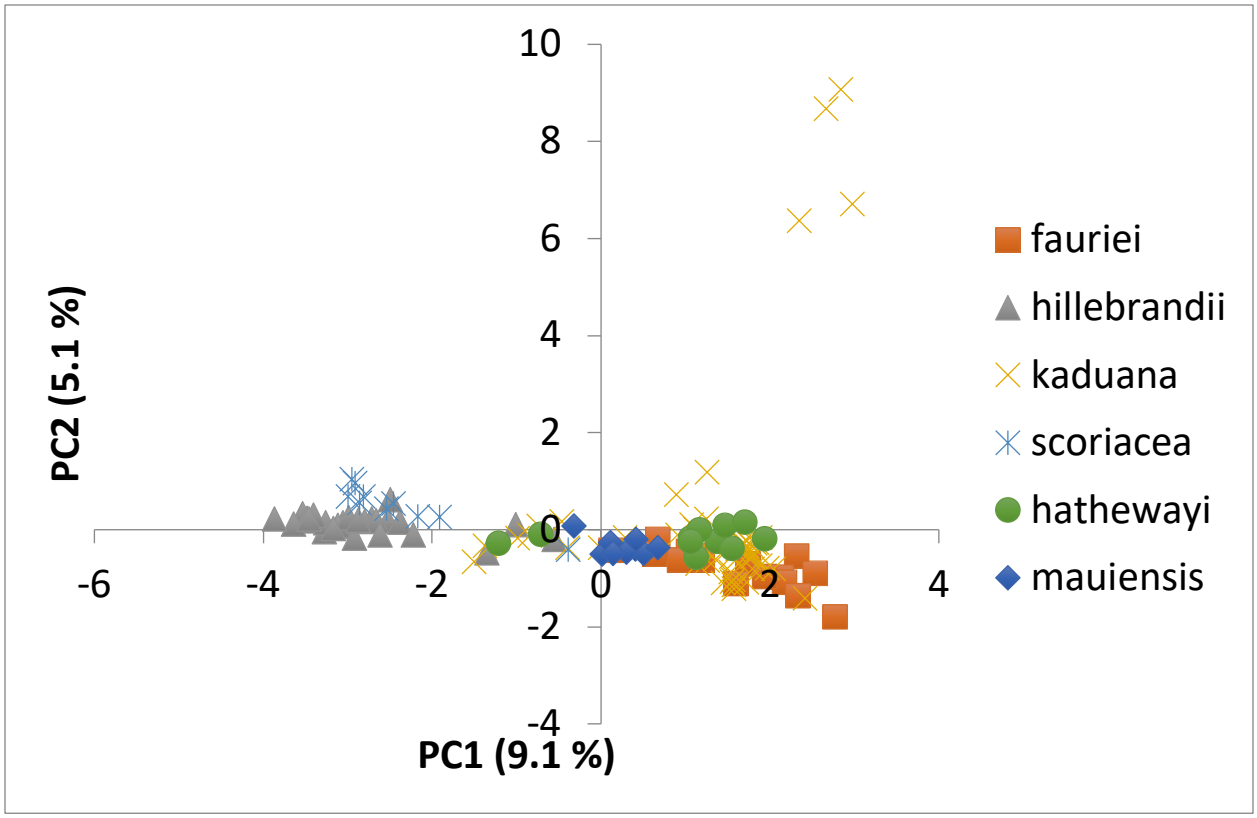

Figure 2.8A. Principal coordinate analysis using MIG-seq data of taxa in the *kaduana* group using a matrix of covariance values calculated from population allele frequencies.

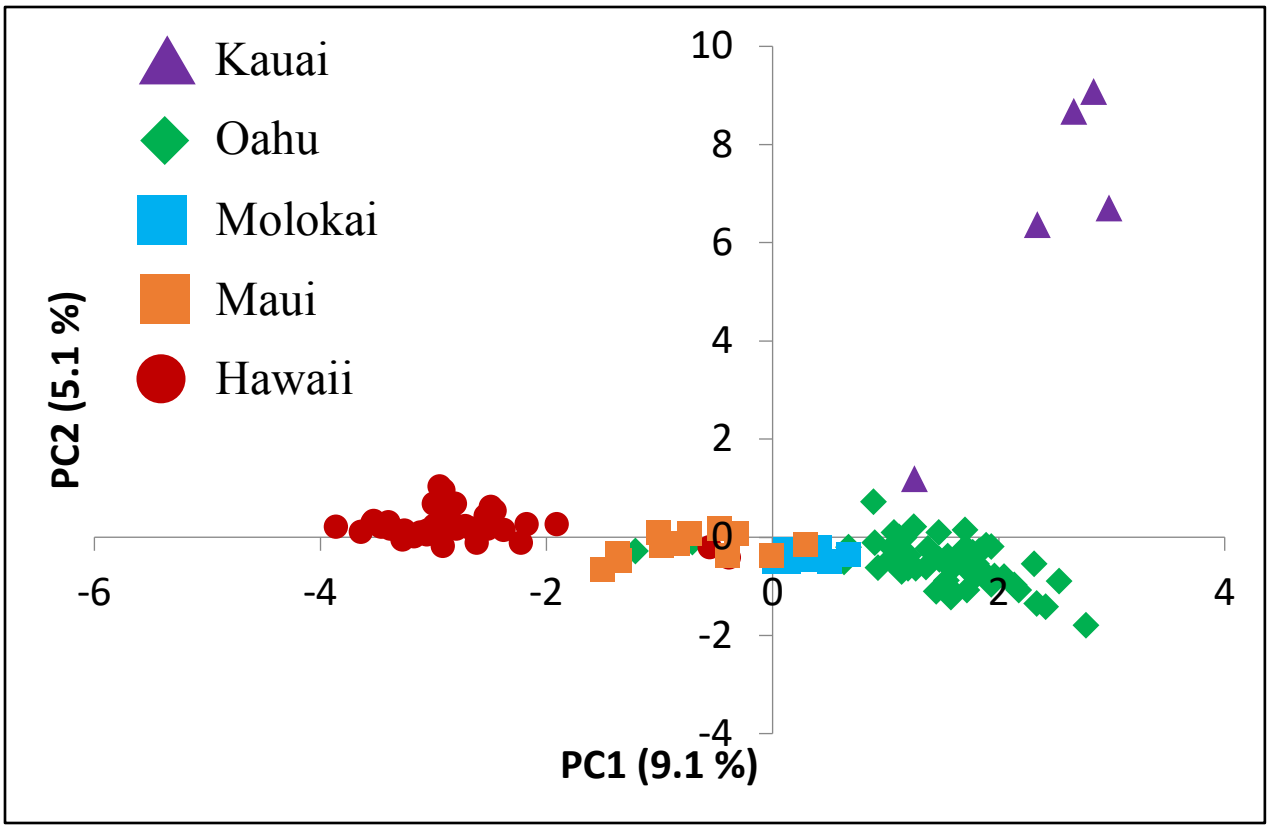

Figure 2.8B. Principal coordinate analysis using MIG-seq data of taxa in the *kaduana* group assigned to island locality using a matrix of covariance values calculated from population allele frequencies.

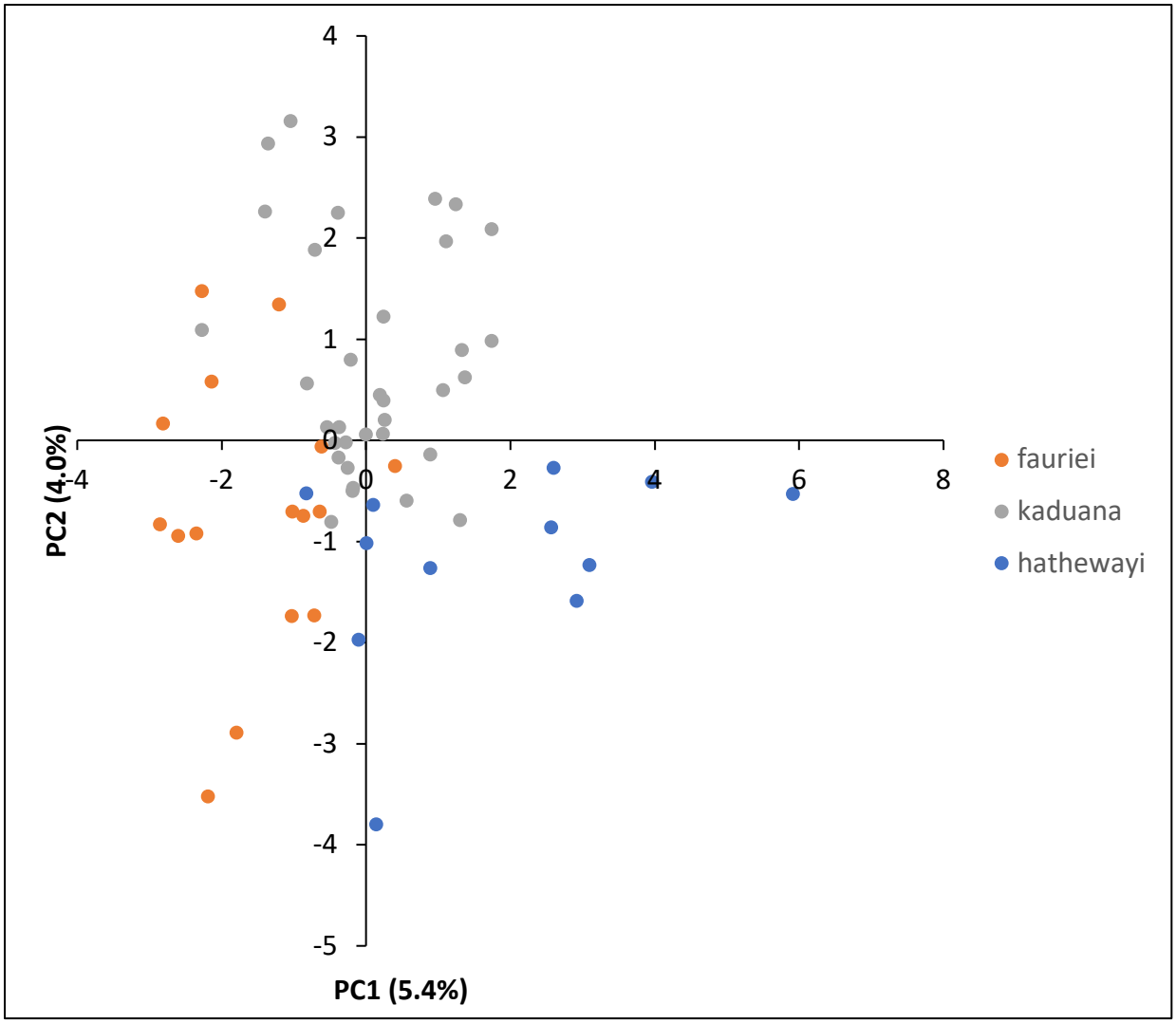

Figure 2.9. Principal coordinate analysis using MIG-seq data of O‘ahu populations of *P. fauriei*, *P. kaduana*, and *P. hathewayi* using a matrix of covariance values calculated from population allele frequencies.

representing populations of *P.kaduana*, *P. mauiensis*, *and P. hawaiiensis* var. *hillerandii* from Maui Nui and two remaining groups represented by individuals of *P. hawaiiensis* var. *hillebrandii* and var. *scoriacea*, respectively (Figure 2.10). Lastly, it was indicated that *P. kaduana* diverges among island populations (Figure 2.11).

*STRUCTURE —*

The STRUCTURE analysis with all populations of taxa in sect. *Straussia* are included demonstrated that the number of populations or clusters (*K*) with the highest Δ*K* was two. When all populations are included, there is strong support for separating members of sect. *Straussia* into the *mariniana* and *kaduana* groups (Figure 2.12). The *mariniana* group exhibited the largest Δ*K* value at *K*=2. Within the *mariniana* group, *P. mariniana* from several islands and *P. hawaiiensis* var. *hawaiiensis* from Hawai‘i island mostly appear to belong to the first genetic cluster and all with some level of admixture (Figure 2.13). However, var. *hawaiiensis* appears to have higher probabilities of belong to the second genetic cluster. The *kaduanna* group also exhibited the largest Δ*K* value at *K*=2. Within the *kaduana* group, *P. hawaiiensis* var. *hillebrandii* and var. *scoriacea* are assigned to a distinct cluster, whereas *P. fauriei*, *P. kaduana*, *P. hathewayi*, and *P. mauiensis* are assigned to a second cluster and some level of admixture is observed between the two clusters (Figure 2.14). Individuals of *P. fauriei*, *P. hathewayi*, and *P. kaduana* on O‘ahu show no differentiation among each other (Figure 2.15). However, there are two individuals that have higher probabilities of belonging to the second genetic cluster, *P. fauriei* from Poamoho summit in the central Ko‘olau Mountains and *P. kaduana* from Maunawili summit in the southern Ko‘olau Mountains, that were growing in sympatry with *P. fauriei*. Individuals of *P. hawaiiensis* var. *hillebrandii*, var. *scoriacea*, *P. kaduana*, and *P. mauiensis* from Maui Nui and Hawai‘i island belong to two genetic clusters

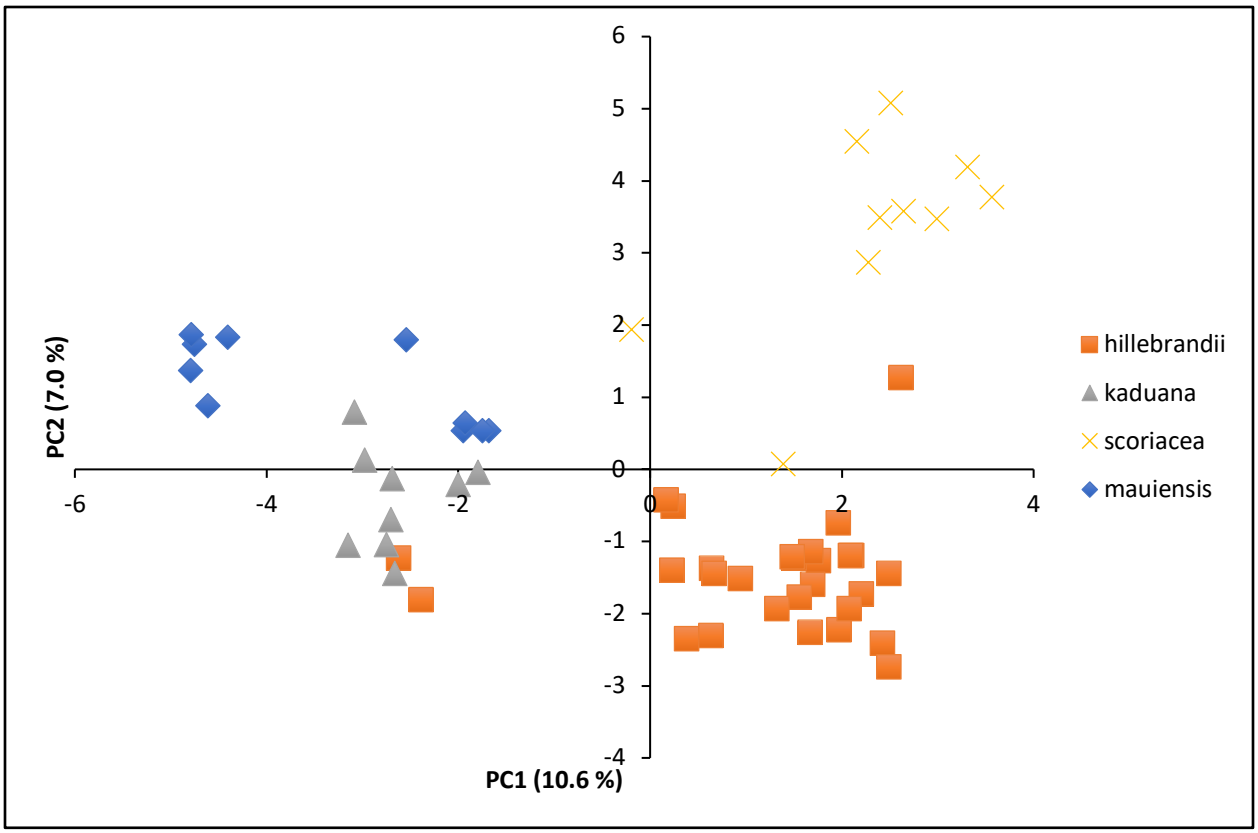


Figure 2.10. Principal coordinate analysis using MIG-seq data of populations of *P. kaduana*, *P. mauiensis*, and *P. hawaiiensis* var. *hillebrandii* and var. *scoriacea* from Molokaʻi, Maui and Hawaiʻi island using a matrix of covariance values calculated from population allele frequencies.

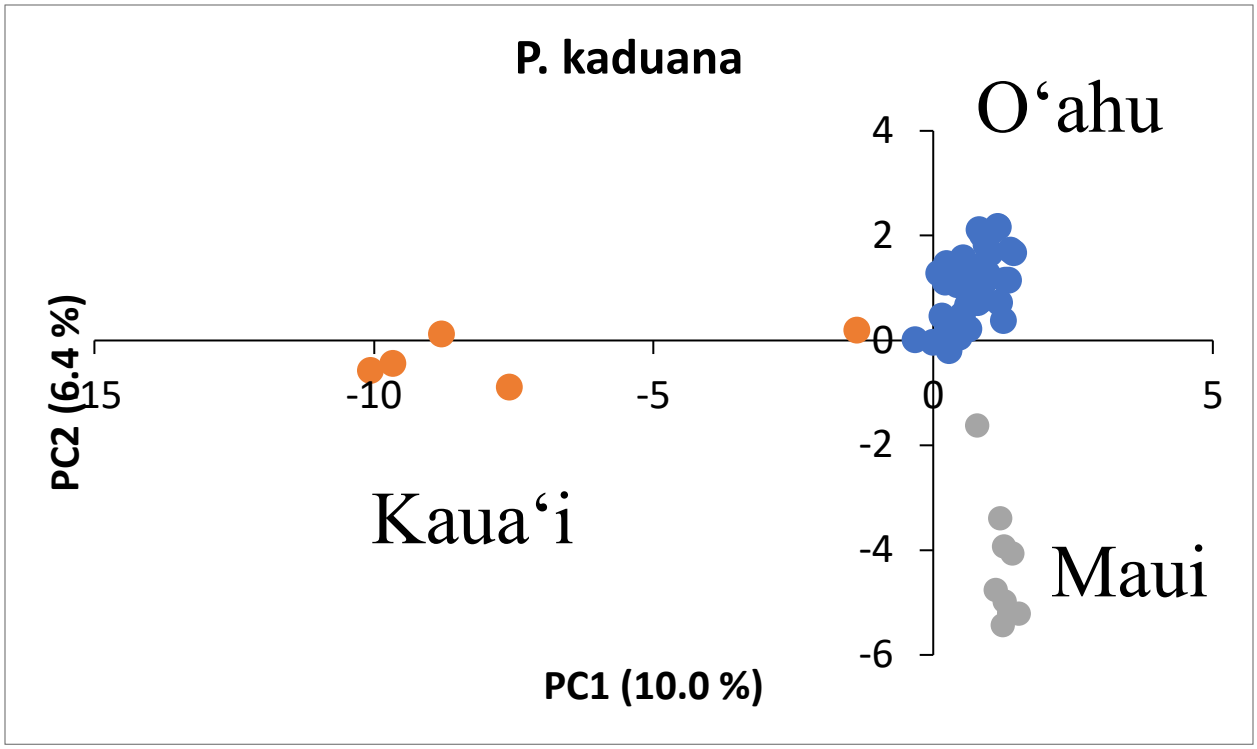

Figure 2.11. Principal coordinate analysis of populations of *P. kaduana* from Kaua‘i (orange), O‘ahu (blue), and Maui (grey) using a matrix of covariance values calculated from population allele frequencies.

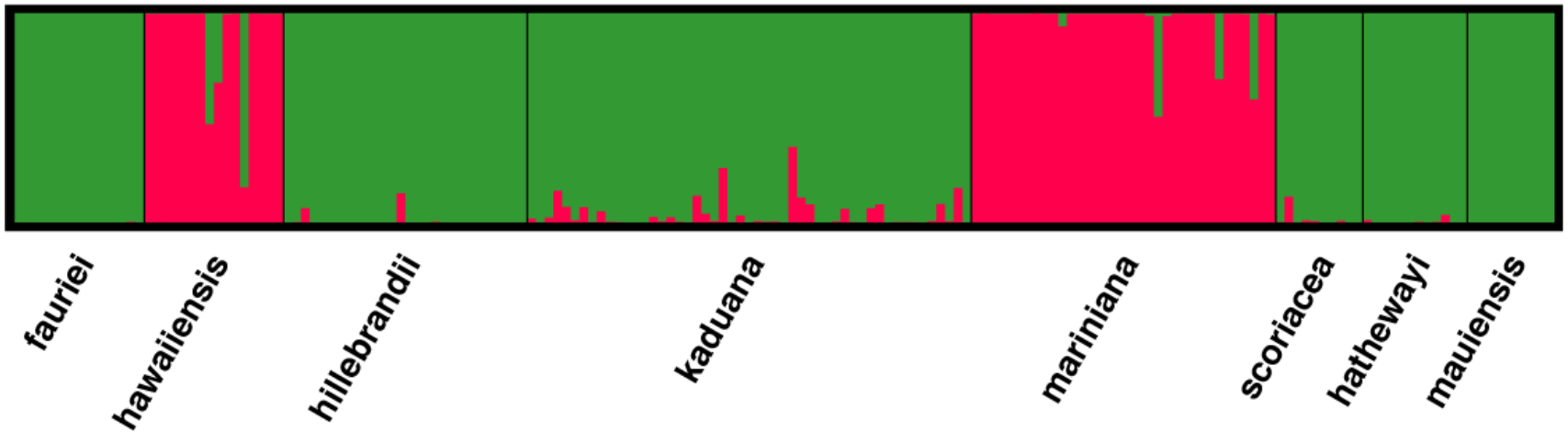


Figure 2.12. Bar plots of inferred population assignment based on MIG-seq data using STRUCTURE with members of sect. *Straussia* (*P. fauriei*, *P. hawaiiensis* var. *hawaiiensis*, var. *hillebrandii*, and var. *scoriacea*, *P. kaduana*, *P. hathewayi*, and *P. mauiensis*) assigned to two clusters. Labels below correspond to taxa.

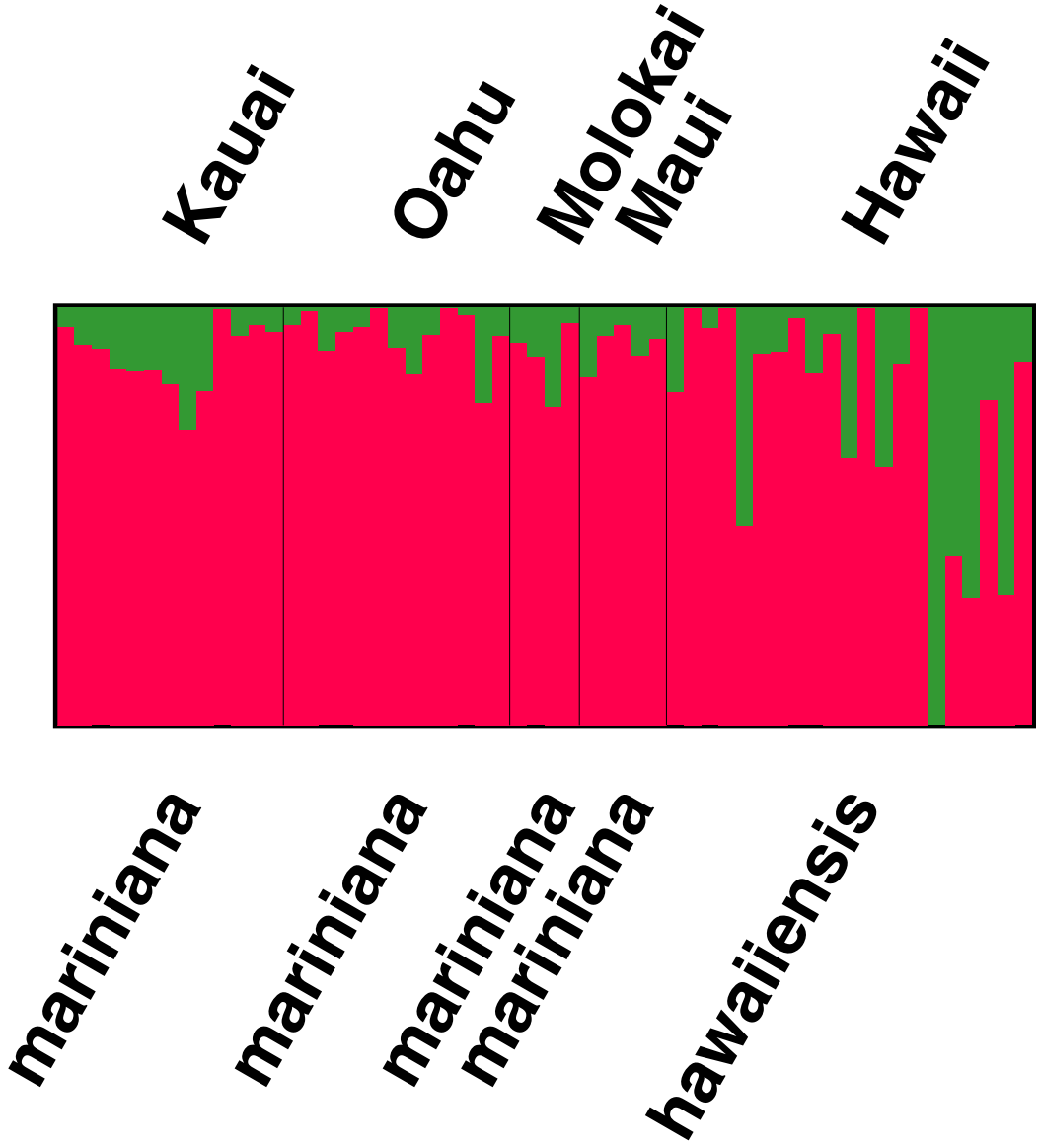


Figure 2.13. Bar plots of inferred population assignment based on MIG-seq data using STRUCTURE with members of the *mariniana* group (*P. mariniana* and *P. hawaiiensis* var. *hawaiiensis*) assigned to two clusters. Labels below correspond to taxa, whereas labels above correspond to island.

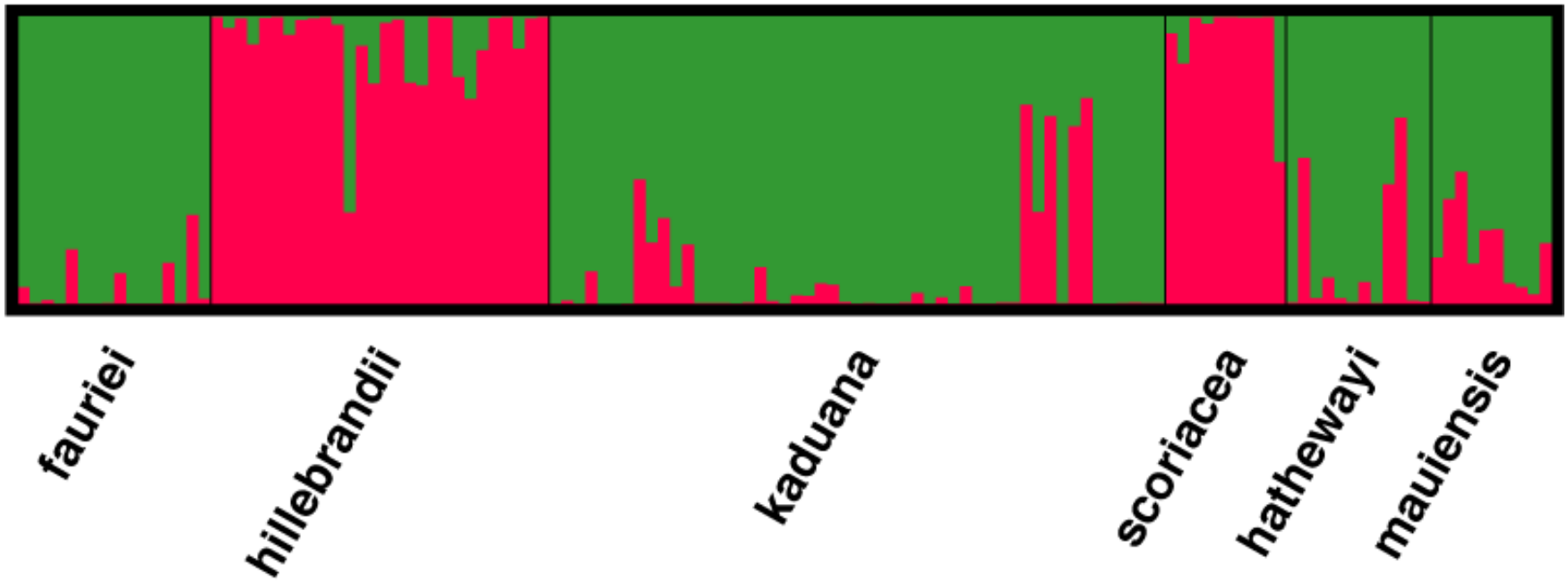


Figure 2.14. Bar plots of inferred population assignment based on MIG-seq data using STRUCTURE with members of the *kaduana* group (*P. fauriei*, *P. hawaiiensis* var. *hillebrandii* and var. *scoriacea*, *P. kaduana*, *P. hathewayi*, and *P. mauiensis*) assigned to two clusters. Labels below correspond to taxa.

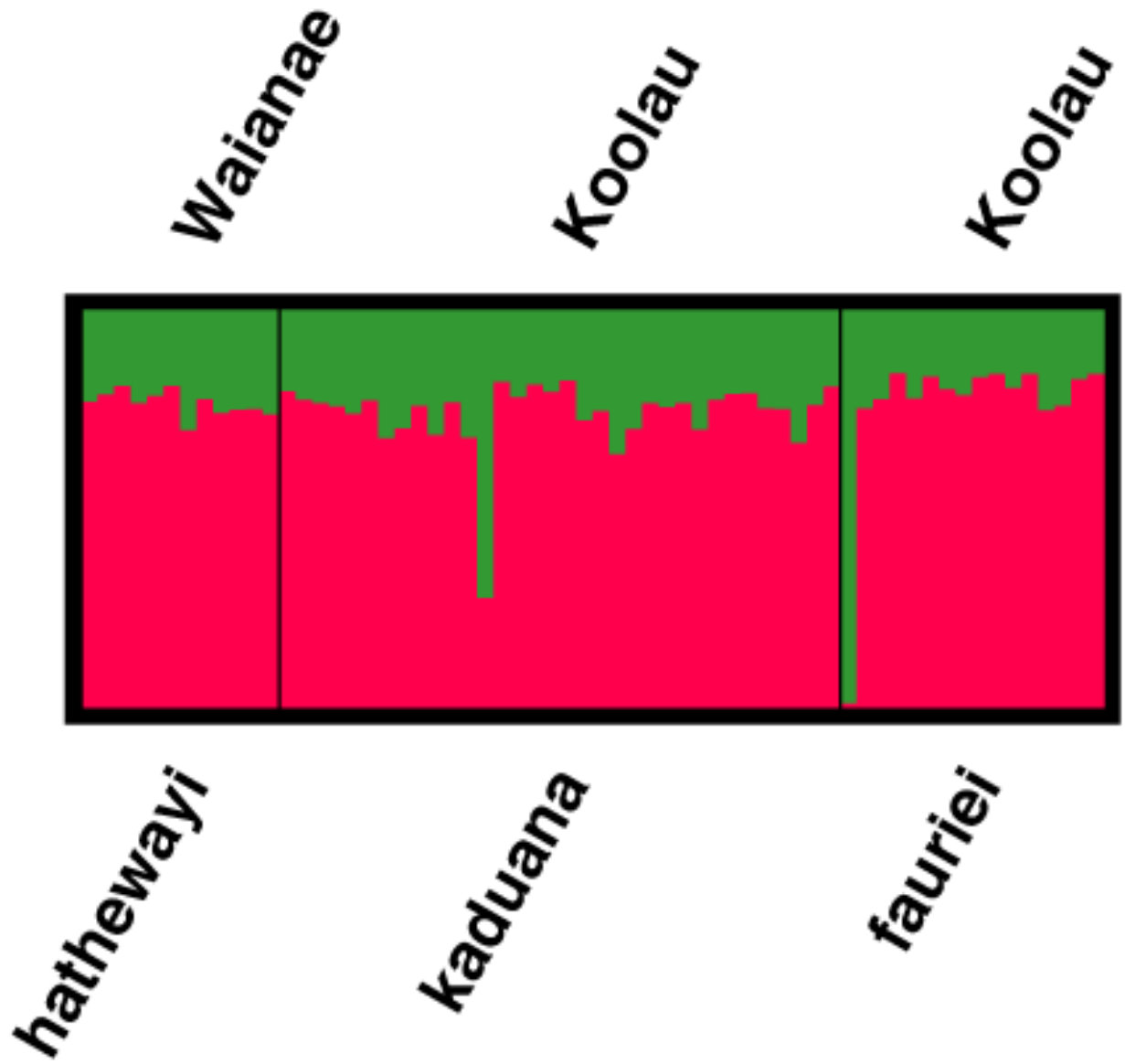


Figure 2.15. Bar plots of inferred population assignment based on MIG-seq data using STRUCTURE of *P. fauriei*, *P. hathewayi*, and *P. kaduana* from the island of O‘ahu assigned to two clusters. Labels below correspond to taxa, whereas labels above correspond to mountain ranges on O‘ahu to which samples were collected from.

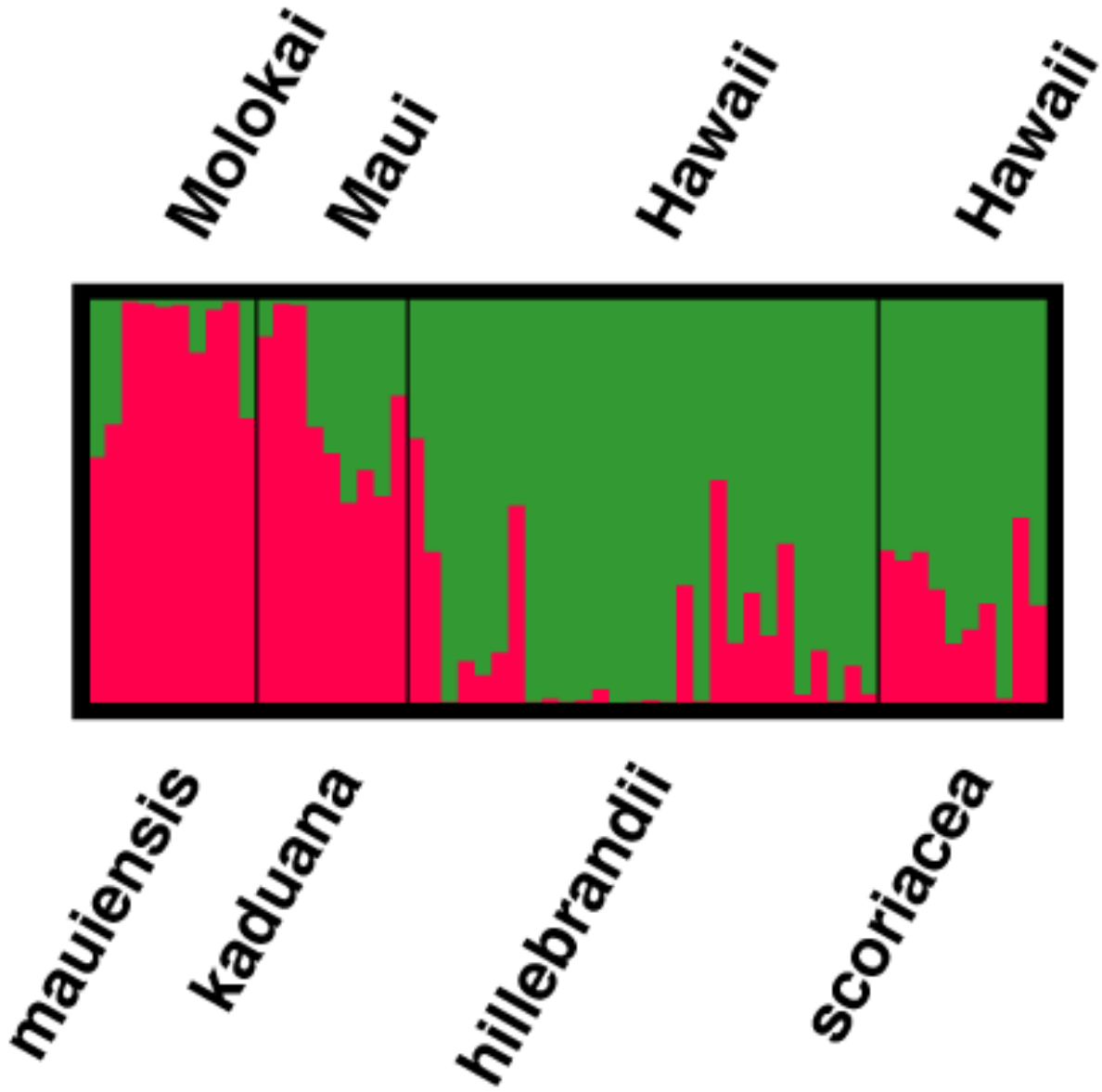


Figure 2.16. Bar plots of inferred population assignment based on MIG-seq data using STRUCTURE of *P. kaduana, P. mauiensis*, and *P. hawaiiensis* var. *hillebrandii* and var. *scoriacea* from the islands of Molokaʻi, Maui, and Hawaiʻi assigned to two clusters. Labels below correspond to taxa, whereas labels above correspond to island.

(Figure 2.16), one corresponding to individuals of *P. kaduana* and *P. mauiensis*, whereas the second corresponding to *P. hawaiiensis* var. *hillebrandii* and *P. hawaiiensis* var. *scoriacea* with some admixture observed between the clusters.

*AMOVA —*

Results of the AMOVAs testing for genetic subdivision revealed in the standard AMOVAs that the highest $F_{ST}$ value was for grouping by taxa ($F_{ST}$ =0.46) and then by island ($F_{ST}$ =0.12). However, more of the variation (88%) is explained by islands than by taxa (Table 2.12). AMOVA was used to assess the distribution of genetic variation on multiple taxonomic and spatial scales. When AMOVA was applied to populations of *P. mariniana* and *P. hawaiiensis* var. *hawaiiensis*, 26.2% of the variation was partitioned among islands, 4.8% among populations within islands and 69.1% within islands (Table 2.13). Furthermore, 6.7% of the variation was partitioned among species, 24.4% among populations within species and 68.9% within species. When AMOVA was applied to populations of *P. mariniana* among islands, 28.1% of the variation was partitioned among islands, 4.4% among populations within islands and 67.5% within islands (Table 2.13). When AMOVA was applied to populations of the *kaduana* group 12.0% of the variation was partitioned among islands, 8.9% among populations within islands and 79.1% within islands (Table 2.14). When AMOVA was applied to populations of the *P. kaduana*, *P. mauiensis*, and *P. hawaiiensis* var. *hillebrandii* and var. *scoriacea* on Maui Nui and Hawai‘i, 14.1% of the variation was partitioned among taxa, 5.6% among populations within islands and 79.3% within islands (Table 2.14). When AMOVA was applied to populations of *P. kaduana*, *P. fauriei*, and *P. hathewayi* on O‘ahu, 3.8% of the variation was partitioned among species, 6.2% among populations within species and 90% within populations (Table 2.14). When AMOVA was applied to populations of *P. kaduana* among islands, 8.5% of the variation was

Table 2.12. AMOVA results testing genetic subdivision based on MIG-seq data among members of sect. *Straussia* by island and taxa

| Main Category | Subdivisions | Variation | Sum of squares | d.f. | Variance Component | Percentage of variation | $F_{ST}$ |
|---|---|---|---|---|---|---|---|
| islands | Kaua'i, O'ahu, Moloka'i, Maui, Hawai'i | Within Population | 6431.941 | 172 | 37.395 | 0.88 | |
| | | Among Population | 783.765 | 4 | 5.122 | 0.12 | 0.12 |
| taxa | *P. fauriei*, *P. hathewayi*, *P. hawaiiensis* vars. *hawaiiensis*, *hillebrandii*, *scoriacea*, *P. kaduana*, *P. mariniana*, *P. mauiensis* | Within Population | 4053.909 | 169 | 23.988 | 54 | |
| | | Among Population | 3155.571 | 7 | 20.422 | 46 | 0.46 |

Table 2.13. AMOVA results testing genetic subdivision based on MIG-seq data among members of the *mariniana* group by (1) taxa, (2) island, (3) *P. mariniana* among islands

| Variation | Sum of squares | d.f. | Variance Component | Percentage of variation | $F_{ST}$/ $F_{SC}$/ $F_{CT}$ |
|---|---|---|---|---|---|
| ***mariniana* group** | | | | | |
| 1. Taxa | | | | | |
| Within Population | 883.133 | 39 | 22.644 | 0.689 | 0.311 |
| Among Population/ within taxa | 510.495 | 10 | 8.016 | 0.244 | 0.261 |
| Among taxa | 156.685 | 1 | 2.205 | 0.067 | 0.067 |
| 2. Islands | | | | | |
| Within Population | 891.283 | 39 | 22.853 | 0.691 | 0.309 |
| Among Population/ within islands | 215.805 | 7 | 1.575 | 0.048 | 0.064 |
| Among islands | 441.813 | 4 | 8.653 | 0.262 | 0.262 |
| 3. *P. mariniana* | | | | | |
| Within Population | 552.683 | 26 | 21.257 | 0.675 | 0.325 |
| Among Population/ within islands | 138.705 | 5 | 1.386 | 0.044 | 0.061 |
| Among islands | 287.469 | 3 | 8.836 | 0.281 | 0.281 |

Table 2.14. AMOVA results testing genetic subdivision based on MIG-seq data among members of the *kaduana* group by (1) taxa, (2) island, (3) *P. fauriei, P. hathewayi, P.kaduana* on O'ahu, (4) *P. kaduana*, *P. hawaiiensis* vars. *hillebrandii*, *scoriacea*, *P. mauiensis* on Maui Nui and Hawai'i island, (5) *P. kaduana* among islands

| Variation | Sum of squares | d.f. | Variance Component | Percentage of variation | $F_{ST}$/ $F_{SC}$/ $F_{CT}$ |
|---|---|---|---|---|---|
| ***kaduana* group** | | | | | |
| 1. Taxa | | | | | |
| Within Population | 1526.068 | 100 | 15.261 | 0.805 | 0.195 |
| Among Population/ within taxa | 471.889 | 20 | 1.874 | 0.099 | 0.109 |
| Among taxa | 307.059 | 5 | 1.821 | 0.096 | 0.096 |
| 2. Islands | | | | | |
| Within Population | 1534.613 | 100 | 15.346 | 0.791 | 0.209 |
| Among Population/ within islands | 510.968 | 21 | 1.726 | 0.089 | 0.101 |
| Among islands | 269.411 | 4 | 2.321 | 0.12 | 0.12 |
| 3. *P. fauriei*, *P. kaduana*, *P. hathewayi* | | | | | |
| Within Population | 797.683 | 50 | 15.954 | 0.900 | 0.100 |
| Among Population/ within taxa | 222.429 | 11 | 1.093 | 0.062 | 0.064 |
| Among taxa | 74.294 | 2 | 0.672 | 0.038 | 0.038 |
| 4. *P. kaduana*, *P. hawaiiensis* vars. *hillebrandii*, *scoriacea*, *P. mauiensis* | | | | | |
| Within Population | 654.702 | 46 | 14.233 | 0.793 | 0.207 |
| Among Population/ within taxa | 142.580 | 7 | 1.005 | 0.056 | 0.066 |
| Among taxa | 153.981 | 3 | 2.714 | 0.151 | 0.151 |
| 5. *P. kaduana* | | | | | |
| Within Population | 647.683 | 40 | 16.192 | 0.834 | 0.166 |
| Among Population/ within islands | 158.681 | 8 | 1.568 | 0.081 | 0.088 |
| Among islands | 112.107 | 2 | 1.653 | 0.085 | 0.085 |

partitioned among islands, 8.1% among populations within islands and 83.4% within islands (Table 2.14).

*Mantel test —*

When Mantel test is performed on all taxa there was a correlation between genetic and geographic distances (r = 0.242, p= 0.013). In addition, the *mariniana* group also showed significant positive correlation between genetic and geographic distances (r = 0.697, p= 0.006). However, there was no correlation between genetic and geographic distances within the *kaduana* group (r = 0.216, p= 0.071). Conversely, populations of *P. kaduana* on several islands showed a significant positive correlation between genetic and geographic distances (r = 0.988, p= 0.001).

## DISCUSSION

*Phylogenetic analyses —*

The phylogenetic analyses in the current study resulted in similar topologies from previous studies but inferred relationships among species in the present study are weakly supported. This is potentially due to recent divergence of some of the species or rapid radiation over a short period of time (Nepokroeff et al., 2003; Zhang, 2016). Moreover, the Hawaiian islands' flora is replete with examples of morphologically diverse radiations with little genetic variation among them (Soltis et al., 1996; Carlquist et al., 2003; Givnish et al., 2009; Knope et al., 2012; Appelhans et al., 2014; Welch et al., 2016). One hypothesis for this situation would be that sect. *Straussia* consist of a few species that possess a great deal of morphological variation potentially due to phenotypic plasticity or differing selection at the population level.

It is becoming apparent that species of sect. *Straussia* are separated into two groups (*marinana* and *kaudana* clades). This is further supported by the distribution of the two

morphological character states (venation visible or obscure, domatia conspicuous or inconspicuous) used in the ancestral character state reconstructions (see below). Although finer scale distinctions cannot be made definitively within the *marinana* and *kaduana* clades as yet, there are some observations that can contribute to a better understanding of their relationships. The only *P. kaduana* individual along with the *P. marinana* samples from the oldest island of Kauaʻi group with the Kauaʻi endemics, *P. greenwelliae* and *P. wawrae*, respectively. These results our concordant with the findings from Zhang (2016) in which taxa group by geography or represent samples that were misidentified or are hybrids. There is some evidence of hybridization at least one individual *P. mariniana* groups with *P. hathewayi*, whereas in chloroplast phylogeny it groups with other individuals of *P. mariniana*. Furthermore, another potential *P. mariniana* hybrid individual maintained an intermediate position between *mariniana* and *kaduana* clades in the phylogeny. Additionally, several samples from Nepokroeff et al. (2003) form a monophyletic group that is separate from each of their respective species; a similar result was observed in Zhang (2016) phylogenetic analyses. These results may be due to the difficulty of aligning their ITS sequences with the rest of the data set and perhaps may need to be excluded from subsequent analyses because the quality of multiple sequence alignments can affect the quality of the inferred phylogenetic tree (Yue et al., 2009; Hossain et al., 2015). Given the clear evidence of two large clades, although with unresolved intra clade relationships, perhaps should be recognized as subsections. However, this needs further investigation before making such a major taxonomic change and is beyond the scope of this study

Disagreements between molecular analyses and morphological taxonomy are common in Hawaiian taxa and make it difficult to determine true evolutionary relationships. This is apparent with the three varieties of *P. hawaiiensis* that have been grouped due to morphological

similarities by previous taxonomic treatments (Fosberg 1964; Sohmer 1977; Wagner et a. 1990), but are homoplasious in the molecular analyses, especially for var. *hillebrandii* and var. *scoriacea*. A similar situation appears to exist with *Psychotria hawaiiensis* var. *hawaiiensis* nested within the P. *mariniana* clade but, in agreement with Fosberg's (1964) observations noting *P. hawaiiensis* var. *hawaiiensis* approaches *P. mariniana* in morphology. Currently, *P. mariniana* is distributed across all the major Hawaiian Islands, except for the youngest island, Hawai‘i, where *P. hawaiiensis* var. *hawaiiensis* is predominantly found. The latter may represent an incipient species (Nepokroeff et al., 2003). In the combined analysis, variety *hillebrandii* individuals from Hawai‘i island group together, whereas individuals from Maui may have been misidentified and could represent other taxa such as *P.kaduana* or *P. mauiensis*. This result is concordant with Sohmer's (1977) obervations noting that individuals of *P. hawaiiensis* on the islands of Maui and Moloka‘i were often silimar in appearance to *P. mauiensis*.

This is the first time *P. hawaiiensis* var. *scoriacea* was used in a phylogenetic study where it shows a close relationship with other members of the *kaduana* clade and in some analyses it groups with *P. hawaiiensis* var. *hillebrandii*. Previous taxonomic treatments proposed that *P. hawaiiensis* and *P. mauiensis* were closely related species (Fosberg, 1964; Sohmer, 1977). However, these molecular analyses indicate that this is not the case and if the varieties *hillebrandii* and *scoriacea* were removed from *P. hawaiiensis*, it would be suspected that the morphological affinities between these taxa cited from previous taxonomic treatments might fade away. Further work is needed to resolve the relationships of *P. hawaiiensis* varieties *hillebrandii* and *scoriacea* with the rest of the taxa in the *kaduana* clade. Additionally, it remains to be resolved *P. hawaiiensis* var. *hawaiiensis* is a distinct species or a representative of *P. mariniana*.

Including more samples per species into phylogenetic analyses may improve species relationships. Such data exists (Zhang, 2016), however, they were not made available at the time of the current study. Additionally, the gene regions used in this study may not be variable enough to infer phylogenetic relationships among closely related species. Future investigations may need to shift to next-generation sequencing (NGS) techniques such as RAD-seq and genotyping-by-sequencing (GBS) which has the potential to yield a large numbers of variable sites (Davey and Blaxter, 2011) and resolve even the most problematic phylogenetic challenges (Wagner et al., 2013; Escudero et al., 2014; Razkin et al., 2016; Hamon et al., 2017).

*Ancestral character state reconstructions —*

Wagner et al. (1990) notes tertiary veins that are often conspicuously reticulate in some of the species are cited in the most recent taxonomic treatment. This character trait while not sufficiently discriminatory for defining species boundaries, it strongly support the presence of two larger clades distinguishing the *mariniana* clade from the *kaduana* clade. Furthermore, these character state differences support the phylogenetic placement of the three varieties of *P. hawaiiensis* (Fig. 2.4A). When Rock (1913) originally described *P. hawaiiensis* var. *hillebrandii*, he mentioned that it resembled *P. hawaiiensis* in one way and *P. mauiensis* in another. Currently, *P. hawaiiensis* is distinguished by several reproductive characters and based on these analyses, it is suspected that its resemble to *P. mauiensis* (i.e. leaves with conspicuously reticulate tertiary veins, and small, inconspicuous or absent domatia) which would support its placement within the *kaduana* clade. Also, *P. hawaiiensis* var. *scoriacea* also possesses this trait and would support its placement within the *kaduana* clade. Some of the *P. mariniana* samples used in this study had to non-priminent reticulate tertiary veins, although they were present, and were suspected of being hybrids upon collection, a finding supported by their grouping with taxa in the *kaduana* clade.

Hybridization with sympatry has also been noted for members of sect. *Straussia* in previous taxonomic treatments as well (Fosberg, 1964; Sohmer 1977; Wagner et al. 1990). Members of the *mariniana* clade may have less visible reticulate tertiary venation due to their thicker leaves, relative to the rest of the section, and that these differences appear more readily in the field than on herbarium specimens (Figure 2.17). In addition, several studies have shown leaf venation can have taxonomic value at different hierarchical levels in other members of Rubiaceae (Hawthorne, 2013; Wagner and Lorence, 1998; Davis and Rakotonasolo, 2001; Pacheco-Trejo et al., 2009). However, it remains to be determined if leaf architecture is truly informative at the species level in sect. *Straussia*.

Presence of domatia on the abaxial leaf surface located between the midrib and secondary veins is also common among members of Rubiaceae (Robbrecht, 1988). These domatia are typically inhabited by mites that are predatory or fungivorous which benefits the plants (Pemberton and Turner, 1989; Richards and Coley, 2011). However, it is assumed that domatia are formed regardless of the presence of the arthropods that inhabit them (Romero & Benson, 2005), and may be a family trait. Ancestral state reconstructions largely supporting field observations, show that species possessing conspicuous tertiary veins typically have small, inconspicuous or absent domatia, whereas those without visible tertiary veins generally having conspicuous domatia. Nevertheless, while domatia size is variable Hillebrand (1888) noted that *P. hawaiiensis* var. *hawaiiensis* and *P. mariniana* have the largest domatia among sect. *Straussia*, supporting the closely relationship in molecular analyses. It is unknown what is driving the variation in domatia size in sect. *Straussia*, but observations of another *Psychotria* species along a rainfall gradient revealed that dry forest individuals had small domatia, whereas.

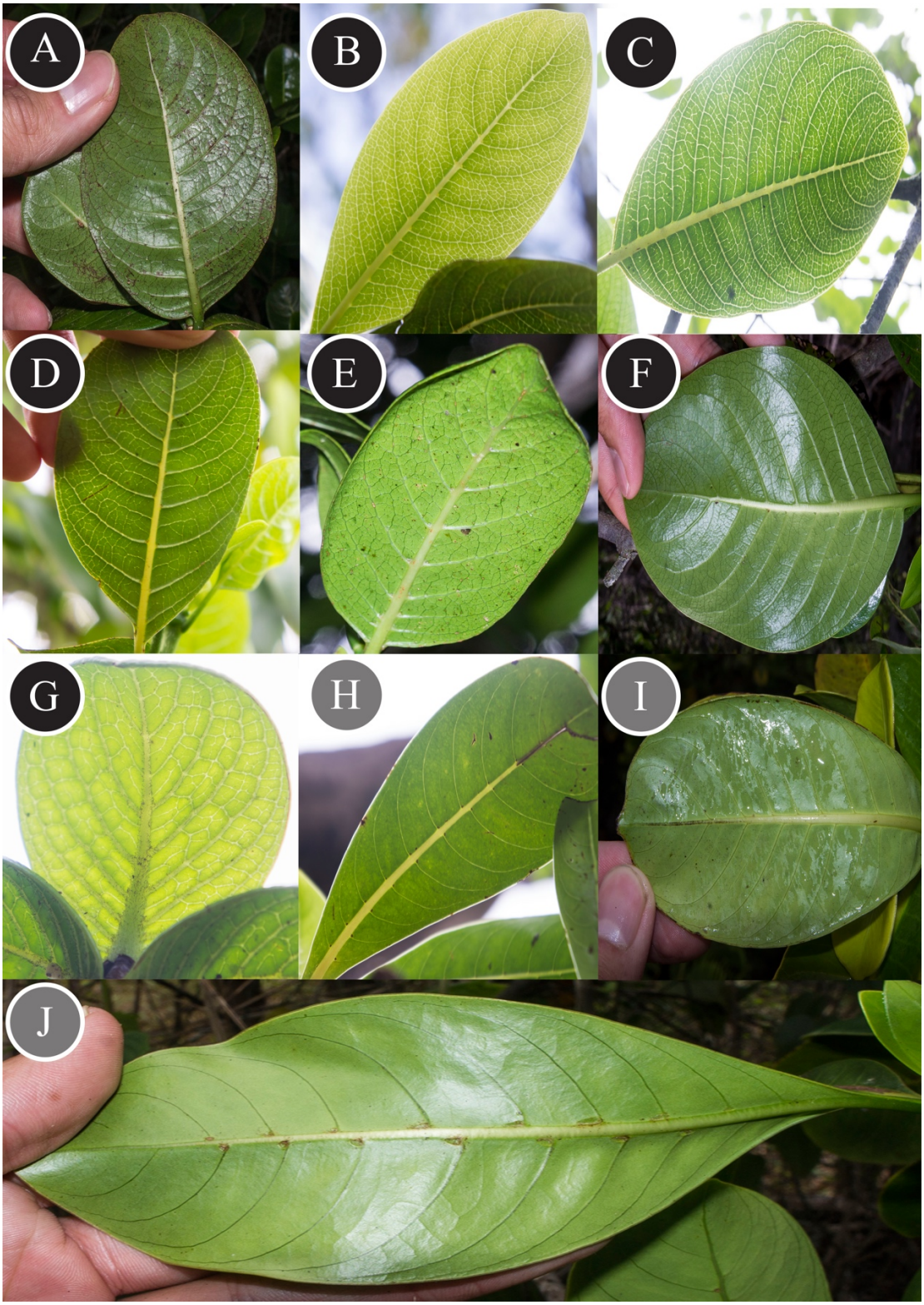


Figure 2.17. Abaxial leaf surfaces of the members of sect. Straussia. Black circles = *greenwelliae* clade (A-G) with reticulate venation and inconspicuous domatia: (A) *P. hathewayi,* (B) *P. kaduana,* (C) *P. hawaiiensis var. hillebrandii* (D*) P. mauiensis,* (E) *P. greenwelliae*, (F) *P. hawaiiensis var. scoriacea*, (G) *P. fauriei.* Gray circles = *mariniana* clade (H-J) with reticulate venation not visible, but with conspicuous domatia, except for *P. wawrae:* (H) *P. mariniana*, (I) *P. wawrae,* (J) *P. hawaiiensis var. hawaiiensis*.

those in wet forests had larger domatia (Richards and Coley, 2011). This may not be the case for Hawaiian *Psychotria*, *P hawaiiensis* var. *hawaiiensis* and var. *scoriacea* both possess large domatia despite inhabiting mesic to wet forests and dry forests, respectively, whereas var. *hillebrandii* inhabits wet to moderately wet forests but possesses small domatia that are almost absent. Presence or absence and type of domatia can have taxonomic value in delineating species (Burch et al. 1975; Moraes et al. 2009; Hawthorne, 2013) The value of this character for Hawaiian *Psychotria* in making taxonomic decisions in delineating members of sect. *Straussia* is, however, beyond the scope of this study.

*SRAP analyses —*

SRAP data largely support the results from the previous phylogenetic analyses (Nepokroeff et al., 2003; Zhang, 2016) validating the separation between the *mariniana* and *kaduana* clades, with some exceptions (i.e., *P. mariniana* individuals that showed a close relationship with other species in sect. *Straussia* and were suspected of being hybrids). Also, the SRAP analyses further supports the phylogenetic analyses separating the varieties of *P. hawaiiensis* from each other. Variety hawaiiensis is most genetically similar to *P. mariniana* which is consistent with the phylogenetic analyses. Variety *hillebrandii* is genetically closest to *P. mauiensis*, congruent with the results from Zhang (2016) and when Rock (1913) originally described this taxon, he stated that it showed some resemblance to *P. mauiensis*. Also, *P. hawaiiensis* var. *scoriacea* is most genetically similar to *P. fauriei* despite differing in their habit and habitat although, this relationship was not apparent in all of the phylogenetic analyses here due to the lack of variation of the gene regions used. Furthermore, variety *scoriacea* also shows a close relationship with *P. mauiensis* to which it was originally described a variety of (Rock, 1913).

*MIG-seq analyses —*

Comparing this analyses with SRAP and phylogenetic data two distinct groups are consistently observed within sect. *Straussia* (i.e. *mariniana* and *kaduana* groups). However, there were some differences with the relationship among taxa within the *kaduana* group between the SRAP and MIG-seq PCO analyses. The MIG-seq data indicated that *P. hawaiiensis* var. *hillebrandii* and var. *scoriacea* are most genetically similar, but this was not observed in the SRAP analysis, perhaps due to the limited samples of var. *scoriacea* used in SRAP analysis. Additionally, The PCO analysis using SRAP data showed greater differentiation among taxa in the *kaduana* group, but there were very low levels of differentiation observed in the MIG-seq analysis.

The PCO analysis of the *mariniana* group revealed a close relationship between *P. mariniana* and *P. hawaiiensis* var. *hawaiiensis* that was observed in previous studies (Nepokroeff et al., 2003; Zhang, 2016). Moreover, *P. mariniana* individuals largely group by island, a finding that is supported by the Mantel test in which individuals that are geographically closer are most genetically similar. However, the STRUCTURE analysis revealed little differentiation among individuals within this group. This is corroborated by the AMOVA analysis that showed 6.7% of the variation was partitioned among these species. Although *P. mariniana* is distributed throughout most of the islands, except for Hawai‘i island where *P. hawaiiensis* var. *hawaiiensis* is predominantly found. Although morphological characters are recognized to support the separation of these two species, *P. hawaiiensis* is not genetically distinct enough from *P. mariniana* to warrant recognition as a separate species. It perhaps represents an incipient lineage and should be reduced to variety of *P. mariniana*.

The PCO analysis revealed no clear distinct grouping among taxa, but most of the individuals did group by island. This was confirmed by the AMOVA analysis that showed 9.6% and 12% of the variation was partitioned among taxa and islands, respectively. The lack of differentiation may explain why the Mantel test found no correlation between geographic and genetic distances because they are all genetically similar. This was supported by the STRUCTURE analysis that indicated that *P. fauriei*, *P. kaduana*, *P. hathewayi*, and *P. mauiensis* belonged to the same genetic cluster. This would support the findings of Skottsberg (1944) who proposed reducing many of the recognized species to *P. kaduana* due to lack of distinct morphological boundaries. However, it was indicated that *P. hawaiiensis* var. *hillebrandii* and var. *scoriacea* appear to be genetically distinct from the rest of this group in the STRUCTURE and PCO analyses and supporting their elevation to specific rank. Further collections of var. *hillebrandii* and var. *scoriacea* on Moloka‘i and Maui are recommended to confirm their presence on those islands or if they are representatives of morphologically similar taxon (i.e. *P. kaduana*). Moreover, future investigations should explore their relationship with *P. mauiensis*, a species underrepresented in this study. Also, two taxa that were previously associated with var. *hillebrandii*: *Straussia hillebrandii* var. *molokaiensis* *S. hillebrandii* and var. *rotundifolia* described by Rock (1913) and Skottsberg (1944), respectively, are currently reduced to synonymy with *P. mauiensis*.

Despite populations of *P. hathewayi* and *P. fauriei* being endemic to the Wai‘anae and Ko‘olau mountain ranges, respectively, on the island of O‘ahu they do not show any clear patterns of genetic differentiation from each other or from *P. kaduana*. This is supported by the STRUCTURE analysis and by AMOVA revealing only 3.8% of the variation was distributed among these species. Together, these results indicate that these species have not diverged

genetically, at least such divergence was not detected with MIG-seq data. The lack of differentiation among these species could be due to recent divergence (Zhang, 2016) or divergence with continuing gene flow. *Psychotria kaduana* has an estimated range that spans the ranges of both *P. fauriei* and *P. hathewayi*. Moreover, *P. fauriei* is known to hybridize with *P. kaduana* in the Koʻolau Mountains (Fosberg, 1964; Sohmer, 1977). Also, *P. fauriei* and *P. hathewayi* exhibit similar morphology, habit, and ecological preference (Sohmer, 1978). It may be that these taxa represent incipient speciation within a genetically variable *P. kaduana* with the *P. fauriei* and *P. hathewayi* forms representing adaptation to different environments.

The characters used to distinguish *P. kaduana* become obscure on the islands of Molokaʻi, Maui, and Lanaʻi where there are no sharp lines demarcating species boundaries (Skottsberg, 1944; Fosberg, 1964). In this study, populations of *P. kaduana*, *P. mauiensis* and *P. hawaiiensis* var. *hillebrandii* from Maui Nui group together and are not well differentiated in the STRUCTURE and PCO analyses. *Psychotria mauiensis* is extremely variable in features such as leaf shape and size, pubescence, and size of the floral parts (Wagner et al., 1990) and it is hypothesized that this taxon has at its basis elements of *P. kaduana* (Sohmer, 1978). Furthermore, *P. kaduana* is already recognized as one the most highly variable species in sect. *Straussia*. Perhaps these sibling species on Maui Nui are adapting from similar gene pools to similar environments available on these islands (Sohmer, 1978). Additionally, the two individuals of *P. hawaiiensis* var. *hillebrandii* collected from Maui in this study may have been misidentified and appear to be representatives of *P. kaduana*, according to the PCO analysis. These individuals possessed abaxial leaf surfaces and inflorescence axes with a reddish pubescence that is characteristic of var. *hillebrandii*. Perhaps, color of pubescence may not be as

informative as previously thought by previous taxonomic treatments because it is not consistently observed in the field (Figure 2.17).

Island-based clades were observed in previous phylogenetic analysis of the chloroplast data (Zhang, 2016) and the MIG-seq data. All Psychotria species have bird dispersed fruits (Nepokroeff et al., 1999). Additionally, the islands have lost many of its native frugivores including crows, thrushes, and several honeycreepers (Foster and Robinson, 2007). This loss could lead to low dispersal ability and gene flow among habitats and islands for native fleshy-fruited species (Givnish et al., 1995; Givnish et al., 1998; Price and Wagner, 2004; Chimera and Drake, 2010). Typically, when an individual's dispersal capacity does not extend to its geographical range, differentiation can be attributed to isolation by distance (Balloux and Lugon-Moulin, 2002). This is supported by the PCO analyses using the MIG-seq data that showed the two most widely distributed species, *P. mariniana* and *P. kaduana*, grouping by island and also showed a significant correlation between geographic and genetic distance of the populations. The islands may be acting as barrier to gene flow, but hybridization and introgression among species may still be occurring within islands (Sohmer, 1977; Wagner et al., 1990). The majority of the species are found occurring in sympatry in the mesic to wet forest habitats and all species were observed flowering during the same period when collections were made (Spring-Summer). A lack of inter-specific crossing barriers is well known in other Hawaiian radiations (Motley and Carr, 1998; Harbaugh et al., 2009; Bacon et al., 2011; Knope et al., 2012).

Hybridization and introgression is thought to be responsible for some of the morphological variation observed within species of sect. *Straussia* (Fosberg, 1964; Sohmer, 1977; Wagner et al., 1990). Nonetheless, when species such as *P. fauriei* and *P. kaduana* occur in sympatry along the Koʻolau summits, *P. fauriei* still maintains its specific identity. This could

Figure 3.18. To showcase the pubescence not consistently being reddish or rusty on the abaxial leaf surface and inflorescence axes of *P. hawaiiensis* var. *hillebrandii* from the type locality at Kipuka Puaulu, Hawai‘i

be due to unilateral incongruity, wherein the success of an inter-specific cross depends on the direction of the cross (Covey et al., 2010) or perhaps pollen may be more likely to be transferred to *P. kaduana* plants in these areas because they are more abundant. Moreover, Currat et al. (2008) predicted that unidirectional cytoplasmic introgression should occur from the local species to the invading species. If introgression is occurring unilaterally, this could provide *P. kaduana* the genetic plasticity to colonize different habitats (i.e., *Dubautia scabra*: Caraway et al., 2001) as this taxon is already known to have a wide ecological amplitude (Sohmer, 1977). Also, *P. kaduana* displayed high genetic variation within populations (83.4%) in the AMOVA analysis. Studies of other widely distributed native forest trees that are highly adaptable to environmentally heterogenous habitats, such as *Acacia. koa* and *Metrosideros polymorpha*, revealed similarly high percentage of variance resulting from within population variation (72%, and 91%, respectively) (Harbaugh et al., 2009; Adamski et al., 2012). Furthermore, karyological data within sect. *Straussia* should be explored further, if species are of different ploidy levels, it could form a natural barrier to prevent hybridization (Rabakonandrianina, 1980). *Psychotria mariniana* and *P. kaduana* are presumed to be hexaploids, whereas *P. greenwelliae* is considered to be an octoploid (Kiehn and Lorence, 1996; Kiehn, 2005).

**Future directions**

The current study shows that P. *hawaiiensis* is polyphyletic and the infraspecific taxa are more closely related to other species within sect. *Straussia* than they are to each other. This suggests that *P. hawaiiensis* may have been described based on features of two or more species. Nomenclatural changes that may be needed in the future include reducing *P. hawaiiensis* var. *hawaiiensis* to varietal rank of *P. mariniana*. In addition, results from the STRUCTURE and

PCO analyses show that most individuals of *P. hawaiiensis* var. *hillebrandii* and var. *scoriacea* form separate genetic groups, indicating that these two species may need to be recognized as separate distinct species. Also, the lack of differentiation among *P. fauriei*, *P. hathewayi*, *P. kaduana*, and *P. mauiensis* is apparent in the phylogenetic, STRUCTURE and PCO analyses. As such, it may be a reasonable conclusion to reduce the taxa in the *kaduana* group to *P. kaduana*. However, other next-generation sequencing (NGS) techniques such as RAD-seq or GBS have the potential to yield a large numbers of variable sites (Davey and Blaxter, 2011) and has shown the potential to disentangle the relationships of even the most problematic taxonomic groups, including recently derived lineages (Wagner et al., 2013; Escudero et al., 2014; Labate et al., 2014; Lexer et al., 2016; Hamon et al., 2017). Furthermore, a more thorough sampling of populations of these taxa on Oʻahu and Maui Nui (Molokaʻi, Maui, and Lanaʻi) should be made to assess the total variation prior to formalizing any nomenclatural changes. It is important to resolve these relationships because currently, *P. hawaiiensis* has a very broad estimated range across multiple islands (Price et al., 2012) but if there are actually multiple species, identified by taxonomic revisions, their conservation status may have to be re-evaluated. This is especially true for variety *scoriacea* which may have a more reduced/restricted range and is confined to the threatened ecosystems of the dry forests of Maui and Hawaiʻi (Sohmer, 1977; Wagner et al., 1990). These dry forests have been heavily impacted by land development, fires, and invasive species and have been reduced to less than 10% of their original area (Bruegmann, 1996).

Using conspicuousness of domatia and visibility of reticulate tertiary venation of the leaves strongly delineated the *mariniana* and *kaduana* clades of sect. *Straussia*. However, no comparative anatomical studies have been undertaken on Hawaiian *Psychotria*, but micromorphological or anatomical features may potentially segregate species within sect.

*Straussia* and have been insightful in other Rubiaceae genera (Kocsis et al., 2004; Moraes et al., 2009; Arruda et al., 2010). Additionally, Sect. *Straussia* has been noted for species that exhibit extreme morphological diversity and a great ecological amplitude (Sohmer, 1977; Sohmer, 1978). Nevertheless, what is causing this variation has remained unexplained, but other studies has provided a more quantitative understanding of such diversity within other Hawaiian plant radiations (Dunbar-Co et al., 2009; Blonder et al., 2016; McKwon et al., 2016).

**A note on field observations**

Some taxa have been characterized as having a colored pubescence on various parts which was treated as taxonomically important, but this was not always observed in the field. In particular, *P. hawaiiensis* var. *hillebrandii* is distinguished by the abaxial leaf surface and inflorescence axes that is covered with a rusty pubescence. However, when collections of this taxon were being made, the majority of the hairs where whitish in color, especially on younger parts of the plant, although perhaps these trichomes turn a reddish or rusty pubescent color as they age (Figure 2.17). Additionally, *P. hawaiiensis* var. *scoriacea* is distinguished from its congeners by its inflorescence axes with a whitish or yellowish-brown pubescence (Sohmer, 1977; Wagner et al., 1990). However, when making collections of this taxon in the type locality, inflorescence axes with yellowish-brown pubescence were not observed (Figure 2.18). Although, this taxon was not sampled throughout its range, the color of the trichomes may change when voucher specimens are dried. This was also observed in another Hawaiian species, *Melicope elliptica* (Rutaceae), in which the capsular hairs often become yellowish when dried. (Wagner et al. 1990, Pg.1204). In short, the color of trichomes may not be of taxonomic value of Hawaiian *Psychotria*.

Figure 2.19. Two photos of *P. hawaiiensis* var. *hillebrandii* inflorescence to note the differences of the pubescence color at different stages. Younger inflorescence with flowers (Left) with whitish pubescent inflorescence axes , whereas a slightly older inflorescence with immature fruits (Right) with inflorescence axes possessing both rusty colored and whitish pubescence. Photos were taken from the type locality at Kipuka Puaulu, Hawai‘i.

Figure 2.20. Two photos of *P. hawaiiensis* var. *scoriacea* inflorescence to note the pubescence color being whitish. These were taken from the type locality at Manuka, Hawai‘i.

# CHAPTER 3. HYPOTHESES REVISITED

The following formal hypotheses were proposed at the beginning of this research based on knowledge of the previous taxonomic treatments and phylogeny of Hawaiian *Psychotria*. Conclusions regarding acceptance or rejection of hypotheses of this study follow.

**Hypothesis one**: Members within sect. *Straussia* have similar morphological character states due to convergent evolution.

This hypothesis is supported to a limited degree. Currently, *P. hawaiiensis* is characterized by fruit 6-8(-10) mm long, including the collar-like persistent calyx at apex and the persistent disk, and inflorescence with 3-4 orders of branching. Phylogenetic and SRAP analyses reveal that the characters used to circumscribe this taxon may be due to convergent evolution. *P. hawaiiensis* varieties *hillebrandii* and *scoriacea* are more closely related to other species within sect. *Straussia* than they are to *P. hawaiiensis* var. *hawaiiensis*.

**Hypothesis two**: Plants referred to as *P. hawaiiensis* var. *hillebrandii* and *P. hawaiiensis* var. *scoriacea* are genetically distinct from *P. hawaiiensis*.

It appears that *P. hawaiiensis* is circumscribed based on elements of two more taxa. Phylogenetic analyses show that species in sect. *Straussia* are divided into two distinct clades. The varieties *hillebrandii* and *scoriacea* are found in the *kaduana* clade, whereas variety *hawaiiensis* is found in the *mariniana* clade. Furthermore, the SRAP analyses also supports the separation of these varieties and it appears that varieties *hillebrandii* and *scoriacea* are more closely related to *P. mauiensis* and *P. fauriei*, respectively. Nonetheless, further work is needed to resolve the relationships of the three varieties with the other members of sect. *Straussia*.

**Hypothesis three**: Plants previously referred to as *Straussia longissima* by Rock (1913) are genetically distinct from *Psychotria kaduana.*

This hypothesis is rejected. Results from the SRAP analysis show that this taxon is not genetically distinct and groups with the rest of the *P. kaduana* individuals. The current phylogenetic analyses do not show any support for this taxon being distinct due to the lack of variation of the gene regions used. Additionally, the recent phylogenetic analysis by Zhang (2016) show this taxon grouping with *P. kaduana*. It appears that this taxon is just an extreme variant connected by intergrading forms as suggested by Sohmer (1977).

**Hypothesis four**: *Psychotria mauiensis* populations on different islands are genetically distinct (Kaua‘i, Maui, Moloka‘i, and Hawai‘i).

This hypothesis is rejected. This was the most difficult taxon to sample from and locate in the field. Only two populations of this species were sampled from Moloka‘i and Maui, but the Maui population is only represented by one individual. The *P. mauiensis* individuals collected from Moloka’i at the Kamakou Preserve are abundant and most closely resemble Rock’s *Straussia oncocarpa* var. *subcordata* which is currently reduced to synonymy of *P. mauiensis*. The one *P. mauiensis* individual collected from Maui was collected from the dry forest of Auwahi which is a completely different habitat from the Moloka‘i population which was collected in a wet forest. Nevertheless, the SRAP analysis indicates that the *P. mauiensis* from different islands are not genetically distinct from each other and share a very close relationship with *P. hawaiiensis* var. *hillebrandii*.

**Hypothesis five**: There are low levels of genetic differentiation among species of *Psychotria* sect. *Straussia*

This hypothesis is partially supported. The phylogenetic analyses revealed poorly resolved relationships and short-branching polytomies within sect. *Straussia*. Low genetic differentiation may be due to recent divergence or rapid radiation over a short period of time (Zhang, 2016). However, SRAP analysis showed some genetic differentiation with individuals grouping by species, except for *P. mauiensis*, *P. hawaiiensis* var. *hillebrandii*, *P. kaduana* and *P. greenwelliae*. Future investigations may need to shift to next-generation sequencing (NGS) techniques to resolve even the most problematic phylogenetic challenges (Wagner et al., 2013; Escudero et al., 2014; Razkin et al., 2016; Hamon et al., 2017).

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