Molecular Phylogeny, Revised Higher Classification, and Implications for Conservation of Endangered Hawaiian Leaf-Mining Moths (Lepidoptera: Gracillariidae: *Philodoria*)[1]

Chris A. Johns,[2,3,5] *Matthew R. Moore*,[4] *and Akito Y. Kawahara*[2,3,4]

Abstract: The leaf-mining moth genus *Philodoria* Walsingham (Lepidoptera: Gracillariidae) is composed of 30 described species, all of which are endemic to the Hawaiian Islands. *Philodoria* is known to feed on 10 families of endemic Hawaiian host plants, with several species recorded only from threatened or endangered hosts. Beyond their dependence on these plants, little is known of their evolutionary history and conservation status. We constructed a molecular phylogeny of *Philodoria* to assess validity of its current subgeneric classification and to help guide future work on this threatened Hawaiian lineage. Mitochondrial and nuclear DNA sequences from three genes (*CO1*, *CAD*, *EF-1α*) combining for a total of 2,041 base pairs, were collected from 11 *Philodoria* species, incorporating taxa from both currently recognized subgenera. These data were analyzed using both parsimony and model-based phylogenetic approaches. Contrary to the most recent systematic treatment of *Philodoria*, our results indicate strongly that the two currently recognized *Philodoria* subgenera are not monophyletic and that morphological characters used to classify them are homoplasious. Based on our robust results, we revised the higher classification of *Philodoria:* the subgenus *Eophilodoria* Zimmerman, 1978 is established as subjective junior synonym of *Philodoria* Walsingham, 1907. We also present new host plant and distribution data and discuss host range of *Philodoria* as it pertains to endangered Hawaiian plants.

Philodoria Walsingham, 1907 (Lepidoptera: Gracillariidae) is a genus of leaf-mining micromoths that currently includes 30 described species, all of which are endemic to the Hawaiian Islands (Zimmerman 1978). The genus is extraordinary in that its larvae mine leaves of 10 plant families from seven orders (Swezey 1954, Zimmerman 1978). Host plant groups include iconic and endangered Hawaiian plant taxa such as the silversword alliance (*Argyroxiphium* DC. and *Dubautia* Gaudich.) and the Hawaiian lobelioids (*Clermontia* Gaudich.). Approximately 80% of *Philodoria* species feed on a single plant host species, and more than three-quarters of these species are restricted to a single Hawaiian island (Zimmerman 1978). The genus *Philodoria* should be considered a conservation priority due to the stringent host specificity and limited geographic range of the majority of its species.

The taxonomic history of *Philodoria* has been unstable, and the group's evolutionary relationships remain unknown despite its distribution across Hawai'i and specialization on distantly related plants. *Philodoria* was origi-

[1] This research was supported by the National Science Foundation (Graduate Research Fellowship to C.A.J.; DEB no. 1354585 to A.Y.K.), the National Geographic Society (no. C283-14 to C.A.J.; no. 9686-15 to A.Y.K.), the Entomological Society of America (2014 SysEB Travel Award to C.A.J.), the University of Florida's Tropical Conservation and Development Program (2014 Field Research Grant to C.A.J.), and the Society for Systematic Biologists (2012 SSB MiniARTS Grant to A.Y.K.). Manuscript accepted 29 March 2016.

[2] Department of Biology, University of Florida, Gainesville, Florida 32611.

[3] Florida Museum of Natural History, Gainesville, Florida 32611.

[4] Department of Entomology and Nematology, University of Florida, Gainesville, Florida 32611.

[5] Corresponding author (e-mail: johns.chris.a@ufl.edu).

Pacific Science (2016), vol. 70, no. 3:361–372
doi:10.2984/70.3.7

nally assigned to Tineidae Latreille (Walsingham 1907), followed by placement in Glyphipterigidae Stainton (Meyrick 1912). Species within *Philodoria* have also been assigned to various other genera, including *Gracillaria* Haworth, *Elachista* Treitschke (Walsingham 1907), and *Parectopa* Clemens (Meyrick 1928). The most recent systematic treatment grouped all Hawaiian species previously assigned to *Elachista*, *Gracillaria*, and *Parectopa* into *Philodoria* (Zimmerman 1978). Zimmerman divided the genus into two subgenera, *Philodoria* (*Eophilodoria*) and *Philodoria* (*Philodoria*), based on the size of the maxillary palpus. Under this classification, Zimmerman assigned 16 *Philodoria* species with the maxillary palps "fully developed" to the subgenus *Eophilodoria* (type species: *P. marginestrigata* Walsingham). Fourteen *Philodoria* species with this structure "greatly reduced, vestigial, or obsolescent" were assigned to the subgenus *Philodoria* (type species: *P. succedanea* Walsingham). In addition, Zimmerman's treatment defines *Philodoria* species based on scale patterns, host plant associations, and distribution. However, no phylogenetic data/analyses have evaluated the usefulness of these characters for defining the subgenera or species. This study represents the first attempt to evaluate the usefulness of the maxillary palp character (i.e., the monophyly of the subgenera) for the subgeneric classification of *Philodoria*.

We constructed the first phylogeny of *Philodoria* that sampled molecular sequence data from one mitochondrial and two nuclear genes from 11 *Philodoria* species (see Table 1) to test the subgeneric classification of Zimmerman (1978). Our results do not support Zimmerman's subgenera, and we discuss patterns of host plant associations among our sampled *Philodoria* species.

MATERIALS AND METHODS

Taxon Sampling, Amplification, and Sequencing

Thirteen samples representing 11 species of *Philodoria* were collected during April 2013 at 13 sites on the islands of O'ahu and Maui (Figure 1). Specimens of the type species of each subgenus defined by Zimmerman (1978), *Philodoria* (*Eophilodoria*) *marginestrigata* and *Philodoria* (*Philodoria*) *succedanea*, were captured in these collections (Table 1). *Philodoria* collection localities were selected based on historical records of Swezey (1954) and Zimmerman (1978). New localities were also surveyed based on the presence of known *Philodoria* host plant species. We visually identified host plants and collected leaves with signs of leaf miner larval activity. Both inactive and active leaf mines were photographed and georeferenced. Leaves with active mines and advanced larval instars were collected and kept in cool, dry conditions in plastic containers for rearing. Successfully reared moths were stored in 100% ethanol for molecular analyses. Larvae that did not successfully pupate and emerge as adults were stored in ethanol for future morphological and molecular analyses. Moths and the leaves from which they were reared were kept as voucher material and are deposited at the McGuire Center for Lepidoptera and Biodiversity (MGCL), Florida Museum of Natural History, Gainesville, Florida. Parasitoids reared from these collections are also stored at MGCL.

Multiple representatives of two species (*Philodoria auromagnifica*, samples CJ-064 and CJ-072; *Philodoria splendida*, samples CJ-049 and CJ-105) were included in the study to determine genetic variation between samples collected from different volcanoes or host plants. All adult moths sequenced in this study were reared from active leaf mines as detailed earlier, with the exception of CJ-049, which was field collected as an adult. *Philodoria* species were identified by comparing adult morphology with specimens determined by Otto H. Swezey or Elwood C. Zimmerman that were stored in the Bishop Museum, Honolulu (BPBM) or the Smithsonian National Museum, Washington, D.C. (USNM). We also aided our identifications by comparing our locality data and larval host plant data with historical records.

Molecular data were obtained by extracting the DNA from the entire adult moth. Extraction methods followed manufacturer's protocols for the Qiagen DNEasy kit (Qiagen, Inc., Valencia, California). Specimens

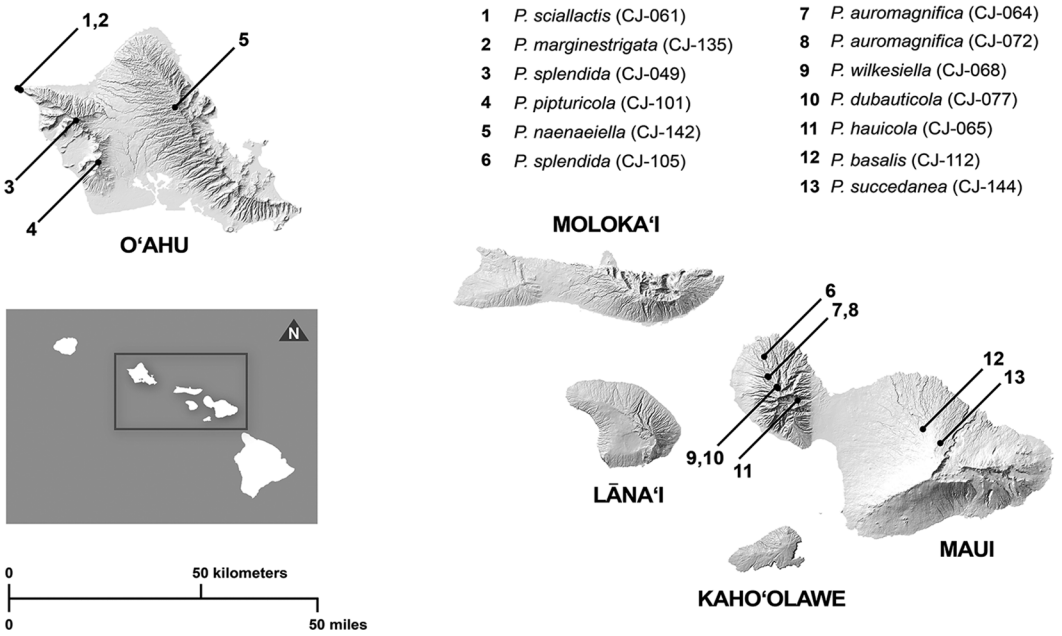

FIGURE 1. Map of the Hawaiian Islands and the collection localities for the taxa sampled in this study. Additional information is available in Table 1.

were sequenced for three genes: mitochondrial *Cytochrome c Oxidase subunit 1* [*CO1;* 603 base pairs (bp)], nuclear *Carbamoylphosphate Synthase* domain of CAD (922 bp), and nuclear *Elongation factor 1-alpha* (EF-1α) (516 bp); the primer sequences for amplification of each fragment are listed in Table 2. We included the same loci for three gracillariids, *Epicephala relictella*, *Parectopa robiniella*, and *Conopomorpha* sp. from the study of Kawahara et al. (2011). These taxa were included as outgroups because they are known to be close relatives of *Philodoria* (Kawahara et al. 2016). Sequences were edited using Geneious Pro v5.5.8 (Biomatters 2013), and sequence alignments were produced using the MUSCLE alignment algorithm with default parameters (Edgar 2004). Each gene alignment was manually concatenated together into a single alignment that totaled 2,041 bp. Supplemental Table S1 lists GenBank accession numbers; the single gene trees, concatenated data set, and photos of sequenced tissue are available from the Dryad data depository (http://datadryad.org).

Authors' Note: Supplemental materials available only on BioOne (http://www.bioone.org/).

Phylogenetic Analyses

Analyses using parsimony (P), maximum likelihood (ML), and Bayesian inference (BI) were first conducted on individual loci to assess congruence among data sets. Parsimony analyses were executed in PAUP* 4.0 (Swofford 2003) using heuristic searches performed with 1,000 random addition replicates and tree bisection-reconnection (TBR) branch swapping. For ML and BI, we first partitioned the concatenated data set by gene region and codon position, and determined the best-fitting models of sequence evolution for each partition in PartitionFinder 1.0.1 (Lanfear et al. 2012) using the Akaike Information Criterion. The models for each partition were used in the following analyses and are listed in Supplemental Table S2. ML analyses were implemented in RAxML 8.1.12 (Stamatakis 2014), with 1,000 bootstrap replicates. Bayes-

TABLE 1

Summary of Current *Philodoria* Classification, Diversity, and Host Plants of Taxa Included in This Study

Moth Taxon	Author	Subgenus	Host Plant Species[a,b]	Host Plant Family	Last Documented Previous to This Study[a]	Study Specimen ID	Moth Dist.[a,c]	Collection Locality (DDD.DDDDD°)
P. splendida	Walsingham, 1907	*Philodoria*	*Metrosideros polymorpha*	Myrtaceae	1943	CJ-049	H, K, **L,** Mo, O	21.504618° N, 158.146989° W
P. sciallactis	(Meyrick, 1928)	*Eophilodoria*	*Melanthera integrifolia*	Asteraceae	1927	CJ-061	**O**	21.572609° N, 158.275253° W
P. auromagnifica	Walsingham, 1907	*Philodoria*	*Myrsine lessertiana*, ***Myrsine sandwicensis***	Primulaceae	1928	CJ-064	H, M†, Mo, **O**	20.930785° N, 156.610357° W
P. hauicola	(Swezey, 1910)	*Eophilodoria*	*Hibiscus tiliaceus*	Malvaceae	1910	CJ-065	H, K, M, O	20.881126° N, 156.546767° W
P. wilkesiella	Swezey, 1940	*Philodoria*	*Argyroxiphium grayanum*	Asteraceae	1940	CJ-068	**M**	20.910519° N, 156.592075° W
P. auromagnifica	Walsingham, 1907	*Philodoria*	*Myrsine lessertiana*	Primulaceae	1928	CJ-072	H, M†, Mo, **O**	20.930585° N, 156.610279° W
P. dubauticola	(Swezey, 1940)	*Eophilodoria*	*Dubautia plantaginea*, ***Dubautia laxa***	Asteraceae	1940	CJ-077	**M**	20.911807° N, 156.592131° W
P. pipturicola	Swezey, 1915	*Philodoria*	*Pipturus* sp.	Urticaceae	1928	CJ-101	M, **O**	21.412447° N, 158.100055° W
P. splendida	Walsingham, 1907	*Philodoria*	*Metrosideros polymorpha*	Myrtaceae	1919	CJ-105	H, K, **L,** M†, Mo, O	20.976205° N, 156.61929° W
P. basalis	Walsingham, 1907	*Philodoria*	*Metrosideros polymorpha*	Myrtaceae	1919	CJ-112	H, **M**	20.818547° N, 156.268527° W
*P. marginestrigata**	Walsingham, 1907	*Eophilodoria*	*Abutilon*, *Dubautia*, *Sida*	Asteraceae, Malvaceae	1990	CJ-135	H, K, Mo, N, **O**	21.572609° N, 158.275253° W
P. naenaeiella	(Swezey, 1940)	*Eophilodoria*	*Dubautia laxa*, ***Dubautia plantaginea***	Asteraceae	1943	CJ-142	M, **O**	21.533236° N, 157.927446° W
*P. succedanea**	Walsingham, 1907	*Philodoria*	*Myrsine sandwicensis*, ***Myrsine lessertiana***	Primulaceae	1896	CJ-144	H, **M**	20.785926° N, 156.230492° W

[a] As indicated by pinned (BPBM and USNM) and published (Zimmerman 1978) data.

[b] Plant species names in **bold** indicate new host plant records observed in this study.

[c] Islands: H, Hawai‘i; K, Kaua‘i; L, Lāna‘i; M, Maui; Mo, Moloka‘i; N, Ni‘ihau; O, O‘ahu. Islands in **bold** denote type-locality islands. Islands underlined denote the collection localities of associated specimens.

* Type species of corresponding subgenus.

† New island record.

TABLE 2

Primer, Primer Nucleotide Sequence, Gene, Size, and Author of Sequences Used in Phylogenetic Analyses of *Philodoria*. (The Underlined Portion of CAD Denotes the Complementary Region of the Primer to the Gene's Sequence.)

Primer	Primer Nucleotide Sequence	Gene	Size	Citation
LepF1	ATTCAACCAATCATAAAGATAT	*CO1*	687	Hebert et al. (2004)
LepR1	TAAACTTCTGGATGTCCAAAAA	*CO1*	—	
ef44	GCYGARCGYGARCGTGGTATYAC	*EF-1α*	~1,100	Monteiro and Pierce (2001)
efrcM4	ACAGCVACKGTYTGYCTCATRTC	*EF-1α*	—	
CADm5F	TAATACGACTCACTATAGGGTGGAARGARGTNGARTAYGARGT	*CAD*	1,056	Kawahara et al. (2013)
CADm1mR	ATTAACCCTCACTAAAGACNGCRCACCARTCRAAYTCNACNGA	*CAD*	—	

ian analyses were conducted in MrBayes 3.2.1 (Ronquist et al. 2012), sampling MCMC chains every 1,000th tree for 20 million generations. Stationarity was evaluated in Tracer 1.6.0 (Rambaut et al. 2014), and 2,000,000 generations (10%) were subsequently discarded as burn-in. No strongly supported topological incongruence was observed between individual gene trees using these methods, and identical tree topologies were obtained for each locus. These parameters were then used to analyze the concatenated data set. Phylogenetic trees were visualized in FigTree 1.4.2 (Rambaut 2009).

Hypothesis Testing

To compare the confidence between our results and Zimmerman's (1978) morphology-based hypothesis, we conducted an analysis in which the two *Philodoria* subgenera were each constrained to be monophyletic. In RAxML, an ML tree was estimated with this constraint enforced, and the likelihood score of this tree was compared to the ML tree obtained from the unconstrained analysis. Statistical comparisons between these trees were made with the Shimodaira-Hasegawa (SH) test implemented in RAxML and the Approximately UnBiased (AU) test of Shimodaira (2002) implemented in CONSEL 0.20 (Shimodaira and Hasegawa 2001). For the AU test, we estimated site likelihoods for both constrained and unconstrained analyses with PAUP* (Swofford 2003) before combining them into a single file for CONSEL.

RESULTS

Sequencing, Phylogenetic Analysis, and Hypothesis Testing

Nuclear and mitochondrial sequences were obtained from 13 gracillariid specimens. Successful amplifications of all genes were obtained for all but one sample (*P. naenaeiella*, CJ-142, *CAD*), and sequence data for *CO1* were missing for one outgroup (*Conopomorpha* sp.). The final data matrix had only 4.8% missing data, and individual gene trees had nearly identical ingroup relationships (topo-

logical discrepancies were caused by missing data for the two taxa just listed). The concatenated data set resulted in trees with entirely congruent ingroup topologies in all subsequent phylogenetic analyses, regardless of optimality criterion.

Parsimony heuristic searches resulted in one most parsimonious tree (length = 1570, CI = 0.7312, RI = 0.6837). ML and BI analyses also resulted in trees with the same topology as the parsimony tree, and branch support was strong (>70% bootstrap for ML, >0.90 PP for BI) for nearly all nodes. All concatenated trees supported the division of the genus into two main clades (Clade A and B), the composition of which was Clade A (*Eophilodoria* + *Philodoria*) and Clade B (remaining *Philodoria*) (Figure 2). Monophyly of subgenera, as previously defined (Zimmerman 1978), was statistically rejected ($P < .0001$) for both SH and AU tests.

DISCUSSION

Subgeneric Classification

Our molecular phylogeny of *Philodoria* does not support the morphology-based classification of Zimmerman (1978), who split the genus into two subgenera, *Philodoria* (*Eophilodoria*) and *Philodoria* (*Philodoria*), based on the development of the maxillary palpus. Zimmerman (1978) classified *Philodoria* species with a fully developed maxillary palpus as subgenus *Eophilodoria*, and species with this structure "greatly reduced, vestigial, or obsolescent" as belonging to subgenus *Philodoria*. Our results confirm that *Philodoria* species are classified into two groups (Figure 2). Shimodaira-Hasegawa and AU tests statistically rejected the monophyly of Zimmerman's subgenera, because *Philodoria wilkesiella* and *P. pipturicola*, species originally described within subgenus *Philodoria*, were consistently nested within the clade containing species belonging to subgenus *Eophilodoria* (Figure 2).

Host plant data corroborate the grouping of *P. wilkesiella* and *P. pipturicola* with the related taxa found in Clade A (Figure 2), suggesting that host ranges for *Philodoria* species may be conserved at the level of plant family. *Philodoria wilkesiella* feeds on the endemic aster *Argyroxiphium grayanum* (Hillebr.) O. Deg., and all sampled species that feed on asters were placed in Clade A. *Philodoria pipturicola* feeds on plant species in the Hawaiian nettle genus *Pipturus* Wedd. (Urticaceae), which is host to seven *Philodoria* species across the Hawaiian Islands. All *Philodoria* species that mine leaves of *Pipturus* are currently placed within the subgenus *Philodoria* (Zimmerman 1978). We postulate that the six other *Pipturus* miners, which were not sampled in this study, are probably incorrectly classified, because they share similarities in morphology and host plant preference with the *Pipturus* miner included in our analyses. Following this pattern, it is likely that the *Philodoria* species that mine other Hawaiian plant genera within Urticaceae (*Neraudia* Gaudich., *Touchardia* Gaudich., and *Urera* Gaudich.) are also incorrectly classified. Currently, Zimmerman's (1978) classification hypothetically places *Philodoria* species that feed on these three plant genera in Clade A. Future phylogenetic studies should include additional *Philodoria* species to comprehensively elucidate evolutionary patterns of host plant shifts.

The phylogeny reported here suggests that the morphological characters used to classify the *Philodoria* subgenera may be homoplasious. These data suggest that the reduction of the maxillary palps is not a reliable character for the subgenus *Philodoria* (*Philodoria*) or that the interpretation of these characters was otherwise flawed [i.e., Zimmerman (1978) based his classification on a compound character or an inadequately defined continuous character]. The two main clades A and B (Figure 2) recovered in these analyses are well supported and could be treated as revised subgenera. However, there are no reliable morphological characters or hypothesized synapomorphies to separate Zimmerman's (1978) subgenera for identification purposes and no obvious ecological differences that define the two main clades in our study. Therefore, the subgeneric rank is here removed. *Philodoria* Walsingham, 1907 is the oldest name, and the genus-group name *Eophilodoria* is here placed in synonymy with it (*Eophilodoria* Zimmerman, 1978, **n. syn.**). Results from the study

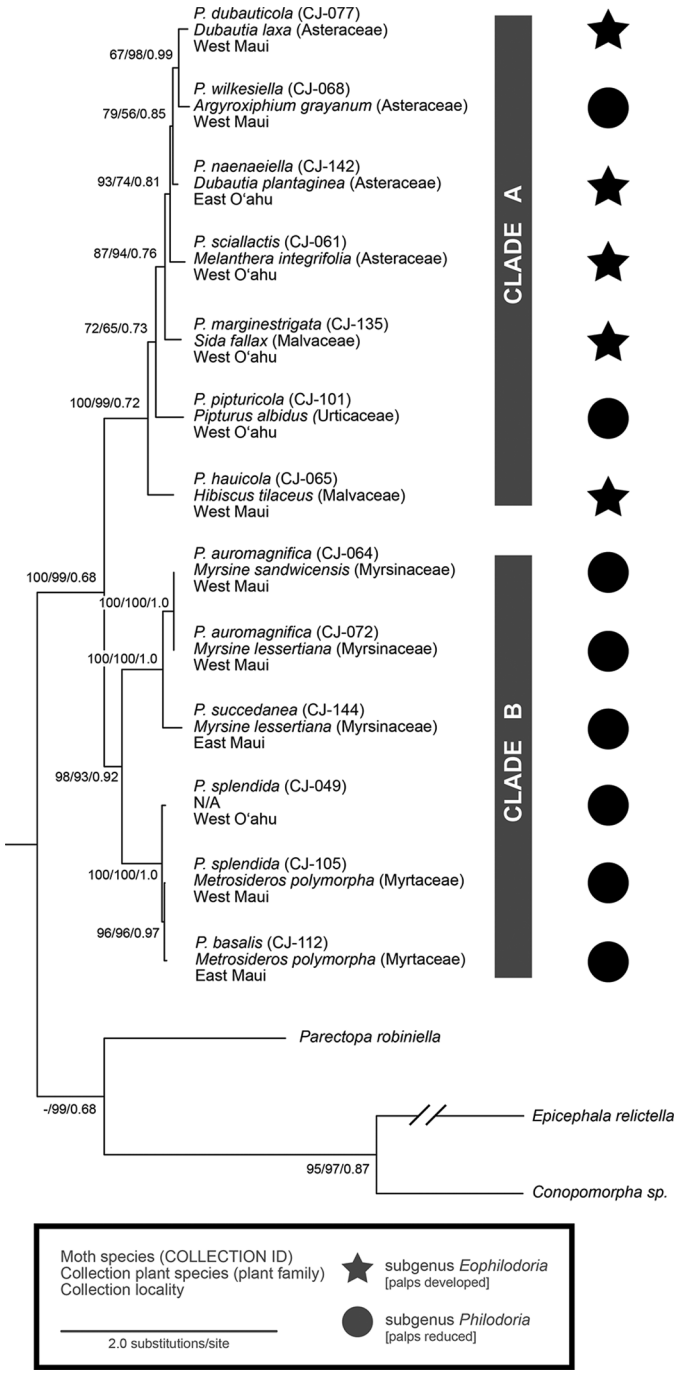

FIGURE 2. Majority rule consensus tree from BI analyses of *Philodoria*. Host plant, collection locality, and subgeneric classification are also displayed. Support values indicate bootstrap values for P, followed by bootstraps for ML, and the posterior probabilities from BI, pertaining to the adjacent node. Our results support the division of *Philodoria* into two clades (referred to as "Clade A" and "Clade B") but differ from the morphology-based classification proposed by Zimmerman (1978).

reported here confirm our poor understanding of *Philodoria* and demonstrate the need for a closer look at the phylogenetic relationships, current distributions, and conservation status of these species.

Philodoria: Implications for Ecology and Conservation

We present new host plant and distribution data that have implications for the ecology and conservation of *Philodoria*. Two species that mine *Myrsine* L. (Ericales: Primulaceae) were collected in this study. *Philodoria succedanea* (CJ-144) was reared from *Myrsine lessertiana* (a previously unrecorded host plant species for this moth species) near the moth's type locality on East Maui (Table 1). On West Maui, *P. auromagnifica* (CJ-064 and CJ-072) was reared from leaves of *M. sandwicensis* and *M. lessertiana*. Although these specimens very closely resemble *P. auromagnifica*, a *Myrsine* miner known from O'ahu, Moloka'i, and Hawai'i Island (Table 1), they differ subtly in wing pattern and may represent an undescribed species. Zimmerman (1978) hypothesized that there might be numerous undescribed species of *Philodoria* on *Myrsine*. In addition, some *Philodoria* specimens dissected by Zimmerman and housed at USNM include label details that indicate he believed they represent new species collected from *Myrsine*. There are no *Myrsine*-feeding *Philodoria* recorded from Kaua'i, suggesting that there is a gap in host plant sampling on that island, especially considering that Kaua'i is home to eight described *Philodoria* species and at least 10 *Myrsine* species (Wagner et al. 1990), three of which are endangered (U.S. Fish and Wildlife Service 2015). Future efforts to collect *Philodoria* on Kaua'i should focus on *Myrsine* species.

Eight *Philodoria* species feed on Asteraceae, and nearly all of these aster feeders are recorded to mine only one host plant genus (Zimmerman 1978). The exception to this pattern is *P. marginestrigata* (included in this study), which is recorded to mine plants in Asteraceae and Malvaceae. The association of this moth species with Asteraceae, however, remains dubious, and it is likely that early observations of an aster host plant for *P. marginestrigata* were incorrect (Zimmerman 1978).

The remaining aster-feeding *Philodoria* species are all single-island endemics, and many are restricted to one volcano within each island. Of the aster-feeding *Philodoria* species included in our study, *P. dubauticola*, *P. naenaeiella*, and *P. wilkesiella* are single-volcano endemics. The high level of endemism and the diverse, yet host-specific nature of *Philodoria* suggests that additional undescribed *Philodoria* species could be mining many Hawaiian asters.

The well-known Hawaiian silversword alliance includes approximately 50 aster taxa in three genera: *Argyroxiphium*, *Dubautia*, and *Wilkesia* A. Gray. The true silverswords and greenswords, *Argyroxiphium*, are some of the most highly protected Hawaiian plants and include four extant and one extinct species (Wagner et al. 1999); *A. grayanum* is the host of *P. wilkesiella* (Swezey 1940). Although it is possible that the other *Argyroxiphium* species may serve as hosts for *Philodoria*, *A. grayanum* is the only extant member of this genus that has glabrous leaves. It remains to be seen whether the other *Argyroxiphium* species, which have dense pubescence on the leaf surfaces, are mined by *Philodoria*.

The Hawaiian endemic plant genus *Dubautia* contains approximately 23 endemic species (Carr 1985). Only three species (Kaua'i's endangered *D. latifolia* and the widespread species *D. laxa* and *D. plantaginea*) are known to serve as *Philodoria* hosts (Zimmerman 1978). We predict that our knowledge of *Philodoria* species that feed on *Dubautia* has been limited by inadequate sampling of rare plant species in this genus. In the same way, the Kaua'i endemic greensword genus *Wilkesia* could feasibly harbor an undescribed *Philodoria* species that has been overlooked by field surveys. Indeed, recent field observations have noted signs of internal feeding on leaves of *Wilkesia gymnoxiphium* (N. Tangalin, pers. comm.); however, it has not yet been confirmed whether this damage is caused by *Philodoria*. A closer examination of *Argyroxiphium*, *Dubau-*

tia, and *Wilkesia*, and local populations on different islands, may yield additional new *Philodoria* species.

There are several Hawaiian aster genera that are not part of the silversword alliance that are absent from the *Philodoria* host plant record or have surprisingly few *Philodoria* species known to feed on them. For example, *Bidens* L. and *Tetramolopium* Nees are diverse Hawaiian aster radiations that lack *Philodoria* feeding records. The closely related genera *Melanthera* Rohr and *Lipochaeta* DC., which together comprise 16 widely distributed endemic Hawaiian plant species, have yielded only two *Philodoria* feeding records (*P. lipochaetaella* and *P. sciallactis*). Many *Melanthera* and *Lipochaeta* species are known from lowland Hawaiian ecosystems, and nearly half of these species have become alarmingly rare (Chau, unpubl. data). *Philodoria sciallactis* mines leaves of *M. integrifolia* only at Ka‘ena Point on O‘ahu (Zimmerman 1978). Ka‘ena Point is now a protected area and contains some of the only remaining intact coastal ecosystem where *M. integrifolia* exists naturally on O‘ahu (Department of Land and Natural Resources, State of Hawai‘i 2009). Based on surveys conducted during this study and collection localities listed in Swezey (1954) and Zimmerman (1978), it is likely that *P. sciallactis* persists only within the small confines of this conservation land. With such a narrow geographic and host plant range, the monophagous *P. sciallactis* is perhaps the most threatened species in the genus and may require immediate and urgent conservation prioritization.

Another aster genus that is likely to harbor *Philodoria* is *Hesperomannia* A. Gray, one of Hawai‘i’s most critically endangered plant genera. This plant genus comprises four species, all of which are federally listed as endangered (Morden and Harbin 2013, U.S. Fish and Wildlife Service 2015). Swezey (1940) noted that he reared a moth similar to *P. naenaeiella* (CJ-142, Clade A, Figure 2) from *H. swezeyi* on Oah‘u, but he did not formally list this plant species as a host in his later synthesis of Hawaiian insect-plant relationships (Swezey 1954). Upon examination of Swezey’s *P. naenaeiella* samples, Zimmerman (1978) emphasized that *Hesperomannia* requires further investigation as a host plant of *Philodoria*. A recent study of dried Hawaiian *Hesperomannia* leaves from the Bernice P. Bishop Museum Herbarium revealed that *H. arborescens* leaves collected on Lāna‘i in 1929 were mined by an unknown *Philodoria* species (Johns et al. 2014). The population of *Hesperomannia* on Lāna‘i, however, is extirpated (Wagner et al. 1990, Morden and Harbin 2013). It is unclear whether additional *Hesperomannia* species serve as host plants of *Philodoria*, but recent field observations revealed signs of endophytophagous insect feeding on Kaua‘i’s *H. lydgatei* and Maui’s *H. arborescens* (N. Tangalin, pers. comm.; K. M. Bustamente, pers. comm.).

Surveys of other endangered Hawaiian plants also provide evidence for *Philodoria* host plant associations. *Remya* Hillebr. ex Benth. is a genus of aster with three described species, all of which are endangered (U.S. Fish and Wildlife Service 2015). *Hibiscadelphus* Rock (Malvales: Malvaceae) includes eight species, six of which are extinct in the wild and the other two are endangered (Oppenheimer et al. 2014, U.S. Fish and Wildlife Service 2015). Herbarium leaves of *Remya mauiensis* and *Hibiscadelphus distans*, both belonging to plant families known to be *Philodoria* hosts, show signs of insect damage that resemble leaf mining (C.A.J., unpubl. notes). Such evidence, even though preliminary, warrants immediate further investigation into the host range of *Philodoria* because many of these plants are critically endangered. Given the small body size of *Philodoria*, its preference for a diversity of host plants, the challenges of sampling from the typically remote locations in which these plants are found, and the evidence of larval mining on rare plants, it seems likely that previous sampling efforts may have failed to record *Philodoria* species that occur on uncommon plant species. Because 13 of the 21 plant genera mined by *Philodoria* contain threatened or endangered species (Table 3), it is important that field surveys by research entomologists be encouraged in Hawai‘i to further elucidate

TABLE 3

Host Plant Genera Mined by *Philodoria* Moths and the Conservation Status of Their Members

Host Plant Genus	Host Plant Family	No. of Native Taxa in Plant Genus Mined by *Philodoria* (Zimmerman 1978)	No. of Native Taxa for Given Plant Genus (No. of Native Taxa Possibly Extinct) (Wagner et al. 1999)	No. of Taxa in Host Plant Genus Threatened or Endangered in Hawai'i (U.S. Fish and Wildlife Service 2015)
Argyroxiphium	Asteraceae	1	6 (1)	3
Dubautia	Asteraceae	3	37 (1)	9
Hesperomannia	Asteraceae	1	4	4
Lipochaeta[a]	Asteraceae	2	16 (4)	7
Xanthium[b]	Asteraceae	1	1	0
Clermontia	Campanulaceae	1	31	8
Abutilon	Malvaceae	2	4	3
Hibiscus	Malvaceae	3	13	4
Sida	Malvaceae	4	1	0
Myoporum	Scrophulariaceae	1	2	0
Myrsine	Primulaceae	1	19	5
Metrosideros	Myrtaceae	1	13	0
Pisonia	Nyctaginaceae	1	5	0
Pittosporum	Pittosporaceae	1	11	3
Lysimachia	Primulaceae	2	14 (1)	8
Neraudia	Urticaceae	1	6	3
Pipturus	Urticaceae	1	4	0
Touchardia	Urticaceae	1	1	0
Urera	Urticaceae	2	2	1

[a] Including plant species currently assigned to *Melanthera* (see Chau, unpubl. data).
[b] Nonnative.

host ranges of *Philodoria* species (Medeiros et al. 2013).

ACKNOWLEDGMENTS

We thank Charmian Dang, Betsy Gagné, and Cynthia King (Hawai'i Department of Land and Natural Resources, Division of Forestry and Wildlife) for permitting; Keahi M. Bustamente (Leeward Haleakalā Watershed Restoration Project) and Natalia Tangalin (National Tropical Botanical Garden) for collection assistance, reports on field observations, and project planning; Margaret J. Sporck-Koehler (Hawai'i Department of Land and Natural Resources) for assistance with fieldwork, land access, and gathering information on host plant conservation statuses; Pat Bily (Nature Conservancy) for land access; Pomaika'i Kanaiaupio-Crozier, Lono Dunn, Daniel Tanaka, and Joe Ward (Pu'u Kukui Watershed Preserve) for land access and field support; William P. Haines (University of Hawai'i at Mānoa), Susan Ching Harbin (Hawai'i Plant Extinction Prevention Program), and Joel Q. C. Lau for helpful suggestions on host plant locations; Daniel Rubinoff (University of Hawai'i at Mānoa) for the initial development of the project; Sheperd Meyers and Barbara Kennedy for allowing examination of insect and herbarium specimens at Bernice Pauahi Bishop Museum; Donald R. Davis (U.S. National Museum) for assisting with specimen identification; two anonymous reviewers for their helpful comments on an early version of the manuscript; and Jesse W. Breinholt, Peter R. Houlihan, David Plotkin, Francesca Ponce, and Lei Xiao of the Florida Museum of Natural History for assistance with laboratory work and data analysis.

Literature Cited

Biomatters. 2013. Geneious version 5.5.8. (Available from www.geneious.com).

Carr, G. D. 1985. Monograph of the Hawaiian Madiinae (Asteraceae): *Argyroxiphium*, *Dubautia*, and *Wilkesia*. Allertonia 4:1–123.

Department of Land and Natural Resources, State of Hawai'i. 2009. Final environmental assessment for the Ka'ena Point Ecosystem Restoration Project. Office of Environmental Quality Control Bulletin.

Edgar, R. C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792–1797.

Hebert, P. D. N., E. H. Penton, J. M. Burns, D. H. Janzen, and W. Hallwachs. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proc. Natl. Acad. Sci. U.S.A. 101:14812–14817.

Johns, C. A., N. Tangalin, K. M. Bustamente, and A. Y. Kawahara. 2014. Evidence of an undescribed, extinct *Philodoria* species (Lepidoptera: Gracillariidae) from Hawaiian *Hesperomannia* herbarium specimens. Proc. Hawaii. Entomol. Soc. 46:55–57.

Kawahara, A. Y., J. W. Breinholt, F. V. Ponce, J. Haxaire, L. Xiao, G. P. Lamarre, D. Rubinoff, and I. J. Kitching. 2013. Evolution of *Manduca sexta* hornworms and relatives: Biogeographical analysis reveals an ancestral diversification in Central America. Mol. Phylogenet. Evol. 68:381–386.

Kawahara, A. Y., I. Ohshima, A. Kawakita, J. C. Regier, C. Mitter, M. P. Cummings, D. R. Davis, D. L. Wagner, J. De Prins, and C. Lopez-Vaamonde. 2011. Increased gene sampling strengthens support for higher-level groups within leaf-mining moths and relatives (Lepidoptera: Gracillariidae). BMC Evol. Biol. 11:182.

Kawahara, A. Y., D. Plotkin, I. Ohshima, C. Lopez-Vaamonde, P. Houlihan, J. W. Breinholt, A. Kawakita, L. Xiao, J. C. Regier, D. R. Davis, T. Kumata, J.-C. Sohn, J. De Prins, and C. Mitter. 2016. A molecular phylogeny and revised higher-level classification for the leaf-mining moth family Gracillariidae and its implications for larval host use evolution. Syst. Entomol. (in press).

Lanfear, R., B. Calcott, S. Y. W. Ho, and S. Guindon. 2012. PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol. Biol. Evol. 29:1695–1701.

Medeiros, M. J., J. A. Eiben, W. P. Haines, R. L. Kaholoaa, C. B. A. King, P. D. Krushelnycky, K. N. Magnacca, D. Rubinoff, F. Starr, and K. Starr. 2013. The importance of insect monitoring to conservation actions in Hawai'i. Proc. Hawaii. Entomol. Soc. 45:149–166.

Meyrick, E. 1912. Lepidoptera, Heterocera, Fam. Gracilariadae. *In* P. Wystman, ed. Genera insectorum, 128. Bruxelles, 36.

———. 1928. Exotic Microlepidoptera. Exotic Microlepidoptera (Marlborough) 3 (13): 385–416.

Monteiro, A., and N. E. Pierce. 2001. Phylogeny of *Bicyclus* (Lepidoptera: Nymphalidae) inferred from *COI*, *COII*, and *EF-1α* gene sequences. Mol. Phylogenet. Evol. 18:264–281.

Morden, C. W., and S. C. Harbin. 2013. Evolution and biogeographic origins of the endemic Hawaiian genus *Hesperomannia* (Asteraceae). Pac. Sci. 67:219–235.

Oppenheimer, H. L., K. M. Bustamente, and S. P. Perlman. 2014. A new species of *Hibiscadelphus* Rock (Malvaceae, Hibisceae) from Maui, Hawaiian Islands. PhytoKeys 75 (39): 65–75.

Rambaut, A. 2009. FigTree version 1.4.2. Institute of Evolutionary Biology, University of Edinburgh. (Available from tree.bio.ed.ac.uk/software/figtree).

Rambaut, A., M. A. Suchard, D. Xie, and A. J. Drummond. 2014. Tracer version 1.6. (Available from beast.bio.ed.ac.uk/Tracer).

Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Hohna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61:539–542.

Shimodaira, H. 2002. An approximately unbiased test of phylogenetic tree selection. Syst. Biol. 51:492–508.

Shimodaira, H., and M. Hasegawa. 2001. CONSEL: For assessing the confidence of

phylogenetic tree selection. Bioinformatics 17:1246–1247.

Stamatakis, A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313.

Swezey, O. H. 1910. Some new species of Hawaiian Lepidoptera. Proc. Hawaii. Entomol. Soc. 2 (3): 103–106.

———. 1915. New species of Hawaiian moths. Proc. Hawaii. Entomol. Soc. 3 (2): 93–97.

———. 1940. New species of Hawaiian Lepidoptera. Proc. Hawaii. Entomol. Soc. 10 (3): 461–465.

———. 1954. Forest entomology in Hawaii: An annotated check-list of the insect faunas of the various components of the Hawaiian forests. Bernice P. Bishop Mus. Spec. Publ. 44.

Swofford, D. L. 2003. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

U.S. Fish and Wildlife Service. 2015. U.S. Fish and Wildlife Service Environmental Conservation Online System (http://ecos.fws.gov/ecp/).

Wagner, W. L., D. R. Herbst, and S. H. Sohmer. 1990. Manual of the flowering plants of Hawai'i. University of Hawai'i Press and Bishop Museum Press, Honolulu.

Wagner, W. L., M. M. Bruegmann, D. M. Herbst, and J. Q. C. Lau. 1999. Hawaiian vascular plants at risk: 1999. Bishop Mus. Occas. Pap. 60:1–58.

Walsingham, L. 1907. Microlepidoptera. Pages 469–759 *in* D. Sharp, ed., Fauna Hawaiiensis. Vol. 1. Cambridge University Press, Cambridge.

Zimmerman, E. C. 1978. Insects of Hawaii. Vol. 9. Microlepidoptera, Part 1. Gracillarioidea. University of Hawai'i Press, Honolulu.