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Origin and macroevolution of micro-moths on sunken Hawaiian Islands

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The origins and evolution of Hawaiian biodiversity are a matter of controversy, and the mechanisms of lineage diversification for many organisms on this remote archipelago remain unclear. Here we focus on the poorly known endemic leaf-mining moth genus *Philodoria* (Lepidoptera, Gracillariidae), whose species feed on a diversity of Hawaiian plant lineages, many of which are critically endangered. We use anchored hybrid enrichment to assemble the first phylogenomic dataset (507 loci) for any Hawaiian animal taxon. To uncover the timing and pattern of diversification of these moths, we apply two frequently used dating calibration strategies, biogeographic calibrations and secondary calibrations. Island calibrations on their own resulted in much younger and unrealistic dates compared to strategies that relied on secondary calibrations. *Philodoria* probably originated on the now partially sunken islands of Laysan or Lisianski, approximately 21 Ma, and were associated with host plants in the families Ebenaceae, Malvaceae or Primulaceae. Major feeding groups associated with specific host-plant families originated soon after the plants colonized the islands. Allopatric isolation and host shifts, in concert and independently, probably play major roles in the diversification of *Philodoria*. Our dating results indicate that *Philodoria* is among the oldest known Hawaiian arthropod lineages, and that island calibrations alone can lead to unrealistically young dates.

1. Introduction

The Hawaiian Islands are a well-known hotspot of biodiversity and endemism [1–5]. The archipelago has a unique and dynamic geological history, with islands forming while situated above the Hawaiian hotspot, a stationary magma plume that penetrates the northwesterly moving Pacific plate [6]. Three segments of the Hawaiian chain mark important geologic periods of biological relevance. The first and youngest segment, the current main 'high' Hawaiian Islands are the closest to the hotspot. These high islands extend from Hawaii Island, presently the largest and still forming over the hotspot, to Kauai, which is about 4.7 Myr old. The second and immediately older segment is composed of rocky pinnacles and atolls that make up the Northwest Hawaiian Islands (NWHI), extending from Nihoa (7.3 Myr) to Kure atoll (29.8 Myr). Beyond the NWHI are sunken landmasses that mark the third segment, the Emperor Seamounts. It is presumed that these three segments delineate geologic periods that each have shaped ancient and contemporary Hawaiian biodiversity [7].

Hawaiian hotspot dynamics have been inconsistent, with periods of reduced volcanic activity where few or no islands existed above the ocean surface [7]. Between 33 Ma and 29 Ma, no islands were subaerial, possibly resulting in the extinction of local biota, and necessitating de novo colonization of the islands via long-distance dispersal [1]. Following this period, the archipelago consisted only of small islands less than 1000 m elevation, until the formation of Laysan and Lisianski, approximately 23 Ma. A second period of

Figure 1. Images of *Philodoria* natural history. (*a*) *Philodoria* sp. 14 adult; (*b*) *Philodoria* caterpillar mining a host-plant leaf; (*c*) *Philodoria* moth and host-plant habitat on Kauai Island.

reduced volcanic activity between the formation of Nihoa 7.3 Ma and Kauai approximately 4.7 Ma led to an archipelago comprised again only of small, distantly spaced islands. This second period of reduced activity is thought to have been an additional barrier limiting dispersal between the NWHI and the main Hawaiian Islands [7].

Early divergence time estimations for endemic Hawaiian lineages suggest that much of the contemporary Hawaiian biota colonized the archipelago after the formation of Kauai, approximately 4.7 Ma [7]. Some recent age estimates of Hawaiian arthropods, however, yield crown ages that predate the formation of Kauai, suggesting that the NWHI are a more significant colonizing source for the main high islands than was previously thought. Examples include the Hawaiian *Ptycta* bark lice, approximately 7 Ma [8], *Megalagrion* damselflies, approximately 9 Ma [9], *Idiomyia* and *Scaptomyza* flies, approximately 10–13 Ma [10,11], *Hyposmocoma* fancy-case caterpillars, approximately 15 Ma [12–14], Hawaiian *Limnoxenus* water beetles, approximately 20 Ma [4], *Rhyncogonus* weevils, approximately 7 Ma [15], and *Xyleborus* beetles, approximately 10 Ma [16]. However, a significant number of these Hawaiian biogeographic studies have relied on the age of biogeographic events to infer absolute divergence times (e.g. [9,12,13,17,18]). Others have relied on published rates of nucleotide substitution often associated with a strict clock model assuming homogeneous rates across lineages [15,19,20] (but see e.g. [4] for a fossil-based analysis of divergence times). Although widely used, calibrations based on biogeographic events are contentious, especially within the context of island biogeography, as they assume that endemic groups on an island cannot be older than the geological age of that island [21], which may not reflect the true history of the organisms and the islands they inhabit [22,23]. Published rates of nucleotide substitution for biogeographic studies are equally problematic, because their use assumes that distantly related clades share similar rates, an assumption that is untenable (e.g. [24]).

The endemic Hawaiian leaf-mining moth genus *Philodoria* (Lepidoptera, Gracillariidae) comprises 32 described species, the majority of which feed on endemic Hawaiian plants [25–27] (figure 1). As the only gracillariid endemic to Hawaii, *Philodoria* larvae have a broad host range, mining the leaves of Hawaiian plants from six families and at least as many plant orders [25]. Nearly all species are monophagous, feeding on the leaves of only one or a select few closely related plant species. *Philodoria* are also highly endemic, with approximately 75% of species restricted to a single island or volcano [25,26].

Despite the intriguing host associations and reliance on threatened Hawaiian plants, little is known about the evolutionary history of *Philodoria*. The only phylogenetic study for *Philodoria* relied on 11 species and three loci, limiting capability to conduct a detailed investigation into the evolution of the genus [28]. Given its broad host range and restricted geographical ranges of individual species, *Philodoria* is an ideal candidate to study mechanisms of lineage diversification and

host evolution on the Hawaiian Islands. Here we use anchored hybrid enrichment (AHE) [29] of up to 507 loci to assemble the first phylogenomic dataset for any Hawaiian animal taxon and infer the evolution of *Philodoria* moths with respect to the geological history of Hawaii. We compare how different molecular calibration strategies, specifically using secondary versus biogeographic calibrations, can affect age estimates. Because of their intimate relationship with plants, this study also examines the evolution of host use in *Philodoria*. We discuss how these results compare to evolutionary patterns found in other endemic Hawaiian plants and insects.

2. Material and methods

(a) Taxon sampling

Philodoria specimens were collected between 2013 and 2016 on the islands of Kauai, Oahu, Molokai, Maui, Lanai and Hawaii. We restricted our sampling to larvae that were actively feeding on plant leaves, so that host association data could be obtained with confidence. These larvae were reared to adulthood following the methods of Johns *et al*. [28]. Successfully reared moths were stored in greater than 96% ethanol and at −80°C. One specimen of *P. hauicola* was collected in RNAlater and sequenced as a transcriptome (see below). Larvae that failed to hatch and reared adult moths were kept as vouchers and are deposited at the McGuire Center for Lepidoptera and Biodiversity (MGCL), Florida Museum of Natural History, Gainesville, Florida, USA. In total, 673 *Philodoria* adults were reared from plants collected at 42 localities across the Hawaiian Islands. Our collecting efforts allowed sampling 26 of the 32 described *Philodoria* species, plus 16, undescribed species [26]. Unfortunately, five species (*P. dubauticola*, *P. kauaulensis*, *P. kolea*, *P. naenaeniella* and *P. pipturicola*), previously sampled by Johns *et al*. [28] and in Kobayashi *et al*. [27], could not be included in the present dataset because these extracts did not yield enough DNA. *Plutella xylostella* (Plutellidae) and six non-*Philodoria* gracillariid species from five subfamilies were included as out-groups based on their phylogenetic proximity to *Philodoria* [30].

(b) Sample preparation, sequencing and data processing

We used the across-Lepidoptera AHE (Lep1) probe set [31] to capture 855 loci from 32 *Philodoria* specimens. One sample, *P. hauicola* (CJ-257; electronic supplementary material, pp. 17–18), was initially sequenced as a transcriptome and the 855 loci were extracted from assembly following methods in Breinholt *et al*. [31]. For all others, DNA was extracted from ethanol-stored *Philodoria* tissue using the OmniPrep Genomic DNA Extraction Kit (G-Bioscience: catalogue no. 786-136; St. Louis, MO, USA). Library preparation followed the protocol of Breinholt *et al*. [31] and were prepared and sequenced by RAPiD Genomics (Gainesville, FL, USA). Illumina data were sequenced on a HiSeq 3000 for paired-end 100 or 150 bp.

The *P. hauicola* transcriptome and outgroups were processed following data cleaning and assembly methods of Breinholt & Kawahara [32]. The bioinformatic pipeline of Breinholt *et al*. [31] was used to process raw AHE sequence data, resulting in the retrieval of a 507-locus starting dataset for phylogenomic analyses (see Results and electronic supplementary material for more information). We constructed two 507-locus datasets, one of the probe region excluding flanking regions (Dataset 1), and another including the probe and flanking regions (Dataset 2). Final concatenated alignments for Dataset 1 (119 323 bp) and Dataset 2 (258 995 bp) are deposited in the Dryad Digital Repository (datadryad.org, doi:10.5061/dryad.qq6hv63).

(c) Phylogenomic analyses

We used PartitionFinder2 [33] to determine the optimal partitioning scheme for loci in Datasets 1 and 2 using the default rcluster search algorithm and Bayesian information criterion (BIC) for model selection. For Dataset 2, all flanking regions were assigned to one partition because these sites were not always contiguous. Model selection and phylogenetic inference were performed in a maximum-likelihood (ML) framework using IQ-TREE v. 1.4.2 [34]. We assessed nodal support using 100 non-parametric bootstrap (BS) replicates, 1000 ultrafast bootstrap (UFBoot) replicates and 1000 SH-aLRT replicates in IQ-TREE.

To account for possible incomplete lineage sorting, species trees were also inferred using the coalescent summary methods in ASTRAL-II v. 4.10.12 [35]. ASTRAL-II was run with full-length loci from Dataset 2. Individual gene trees were estimated using the GTRGAMMA model for nucleotide evolution for each locus and 100 rapid bootstraps calculated in RAxML v. 8.2.3 [36]. Branch support for the ASTRAL-II species trees were calculated by the quartet score.

(d) Divergence time estimation

To obtain divergence times of *Philodoria*, we subsampled Dataset 1 to make it more tractable for Bayesian relaxed-clock methods by reducing the dataset using the Robinson–Foulds (RF) distance [37] between individual ML gene trees and the ML tree inferred based on Dataset 1 using HashRF [38]. The 50 loci whose gene trees were closest in RF distance to the Dataset 1 ML tree were concatenated into a new matrix, which was used as input for our divergence time estimation analyses. To ensure that the 50-locus subsampled dataset accurately represented Dataset 1, we selected the optimal partitioning scheme in PartitionFinder2 using the greedy algorithm and BIC. We used the best resulting partitioning scheme to conduct an ML phylogenetic tree search in IQ-TREE 1.4.2 with 100 non-parametric bootstrap replicates.

We tested multiple calibration strategies on the 50-locus subsampled dataset, applying secondary and biogeographic calibrations independently and in concert. One form of secondary calibration (SC1) constrained the node *Caloptilia* + *Phyllocnistis* with a uniform prior distribution of 88.5476–115.9883 Myr following Wahlberg *et al*. [39], which estimated divergence times for Lepidoptera using six fossil calibrations and included representatives of these two gracillariid genera. An additional type of secondary calibration (SC2), also from Wahlberg *et al*. [39], constrained the maximum root age with a uniform prior distribution of 88.5476–141.2642 Myr to reflect the upper bound estimated for the most recent common ancestor (MRCA) of *Plutella* and Gracillariidae.

In one type of biogeographic calibration (ISL1), we calibrated four nodes in our tree based on the age of Oahu and the Maui Nui island group. We treated islands that comprise Maui Nui as a single group because they were geologically connected for much of their history [6,40]. Two nodes suggesting dispersal from Kauai to a recently formed Oahu (filled arrows, figure 2) were calibrated using a normal prior distribution set to 3.0 Ma (s.d. = 1.0), and two nodes representing dispersals from Oahu to a recently formed Maui Nui (unfilled arrows, figure 2) were also calibrated using a normal prior distribution set to 2.2 Ma (s.d. = 0.73). Standard deviations of these priors were conservatively set to one-third the island age, to help narrow the island age to a timescale most biologically relevant for colonization.

We also tested a conceptually distinct biogeographic calibration (ISL2), in which we constrained a node representing the MRCA of a *Philodoria* clade confined to Maui Nui (hash marked arrow, figure 2). The host plants of this clade are also endemic to Maui Nui. Whereas ISL1 calibrations hinge on phylogenetic patterns showing dispersal from older islands to newly formed

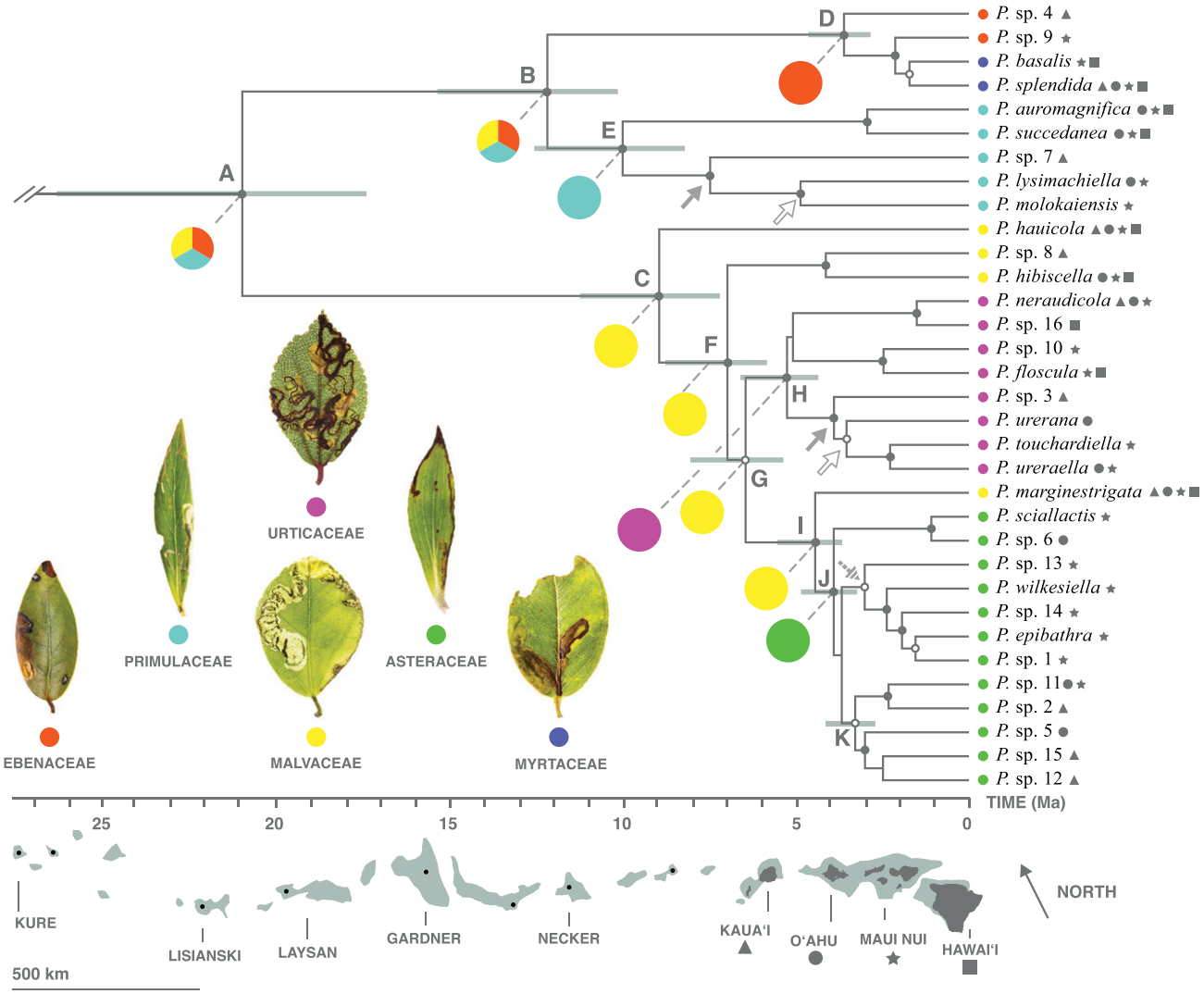

Figure 2. MCC tree from the most likely divergence time estimate (A3) that used the 507-locus topology as a constraint. Grey bars at select nodes indicate 95% highest posterior density interval. Shapes next to tip labels indicate island distributions and correspond to the map. Small coloured circles at tips show host-plant family of the associated *Philodoria* species. Large circles at internal nodes represent reconstructed host-plant family associations. Filled arrows point to calibrated nodes representing dispersals from Kauai to Oahu, and unfilled arrows point to calibrated nodes representing dispersals from Oahu to Maui Nui (ISL1 calibration). Hash marked arrow shows ISL2 calibration. Node bootstrap support is shown for Dataset 1; filled circles = 100%; open circles = 99–70%; unlabeled nodes less than 70%. A reconstruction of the Hawaiian archipelago is redrawn from Price & Clague [7]. Geographical distances do not directly correlate to ages on the *x*-axis.

younger islands, the ISL2 calibration was applied to a species-rich island-endemic clade that presumably originated when the island formed. Thus, for the ISL2 calibration, we used the prior distribution indicated above for the age of Maui Nui. We used these approaches to maintain consistency and allow comparison with the calibration methods in other recent studies of diversification in endemic Hawaiian lineages (e.g. [12,13]).

We analysed the four calibration types (SC1, SC2, ISL1 and ISL2) separately and in combinations to determine the best constraint scheme. We did not run analyses that combined the two biogeographic calibrations (i.e. ISL1 + ISL2) to preserve their conceptual independence. In addition, we examined the effects of the speciation process (birth–death versus Yule models), data partitioning (partitioning versus no partitioning) and topology (the ML tree based on 50-loci ML versus the ML tree from Dataset 1) on divergence time estimates. We used PartitionFinder2 [33] to determine the optimal partitioning scheme for the 50-locus dataset. All loci determined to be partitioned together were concatenated. Because all initial tests favoured partitioned datasets, we chose to run only partitioned datasets for the majority of our divergence time estimation analyses. Dating analyses were conducted in BEAST v.1.8.4 [41]. Each analysis used an uncorrelated relaxed log-normal clock prior and was run for 50 million generations, sampling every 5000th generation. We estimated the marginal likelihood (MLE) for each run using the path/stepping-stone sampling algorithm with default settings. Convergence was assessed by checking the effective sample size (ESS) for each parameter in Tracer v. 1.7 [42].

(e) Ancestral host-plant associations

Ancestral host-plant associations across *Philodoria* were constructed using the ultrametric maximum clade credibility tree derived from the best BEAST analysis (based on MLE comparisons). Host-plant data were coded by family and are listed in table 1. Host range evolution was estimated using parsimony in Mesquite v. 3.31 [43].

3. Results and discussion

The present study provides the first molecular phylogeny constructed from next-generation sequencing data for an endemic Hawaiian animal lineage, and the first AHE Lepidoptera

Table 1. Divergence time analysis results, with ages in millions of years with 95% highest posterior density range in parentheses. Part. = data partitioned or unpartitioned; 50/507 = 50- or 507-locus topology used as a constraint; BD/Yule = speciation process using birth–death or Yule prior; MLE score SS/PS = stepping-stone versus path sampling. The 11 analyses at the bottom did not reach convergence.

analysis	part.	calibration strategy	constr.	spec. proc.	MLE score (SS)	MLE score (PS)	*Philodoria* crown age
A1	Y	SC1	50	BD	−120020.3611	−120237.1611	23.33 (18.93, 27.71)
A2	Y	TOTAL2	50	Yule	−120023.2756	−120187.9523	22.34 (18.85, 26.27)
A3	Y	SC2 + ISL2	507	BD	−120073.886	−120275.6336	20.95 (17.37, 26.3)
A4	Y	SC2 + ISL2	50	Yule	−120107.2133	−120327.1983	22.21 (18.74, 27.22)
A5	Y	TOTAL1	50	Yule	−120343.7631	−120389.8734	19.74 (17.49, 22.07)
A6	Y	TOTAL2	507	Yule	−120362.7128	−120405.2818	21.42 (18.06, 25.69)
A7	Y	SC1 + SC2	507	Yule	−120375.0442	−120393.8155	22.49 (18.55, 26.8)
A8	Y	TOTAL2	50	BD	−120376.4058	−120375.9686	21.98 (18.52, 26.11)
A9	Y	SC1 + SC2	50	Yule	−120377.4512	−120391.2643	23.93 (19.62, 28.41)
A10	Y	SC2 + ISL2	50	BD	−120377.9458	−120377.6145	21.68 (18.33, 26.74)
A11	Y	SC1 + SC2	50	BD	−120381.479	−120381.3535	23.4 (19.35, 27.85)
A12	Y	SC1 + ISL1	50	BD	−120387.1516	−120387.1459	19.33 (17.25, 21.7)
A13	Y	SC2 + ISL1	50	BD	−120389.5796	−120389.3839	19.01 (16.81, 21.32)
A14	Y	TOTAL1	50	BD	−120390.6177	−120390.2294	19.38 (17.22, 21.74)
A15	Y	ISL1	50	Yule	−120391.9453	−120391.3894	9.79 (5.83, 13.42)
A16	Y	SC2 + ISL1	50	Yule	−120398.4324	−120397.9939	19.26 (17.1, 21.54)
A17	Y	SC1 + ISL1	50	Yule	−120399.6258	−120399.493	19.71 (17.58, 21.94)
A18	Y	TOTAL2	507	BD	−120399.8118	−120399.3178	21.02 (17.93, 25.34)
A19	Y	ISL1	507	BD	−120403.268	−120402.8494	9.74 (5.85, 13.53)
A20	Y	SC1	507	BD	−120404.0802	−120403.6846	21.86 (18.1, 26.16)
A21	Y	SC1 + SC2	507	BD	−120404.5646	−120404.4935	21.96 (17.98, 26.08)
A22	Y	SC2 + ISL1	507	BD	−120405.1282	−120404.9004	18.12 (15.81, 20.4)
A23	Y	TOTAL1	507	BD	−120405.141	−120404.5136	18.29 (16.11, 20.63)
A24	Y	SC2 + ISL2	507	Yule	−120406.241	−120405.8712	21.38 (17.68, 27.14)
A25	Y	SC1 + ISL1	507	BD	−120407.7661	−120407.8736	18.26 (16.26, 20.84)
A26	Y	ISL1	507	Yule	−120411.8199	−120411.763	9.73 (5.73, 13.56)
A27	Y	SC1 + ISL1	507	Yule	−120415.2003	−120415.2794	18.62 (16.53, 20.91)
A28	Y	SC2 + ISL1	507	Yule	−120417.3065	−120417.3182	18.48 (16.17, 20.78)
A29	Y	TOTAL1	507	Yule	−120419.4154	−120419.0579	18.71 (16.46, 21.08)
A30	N	SC2 + ISL1	50	Yule	−120523.2929	−120718.1035	19.9 (15.13, 25.01)
A31	N	SC1 + SC2	50	Yule	−120571.1336	−120805.7212	28.67 (21.63, 36.37)
A32	N	SC1 + SC2	50	BD	−120960.0109	−120960.3148	26.77 (20.09, 34.24)
A33	N	SC2 + ISL1	50	BD	−120965.2759	−120965.3651	18.83 (14.61, 23.65)
A34	N	SC1 + SC2	507	BD	−120970.0236	−120970.3298	23.83 (17.82, 30.23)
A35	N	SC2 + ISL1	507	BD	−120973.1813	−120973.5058	18.19 (13.26, 22.79)
A36	N	SC1 + SC2	507	Yule	−120977.8932	−120978.1676	25.85 (19.32, 32.78)
A37	N	SC2 + ISL1	507	Yule	−120980.6909	−120980.9394	18.56 (14.11, 23.34)
A38	Y	ISL1	50	BD	—	—	—
A39	Y	ISL2	50	BD	—	—	—
A40	Y	ISL2	50	Yule	—	—	—
A41	Y	ISL2	507	BD	—	—	—
A42	Y	ISL2	507	Yule	—	—	—
A43	Y	SC1	50	Yule	—	—	—
A44	Y	SC1	507	Yule	—	—	—
A45	Y	SC1 + ISL2	50	BD	—	—	—
A46	Y	SC1 + ISL2	50	Yule	—	—	—
A47	Y	SC1 + ISL2	507	BD	—	—	—
A48	Y	SC1 + ISL2	507	Yule	—	—	—

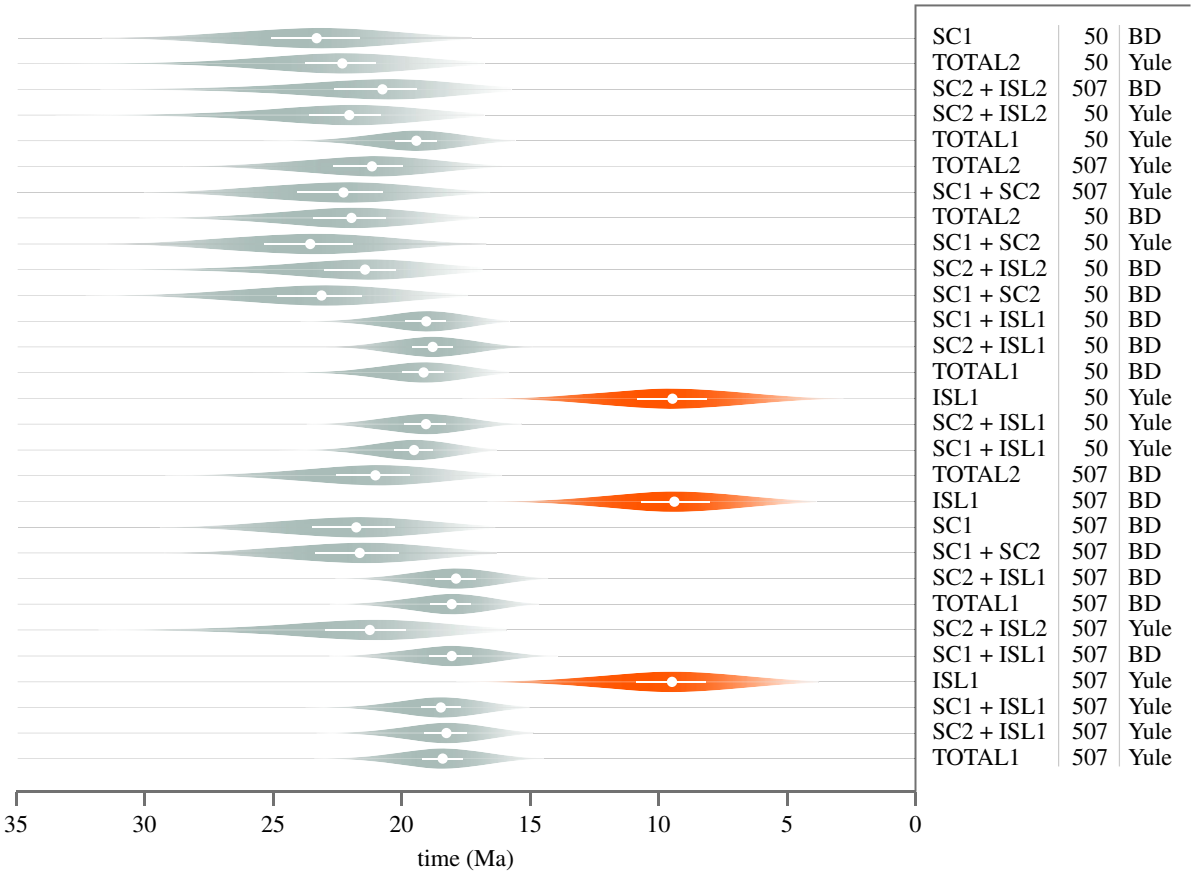

Figure 3. Violin plots of *Philodoria* crown age from 29 most likely divergence time analyses based on partitioned data. Analyses based on only island calibrations result in younger crown age estimates than analyses incorporating fossil-based constraints. BD, birth–death. Numbers refer to locus count referencing constraint topology.

phylogenetic study for a genus. Phylogenetic trees were strongly supported for many relationships within *Philodoria*. These robust genus-level results show that the Lep1 probe set can be effective at taxonomic levels across Lepidoptera (see [31,44,45] for other recent studies that used this probe set and similar ones). Phylogenetic trees were largely congruent in topology between all analyses, each finding strong support for *Philodoria* feeding groups associated with host-plant families. Topologies only differed between Dataset 1 and Dataset 2 in the relationships between *Philodoria* species that feed on the plant genus *Pipturus* (Urticaceae), and the relationships between *P.* sp. 15, *P.* sp. 12 and *P.* sp. 5. Nodal support for *Pipturus* feeders increased with the inclusion of flanking regions (Dataset 2). Dataset 2, based on probe and flanking regions, had higher branch support (BS $\geq$ 80% and UF-Boot/SH-aLRT $\geq$ 95/80) for 29 of 32 (90.6%) in-group nodes, compared with the tree from Dataset 1 (probe only) which had 28 of 32 in-group nodes with high branch support (electronic supplementary material, p. 6–9). Results from the ASTRAL-II analyses largely agree with ML topologies of both Datasets 1 and 2, with all *Philodoria* host-plant family feeding groups remaining together and only minor differences in the placement of species within those groups among trees (electronic supplementary material, p. 12, 13). The ML tree inferred from the trimmed 50-locus (19 077 bp) dataset was also nearly identical to the ML tree from Datasets 1 and 2 (507 loci, with and without flanking regions), only differing in the relationships between *Philodoria* species within each host-plant family feeding group. Although topologies were largely identical between trees from these analyses, we focus our discussion on results from Dataset 2 because that analysis was based on more data (included flanking regions) and provided more conclusive results.

Our results strongly support the monophyly and many deep divergences in *Philodoria*, and are in agreement with previous work based on fewer taxa and loci [28]. Major host-plant feeding clades were well supported (nodes D–I, figure 2). Relaxed molecular clock estimates for the crown age of *Philodoria* from each of the divergence time analyses that used secondary calibrations, regardless of speciation process or constraint topology, largely agreed, with overlapping credibility intervals. *Philodoria* crown age estimates that were based exclusively on island calibrations, however, were consistently younger (figure 3). The marginal likelihood score was highest for analyses that used the trimmed 50-locus dataset topology as a constraint, except for A3, A6 and A7 (table 1), which used the ML topology from Dataset 1 as a constraint. Among analyses that used the 507-locus topology as a constraint, A3 was preferred. This analysis used one secondary and one biogeographic calibration (SC2 + ISL2), and estimated the crown age divergence between *Philodoria* and its closest relatives at approximately 21 Ma, in the early Miocene (95% HPD = 17.37–26.30; node A, figure 2). In order to estimate objective and reliable divergence times, it is critical to use independent calibration strategies, especially for insular endemic lineages such as those that form the Hawaiian biota. Island age-based calibrations are frequently used alone in Hawaiian biogeographic studies, but this approach is well known to rely on circular assumptions [21]. Studies that rely on published rates of nucleotide substitution assume evolutionary stasis

across lineages of the tree, which is often unrealistic [24]. Our results demonstrate that, when possible, applying both secondary and biogeographic calibrations should be preferred.

Price & Clague [7] depicted two 'peak periods' for putatively higher rates of colonization in Hawaii, the first between 8 and 18 Ma and the second between 3 Ma and the present. They attributed the majority of extant diversity to the second colonization period, which post-dated the formation of the current high islands. However, most studies have dated the colonization of Hawaiian arthropod lineages prior to the formation of Kauai (e.g. [8,9,12]; but see [4]). The megadiverse Hawaiian case-building caterpillar genus *Hyposmocoma* is an extreme example of a pre-Kauai origin [12] with over twenty independent dispersal events from the NWHI to the current high islands. Only one Hawaiian plant lineage (the Hawaiian lobelioids [Campanulaceae]) is thought to have colonized the current high islands from the NWHI [46]. Our results, along with recent studies that use rigorous Bayesian relaxed-clock methods to date divergences of Hawaiian taxa [4], reflect mounting evidence suggesting that a larger fraction of contemporary Hawaiian biodiversity originated in the NWHI than proposed by Price and Clague [7]. Based on clear discrepancies observed in secondary versus island calibration strategies for *Philodoria*, we predict that other studies that have used exclusively island calibrations in the past may present underestimated divergence times for Hawaiian lineages. As a result, our historical understanding of the origin and evolution of Hawaiian biodiversity is probably far less complete than previously thought.

Our dating results, regardless of calibration strategy (table 1), show that *Philodoria* originated before the formation of Kauai, but prior to Price & Clague's [7] first peak period for colonization when Laysan and Lisianski were formed. Our most likely result depicts *Philodoria* colonizing Hawaii approximately 21 Ma (95% HPD: 17.37–26.30; node A, figure 2) when Laysan and Lisianski were the largest landmasses in the archipelago, exceeding 1000 m in elevation [1], and probably harbouring a diversity of forest ecosystems and possible larval host plants. These once high but now sunken islands are thought to have received more rainfall and thus could support a greater diversity of terrestrial ecosystems at their peak heights [7]. While our results suggest that *Philodoria* inhabited the NWHI at one point in the past, it is unlikely that the *Philodoria* species that depend on large forest trees still exist in the NWHI today, due to the recent reductions in habitat and host-plant availability [7]. Biodiversity surveys on several NWHI have never recorded *Philodoria* [47]. Our findings are in line with the hypothesis that Lisianski was the first island in the chain that the contemporary Hawaiian biota could colonize, because prior to its existence, there was a period where no islands were subaerial [1].

Price & Clague [7] proposed that a period of reduced volcanic activity between when Necker formed (approx. 11 Ma) and before the emergence of Kauai (approx. 5.1 Ma) significantly shaped contemporary Hawaiian biodiversity. During this geological period, the subaerial terrains of the archipelago were distantly spaced and greatly reduced, resulting in a decline in complex terrestrial habitats. This reduction of ecosystems is thought to have been a significant colonization barrier for taxa on islands that predate Kauai to disperse to the current high islands. Our results suggest that *Philodoria* survived this period of low volcanic activity and the eight lineages in the genus successfully dispersed to the current high islands from older, now submerged Hawaiian Islands (figure 2).

Our divergence time estimates indicate that *Philodoria* located on older Hawaiian Islands in the early Miocene colonized younger islands within the archipelago as they formed over the hotspot during the first peak period, a pattern of diversification that is consistent with the progression rule [48]. The origin of this clade (node B, figure 2) around 12 Ma (95% HPD: 10.12–15.32) indicates that divergence of these species occurred approximately when Gardner, LaPerouse and Necker were islands with at least one high (greater than 1000 m) mountain peak, and supported diverse ecosystems [7]. Another group follows the same pattern, originating before the formation of Kauai and within the first peak period (9 Ma; 95% HPD: 7.12–11.22; node C, figure 2; table 1). Major feeding clades in *Philodoria* originated near the formation of Kauai approximately 4.7 Ma, a time that is thought to have seen a proliferation of ecosystem types for diversification and potential host plants for *Philodoria* [7,49,50].

The ancestor of extant *Philodoria* may have been polyphagous, with host plants belonging to the families Ebenaceae, Malvaceae and/or Primulaceae (nodes A and B, figure 2). Although the probability that each of these families is the ancestral host is approximately the same (electronic supplementary material, p. 15), the certainty of ancestral nodes of *Philodoria* was high (figure 2). This suggests that these host-plant families once existed on the NWHI, and that the relationships of extant *Philodoria* are conserved at the host-plant family level. Several lines of evidence, independent of our phylogenetic results, support the hypothesis that *Philodoria* were possibly feeding on plants on the NWHI. For instance, *Sida fallax* (Malvaceae), the host plant for *P. marginestrigata*, is presently found in the NWHI on both Midway and Nihoa [51]. The pollen record shows that this plant was also found on Laysan [52], but has since been extirpated with the introduction of non-native herbivores [53]. Similarly, pollen from *Hibiscus* (Malvaceae), on which at least three extant *Philodoria* species feed [26], was also found on Laysan, but is now extirpated [52]. *Philodoria* may have faced a similar extirpation on Laysan, much like the well-documented extinction of the endemic Laysan weevil (*Rhyncogonus bryani*) due to defoliation of its host plant by non-native herbivores [47,54]. Because Ebenaceae, Malvaceae and Primulaceae are represented on the current high islands by members that inhabit coastal, dry-land and rock habitats, it is within reason that relatives of these plants may have once served as host to *Philodoria* on the NWHI.

The origins of several plant-specific feeding groups broadly correspond to the arrival of their respective hosts. The Primulaceae-feeding clade was estimated to have originated approximately 10 Ma (95% HPD = 8.19–12.53; node E, figure 2). The first colonization time of extant Hawaiian *Lysimachia* (Primulaceae) is reported as 1.9 Ma [55], younger than the three *Philodoria* species that feed on this plant genus (7.5 Ma [95% HPD: 5.97–9.37]). The stem age (7.8 Ma) of this endemic plant lineage, however, is closer to the origin of *Lysimachia*-feeding *Philodoria*, suggesting association with extinct members of the plant genus. Our analyses show that the ancestor of Urticaceae–Asteraceae feeding *Philodoria* arose approximately 6 Ma (95% HPD = 5.35–8.03; node G, figure 2), before the formation of Kauai. The Urticaceae-feeding *Philodoria* (5 Ma [95% HPD: 4.34–6.59]) evolved

after the formation of Kauai, probably tracking their host plants as new islands formed over the hotspot, following the general trend of the progression rule [48]. The most diverse *Philodoria* species group, the Asteraceae miners, have a crown age estimate of approximately 4 Ma (95% HPD: 3.22–4.84; node J, figure 2), contemporaneous with the formation of Kauai. Interestingly, this age is also largely consistent with the approximate colonization date for plants in the endemic silversword alliance, approximately 5 Ma [49]. Many silversword species are rare and endangered, and restricted to mountaintops [56]. *Philodoria* appear to have either tracked these host plants to other islands as they formed and diversified in the process, or were previously more generalist feeders, and became specialists once a new island was colonized. *Philodoria* feed on two younger Hawaiian aster genera, *Hesperomannia* and *Lipochaeta*, which are not part of the silversword alliance, and the origin times for these *Philodoria* are also compatible with colonization times for their respective hosts (figure 2; electronic supplementary material, pp. 17–18) [57,58]. Similarly, radiation onto Myrtaceae occurred at 1.7 Ma (95% HPD: 1.30–2.23), which is consistent with age estimates of *Metrosideros polymorpha* in Hawaii (1.4–6.3 Ma [50]), the dominant canopy plant and a colonist of young lava flows.

Our ancestral state reconstruction analysis suggests that the six main host-plant families were each colonized only once throughout the evolution of *Philodoria* (figure 2). The colonization onto Malvaceae was followed by secondary host-plant switches to Asteraceae and Urticaceae (approx. 4 Ma and approx. 5 Ma, respectively), two plant families that many *Philodoria* species use as their larval host plant [26]. It is remarkable that there were no back-switches to a host plant that was used previously. These Hawaiian host plants are strikingly different morphologically [51] and it could be that larval specialization onto a particular plant morphotype limits host switching. Other herbivorous insect radiations on islands also show limited host switching compared to continental counterparts [59].

Similar to other phytophagous insects on islands [59,60,61], both host switching and allopatric isolation are significant mechanisms influencing the diversification of *Philodoria*. Of the 11 sister species pairs in our phylogeny (figure 2), six diverged by shifting to a related host on a different island and four involved one mechanism but not the other. Only one pair diverged without changing host species or island distribution, suggesting evolutionarily significant events at finer geographical or ecological scales (e.g. niche partitioning within an individual host plant [27]).

Philodoria diversity within plant families appears to be linked, at least in part, to host abundance and distribution. A widespread host species may facilitate population connectivity for *Philodoria*, thereby reducing opportunities for divergence through spatial isolation [62]. For example, only two *Philodoria* species feed on the canopy-dominant *Metrosideros polymorpha* (Myrtaceae), which occurs on all main islands [51]. This pattern is also evident with respect to *Myrsine lessertiana* (Primulaceae), *Hibiscus tiliaceus* (Malvaceae) and *Sida fallax* (Malvaceae), each of which are common on every island [51] and host to no more than two *Philodoria* species (electronic supplementary material, pp. 17–18). By contrast, the large number of aster feeders (12 species; node K, figure 2) may be a product of host species with narrow ranges. Of the aster-feeding *Philodoria*, 83% are confined to plants that are themselves single island endemics [51]. This phenomenon might also be influenced by hybridization between *Philodoria* host plants, particularly those in the silversword alliance, resulting in 'hybrid bridges' [63]. In this case, hybridization may facilitate host switching for *Philodoria*, which has occurred in other phytophagous insects feeding from these plants [64].

Like other insular phytophagous insect radiations (e.g. [60,64]; but see [65]), *Philodoria*, as a whole, feeds on a diversity of plant families, but individual species are specialists. This lends support to the idea that phytophagous insects colonize remote islands as more generalist feeders, and then become specialized over time [5]. While *Philodoria* do feed from many dominant Hawaiian plant families, compared with other, sometimes younger Hawaiian phytophagous insects, the genus as a whole uses relatively few hosts. The Hawaiian endemic leaf-hopper genus *Nesophrosyne* (Hemiptera, Cicadellidae), which originated approximately 3.4 Ma, comprises over 200 species and uses 21 plant families [66]. Similarly, many Hawaiian members of the bug genus *Orthotylus* (Hemiptera, Miridae) are host-specific, but in total, members of the genus use 16 plant families [67]. Extant *Philodoria* are notably absent from several dominant plant families in Hawaii. One explanation for a limited host range may be that *Philodoria* diversity was reduced when volcanism subsided just prior to the formation of Kauai, leaving a diminished archipelago with fewer habitats and presumably fewer hosts. Competitive exclusion has been a factor in niche partitioning in other phytophagous insects in Hawaii [66] and it may be that other leaf miners pre-empted plant family colonization by *Philodoria* (e.g. *Coprosma* and *Kadua* [Rubiaceae], both host to *Aristotelia* leaf miners [25]). Their absence from dominant plant families could also be attributed to host-plant morphology or herbivory defenses (e.g. leaf pubescence in Gesneriaceae, latex in Campanulaceae [51]). Considering the lack of back-switches in *Philodoria* host evolution and their absence from presumably inhospitable host families, the adaptation abilities of endophytophagous larvae may be a significant factor limiting host range.

We used phylogenomic data, with extensive taxon sampling and host-plant data, to investigate the timing and pattern of *Philodoria* evolution in Hawaii. Our results strongly conclude that *Philodoria* originated in the NWHI and spread through the younger high islands, tracking their host plants on those islands. Six independent switches to new host families occurred, without any instance of *Philodoria* species reverting to previously used hosts. This study, which includes a comprehensive sampling of genes, taxa and host-plant data, represents a significant contribution to our understanding of phytophagous insect evolution in the Hawaiian archipelago. With many of these host plants and their habitats threatened [56], this timely investigation will help inform critical studies needed to prioritize conservation areas for this unique Hawaiian fauna. Given the intimate relationship between *Philodoria* and their Hawaiian host plants, future studies should investigate the mechanisms that led to these phytophagous insects switching hosts and its implications for the conservation of the group.

Ethics. Collection of insect specimens was allowed under legal permit from the State of Hawaii Department of Land and Natural Resources, the Nature Conservancy and the National Park Service.

Data accessibility. The data and metadata associated with this article are available at Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.qq6hv63 [68].

Authors' contributions. C.A.J. and A.Y.K. designed the project and sampled for specimens. C.A.J., J.W.B. and E.F.A.T. conducted analyses. All the authors wrote and read the final version of the manuscript.

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