



# Host-plants shape insect diversity: Phylogeny, origin, and species diversity of native Hawaiian leafhoppers (Cicadellidae: *Nesophrosyne*)

Gordon M. Bennett\*, Patrick M. O'Grady

University of California, Department of Environmental Science, Policy and Management, 137 Mulford Hall #3114, Berkeley, CA 94720, United States

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## ABSTRACT

Herbivorous insects and the plants on which they specialize, represent the most abundant terrestrial life on earth, yet their inter-specific interactions in promoting species diversification remains unclear. This study utilizes the discreet geologic attributes of Hawai'i and one of the most diverse endemic herbivore radiations, the leafhoppers (Hemiptera: Cicadellidae: *Nesophrosyne*), as a model system to understand the role of host-plant use in insect diversification. A comprehensive phylogeny is reconstructed to examine the origins, species diversification, and host-plant use of the native Hawaiian leafhoppers. Results support a monophyletic *Nesophrosyne*, originating from the Western Pacific basin, with a sister-group relationship to the genus *Orosius*. *Nesophrosyne* is characterized by high levels of endemism according to individual islands, volcanoes, and geologic features. Clades demonstrate extensive morphologically cryptic diversity among allopatric species, utilizing widespread host-plant lineages. *Nesophrosyne* species are host-plant specific, demonstrating four dominant patterns of specialization that shape species diversification: (1) diversification through host switching; (2) specialization on widespread hosts with allopatric speciation; (3) repeated, independent shifts to the same hosts; and, (4) absence or low abundance on some host. Finally, evidence suggests competing herbivore radiations limit ecological opportunity for diversifying insect herbivores. Results provide evolutionary insights into the mechanisms that drive and shape this biodiversity.

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## 1. Introduction

The relationship between plants and insects is one of the greatest evolutionary stories in the history of life on earth. Their importance in global terrestrial ecosystem functioning is self evident, as both represent the most abundant life on the planet (Schoonhoven et al., 2005). At least half of all known insects feed on plants; however, this number is an underestimate, as up to 90% of insect life has yet to be described (Strong et al., 1984; Grimaldi and Engel, 2005). While plant-insect interactions have received much attention and are easily manipulated in experimental studies, there have been few broad-scale phylogenetic studies for circumscribed herbivorous groups and their ecological associations (Schoonhoven et al., 2005; Janson et al., 2008). As a result, the evolutionary role of interspecific interactions in promoting herbivorous insect and plant diversification, at both the global and local scales, remains unclear (Schoonhoven et al., 2005; Janson et al., 2008; Roderick and Percy, 2008).

In order to assess the evolutionary importance of plant-insect interactions in the evolution of both groups, well-developed phylo-

genetic hypotheses and circumscribed host-plant information are necessary for specific taxonomic groups. These data can be difficult to acquire in broadly distributed, diffuse systems with continental distributions. Oceanic islands offer unique, tractable natural laboratories to circumscribe and study plant-insect evolution, due to their discrete, isolated, and replicated nature (MacArthur and Wilson, 1967; Grant, 1998). The Hawaiian archipelago is unrivaled in this regard, with its biota derived almost entirely *in situ*, resulting in unparalleled rates of endemism: 99% for arthropods and 89% for plants (Wagner and Funk, 1995; Miller and Eldridge, 1996; Wagner et al., 1999). These factors provide the opportunity, especially with plant-insect interactions, to develop an understanding of the forces that drive and shape biodiversity. Furthermore, the insular nature of island radiations offer the ability to biologically and evolutionarily contextualize archipelagic species diversification by locating the source of origin for endemic lineages (Carlquist, 1966). Expanding this knowledge for island groups yields a model for understanding how regional biodiversity is evolutionarily assembled and maintained.

Previous studies on herbivorous insects have generally focused on several iconic model systems, including Lepidoptera (Ehrlich and Raven, 1964; Pellmyr, 2003), Coleoptera (Farrell and Mitter, 2008; Farrell and Sequeira, 2004), Psyllidae (Percy et al., 2004), *Rhagoletis* fruit flies (Berlocher, 2000), and fig wasps (*Silvius*

\* Corresponding author.

E-mail address: [gbennett@berkeley.edu](mailto:gbennett@berkeley.edu) (G.M. Bennett).

et al., 2008). While the developed knowledge of these systems provides invaluable insights and a comparative framework, results may not be generally applicable to other groups (Janson et al., 2008). Remarkably, one of the largest gaps in our understanding includes the hemipteroid lineages in Auchenorrhyncha, which contain some of the largest obligate host–plant restricted groups known (Grimaldi and Engel, 2005). In particular, the leafhoppers (Cicadellidae) provide a unique, and heretofore overlooked, system with which to examine host–insect evolution. Cicadellids represent one of the largest insect families and compose a dominant element of terrestrial ecosystems globally (Oman et al., 1990; Zahniser and Dietrich, 2008). Leafhoppers have been considered excellent models to understand ecological and biogeographic mechanisms of species diversification due to their high levels of host–plant specificity, limited dispersal, and high rates of local endemism (Hamilton, 1997; Nielson and Knight, 2000; Felix and Mejdalani, 2011). Yet despite their ecological dominance, and highly adapted phytophagous lifestyle, species-level diversification patterns and ecological associations remain largely unknown, particularly in the largest subfamily, Deltocephalinae (Zahniser and Dietrich, 2010).

The presented research focuses on the endemic Hawaiian leafhopper genus *Nesophrosyne* (Deltocephalinae; Fig. 1). *Nesophrosyne* represents one of the most diverse and ecologically dominant herbivore radiations on Hawai'i. The genus contains 72 described species; however, its total diversity remains mostly undescribed (Zimmerman, 1948; Bennett and O'Grady, 2011). Species are distributed across all high islands, filling niches in coastal to subalpine habitats. *Nesophrosyne* are obligate phloem feeders, ovipositing and developing on particular host–plants, and are highly host specific, utilizing 75% of the most ecologically dominant and species-rich endemic genera (Kirkaldy, 1907, 1910; Osborn, 1935; Zimmerman, 1948; Bennett and O'Grady, 2011; Wagner et al., 1999). They

further demonstrate dramatic morphological adaptations both to local climate and to host–plant morphology (Zimmerman, 1948). The endemic nature and host specificity of *Nesophrosyne* offers a discrete and diverse system to examine the role of host–plant associations in insect diversification.

This study investigates the origin, species diversity, and host–plant use of *Nesophrosyne* in a molecular phylogenetic context. The principal question guiding this work is the roll of host–plant specialization in shaping ecological and species diversification in phytophagous insects. This study is one of the first aimed at understanding genus-wide host–plant associations in a molecular systematic framework within Cicadellidae and for native Hawaiian hemipteroid radiations. Results establish a general model for contextualizing species diversification and the role of host–plant associations in the radiations of herbivorous insects.

## 2. Methods

### 2.1. Material collected

Ingroup taxon sampling and species delimitation for phylogenetic analyses was conducted under the following criteria (in the following order): (i) morphological diagnosis of described species and identified undescribed species, (ii) ecological species diagnosis based on host–plant associations, and (iii) geographical diagnosis of species distributed across the biogeographic provinces of the Hawaiian Islands. These criteria were used to delimit initial species hypothesis, which were then objectively evaluated with the Cytochrome Oxidase II (COII) locus. The use of COII for species delimitation in *Nesophrosyne* was tested in Bennett and O'Grady (2011) for the *kanawao* species group (Clade M in this study; see Results, Fig. 3). They found that COII reliably reconstructs highly supported, reciprocally monophyletic clades for both morphologically diagnosable species and cryptic sibling species with large disjunct ranges (e.g., the volcanoes of Maui and Hawai'i Island). Intra-specific species variation was found to fall within 2–3% divergence across large species' ranges (e.g., *Nesophrosyne aakokohaika* endemic to Mauna Loa). Thus, individuals in this study with identical COII haplotypes or <2–3% difference, were removed from further molecular analyses. Final multi-gene, phylogenetic results were used as a fourth objective criterion to evaluate species hypotheses (see Results). In total, 363 individuals were screened, in order to delineate species boundaries and to test host–plant associations (data not shown). A total of 191 *Nesophrosyne* species among 198 samples were identified in this study, representing 48 of the 72 currently described species, and 143 potentially new species (SI Table 1).

Material included in molecular analyses was field collected by sweep-netting individual plants and placed directly into 95% ethanol and labeled with a four-digit field barcode (e.g., GB-001.7). Species identifications followed Zimmerman (1948) and original descriptions (Kirkaldy, 1907, 1910; Osborn, 1935; Bennett and O'Grady, 2011). It is important to note that *Nesophrosyne* has not heretofore received systematic treatment, or a focused taxonomic effort, and reliable identifications for much of the described diversity are difficult and often impossible (Zimmerman, 1948; Bennett and O'Grady, 2011). Furthermore, species described by Kirkaldy (1907, 1910) and Osborn (1935) are based on inconsistent premises (e.g., single specimens, males, females, or teneral specimens) and further consist of short, often uninformative descriptions (see Kirkaldy, 1910). Species unable to be positively identified are considered to be new, and are designated with an appended DNA barcode (e.g., “*N. sp.123*”). Material for DNA extraction followed a non-destructive vouchers protocol in order to preserve specimens for designation as type material. Morphotype vouchers have



**Fig. 1.** Native Hawaiian leafhopper species (Cicadellidae: *Nesophrosyne*). Species names from top left to right: “*N. sp.125*”, “*N. sp.253*”, “*N. sp.296*”, *N. anguilifera*, and *N. cinera*; bottom left to right: *N. craterigena*, “*N. sp.23*”, *N. oreadis*, *N. notatula*, and “*N. sp.293*”.

been designated for each species from the material used in phylogenetic analyses, and include the dorsal habitus and genitalia.

In order to determine the geographic and taxonomic origin for *Nesophrosyne*, taxon sampling included a total of 12 genera (16 individuals) in Deltocephalinae subfamily from around the Pacific Basin (SI Table 1). Taxon selection was informed by (a) previous systematic work on the Deltocephalinae subfamily, targeting members of the Opsiini tribe and related genera (Zahniser and Dietrich, 2010); and, (b) proposed sister genera to *Nesophrosyne*, including *Scaphoideus* (Osborn, 1935) and *Osbornellus* (Zimmerman, 1948). The strongest candidate for the closest relative of *Nesophrosyne* is the genus *Orosius*, which shares membership in the Opsiina subtribe (Dai et al., 2010; reviewed in Bennett and O'Grady, 2011). *Orosius* has Western Pacific Rim distribution (e.g. Oceania, South East Asia, and Africa), and was sampled from Australia for this study (SI Table 1). Species in the genus *Deltocephalus* (Tribe: Deltocephalini) were specified as phylogenetic outgroups.

## 2.2. Host–plant associations

The host–plant associations of *Nesophrosyne* were resolved in the field from collections conducted by the authors. Field collected data were combined with historical collections housed in the Bernice P. Bishop Museum, Honolulu, HI; the University of Hawai'i, Manoa Insect Museum; original literature describing species and paratype collections, including material in the British Museum of Natural History, London (Kirkaldy, 1907, 1910; Osborn, 1935); and, a large collection (~1500 specimens) provided by D. Polhemus from the Smithsonian Institution's National Museum of Natural History, Washington, D.C. The dominant host–plant of a leafhopper species was inferred under the following criteria: (a) a positive host identification to the plant genus level; (b) confirmation of host associations through repeated collection of a leafhopper species from the same plant (i.e., singletons are generally treated as unknown); and (c) phylogenetic relationships support a proposed host, unless contradicted by field data. Some species may be capable of utilizing secondary hosts and were thus considered; however, there is considerable evidence that most if not all species are host–plant specific (Kirkaldy, 1907, 1910; Osborn, 1935; Zimmerman, 1948; Bennett and O'Grady, 2011), which is a common pattern among other herbivorous insects on Hawai'i (Asche, 1997; Roderick and Percy, 2008; Polhemus, 2011), and a general expectation for herbivorous insects world-wide (Schoonhoven et al., 2005).

Host–plants were field identified to family and genus according to current taxonomic standards provided in the Manual of the Flowering Plants of Hawai'i (Wagner et al., 1999). Plants that could not be field determined were photographed and later identified. Species-level identifications were made when possible; however, this requires plants to be in flower or fruit, which was not always available. Furthermore, the species-level taxonomy for some Hawaiian plant lineages is not well worked out (Wagner et al., 1999).

## 2.3. Sequencing and alignment

Total genomic DNA was extracted from a cross section of the anterior abdominal segments, using Qiagen DNeasy extraction kit (Qiagen Corps). A total of six genes were amplified from mitochondrial (*Cytochrome Oxidase I & II*, and 16S), nuclear (*Histone 3* and *Wingless*), and obligate betaproteobacterial endosymbiont (16S: CoBE-16S) genomes. Bacterial endosymbiont genomic data were selected because they have been increasingly incorporated in inference of non-model organism evolution since obligate symbionts are transovarially transmitted and show ancient co-cladogenesis (Lozier et al., 2007; Andersen et al., 2010; Moran et al., 2008).

Specifically, bacterial endosymbionts have been demonstrated to show strict co-phylogenetic correspondence in other leafhopper–bacteria systems (Takiya et al., 2006). The obligately associated betaproteobacteria in *Nesophrosyne* is related to other betaproteo lineages found throughout Auchenorrhyncha, including '*Candidatus* Zinderia insecticola' from spittlebugs also in the Cicadomorpha infraorder (Bennett unpub. data; McCutcheon and Moran, 2010), as well as in multiple genera from across the Deltocephalinae subfamily (presented here and see Noda et al., 2012), further supporting the ancient co-cladogenic association for these lineages. Polymerase Chain Reaction conditions and primers were adapted from previous studies (see Table 1) and optimized for *Nesophrosyne*. Reactions began with an initial 5 min denaturation step at 95 °C; followed by a 35-cycle regime of 95 °C for 30 s, 54–64 °C for 1 min (annealing optimized for each gene: Table 1), and 72 °C for 1 min (extension phase); and, a final 72 °C extension for 5 min. For COI, a stepwise PCR reaction was used with a three-stage cycle regime as follows: 95 °C for 30 s, 60 °C for 1 min, and 72 °C for 1 min, for 10 cycles; 95 °C for 30 s, 56 °C for 1 min, and 72 °C for 1 min, for 10 cycles; and 95 °C for 30 s, 54 °C for 1 min, and 72 °C for 1 min, for 5 cycles. Primer pairs that did not work for some taxa were redesigned and optimized using Primer3 (Table 1; Rozen et al., 2000). Successful PCR amplifications were cleaned using the ExoSAP-IT protocol (USB). Both forward and reverse directions were sequenced at the U.C. Berkeley, Barker Sequencing Center, using an ABI 3730 capillary sequencer. Sequences were submitted to GenBank for public archiving (SI Table 1).

Sequenced contigs were aligned and edited in Geneious v5.1 (Drummond et al., 2010) and identity checked on GenBank. Different alignment strategies were employed based on the genomic locus of each gene. All protein-coding genes were aligned using MUSCLE v3.5 (Edgar, 2004), conceptually translated into amino acid sequences, and checked for frame-shift mutations and internal stop codons. 16S mtDNA contains highly variable loop regions and was aligned using Probalign v1.2, which has demonstrated superior performance over other available alignment methods (Roshan and Livesay, 2006). The CoBE-16S locus was aligned using the RDP 10, which informs alignment strategy with secondary ribosomal folding structure (Cole et al., 2008). Sequence alignments of both 16S and CoBE-16s revealed highly variable loop regions containing large insertion-deletions for which homologous statements were not trivial, and were removed as follows: 16S base pair regions 30–38, 165–195, 290–305, 325–361, and 590–604; and, CoBE-16S regions 72–78 and 778–792. Preliminary phylogenetic analyses further indicated that the inclusion of the loop regions reduced support at more basal node, indicating conflict between these regions and the larger alignment (data not show). Aligned gene matrices were concatenated into a full data matrix, pending assessment of phylogenetic congruence (see below).

## 2.4. Phylogenetic analyses

Phylogenetic analyses were performed on a data matrix consisting of 214 taxa and 6 loci, comprising 3520 characters. Species relationships were inferred using maximum likelihood (ML) and Bayesian methods. All loci were initially examined individually and in combination by genome to determine their suitability for concatenation into a single data matrix. In particular, we evaluated the influence of the CoBE-16S on the inference of phylogeny, since it offers a novel locus for cicadellid systematics. Supplementary information Figs. 1 and 2, show the phylogenetic results for the alignment excluding the CoBE-16S locus and for the CoBE-16S gene tree, respectively. Both recover the same general clade and species level relationships found in the complete data alignment, although basal and mid-level support is greatly reduced relative to the total



**Table 1**  
Genomic loci used in phylogenetic analysis of *Nesophrosyne*.

Gene	Genome	# Of characters (parsimony informative chars.)	Model <sup>h</sup> : AIC BIC	Primer name (annealing temp.)	Primer sequence 5'–3'
Cytochrome Oxidase II (COII)	mtDNA	714 (434)	TVM + I + G TVM + I + G	TL2-J-3037 <sup>a</sup> (53 °C)	TAGTATGGCAGATTAGTCAATGAA
Cytochrome Oxidase I (COI)	mtDNA	675 (319)	GTR + I + G TVM + I + G	C2-N-3661 <sup>a</sup> LCO <sup>e</sup> (3-Step) HCO <sup>e</sup>	CCRCAAAATTCWGARCATTGACCA GCTCAACAAATCATAAAGATATTGG TAAACTTCAGGGTGACCAAAAAATCA
Nuclear Histone 3 (H3)	nucDNA	309 (101)	GTR + I + G GTR + I + G	H3F <sup>b</sup> (62 °C) H3R <sup>b</sup>	ATGGCTCGTACCAAGCAGACGGC ATATCCTTGGGCATGATGGTGAC
Wingless (wg)	nucDNA	303 (93)	TRN + I + G K80 + I + G	wg-1.5f <sup>c</sup> (62 °C) wg-7.5B <sup>c</sup> wg-4F <sup>g</sup> (62 °C) wg-4R <sup>g</sup>	GTSAAACBTGYTGGATGCG GTCCTGTAMCCVCGUCCACAACACAT CCGTGTGGTCGGGGACAACC CCCGTCCACCCGATGCTTG
16S	mtDNA	519 (243)	TRN + I + G TRN + I + G	LR-J-12887 <sup>d</sup> (53°) LR-N-13398 <sup>d</sup>	CCGGTYTGAACCTCARATCA CRMCTGTTAWCAAAAACAT
16S Betaproteobacteria (CoBE-16S)	endosymbiont	1000 (245)	GTR + I + G GTR + I + G	10F <sup>f</sup> (58°) 1507R <sup>f</sup> Beta_300 <sup>g</sup> (60°) Beta_1352 <sup>g</sup>	AGTTTGATCATGGCTCAGATTG TACCTTGTACGACTTCACCCAG GCGGATGTATAGCCACTGGGA ATAAACTTACTCCATGGTG

<sup>a</sup> Takiya et al. (2006).

<sup>b</sup> Ogden and Whiting (2003).

<sup>c</sup> Cryan et al. (2004).

<sup>d</sup> Dietrich et al. (1997).

<sup>e</sup> Folmer et al. (1994).

<sup>f</sup> Munson et al. (1991).

<sup>g</sup> Primers designed in this study.

<sup>h</sup> Likelihood model of nucleotide evolution determined in Modeltest (Posada and Crandall, 1998).

dataset. The CoBE-16S locus is a relatively conserved gene, increasing mid-level and basal nodal support (e.g., see Results Fig. 3 and SI Fig. 1, Clades A, M, K, and I, among others). The effect of missing data was also assessed by reconstructing phylogenetic relationships with data matrices that progressively removed loci with the highest percent missing data (see SI Table 1 for gene sampling). All data assessments and verifications were performed in RAXML v7.2.7 (Stamatakis et al., 2008), described below (results not shown). In general, species-level relationships are resolved for fast evolving mtDNA loci (COI and COII), but deeper relationships are unresolved. Conversely, nucDNA and ribosomal genes show mixed resolution at deeper nodes and are unable to resolve species-level relationships. Removal of loci with missing data did not change species or clade level relationships, but basal support progressively declined. Thus, all loci were concatenated into a single data matrix for full phylogenetic analyses.

Gene partitioning has become a required consideration for properly analyzing large multi-gene datasets and directly optimizes information contained within large complex data matrices, which is particularly useful for rapid radiations such as those on islands (Brandley et al., 2005; Li et al., 2008). The optimal partitioning strategy for this study was determined in a ML framework, using RAXML v7.2.7 on Cipres (Stamatakis et al., 2008; Miller et al., 2009). RAXML was chosen due to the computational complexity of the dataset and available computing power (Stamatakis et al., 2008). Molecular data were increasingly partitioned, according to the biological elements from a single partition (concatenated) to fourteen partitions (codons, 16S, and CoBE-16S: Table 2). RAXML was run under the GTR-GAMMA model for 1000 bootstrap iterations and final ML search. For each ML reconstruction, log likelihood scores were used to evaluate partition performance with the Akaike Information Criterion (AIC) and AIC

weights (Table 2: Burnham and Anderson, 2002). The AIC overwhelmingly selected a fully partitioned analysis, which contributes to a growing body of literature that suggests codon and gene identity are important considerations in phylogenetic analyses of multi-gene datasets (Brandley et al., 2005).

Bayesian phylogenetic inference was completed using MPI-MrBayes v3.1.2 on the Abe TeraGrid in Cipres (Ronquist and Huelssenbeck, 2003; Miller et al., 2009). Likelihood models of base-pair evolution were determined for each partition element with the Bayesian Information Criterion in MODELTEST v3.7 (Posada and Crandall, 1998). Since MrBayes is unable to incorporate some models of evolution, the closest model to the one selected with Modeltest that could be implemented, with the necessary parameters (Table 3), was used for each Bayesian phylogenetic analysis. All data partitioned elements were unlinked, assigned the appropriate model of molecular evolution, and branch lengths set to vary proportionally across partitions. Based on preliminary analyses, chain temperature was adjusted down ( $t=0.1$ ) to facilitate convergence on the posterior optima. Two independent searches of the posterior distribution were run with four chains each for a total of  $17.322 \times 10^6$  generations, sampled every 1000th iteration. Runs were monitored throughout their length, using the average standard deviation of the split frequencies (ASDSF) and the cumulative function in AWTY (Nylander et al., 2008). The run was terminated after achieving an ASDSF of 0.03 (an ASDSF=0.05 was used as an approximation of convergence of large datasets; Ronquist et al., 2011). Final convergence and burn-in were determined by plotting outputs in Tracer v1.5 and AWTY, and assessing the potential scale reduction factor (Nylander et al., 2008; Rambaut and Drummond, 2009). A 50% majority rule consensus tree was constructed from post burn-in iterations.

**Table 2**

Akaike Informations Criterion (AIC) summary statistics for data partitioning strategies reconstructed in RAxML v7.2.7 (Stamatakis et al., 2008).

Partitions	Partition elements	$l^a$	$K^b$	AIC <sup>c</sup>	$\Delta AIC^d$	$w^e$	Rank <sup>f</sup>
1	Concatenated	−110374.19	434	221616.39	7838.40	0	10
2	Gene type	−109814.63	443	220515.27	6737.28	0	9
3	Genome	−108662.27	452	218228.55	4450.55	0	8
4	Universal codons and single ribosome	−108413.90	461	217749.80	3971.80	0	7
5	Universal codons, 16S, and CoBE-16S	−107825.71	470	216591.43	2813.44	0	5
6	Individual gene	−108291.86	479	217541.72	3763.73	0	6
9	Individual gene codons 1 + 2, 3 and single ribosome	−107188.59	506	215389.18	1611.18	0	4
10	Individual gene codons 1 + 2, 3, 16S, and CoBE-16S	−106729.23	515	214488.47	710.48	5.257E−155	2
13	Individual gene codons and single ribosome	−106926.69	542	214937.39	1159.40	1.7347E−252	3
14	Individual gene codons, 16S, and CoBE-16S	−106337.99	551	213777.99	0	1	1

<sup>a</sup> log likelihood (lnL) of the ML phylogenetic hypotheses under each partitioning scheme.<sup>b</sup> Number parameters = # of branch lengths ( $2n - 3$ ;  $n = \text{taxa}$ ) plus 9 parameters for each implementation of the GTR + GAMMA model.<sup>c</sup>  $AIC = -2(\ln L) + 2K$ .<sup>d</sup>  $\Delta AIC = AIC - AIC_{\min}$ .<sup>e</sup> AIC weight estimated as the relative likelihood =  $\exp(-0.5\Delta AIC) / \sum \exp(-0.5\Delta AIC)$ .<sup>f</sup> Rank based on  $\Delta AIC$ .**Table 3**

Summary of nucleotide models determined for individual partitions using the BIC in Modeltest (Posada and Crandall, 1998). Implemented models are the closest nucleotide models incorporating the necessary parameters and settings applied in MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003).

Gene	Codon partition	BIC	Implemented model
COII	1st	TrN + I + G	GTR + I + G
	2nd	TVM + I + G	GTR + I + G
	3rd	GTR + I + G	GTR + I + G
COI	1st	Sym + I + G	GTR + I + G (Modified)
	2nd	TrN + G	GTR + G
	3rd	GTR + G	GTR + G
H3	1st	TrNef	GTR (Modified)
	2nd	JC	JC
	3rd	TVM + G	GTR + G
wg	1st	JC + G	JC + G
	2nd	JC	JC
	3rd	TVM + I + G	GTR + I + G
16S	–	TrN + I + G	GTR + I + G
CoBE-16S	–	GTR + I + G	GTR + I + G

### 3. Results

#### 3.1. Host–plant associations

A total of 4118 specimens with positive host–plant information were examined. The dominant host–plant associations were determined for 175 *Nesophrosyne* species, which utilize 33 plant genera in 21 families (SI Tables 1 and 2). Species are highly host–plant specific. The four most dominant host–plant associations broken down by plant family and genera are the following: Rubiaceae (*Psychotria*, *Coprosma*, *Bobea*, *Hedyotis*), Urticaceae (*Pipturus*, *Urera*, *Boehmeria*, and *Touchardia*), Hydrangeaceae (*Broussaisia*), and Sapindaceae (*Dodonaea*). There is evidence that a few *Nesophrosyne* species may use more than one host, including the following: *N. furculata* on *Lobelia* sp. (Campanulaceae) and *Coprosma* sp.; “*N. sp.26*” and *N. umbratilis* on *Hedyotis* sp. and *Psychotria* sp.; and, *N. pele* on *Melicope hawaiiensis* (Rutaceae) and *M. radiata*. Results also indicate that “*N. sp.132*” is associated with the fern genus *Sadleria* (Blechnaceae), which is a unique association for the genus. The dominant host–plant associations were mapped across the tips of phylogenetic results in order to determine the relationships of *Nesophrosyne* species and their associations with particular hosts.

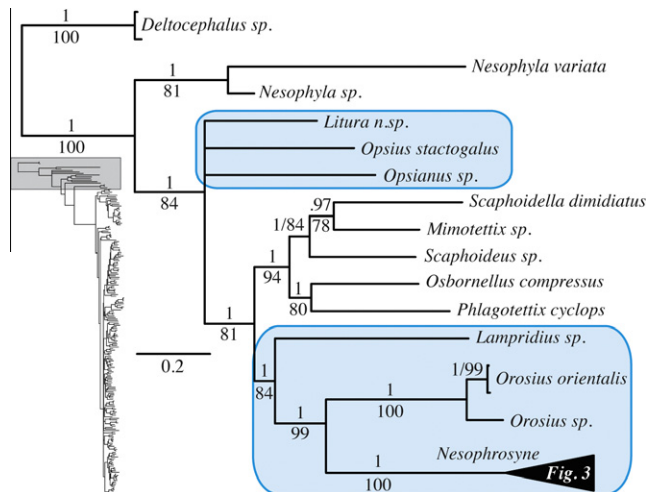
#### 3.2. Phylogenetic results

Phylogenetic results from ML and Bayesian trees were combined using Archaeopteryx v0.957 beta, which provides a visual

tool for comparison of tree topologies (Han and Zmasek, 2009). Both methods yielded similar topologies, providing high support for a monophyletic *Nesophrosyne* and the relationships among other genera (Fig. 2). Basal nodes are generally well supported (BS = 68–81, PP = 1); however, there is a large unresolved polytomy at mid-depth in the tree. Differences between trees were confined to branches that received low or no support with both methods, except in one instance where ML supported a bifurcation between taxa “*N. sp.126*” and *N. giffardi* + *N. giffardi interrupta* that was not supported by the Bayesian analysis. Bayesian results provide higher support for clades at deeper nodes, and are selected as the reference tree for inference of relationships between *Nesophrosyne* species (Figs. 2–5).

#### 3.3. Origin of *Nesophrosyne* and higher taxonomic relationships

Fig. 2 shows the relationships of *Nesophrosyne* and Deltocephalinae genera. Both ML and Bayesian analyses provided strong support for the sister relationship of *Nesophrosyne* to the genus *Orosius* from Australia. The Opsiini genus *Lampridius* from Thailand, is placed sister to the *Nesophrosyne* + *Orosius* clade. The monophyly of Opsiini is not supported by the inclusion of a large clade of Deltocephalinae genera currently assigned to the Athysanini tribe (*Scaphoidella*) or of unplaced tribal status (*Mimotettix*, *Scaphoideus*, *Phlagotettix*, *Osbornellus*), which is additionally supported by the convergent and variable nature of the proposed synapomorphic bifurcate aedeagus at higher taxonomic levels (Dai et al., 2010).

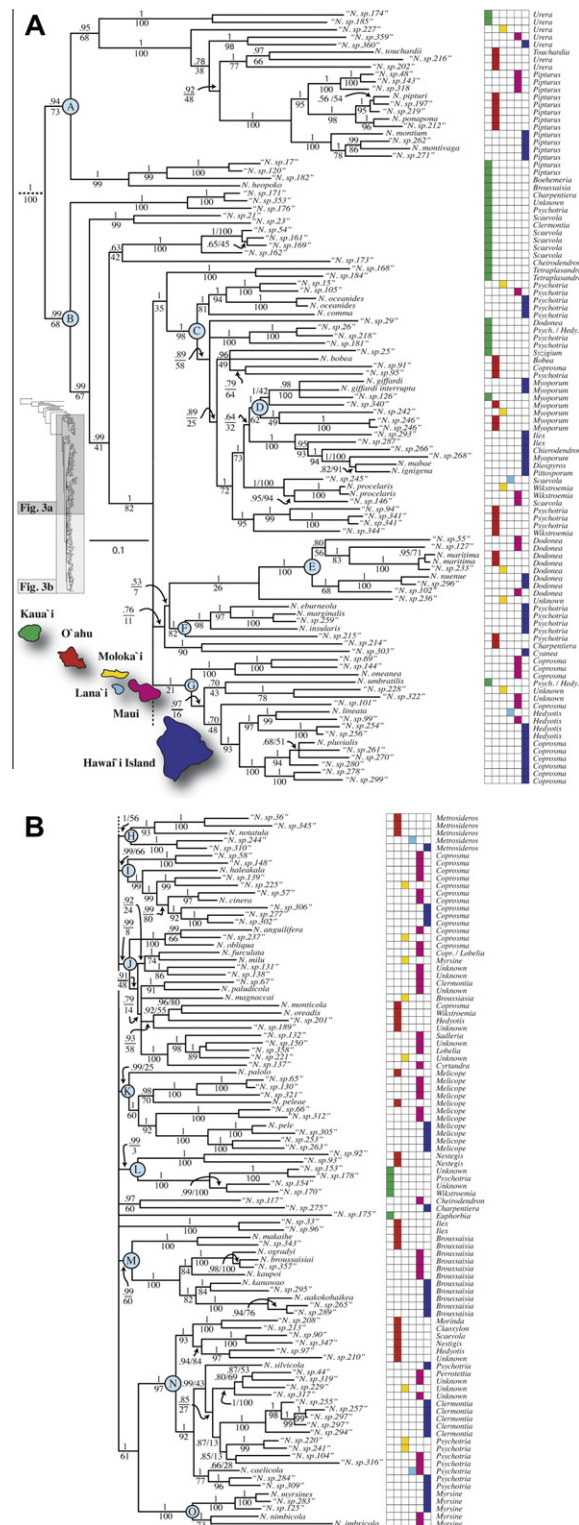


**Fig. 2.** Bayesian phylogram for fully partitioned analyses, highlighting relationships among taxa of tribe Opsiini (in blue boxes) and other members of the Deltocephalinae subfamily. See SI Table 1 for collection locality information. Inset phylogeny shows placement of the featured genera in the full phylogeny. Lineages within *Nesophrosyne* are collapsed here, and presented in detail in Fig. 3. *Deltocephalus* sp. is designated as the outgroup. Numbers above and below the branches represent nodal posterior probabilities and ML bootstrap support values, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 3.4. Species relationships and host-plant use

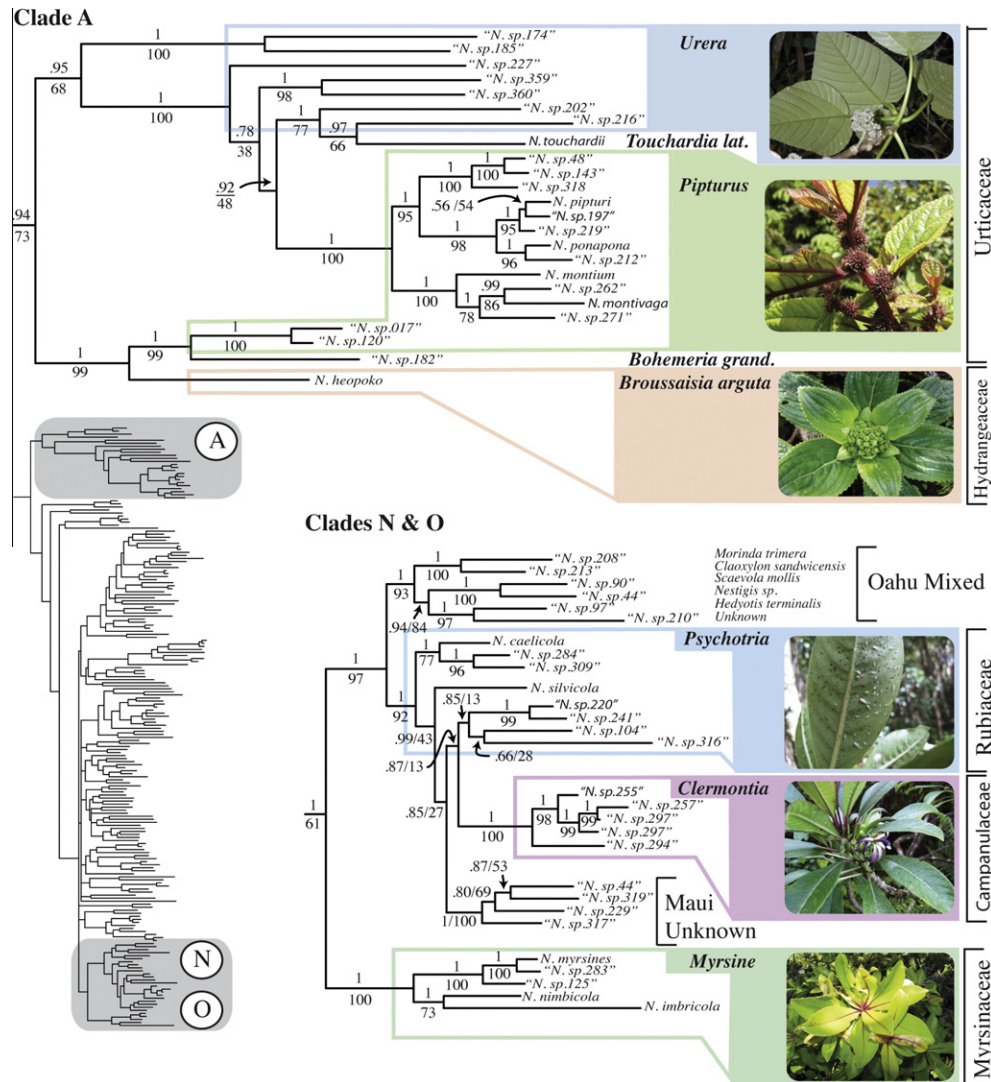
*Nesophrosyne* species-level relationships are well supported and assignable to clades, providing adequate resolution to assess host-plant associations, species diversity, and areas of endemicity (Fig. 3). Aside from Bennett and O'Grady (2011), there has been no previous systematic or taxonomic effort treating the relationships within *Nesophrosyne*, and no precedence exists for comparison of species relationships. *Nesophrosyne* forms large multi-island clades according to host-plant associations and morphological types, which will be described as species groups and subgroups in an upcoming revision. In order to simplify discussion of results, well-supported clades according to the Bayesian analyses are assigned alphanumeric names (Clade A–N; Fig. 3). Broadly, island species distributions are complex, with species occurring on older islands (Kaua'i or O'ahu), representing basally diverging lineages for some clades (A, C, F, H, K, L, M), but more nested lineages in others (D, G, J, N). Host-plant specialization can be summarized as clades characterized by multiple host transitions across plant genera and families (A, C, G, J, L, O); or, clades associated with single widespread host-plant lineages (A, Urticaceae: *Pipturus* and *Ureca*; E, Sapindaceae: *Dodonaea*; I, F, C; Rubiaceae: *Coprosma* and *Psychotria*; K, Rutaceae: *Melicope*; M, Hydrangeaceae: *Broussaisia*; H, Myrtaceae: *Metrosideros*; O, Myrsinaceae: *Myrsine*; and, D, Scrophulariaceae: *Myoporum*).

*Nesophrosyne* is basally split into two parallel radiations (Fig. 3): Clade A (Figs. 3 and 4: BS = 73, PP = .94) and its sister group Clade B (BS = 68, PP = .99). Clade A comprises all species associated with genera in the plant family Urticaceae (*Ureca*, *Touchardia*, *Boehmeria*, and *Pipturus*) throughout all high islands, and a single well-supported transition by *N. heopoko* to *Broussaisia arguta* (Hydrangeaceae) on Kaua'i (Figs. 3 and 4: BS = 99, PP = 1). Species-level relationships are well supported, with the exception of species associated with *Ureca*. Species specializing on *Pipturus* are paraphyletic, including a clade comprising three monophyletic groups restricted to O'ahu, Maui, and Hawai'i Island (BS = 100, PP = 1), and a clade comprising "*N. sp.17*" and "*N. sp.120*" on Kaua'i that is sister to species associated with *Boehmeria* and *Broussaisia*.



**Fig. 3.** Bayesian phylogram for the fully partitioned analyses, showing the ingroup relationships for 191 *Nesophrosyne* species across 198 individuals (SI Table 1 for species information). Ingroup topology is split into A and B (see inset phylogeny), and outgroups have been removed (see Fig. 2). Nodes with circled letters refer to clades discussed in the text. The color-coded grid specifies the islands of endemicity for each taxon and correspond to the colored Hawaiian Islands. Dominant host-plant (Genus) for each species is given adjacent to cells (See SI Table 2 for more host taxonomic information). Numbers above and below the branches represent nodal posterior probabilities and ML bootstrap support values, respectively.





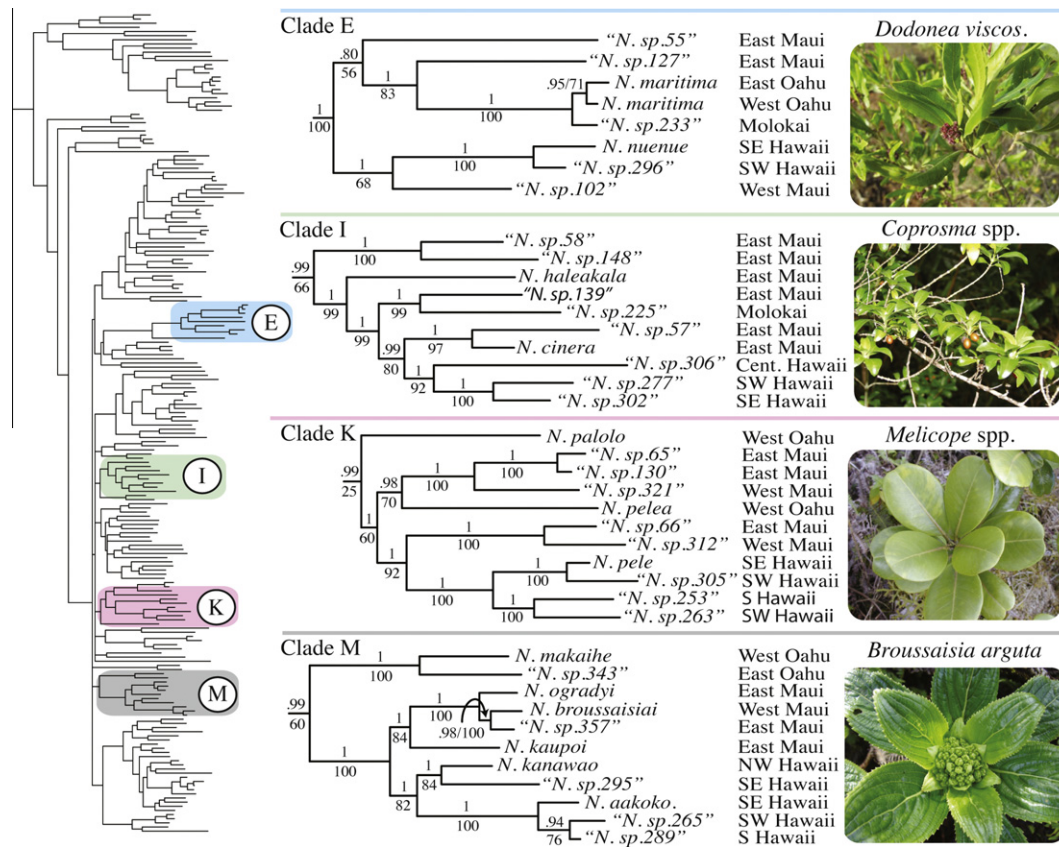
**Fig. 4.** Selected clades from *Nesophrosyne* Bayesian phylogenetic tree shown in Fig. 3. Clade A demonstrates host transitions between four closely related endemic plant genera in the family Urticaceae, and a single transition to the host family Hydrangeaceae. Clade N demonstrates host transitions across multiple distantly related, endemic host-plant families. Clade O is strictly associated with *Myrsine* (Myrsinaceae).

Clade B represents a large radiation associated with all other plant groups (Fig. 3). The basally diverging Kaua'i lineages of that radiation are associated with several host-plant genera and families (e.g., Rubiaceae: *Psychotria*, Goodeniaceae: *Scaevola*, and Campanulaceae: *Clermontia*), most with high support (BS = 41–82, PP = .63–1). The remainder of Clade B comprises all other lineages, which form clades emerging from a large mid-depth polytomy. The remaining clades are described below, to illustrate species relationships and unique patterns among *Nesophrosyne* lineages and clades.

**Clade C** (Fig. 3): Clade C (BS = 35, PP = 1) comprises a large group of small-bodied leafhoppers spread throughout the islands. Support for species relationships are mixed, with most receiving high support (BS = 32–100, PP = .64–1). Host-plant transitions in the group are common, and species are associated with 15 plant genera in 11 families (Fig. 3; SI Table 1). The basally diverging lineages are endemic to Kaua'i, and are associated with plant genera *Tetraplasandra* and *Cheirodendron* (Araliaceae: BS = 100, PP = 1). The remaining species have diverse host associations and are distributed across all islands, which includes: A multi-island clade (e.g., *N. comma* and *N. oceanides*) associated with sister species, *Psychotria mariana* and *P. hawaiiensis* (Rubiaceae: BS = 81–100, PP = 1); a

Kaua'i clade restricted to *Psychotria* and *Hedyotis* (Rubiaceae), including the multi-host associated "*N. sp.26*" (BS = 100, PP = 1); a Kaua'i and O'ahu clade, with basally diverging Kaua'i species associated with *Syzygium* (Myrtaceae), and O'ahu species associated with Rubiaceae genera (*Bohea*, *Coprosma*, and *Psychotria*: BS = 49–64, PP = .79–.96); Clade D, which is found on all islands associated with *Myoporum sandwicense* (Scrophulariaceae: BS = 61, PP = 1), comprising morphologically cryptic species; a diverse clade of Hawai'i Island species associated with 5 families (e.g. *N. ignigena* on Aquifoliaceae: *Ilex*, "*N. sp.268*" on Araliaceae: *Cheirodendron*, and *N. mabae* on Ebenaceae: *Diospyros*, etc.: BS = 91–100, PP = .82–1); a Maui Nui (Moloka'i, Lana'i, and Maui) group associated with *Scaevola* (Goodeniaceae,) and *Wikstroemia* (Thymelaeaceae: BS = 94–100, PP = .95–1); and, finally, an O'ahu associated clade with its basally diverging lineage associated with *Wikstroemia* and other with *Psychotria* (BS = 95–100, PP = 1).

**Clade E** (Figs. 3 and 5): All species in Clade E are restricted to the widespread host-plant, *Dodonaea viscosa* (Sapindaceae). Taxa associated with *D. viscosa* do not constitute a monophyletic group, with an independent transition of "*N. sp.29*" on Kaua'i (Fig. 3, Clade C: BS = 91, PP = 1). Lineages are divided into two parallel groups, including one on Maui, Moloka'i, and O'ahu (BS = 56, PP = .8), and



**Fig. 5.** Selected clades from *Nesophrosyne* Bayesian phylogenetic tree shown in Fig. 3. Clades demonstrate monophyletic assemblages of species specializing on widespread host-plant lineages throughout multiple Hawaiian Islands. Island and area of endemicity for each leafhopper species is given. Islands are sub-divided according to region. Abbreviations for Hawai'i Island correspond to relative compass direction of each region: N = North, South = South, NW = North West, SW = South West, SE = South East, Cent. = Central.

the other on Maui and Hawai'i Island (BS = 68, PP = 1); Maui species are basally divergent in both cases. Lineages occurring on Moloka'i and O'ahu are closely related (BS = 100, PP = 1), with *N. maritima* forming a low elevation, multi-volcano endemic (BS = 71, PP = .95). Gross morphology is similar in all species, but forms two morphologically cryptic clades, one light and the other dark, corresponding to Maui-Oahu group and Maui-Hawai'i Island group, respectively.

**Clade F** (Fig. 3): Clade F (BS = 82, PP = 1) comprises several species in the former *Nesophrosyne* (*Nesoreias*) subgenus. All species are associated with the plant genus *Psychotria* (Rubiaceae). The basally diverging species, "N. sp.215", is endemic to O'ahu, while the rest are endemic to Hawai'i Island (e.g., *N. insularis*, *N. eburneola*, and *N. marginalis*: BS = 98, PP = 1).

**Clade G** (Fig. 3): Clade G, aside from two species with unknown host associations, is restricted to plant genera in the plant family Rubiaceae. The genus level host associations are predominantly split between *Coprosma* and *Hedyotis*. Species are spread throughout all high islands, with basally diverging lineages found on Maui and restricted to *Coprosma* (BS = 100, PP = 1). The Kaua'i species, *N. umbratilis*, is evidently associated with *Psychotria* and *Hedyotis*, and placed in a clade with Moloka'i and Maui species with unknown host associations (BS = 43, PP = .70). The remainder of Clade G forms highly supported, reciprocally monophyletic groups comprising parallel radiations associated with *Hedyotis* (BS = 97, PP = 1) and *Coprosma* (BS = 100, PP = 1). The *Hedyotis* associated clade has a Maui Nui and Hawai'i Island distribution, while the *Coprosma* associated clade is restricted to Hawai'i Island. Species in the *Coprosma* associated clade (e.g., *N. pluvialis*, "N. sp.278",

and "N. sp. 280") form morphologically cryptic species restricted to single volcanoes and volcanic features (e.g., volcanic flanks).

**Clade H** (Fig. 3): Clade H comprises the only species associated with *Metrosideros polymorpha* (Myrtaceae: BS = 56, PP = 1). Species are spread from O'ahu through Hawai'i Island. O'ahu species demonstrate endemicity according to volcanic ranges (BS = 93, PP = 1). Species further demonstrate dramatic external morphological evolution: *N. notatula* is a uniquely brown speckled species, while "N. sp.36" and *N. sp.345* are reddish in color without extensive color patterning.

**Clade I** (Figs. 3 and 5): Clade I comprises species associated with plant genus *Coprosma* (Rubiaceae: BS = 69, PP = .99), occurring on Moloka'i, Maui, and Hawai'i Island. Species relationships are all highly supported (BS = 80–100, PP = .99–1). Maui species are rendered paraphyletic by "N. sp.225" on Moloka'i and the Hawai'i Island endemics. Hawai'i Island species constitute a monophyletic group (BS = 92, PP = 1) and are single-volcano endemics. Species in Clade I are similar morphologically, and indistinguishable in some instances (e.g., "N. sp.302", "N. sp.306", and "N. sp.277"). *Nesophrosyne haleakala*, restricted to the subalpine Haleakala crater floor on Maui, is the only brachypterous species with a thickened cuticle.

**Clade J** (Fig. 3): Species in Clade J are spread from O'ahu through Hawai'i Island (BS = 48, PP = 91). Species demonstrate extensive host transitions among 10 plant genera in 7 families (e.g., Rubiaceae: *Coprosma* & *Hedyotis*, Campanulaceae: *Lobelia* & *Clermontia*, Hydrangeaceae: *Broussaisia*, Gesneriaceae: *Cyrtandra*, etc.). Branch support is mixed, with many branches receiving low support, resulting in a polytomy that contains species endemic to O'ahu,



Moloka'i, and Maui (e.g., *N. monticola*, “*N. sp.189*”, and “*N. sp.137*”). Several clades receive moderate to high support, which include: A *Coprosma* associated group, endemic to Moloka'i and Maui, including the previously described *N. anguilifera* and *N. obliqua*, which share unique external markings, but differ dramatically in head morphology (BS = 100, PP = 1); a low-moderately supported Maui Nui clade with diverse host associations including *N. furculata*, which has a multi-host association with *Coprosma* and *Lobelia* (BS = 24, PP = .92); a moderately supported O'ahu endemic clade (BS = 58, PP = .93) with multiple host associations (e.g., *N. oreadis* on the host-plant Thymelaeaceae: *Wikstroemia*, and *N. monticola* on *Coprosma*); and, finally, a highly supported Maui Nui Clade (BS = 100, PP = 1), with extremely unique host associations, including the single association of “*N. sp.132*” with *Sadleria* fern (Blechnaceae), “*N. sp.358*” with *Lobelia*, and “*N. sp.137*” with *Cyrtandra*, each with unique adaptive morphology (Zimmerman, 1948).

**Clade K** (Figs. 3 and 5): Species in Clade K extend throughout the Hawaiian archipelago, except Kaua'i, and are associated with the genus *Melicope* (Rutaceae: BS = 28, PP = .99). Species level relationships are highly supported (BS = 68–100, PP = .98–100). O'ahu endemic, *N. palolo*, forms the most basally diverging lineage for the group (BS = 28, PP = .99). Species on O'ahu and Maui do not constitute single clades by island (BS = 70–92, PP = .98–1), while species occurring on Hawai'i Island are a monophyletic group and have radiated among volcanic peaks (BS = 100, PP = 1). Members of this group have transitioned among different *Melicope* species in this range. External morphological coloration varies extensively between islands, but is cryptic within islands.

**Clade L** (Fig. 3): Species in Clade L are endemic to the older Islands, Kaua'i and O'ahu (BS = 3, PP = .99). Clade and species support is high, with host-plant associations mixed and partly unknown. The O'ahu species are placed sister to each other (BS = 100, PP = 1), and both associated with *Nestegis* (Oleaceae). Species endemic to Kaua'i form a highly supported clade (BS = 100, PP = 1), and the two known host associations include *Psychotria* (Rubiaceae) and *Wikstroemia* (Thymelaeaceae).

**Clade M** (Fig. 3 and 5): Clade M, the *kanawao* species group, is the only host-associated clade that has been described in *Nesophrosyne* (Bennett and O'Grady, 2011). Species in this group are restricted to the multi-island endemic plant species, *Broussaisia arguta* (Hydrangeaceae) across all high islands (BS = 68, PP = .99), and are the only taxa of *Nesophrosyne* known from *B. arguta* except *N. heopoko* on Kaua'i (Figs. 3 and 4, Clade A) and *N. magnacai* on Moloka'i (Fig. 3, Clade J), which represent independent host transitions. All species constitute single-volcano endemics (BS = 76–100, PP = .94–1). Species form monophyletic, morphologically cryptic groups according to island (Bennett and O'Grady, 2011).

**Clade N and O** (Figs. 3 and 4): Species in Clade N form a multi-island radiation, excluding Kaua'i, in which host-plant transitions are common (BS = 61, PP = 1). Internal species-level support is mixed (BS = 13–100, PP = .66–1), with most relationships receiving high support. Clade N comprises three groups with unique associations across multiple distantly related host-plant genera, including a paraphyletic multi-island association with *Psychotria* (Rubiaceae: BS = 28–77, PP = .85–1), a Maui restricted clade with mostly unknown host associations (a single association with Dipentodontaceae: *Perrottetia*: BS = 100, PP = 1), and a Hawai'i Island restricted radiation on *Clermontia* (Campanulaceae: BS = 100, PP = 1). Clade O represents a monophyletic association of species associated strictly with the plant genus *Myrsine* (Myrsinaceae: BS = 100, PP = 1). Species in each group form single-volcano endemics. A highly supported fifth group, restricted to O'ahu, has specialized on at least four different plant genera: *Melicope* (Rutaceae), *Scaevola* (Goodeniaceae), *Hedyotis* (Rubiaceae), and *Cloaxylon* (Euphorbiaceae), all with high support (BS = 93–100, PP = .94–100).

## 4. Discussion

### 4.1. *Nesophrosyne* origin and higher taxonomic relationships

Determining the origin for endemic Hawaiian radiations is difficult due to the geographic and temporal scale of the problem, which encompasses at least the entire Pacific Basin and millions of years. As a result, the origins for several of the largest endemic Hawaiian insect radiations remain unknown (e.g., *Drosophila* and *Hypomocoma* moths: Rubino, 2008; O'Grady and DeSalle, 2008). Phylogenetic results presented here, support the hypothesized sister relationship and shared origin between *Orosius* and *Nesophrosyne* (Fig. 2: Ghauri, 1966; Linnavuori, 1975). The genitalic characters for species in each genus are morphologically similar, which led to a temporary synonymy of the two (reviewed in Bennett and O'Grady, 2011). Previous Hawaiian workers suggested other potential sister groups to *Nesophrosyne*, including *Osbornellus*, *Scaphoideus*, and *Nephotettix* (Kirkaldy, 1910; Osborn, 1935; Zimmerman, 1948), which do not share internal genitalic characters with *Nesophrosyne* and are placed in distantly related tribes or unnamed groups (Ghauri, 1971; Barnett, 1976; Dominguez and Godoy, 2010; Zahniser and Dietrich, 2010).

The inferred shared ancestry of *Nesophrosyne* and *Orosius* provides the potential to biologically and evolutionarily contextualize species diversification on Hawai'i. *Orosius* is widely distributed throughout Pacific-Oceania region, South East Asia, and Africa (Ghauri, 1966). Many species are highly dispersive, with geographically wide distributions (e.g., *O. argentatus*: Oceania through Africa), and are polyphagous economic pests, vectoring a number of plant diseases (Larsen and Walter, 2007). Plant associations are largely unknown for *Orosius* species; however, the economically important species, *O. argentatus* and *O. orientalis*, feed broadly across many plant families (Ghauri, 1966; Grylls, 1979; Larsen and Walter, 2007). These traits, shared among of some *Orosius* species (Ghauri, 1966), increase the likelihood of long-distance dispersal to remote archipelagos and post-colonization success. Thus, evidence suggests *Nesophrosyne* may have originated from a widespread, polyphagous ancestor.

An inferred Western Pacific origin for *Nesophrosyne*, based in part on the natural distribution of *Orosius*, is further supported by the placement of the genus *Lampridius* (Tribe: Opsiini) from Thailand, sister to the *Orosius*-*Nesophrosyne* clade (Fig. 2). Arrival to Hawaii from the West suggests two potential routes for long-distance dispersal: aerial dispersal along the East-West Sub Tropical Jet Stream (STJS), or oceanic dispersal through flotation. Wind-dispersal along the STJS is predicted for taxa that can be easily swept into and carried by high-altitude winds (Geiger et al., 2007; Gillespie et al., 2011). While cicadellid species demonstrate low dispersal rates (Nielson and Knight, 2000), they are capable of flight, easily swept up in major wind currents, and some are known to be migratory (Nault and Rodriguez, 1985). Gillespie et al. (2011) argue that aerial dispersal is unlikely in animal taxa due to harsh conditions, and lineages with a western origin alternatively relied on dispersal via oceanic currents. This route is available to insects like members of *Nesophrosyne*, which deposit their eggs directly into plant leaves or otherwise spend part of their life stages in vegetation. This medium can provide a buffer against harsh seawater, especially if the leaves are part of a larger mass of vegetative material. However, this mode of dispersal requires a potentially prohibitive 2–3 week journey by sea, whereas aerial dispersal along the STJS can be accomplished in 2–4 days (Geiger et al., 2007; Gillespie et al., 2011).

### 4.2. *Nesophrosyne* diversity and endemism

*Nesophrosyne* is a monophyletic radiation of at least 190 species identified in this study, which represents the most comprehensive

assessment to date. Given that some described species were not collected here, the extant species diversity is likely to be well over 200. Historically, 72 species have been described, but unfortunately much of the original taxonomic work is flawed, which has confused previous understanding of *Nesophrosyne* diversity and classification (Zimmerman, 1948; Bennett and O'Grady, 2011). Results from this study illuminate previously unknown species diversity, providing clear ecological and evolutionary background for species delimitation and genus-wide revision. *Nesophrosyne* is the only endemic leafhopper group to have radiated into hundreds of species.

Among the herbivorous hemipteroid groups endemic to Hawai'i, *Nesophrosyne* is perhaps the most diverse, exceeding endemic Fulgoroidea planthoppers (Roderick and Percy, 2008; Hoch, 2006; Asche, 1997) and the largest planthopper genus, *Orthotylus* (Miridae: Polhemus, 2011). Levels of endemism are exceptionally high, with species forming single-island endemics, with the exception of only a few that are more widespread. Lineages further demonstrate high levels of local endemism, according to geologic sub-partitioning of islands vis-à-vis volcanic peaks and shield flanks, landslides, and eroded valleys. This result provides insights into the unique biogeographic provinces of the Hawaiian Islands. For example, Haleakala Crater (Maui) has a diversity of locally endemic species, including *N. kaupoi*, *N. haleakala*, and "*N. sp.48*", among others, each specializing on unique host-plants (Fig. 3 and SI Tables 1 and 2). The high degree of local endemism exhibited by *Nesophrosyne* is found in other taxonomic groups of arthropods (e.g., *Hyposmocoma*, *Orthotylus*, and *Mecyclothorax* beetles: Rubinoff, 2008; Polhemus, 2011; Liebherr, 2011), and is of critical importance in understanding and conserving Hawaiian biodiversity.

#### 4.3. Cryptic sibling species

The occurrence of cryptic sibling species in *Nesophrosyne* has been previously demonstrated in the *kanawao* species group associated with *Broussaisia arguta* (Figs. 3 and 5, Clade M: Bennett and O'Grady, 2011). Species in this group are morphologically indistinguishable, demonstrating high levels of local endemism and genetic divergence. This pattern is repeated among *Nesophrosyne* clades specializing on other widespread host-plants, occurring in nearly every clade (Fig. 3), including A (*Pipturus*), D (*Myoporum*) E, (*Dodonaea*), G (*Hedyotis* and *Coprosma*), I (*Coprosma*), K (*Melicope*), M (*Broussaisia*), and N (*Psychotria* and *Clermontia*). Cryptic species groups comprise sibling species distributed between islands and volcanoes, generally restricted to plant lineages. The existence of cryptic species has been established for few other Hawaiian groups, especially to this degree and scale (but see Mendelson and Shaw, 2005). To our knowledge, the presence of replicated clades of cryptic species, as a result of the confluence of ecological and biogeographic processes, has not been demonstrated among other leafhopper genera. Undoubtedly, cryptic sibling species are much more common than currently understood in cicadellids and Hawaiian insects.

Several operating forces may drive the formation and maintenance of cryptic groups on island archipelagoes, including maintained selection for host-plant specialization across a geographic range, and behavioral mating cues (Bickford et al., 2006). Specialization on host-plants requires complex morphological, behavioral, and physiological adaptations to establish host preference and to overcome host defenses (Schoonhoven et al., 2005; Futuyma and Agrawal, 2009; Denno and Kaplan, 2007). Thus, selection to maintain host specialization can sustain morphological stasis in lineages that have secondarily dispersed to different islands or volcanoes, but occupy the same fundamental niche (Bickford et al., 2006; Schonrogge et al., 2002). On the other hand, behavioral selection on non-visual mating cues can also sustain morphological

stasis in speciating lineages by creating rapid and lasting barriers between established populations, requiring little or no morphological change (Henry, 1994). This pattern has been documented in other hemipteroid groups, like *Nesophrosyne*, which use abdominal membranes to sing (Henry, 1994; Sueur and Puissant, 2007). While undoubtedly important, further study is required to tease apart sexual mechanisms in *Nesophrosyne* diversification.

#### 4.4. Host-plant use in *Nesophrosyne*

Osborn (1935) noted the importance of the native Hawaiian flora in forming ecological opportunities for established native herbivorous insects. *Nesophrosyne* species are highly host-plant specific and have diversified extensively among endemic dicotyledons, which is unique in the leafhopper subfamily Deltocephalinae that is largely known for associations with grasses and sedges (Zahniser and Dietrich, 2010). Patterns observed in *Nesophrosyne* are consistent with what is known about other endemic Hawaiian herbivorous insect lineages. The genus *Nesosydne* (Delphacidae) demonstrates similarly high host-plant specificity, and has radiated among 28 plant families from a hypothesized monocot-restricted ancestor (Roderick and Percy, 2008; Roderick, 1997). The genus *Orthotylus* (Miridae) contains many highly host-plant specific species, utilizing at least 16 plant families (Polhemus, 2011). Other mirid genera, *Sarona*, *Nesimiris*, and *Cyrtolepis*, are all thought to have expanded their host breadth from a host specific origin (reviewed in Roderick and Percy, 2008).

The broad patterns of host-plant specialization are complex in Hawaiian herbivores, resulting from a complicated interplay between host-plant specialization, host-plant transitions, and island geography. Host specialization requires morphological, behavioral, and physiological adaptations in response to plant defenses, morphology, chemistry, inter-specific interactions with other insect groups, and local environmental conditions, requiring careful study to disentangle (reviewed in Schoonhoven et al., 2005; Futuyma and Agrawal, 2009; Denno and Kaplan, 2007). Unfortunately, the interplay between host-plant use and island geography remains unclear with the lack of resolution of many of the endemic plant and insect lineages for comparison with results presented here (Roderick and Percy, 2008). Despite this situation, four dominant patterns emerge from species diversification and host specialization of *Nesophrosyne* that can provide novel insights into Hawaiian herbivore evolution: (1) diversification through host-plant switching; (2) allopatric diversification within a host-plant lineage; (3) repeated, independent shifts to the same host-plant lineages; (4) and, absence or low abundance on some host-plant lineages.

##### 4.4.1. Diversification through host-plant switching

Transitions to different native host-plant families, genera, and species play a significant role in the diversification and distribution of *Nesophrosyne* species. *Nesophrosyne* shows little evidence of being broadly restricted to evolutionarily similar plant lineages as seen in some other island groups (e.g., psyllids and *Epicephala* moths: Percy et al., 2004; Hembry et al., 2011), or to have co-evolved with particular host taxa (Thompson, 1989). Instead, host-plant switching in *Nesophrosyne* operates across geographic and temporal scales, and at the clade and species levels (Figs. 3–5). Host shifting can result in dramatic and rapid species diversification, extensive morphological diversity, and the expansion of species' biogeographic distributions (Cocroft et al., 2008) as seen in *Nesophrosyne*.

Percy et al. (2004) posited three potential factors that govern host-plant switching in island taxa: (1) open niche space, (2) host-plant abundance, and (3) geographic proximity of host lineages. Oceanic islands are formed *in situ* and therefore present successfully colonizing lineages with open dynamic niche space

(Wagner and Funk, 1995). Over time, these landscapes evolve into lush species-rich forests, providing a diverse array of potential host–plants for radiating insect herbivores. The endemic Hawaiian flora has arrived periodically throughout the formation of the islands, radiating into exceptionally diverse groups that have secondarily lost dispersal abilities and plant defenses, thus providing novel opportunities for insect adaptation (Givnish, 1998; Price and Clague, 2002). This dynamic landscape of host niches presents unparalleled opportunity for ecological release in island insects, and endemic herbivores have routinely capitalized on this opportunity (Roderick and Percy, 2008).

Host–plant abundance and proximity are both important factors in the diversification of *Nesophrosyne*. Species have diversified among some of the most dominant plant families, genera, and species with widespread distributions throughout Hawai'i (e.g., *Psychotria*, *Coprosma*, *Broussaisia*; Wagner et al., 1999). For example, many of the basal Kaua'i lineages demonstrate localized host switching among co-occurring, locally dominant plant genera in the plant family Urticaceae (Figs. 3 and 4, Clade A) and between seven proximally abundant host–plant families (Fig. 3, Clade B). Similar patterns occur extensively among locally abundant plant groups on the younger island, O'ahu (Fig. 3, Clades D and N). On the youngest island, Hawai'i, multiple clades demonstrate local host shifts between ecologically dominant hosts, including Clade N (shifts from *Psychotria* to *Clermontia*, which co-occur in similar habitats) and Clade C (host transitions between five plant genera each in different families). The repeated pattern of host–plant transitions on both old and young islands, and especially across large evolutionary distances between hosts, indicates that this pattern is a continually operating driver of diversification in *Nesophrosyne*.

#### 4.4.2. Specialization on widespread host lineages and allopatric speciation

Osborn (1935) predicted that *Nesophrosyne* lineages initially adapt to novel hosts, and subsequently diversify in allopatry according to island geography. Under this hypothesis, species specializing on the same host lineage throughout multiple islands are expected to be closely related and was found among the *kanawao* species group associated with *Broussaisia arguta* (Bennett and O'Grady, 2011). This prediction is born out in other large *Nesophrosyne* clades associated with widespread, monotypic host–plant lineages, including *Dodonaea viscosa* (Clade E), *Pipturus albidus* (A), and *Myoporum sandwicense* (D). This pattern further emerges among the widely diversified host genera *Melicope* (K), *Coprosma* (I), *Psychotria* (C, F, and N), and *Myrsine* (O). This pattern highlights the important role of island geography in species diversification, as Island topography has promoted allopatric species diversification across volcanic ranges. Several clades have basally diverging lineages on younger islands (e.g., Clades I and E), while others have descended down the chain from older islands in a progression-rule pattern (e.g., Clades A and E; Wagner and Funk, 1995), further demonstrating the ongoing, dynamic diversification of *Nesophrosyne* among the Hawaiian biota. This process apparently requires little morphological change and has resulted in multiple clades of genetically distinct, morphologically cryptic species described above.

#### 4.4.3. Repeated, independent specialization on host lineages

*Nesophrosyne* has transitioned and specialized on the same or similar host–plant types multiple independent times and on different islands. This pattern is observed in several widespread host lineages, including *Clermontia* spp., *Dodonaea viscosa*, and *Broussaisia arguta*, among others (Figs. 3–5, Clades N, E, M). *Nesophrosyne* species have transitioned to wet forest species in the plant genus *Clermontia* at least three independent times, each on different islands. Species specializing on *Dodonaea* and *Broussaisia* form clades

spanning O'ahu through Hawai'i Island, but demonstrate independent transitions to that niche on Kaua'i. This pattern may be the result of the relative isolation of Kaua'i from other islands (approximately 128 km versus a maximum of 48 km for other inter-island distances), indicating a possible barrier limiting dispersal to Kaua'i. However, this pattern needs to be more closely examined in other groups.

The repeated, independent specialization among host–plants is analogous with the 'ecomorph' concept, which has been invoked in an adaptive radiation context to describe congeneric species that independently evolve to fill or specialize in similar ecological niches, such as the same host–plant lineages on different islands (Gillespie, 2004; Yoder et al., 2010). Ecomorph patterns are also observed in the endemic Hawaiian *Tetragnatha* spider radiation (Gillespie, 2004), and suggest that local adaptation is more easily accomplished to fill niches than dispersal by specialized lineages from different islands. In low dispersing groups with high levels of endemism, this pattern is expected between islands, especially if niche space is open and abundant, as are many host–plant lineages (e.g., *Dodonaea* and *Broussaisia*). Ecomorph formation in island herbivores may be facilitated by inter-specific interactions with other insects, host–plant morphology, and host chemistry (Schoonhoven et al., 2005; Futuyma and Agrawal, 2009), requiring locally sustained interactions for adaptation to occur. This process would preclude dispersal from adjacent islands to fill that niche.

#### 4.4.4. Absence or low abundance on host–plants

*Nesophrosyne* demonstrates a general absence from the plant families Asteraceae and Fabaceae, and a comparatively low abundance on Campanulaceae, the genus *Cyrtandra* (Gesneriaceae), and *Metrosideros polymorpha*. These lineages represent some of the oldest, most species-rich, or most ecologically dominant endemic Hawaiian plant groups (Wagner et al., 1999; Givnish et al., 2009). While *Nesophrosyne* may utilize these hosts undetected, collections (or lack thereof) made here corroborate a similar pattern among historical collections of observed absence and low relative abundance when compared to specialization on other hosts (SI Table 2). Direct comparison of host-specialization patterns between *Nesophrosyne* and other endemic herbivore lineages suggests the intriguing possibility that insect–insect interactions play a large role in the exclusion of *Nesophrosyne* from some hosts.

Competition occurs between lineages requiring similar resources, with recent work suggesting that plant-mediated interactions (e.g., plant defenses) can facilitate indirect competition between non-interacting organisms (reviewed in Denno and Kaplan, 2007). This process can lead to the asymmetric exclusion of insects from some plant species or entire groups (Denno and Kaplan, 2007). The potential for competition among Hawaiian herbivores is strong, given that several other hemipteroid radiations have specialized broadly on overlapping host–plants. For example, *Nesophrosyne* is surprisingly rare and restricted in its association with *Metrosideros polymorpha*, one of the most ecologically dominant plants throughout the archipelago (Wagner et al., 1999). Although *Nesophrosyne* does specialize on *M. polymorpha*, it occurs in localized populations, often on only a single plant. *Metrosideros polymorpha* is one of the dominant hosts for endemic gall-forming psyllids (Nishida et al., 1980), and the mosaic exclusion of *Nesophrosyne* can be explained by the impact of gall formation, which alters leaf chemistry, protein content, and leaf morphology (Bagatto et al., 1996).

Of the endemic Hawaiian herbivore groups, only *Nesophrosyne* occupies the same basic niche as *Nesophrosyne* (phloem feeding, oviposition, and nymphal development: Denno and Roderick, 1990), which can lead to direct competition and asymmetric exclusion of both genera from some hosts (Denno and Kaplan, 2007). As predicted under this hypothesis, *Nesophrosyne* species have specialized



broadly on the elements of the Hawaiian flora that *Nesophrosyne* is not known to occupy or has not radiated on extensively, including Asteraceae, Fabaceae, Campanulaceae, and Gesneriaceae (Zimmerman, 1948; Roderick and Percy, 2008). Conversely, *Nesophrosyne* has diversified on plant families that are complementary to those occupied by *Nesosydne*, including Rubiaceae, Goodeniaceae, Myrsinaceae, Rutaceae, Sapindaceae, and Hydrangeaceae, among others (Zimmerman, 1948; Roderick and Percy, 2008). The underlying mechanisms and the degree to which this observation holds across islands are currently unknown, but the patterns help in understanding the distribution and diversification of Hawaiian herbivores, and warrant further study.

## 5. Conclusion

*Nesophrosyne* is perhaps the most diverse herbivore radiation endemic to the Hawaiian Islands, and heretofore one of the least known. Results demonstrate that this exceptional diversification is due to a confluence of processes associated with host–plant specialization. In an adaptive radiation context, it is clear that the ecological opportunity offered by the dynamic diversity of host-plants, drives the diversification of specializing insects, which has remained previously unclear (Yoder et al., 2010). Host–plant associations have promoted at least three parallel processes: (1) extensive host switching, resulting in ecological and morphological diversification; (2) adaptation and retention of host lineages, resulting in extensive allopatric cryptic diversity; and (3) the parallel formation of ecomorphs. Finally, evidence suggests that competing herbivore radiations may restrict and limit ecological opportunity. The result that, in diverse herbivorous groups, multiple evolutionary processes play fundamental roles in species diversification is fundamental for understanding of how adaptive radiations proceed and how biodiversity is organized and maintained. More studies of groups like *Nesophrosyne* are required to determine the extent to which these processes govern herbivorous insect diversity globally.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2012.07.024>.

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