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Diversity despite dispersal: colonization history and phylogeography of Hawaiian crab spiders inferred from multilocus genetic data

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Abstract

The Hawaiian archipelago is often cited as the premier setting to study biological diversification, yet the evolution and phylogeography of much of its biota remain poorly understood. We investigated crab spiders (Thomisidae, *Mecaphesa*) that demonstrate contradictory tendencies: (i) dramatic ecological diversity within the Hawaiian Islands, and (ii) accompanying widespread distribution of many species across the archipelago. We used mitochondrial and nuclear genetic data sampled across six islands to generate phylogenetic hypotheses for *Mecaphesa* species and populations, and included penalized likelihood molecular clock analyses to estimate arrival times on the different islands. We found that 17 of 18 Hawaiian *Mecaphesa* species were monophyletic and most closely related to thomisids from the Marquesas and Society Islands. Our results indicate that the Hawaiian species evolved from either one or two colonization events to the archipelago. Estimated divergence dates suggested that thomisids may have colonized the Hawaiian Islands as early as ~10 million years ago, but biogeographic analyses implied that the initial diversification of this group was restricted to the younger island of Oahu, followed by back-colonizations to older islands. Within the Hawaiian radiation, our data revealed several well-supported genetically distinct terminal clades corresponding to species previously delimited by morphological taxonomy. Many of these species are codistributed across multiple Hawaiian Islands and some exhibit genetic structure consistent with stepwise colonization of islands following their formation. These results indicate that dispersal has been sufficiently limited to allow extensive ecological diversification, yet frequent enough that interisland migration is more common than speciation.

Keywords: Hawaii, mitochondrial DNA, nuclear DNA, phylogeny, phylogeography, Thomisidae

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Introduction

Oceanic islands formed *de novo*, such as those produced by volcanic activity, accumulate biological diversity solely by colonization and subsequent within- or between-island diversification (Gillespie & Roderick 2002; Whittaker & Fernández-Palacios 2007). The relative importance of these evolutionary processes is largely determined by an island's degree of isolation. Situated approximately 3200 km from the nearest continent, the Hawaiian archipelago is the world's most isolated landmass, with natural colonization being rare and restricted to organisms possessing exceptional dispersal capabilities (Gressitt 1956). This isolation appears to have provided available 'ecological opportunity' for the limited number of organisms that successfully colonized and established populations within the remote Hawaiian archipelago (Carlquist 1974). The ecological opportunity in turn has allowed some successful colonists to undergo lineage diversification in association with adaptive divergence of morphological traits that permit the descendents to exploit a variety of resources (Schluter 2000; Gillespie *et al*. 2001). Consequently, assemblages of closely related, yet phenotypically and ecologically diverse, species dominate the archipelago, as illustrated by

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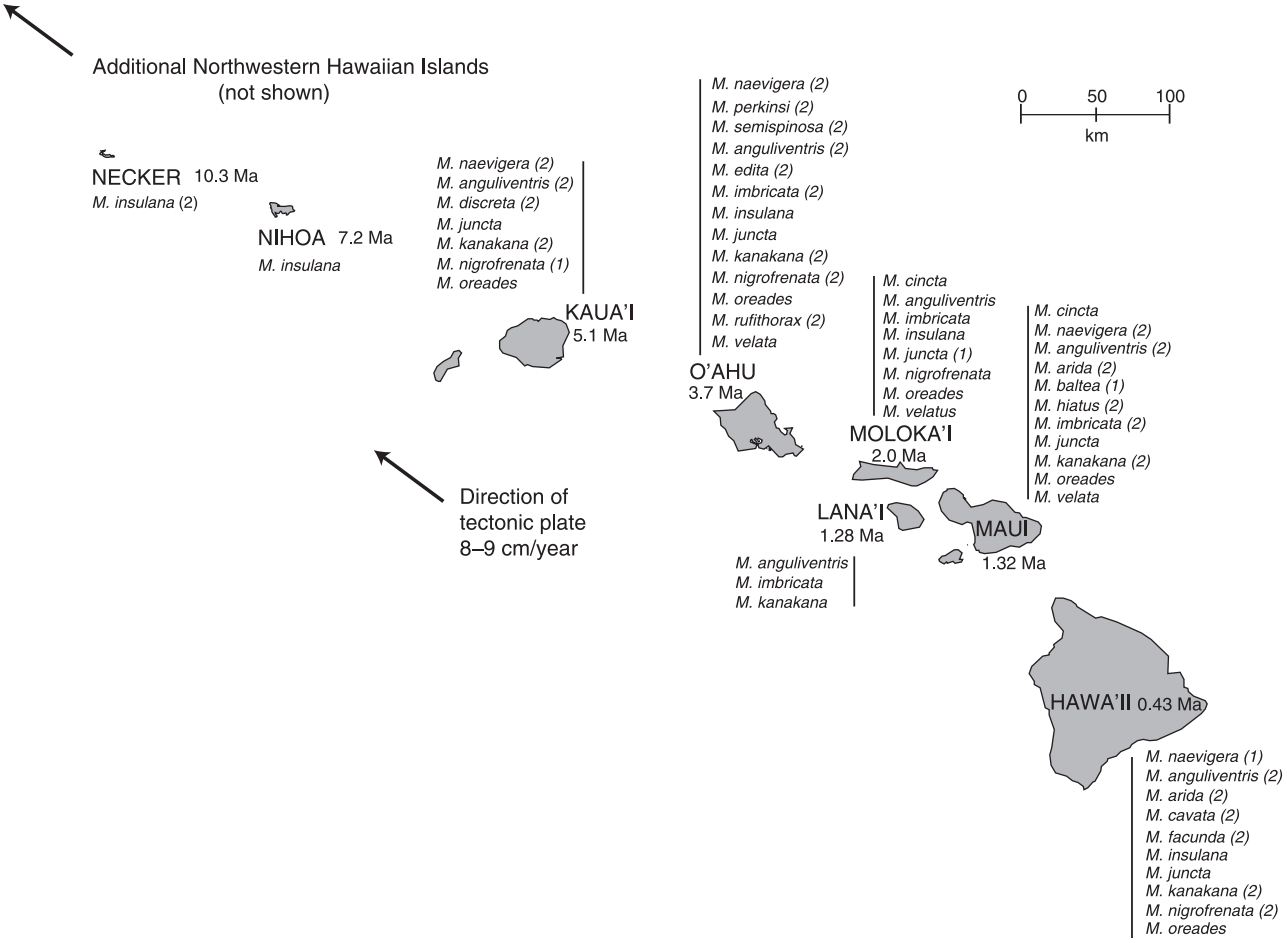

Fig. 1 Distributional map of the Hawaiian *Mecaphesa* (Thomisidae) after Suman (1970) and Garb (2003). Numbers following species names indicate number of individuals sampled for this study from each island. Island ages as reported in Clague (1996).

the spectacular radiations of Hawaiian drosophilid flies (O'Grady & DeSalle 2008), drepanidine honeycreepers (Tarr & Fleischer 1995), silverswords (Baldwin & Robichaux 1995), achatinelline tree snails (Holland & Hadfield 2004), and *Tetragnatha* spiders (Gillespie 2004).

A variety of geological processes have caused the Hawaiian archipelago to become richly dissected by geographic boundaries, such that lineage splitting is also strongly influenced by repeated colonization or vicariance events within and across islands (Funk & Wagner 1995; Vandergast *et al*. 2004). Islands of the archipelago began forming in an assembly-line manner approximately 70 million years ago at a fixed volcanic plume or 'hot spot' (Clague 1996). As the Pacific tectonic plate moves northwestward, magma extruded at the hot spot repeatedly produces new islands (Fig. 1). Because of their sequential arrangement the Hawaiian Islands provide a temporal framework for investigating consecutive stages of evolution, a general premise being that islands are successively colonized in a stepping-stone fashion following their formation, resulting in a phylogeographic pattern termed the 'progression rule' (Funk & Wagner 1995). The availability of known ages of the Hawaiian Islands, obtained from potassium-argon dating (e.g. Clague 1996), also supplies time ranges for estimating colonization dates and speciation rates from phylogenetic hypotheses (Fleischer *et al*. 1998; Hormiga *et al*. 2003; Jordan *et al*. 2003).

The present study examines evolutionary relationships among Hawaiian crab spiders (Thomisidae), one of the few species-rich spider lineages in the archipelago (Gillespie *et al*. 1998). Thomisids are characterized as ambush predators that employ colour camouflage for substrate matching, presumably for prey capture and/or predator avoidance (Oxford & Gillespie 1998). Perhaps the best-known thomisid species are flower-specialists that can reversibly change their coloration to match different floral colours and prey on pollinating insects (Heiling *et al*. 2003). Although the ability of individuals to change coloration has never been observed in Hawaiian thomisids, a wide range of pigmentation is exhibited across these species (e.g. green,

brown, white, black or yellow; Suman 1970). This interspecific colour variation appears to reflect specialization to mimic different microhabitats. For example, certain Hawaiian species are restricted to mosses, others to lichens, leaves, etc., and are cryptically coloured against these substrates (Garb 2003).

Thomisid crab spiders are represented in the Hawaiian Islands by 20 endemic species that occupy a complex geographic distribution. Compared to the majority of terrestrial lineages in which species tend to be single-island endemics (Cowie & Holland 2008), thomisids are somewhat unusual in that ~50% of all species occur on two or more islands (Fig. 1), implying frequent movement between islands. At the same time, the ranges of many species overlap, with apparent coexistence of multiple species at several localities (Garb 2003), suggesting that dispersal has been sufficiently restricted as to allow differentiation and adaptation (Givnish *et al*. 2009). Indeed, the substantial interspecific morphological variation led earlier systematists to assign the species to multiple genera (Simon 1900; Suman 1970). The current study addresses this puzzle by examining the following hypotheses to explain the high diversity yet widespread distributions of Hawaiian crab spider species: (i) species designations have been confounded by convergence, such that species that appear to be widespread are not; (ii) thomisids have colonized the archipelago multiple times and several independently derived lineages subsequently became widespread; or (iii) thomisids have colonized the archipelago once, but intra-archipelago migration has been more frequent than speciation. We address these hypotheses using mitochondrial (ribosomal 16S, tRNA leu, NADH1 and cytochrome oxidase I) and nuclear (elongation factor 1-α) gene sequences densely sampled within and among species to examine inter- and intraspecific phylogenetic relationships, genetic distance and divergence dates from relaxed molecular clock methods. Further, we reconstruct the ancestral distributions of Hawaiian thomisids to infer geographic points of colonization and to assess the temporal sequence implied by estimated divergence dates.

Materials and methods

Sampling

The spider family Thomisidae comprises over 2000 species worldwide and has been divided into seven subfamilies, the largest being Thomisinae. The subfamily Thomisinae again is divided into 13 tribes, the largest being Misumenini (*sensu* Ono 1988), which contains 19 genera, including *Mecaphesa*, *Misumenops*, and *Misumenoides*. The genus *Mecaphesa* is comprised of 48 species and is distributed in the Hawaiian Islands (20 spp.), North and Central America (26 spp.), the Galapagos (1 sp.) and Juan Fernandez Islands (1 sp.) (Platnick 2008). Lehtinen (1993) proposed that all Hawaiian species of *Mecaphesa* represented an adaptive radiation resulting from 'explosive' speciation in the archipelago. Previous molecular phylogenetic studies have supported this hypothesis of intra-archipelago radiation (Garb 1999; Garb & Gillespie 2006). However, conclusions regarding the degree of insular radiation and interspecific relationships were uncertain due to inadequate sampling of characters and taxa. Here, we sampled 82 specimens including 58 from the Hawaiian Islands, corresponding to 17 of the 20 Hawaiian *Mecaphesa* species (Table 1). Two Hawaiian specimens (nos137 and 705; Table 1) could not be diagnosed as any of the described species using Suman's (1970) key and are putatively considered one or two undescribed species. For each species, we included two individuals from every island where it occurs whenever possible. Garb & Gillespie (2006) found evidence for a close relationship between Hawaiian *Mecaphesa* and *Misumenops* from the Marquesas (1 sp.) and Society Islands (2 spp.). We included these *Misumenops* species and six North American *Mecaphesa* species. Selection of outgroups relied on phylogenetic hypotheses in Garb & Gillespie (2006) that included a greater diversity of thomisid species from the same tribe (Misumenini), and was rooted with a species in a different tribe (Coriarachnini *sensu* Ono 1988) from the same subfamily. Garb & Gillespie (2006) found strong support for *Misumenops pallidus* (Argentina), *Misumenops rapaensis* (Austral Islands), *Misumenoides formosipes* (North America) and *Diaea praetexta* (Tonga) being outside of the ingroup taxa. These species are included as outgroups in our analyses. For convenience, we refer to species of *Misumenops* and *Mecaphesa* as '*Mi*.' and '*Me*.,' respectively.

DNA sequence data

Mitochondrial (mt) and nuclear genomic DNA was extracted using the phenol-chloroform preparation of Palumbi *et al*. (1991) or the QIAGEN DNeasy Tissue kit (QIAGEN, Inc.). Specimens were retained as vouchers in 70% ethanol and deposited in the University of California Berkeley Essig Museum of Entomology. Two fragments of the mitochondrial genome were amplified by polymerase chain reaction (PCR): (i) an ~820-bp section of cytochrome oxidase I (COI), and (ii) an ~600-bp fragment spanning part of ribosomal RNA 16S, tRNA leu and NADH dehydrogenase subunit I (henceforth referred to as 16S-ND1). Primers and conditions used to amplify these fragments are listed in Table 2 and detailed in Garb & Gillespie (2006). We obtained sequences of elongation factor 1-∝ (EF1-∝), a nuclear protein-coding gene frequently utilized in arthropod molecular systematics due to its conserved nature (e.g. Hedin & Maddison 2001; Magnacca & Danforth 2007). Some arthropods possess a second copy of the EF1-∝ gene (Danforth & Ji 1998). In jumping spiders (Salticidae) one

Table 1 Specimens sampled in this study, collecting locality and GenBank Accession numbers

ID no.*	Species	Collecting locality	r16S-ND1	COI	EF1-α
Hawaiian taxa					
007	*Mecaphesa anguliventris*	Kauai Island: Alakai Swamp	FJ590878	FJ590772	NA
024	*Mecaphesa anguliventris*	Maui Island: West Maui, Pu'u Kukui	FJ590879	FJ590773	FJ590817
097	*Mecaphesa anguliventris*	Hawaii Island: Mauna Kea, Hakalau NWR	FJ590885	FJ590779	FJ590823
099	*Mecaphesa anguliventris*	Oahu Island: Waianae Mountains, Mount Ka'ala Summit	DQ174333	DQ174376	FJ590824
104	*Mecaphesa anguliventris*	Hawaii Island: Mauna Kea, Hakalau NWR	FJ590887	FJ590781	FJ590828
112	*Mecaphesa anguliventris*	Oahu Island: Mount Ka'ala Summit	FJ590891	FJ590785	FJ590832
490	*Mecaphesa anguliventris*	Maui Island: West Maui, Pu'u Kukui	FJ590910	FJ590801	FJ590855
650	*Mecaphesa anguliventris*	Kauai Island: Hono'nonapali NARS	FJ590923	FJ590813	FJ590871
261	*Mecaphesa arida*	Maui Island: East Maui, Auwahi	FJ590901	FJ590793	NA
542	*Mecaphesa arida*	Maui Island: East Maui, Auwahi	DQ174342	DQ174385	FJ590860
651	*Mecaphesa arida*	Hawaii Island: Mount Hualalai, Pu'uwa'awa'a	FJ590924	FJ590814	NA
652	*Mecaphesa arida*	Hawaii Island: Mount Hualalai, Pu'uwa'awa'a	FJ590925	FJ590815	NA
557	*Mecaphesa baltea*	Maui Island: East Maui, Auwahi	FJ590914	FJ590805	NA
101	*Mecaphesa cavata*	Hawaii Island: Mauna Kea	DQ174334	DQ174377	FJ590825
107	*Mecaphesa cavata*	Hawaii Island: Mauna Kea	FJ590888	FJ590782	FJ590829
067	*Mecaphesa discreta*	Kauai Island: Alakai Swamp	FJ590881	FJ590775	FJ590819
095	*Mecaphesa discreta*	Kauai Island: Alakai Swamp	DQ174332	DQ174375	FJ590822
103	*Mecaphesa edita*	Oahu Island: Mount Ka'ala Summit	DQ174335	DQ174378	FJ590827
111	*Mecaphesa edita*	Oahu Island: Mount Ka'ala Summit	FJ590890	FJ590784	FJ590831
238	*Mecaphesa facunda*	Hawaii Island: Mauna Loa/Mauna Kea Saddle, Kipuka 3	DQ174338	DQ174381	FJ590838
613	*Mecaphesa facunda*	Hawaii Island: Kohala Mts	FJ590916	FJ590807	FJ590864
263	*Mecaphesa hiatus*	Maui Island: East Maui, Auwahi	FJ590902	NA	NA
554	*Mecaphesa hiatus*	Maui Island: East Maui, Auwahi	DQ174343	DQ174386	FJ590862
153	*Mecaphesa imbricata*	Oahu Island: Ko'olau Mountains, Pauoa Flats	DQ174337	DQ174380	FJ590836
155	*Mecaphesa imbricata*	Oahu Island: Ko'olau Mountains, Pauoa Flats	FJ590895	FJ590789	FJ590837
272	*Mecaphesa imbricata*	Maui Island: West Maui, Iao Valley State Park	FJ590903	FJ590794	NA
348	*Mecaphesa imbricata*	Maui Island: East Maui, Kuhiwa Valley	FJ590904	FJ590795	NA
536	*Mecaphesa insulana*	Necker Island: Summit Hill	FJ590913	FJ590804	FJ590858
537	*Mecaphesa insulana*	Necker Island: Summit Hill	DQ174341	DQ174384	FJ590859
072	*Mecaphesa juncta*	Molokai Island: Pu'u Kolekole	DQ174345	DQ174388	FJ590820
043	*Mecaphesa kanakana*	Oahu Island: Ko'olau Mountains, Wa'ahila Ridge	FJ590880	FJ590774	NA
102	*Mecaphesa kanakana*	Hawaii Island: Mauna Kea	FJ590886	FJ590780	FJ590826
108	*Mecaphesa kanakana*	Hawaii Island: Mauna Kea	FJ590889	FJ590783	FJ590830
237	*Mecaphesa kanakana*	Kauai Island: Mount Kahili	FJ590896	FJ590790	NA
547	*Mecaphesa kanakana*	Oahu Island: Ko'olau Mountains, Manoa Cliff Trail	DQ174347	DQ174390	FJ590861
617	*Mecaphesa kanakana*	Maui Island: East Maui, Lower Waikamoi	FJ590917	FJ590808	FJ590865
618	*Mecaphesa kanakana*	Maui Island: East Maui, Lower Waikamoi	FJ590918	FJ590809	FJ590866
644	*Mecaphesa kanakana*	Kauai Island: Kokee State Park	FJ590919	FJ590810	FJ590867
066	*Mecaphesa naevigera*	Maui Island: missing further information	DQ174336	DQ174379	FJ590818
240	*Mecaphesa naevigera*	Kauai Island: Kokee State Park, Nualolo Trail	FJ590897	NA	NA
241	*Mecaphesa naevigera*	Kauai Island: Kokee State Park, Nualolo Trail	FJ590898	NA	NA
340	*Mecaphesa naevigera*	Oahu Island: Waianae Mountains, Pahole NARS	DQ174344	DQ174387	FJ590841
513	*Mecaphesa naevigera*	Hawaii Island: Mauna Loa, Kipahoehoe NARS	FJ590911	FJ590802	FJ590857
582	*Mecaphesa naevigera*	Maui Island: West Maui, Pu'u Kukui	FJ590915	FJ590806	FJ590863
654	*Mecaphesa naevigera*	Oahu Island: Waianae Mountains, Pahole NARS	FJ590926	NA	NA
068	*Mecaphesa nigrofrenata*	Oahu Island: Waianae Mountains, Pu'upane Ridge	FJ590882	FJ590776	NA
074	*Mecaphesa nigrofrenata*	Hawaii Island: Mount Hualalei	FJ590883	FJ590777	NA
086	*Mecaphesa nigrofrenata*	Kauai Island: Ku'ia NARS	FJ590884	FJ590778	FJ590821
343	*Mecaphesa nigrofrenata*	Hawaii Island: Mount Hualalai, Pu'uwa'awa'a	DQ174340	DQ174383	FJ590842
531	*Mecaphesa nigrofrenata*	Oahu Island: Waianae Mountains, Palikea Ridge	FJ590912	FJ590803	NA
120	*Mecaphesa perkinsi*	Oahu Island: Ko'olau Mountains,Wa'ahila Ridge	FJ590892	FJ590786	FJ590833
132	*Mecaphesa perkinsi*	Oahu Island: Waianae Mountains, Mokule'ia Trail	FJ590893	FJ590787	FJ590834
500	*Mecaphesa rufithorax*	Oahu Island: Ko'olau Mountains, Manoa Cliff Trail	DQ174346	DQ174389	FJ590856
645	*Mecaphesa rufithorax*	Oahu Island: Ko'olau Mountains, Manoa Cliff Trail	FJ590920	FJ590811	FJ590868
245	*Mecaphesa semispinosa*	Oahu Island: Waianae Mountains, Mount Ka'ala Summit	FJ590899	FJ590791	FJ590839
653	*Mecaphesa semispinosa*	Oahu Island: Waianae Mountains, Mount Ka'ala Summit	DQ174339	DQ174382	FJ590872
137	*Mecaphesa* sp.	Maui: West Maui, Mount Eke	FJ590894	FJ590788	FJ590835
705	*Mecaphesa* sp.	Maui: East Maui	FJ590927	FJ590816	NA

Table 1 *Continued*

ID no.*	Species	Collecting locality	r16S-ND1	COI	EF1-α
Non-Hawaiian taxa					
396	*Misumenops melloleitaoi*	Society Archipelago: Mo'orea Island	FJ590906	FJ590797	FJ590845
484	*Misumenops melloleitaoi*	Society Archipelago: Mo'orea Island	DQ174330	DQ174373	FJ590854
369	*Misumenops melloleitaoi*	Society Archipelago: Tahiti Island	FJ590905	FJ590796	FJ590844
368	*Misumenops melloleitaoi*	Society Archipelago: Tahiti Island	DQ174331	DQ174374	FJ590843
460	*Misumenops temihana*	Society Archipelago: Raiatea Island	FJ590908	FJ590799	FJ590849
461	*Misumenops temihana*	Society Archipelago: Raiatea Island	DQ174328	DQ174371	FJ590850
701	*Misumenops temihana*	Society Archipelago: Huahine Island	DQ174329	DQ174372	NA
482	*Misumenops delmasi*	Marquesan Archipelago: Tahuata Island	DQ174326	DQ174369	FJ590853
408	*Misumenops delmasi*	Marquesan Archipelago: Hiva Oa Island	FJ590907	FJ590798	FJ590846
412	*Misumenops delmasi*	Marquesan Archipelago: Hiva Oa Island	DQ174325	DQ174368	FJ590848
473	*Misumenops delmasi*	Marquesan Archipelago: Nuku Hiva Island	FJ590909	FJ590800	FJ590851
479	*Misumenops delmasi*	Marquesan Archipelago: Nuku Hiva Island	DQ174324	DQ174367	FJ590852
646	*Misumenops delmasi*	Marquesan Archipelago: Fatu Hiva Island	FJ590921	FJ590812	FJ590869
657	*Misumenops delmasi*	Marquesan Archipelago: Fatu Hiva Island	DQ174327	DQ174370	FJ590873
647	*Misumenops pallidus*	Argentina: Carrizo Plain	DQ174354	DQ174397	FJ590870
256	*Mecaphesa asperata*	USA: South Dakota	FJ590900	FJ590792	FJ590840
410	*Mecaphesa rothi*	USA: California, San Luis Obispo County	DQ174348	DQ174391	FJ590847
661	*Mecaphesa celer*	USA: California, Riverside County	DQ174350	DQ174393	FJ590876
658	*Mecaphesa importuna*	USA: California, San Diego County	DQ174349	DQ174392	FJ590874
660	*Mecaphesa devia*	USA: California, Riverside County	DQ174352	DQ174395	NA
659	*Mecaphesa* sp.	USA: California, Riverside County	DQ174351	DQ174394	FJ590875
076	*Misumenoides formosipes*	USA: Maryland	DQ174353	DQ174396	FJ590877
649	*Misumenops rapaensis*	Austral Archipelago: Rurutu Island	FJ590922	DQ174427	NA
703	*Diaea praetexta*	Tonga: Vava'u Island	DQ174365	DQ174401	NA

*Code number refers to specimen specific code catalogued in authors' collection database; 'NA' indicates sequences that were not obtained due to PCR/sequencing failure.

Table 2 Primer combinations used in this study

Locus	Primer	Sequence (5′–3′)	Reference
Cytochrome oxidase I	LCOI 1490 (F)	GGTCAACAAATCATAAAGATATTGG	Folmer *et al*. 1994
	C1-N- 2568 (R)	GCTACAACATAATAAGTATCATG	Hedin & Maddison 2001
	LCOI 2198 (R)	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al*. 1994
	C1-J-1718 (F)	GGAGGATTTGGAAATTGATTAGTTCC	Simon *et al*. 1994
	C1-N-2191 (R)	GGTAAAATTAAAATATAAACTTC	Simon *et al*. 1994
16S-ND1	LR-N-12945 (F)	CGACCTCGATGTTGAATTAA	Hedin 1997
	ND1Thom (R)	GAGCTACTCTTCGAATTGATCC	Garb & Gillespie 2006
EF1-∝	efF172 (F)	GAAGAAATCAAGAAGGAAGTTTC	This study
	efR912 (R)	CCTCAGTCAAAGCTTCATGATGC	This study

F, forward; R, reverse.

copy contains introns, while the second does not (Hedin & Maddison 2001). The latter is presumed to be nonfunctional because it contains premature stop codons. We designed thomisid-specific primers efF172 and efR912 (Table 2) to amplify a section of EF1-∝ containing two exons flanking one intron. PCR amplification used a touch-down protocol of 16 cycles of 30 s at 95 °C, followed by 58 °C for 60 s, and 60 s at 72 °C, lowering the temperature 2 °C every three cycles. This was followed by 18 cycles of a 95 °C at 30 s, 60 s at 42 °C, and 60 s at 72 °C. The majority of amplified products corresponded to the intron-containing copy of the EF1-∝, but an intron-containing and intron-less copy were intermittently co-amplified. The larger product was gel-purified and directly sequenced. Occasional difficulties associated with direct sequencing of EF1-∝, likely due to co-amplification of length-variable alleles, required cloning. For these samples, ~5 recombinant clones were screened for positive inserts by PCR followed by

plasmid purification. PCR products were purified using the QIAquick PCR purification kit (QIAGEN, Inc.) and each sample was sequenced in both directions. EF1-∝ chromatograms directly sequenced from PCR products contained some sites exhibiting two peaks of equivalent intensity in forward and reverse directions. Such sites were interpreted as heterozygous positions and were recoded using IUPAC ambiguity codes.

Phylogenetic analyses

Sequences of the different genes were manually aligned. All protein-coding sequences were equal in length and conserved at the amino acid level, making their alignment trivial. 16S and tRNA leu sequences exhibited length variation and included three short sections that could not be unambiguously aligned (31 bp in total) that were excluded from phylogenetic analyses. EF1-∝ intron sequences contained minimal length variability (159–168 bp) and were easily aligned except the intron from *Misumenoides formosipes*, which was substantially longer (280 bp). This species' intron was recoded as missing data, while retaining its exon sequence in analyses. For the three sequence types (16S-ND1, COI and EF1-∝) we computed pairwise uncorrected ('p') and maximum-likelihood (ML) corrected sequence divergence. Homogeneity of nucleotide composition across taxa was tested in PAUP* version 4.0b10 (Swofford 2002) excluding invariant sites.

Phylogenetic trees were estimated using parsimony and Bayesian methods for mitochondrial and nuclear data sets separately and combined. Heuristic parsimony searches were performed in PAUP*, with 10 000 random-taxon-addition replicates of stepwise addition followed by tree-bisection and reconnection branch swapping. All characters were treated as equally weighted and reversible. The mitochondrial data contained three characters with gaps, each uninformative, which were treated as missing data. The majority of variable nuclear characters contained informative gaps and were included as recoded presence/absence characters using the 'simple coding' methods of Simmons & Ochoterena (2000). Branch support was estimated from 1000 bootstrap (BS) replicates and decay indices (DI) with TreeRot version 3 (Sorenson & Franzosa 2007). Substitution models for eight character partitions (1: 16S+tRNA leu; 2–4: mt codon positions 1, 2 and 3; 5–7: EF1-∝ codon positions 1, 2 and 3; 8: EF1-∝ intron) were determined by the likelihood-ratio test in ModelTest 3.7 (Posada & Crandall 1998) and implemented in Bayesian analyses with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). Bayesian analyses including the nuclear data also employed a binary model for recoded gapped characters. MrBayes runs consisted of four chains for at least 5×10^6 generations, sampling a tree every 1000 generations and continued until split frequencies fell below 0.01. Clade posterior probability values were computed from a 50% majority rule consensus of post burn-in trees. Heuristic ML tree searches were conducted in PAUP* for the combined data matrix with 10 random taxon addition replicates, employing one substitution model. The resulting tree was used to test alternative hypotheses, by performing additional ML searches while enforcing topological constraints. Resulting trees were compared to the optimal tree using the S–H test (Shimodaira & Hasegawa 1999), with full optimization and 1000 bootstrap replicates.

Conflict among mitochondrial and nuclear genes was assessed by the incongruence length difference (ILD) (Farris *et al*. 1994), with the partition homogeneity test implemented in PAUP*. Null distributions were created from 1000 replicates, with 10 random taxon addition searches per replicate, removing invariant characters (Cunningham 1997). Trees from mitochondrial and nuclear data sets were compared visually to identify well-supported yet conflicting nodes (i.e. supported > 0.95 PP in Bayesian trees, or BS > 70 in parsimony trees) to detect areas of topological disagreement (Wiens 1998). For the parsimony tree(s), partitioned Bremer support (PBS) values (Baker *et al*. 1998) were computed as another way to assess character conflict among the data.

Molecular dating

Homogeneity of nucleotide substitution rate across lineages was assessed by the likelihood ratio test statistic (Huelsenbeck & Rannala 1997), comparing ML scores with and without an enforced molecular clock. Node ages of the unconstrained ML tree were estimated using the program r8s (Sanderson 2003), which has been extensively utilized to obtain divergence dates for insular radiations (e.g. Clark *et al*. 2008; Givnish *et al*. 2009). ML tree topology and branch lengths were supplied with three calibration points to scale branch lengths with time. Outgroup taxa and zero-length terminal branches of the ML tree were pruned. We conducted the cross-validation procedure in r8s for the penalized likelihood (PL) method with the truncated Newton algorithm over a range of values for the smoothing parameter (λ). The value of λ producing the minimum cross-validation score (lowest chi-square error) was used as the optimal level of smoothing for penalized likelihood estimates of node ages. We estimated uncertainty of node ages by generating 500 bootstrapped matrices from the ML tree data using the SeqBoot program in the PHYLIP version 3.6 package (Felsenstein 2004). ML branch lengths of the original topology were computed over each of the 500 bootstrapped matrices in PAUP. The resulting 500 tree files with identical topology but varying branch lengths were analysed in r8s using optimal PL smoothing values. Mean and standard deviation of node ages were estimated in r8s using the 'profile' command.

Table 3 Summary statistics for aligned sequence data

Data type	Sites	Variable	Inform.	Max. dist.	%A	%C	%G	%T	Bias	Ti/Tv	Model
mt 16S- tRNA leu	166	49	20	17.9/60.7	0.35940	0.12626	0.13259	0.38175	n.s.	1.03	K81uf + I + G
mt ND1	395	176	154	23.5/99.9	0.33519	0.12752	0.08479	0.45250	n.s.	3.31	TVM + I + G
ND1 1st	132	43	35	—	0.38115	0.12413	0.12422	0.37051	n.s.	—	HKY + G
ND1 2nd	132	17	14	—	0.21756	0.16450	0.10873	0.50920	n.s.	—	F81 + G
ND1 3rd	131	116	106	—	0.40736	0.09367	0.02090	0.47806	n.s.	—	TrN + G
mtCOI	820	292	247	13.4/40.6	0.26258	0.13089	0.18418	0.42235	n.s.	3.13	GTR + I + G
COI 1st	274	52	34	—	0.27247	0.12726	0.28995	0.31032	n.s.	—	TrN + G
COI 2nd	273	13	6	—	0.13430	0.25147	0.17754	0.43669	n.s.	—	F81 + G
COI 3rd	273	227	207	—	0.38107	0.01384	0.08464	0.52045	n.s.	—	TIM + G
All mtDNA	1381	517	430	17.2/62.9	0.29738	0.12926	0.14682	0.42655	n.s.	2.96	GTR + I + G
EF1-∝	523	141	66	10.9/14.5	0.27670	0.18745	0.20148	0.33437	n.s.	1.70	HKY + G
EF1-∝ 1st	117	8	3	—	0.32612	0.19663	0.34087	0.13638	n.s.	—	F81
EF1-∝ 2nd	117	8	3	—	0.26504	0.27889	0.18054	0.27552	n.s.	—	JC
EF1-∝ 3rd	117	42	22	—	0.21316	0.19400	0.16090	0.43193	n.s.	—	HKY
intron	172	83	38	—	0.29533	0.10976	0.14499	0.44992	n.s.	—	HKY
All data	1904	612	448	11.5/27.9	0.29115	0.14535	0.16287	0.40062	n.s.	2.80	GTR + I + G

mt, mitochondrial; Inform., number of informative characters; Max. dist., maximal genetic distance for data, with uncorrected (p) distance left of diagonal and maximum likelihood corrected distance to right; n.s., non-significant; mitochondrial + ef1 variable and Inform sites do not sum to 'All data,' as some sequences in mt analyses did not have corresponding nuclear sequence. Twenty-three recoded gapped characters from EF1-∝ intron also included in parsimony and Bayesian analysis of nuclear data (Figs S2 and S3) and combined (Figs S3 and S4) data set. Models for all mitochondrial first, second, and third codon positions were TrN + I + G, F81 + I and TIM + G, respectively.

Biogeographic inference

Ancestral geographic distributions of Hawaiian taxa were inferred at tree nodes in the combined data Bayesian tree using stochastic mapping (SM). SM is a Bayesian method of estimating ancestral character states that accounts for variation in branch lengths as well as topological uncertainty from trees sampled from a posterior distribution (Nielsen 2002). Clark *et al*. (2008) compared four methods of reconstructing ancestral ranges for insular taxa and found that SM provided inferences that were most compatible with assumptions about Pacific geology and dispersal mediated speciation. We performed SM analyses using the program SIMMAP (Bollback 2006). Taxa were coded as occurring in one of the following area states: (i) North and South America; (ii) Eastern Polynesia, i.e. Marquesas and Society Islands; (iii) Necker; (iv) Kauai; (v) Oahu; (vi) Maui-Nui (Maui, Lanai and Molokai); and (vii) Hawaii Island (SIMMAP is limited to seven states). A subsample of 1000 post burn-in trees with branch lengths from the MrBayes analysis was supplied as the posterior distribution of topologies. Ancestral states were also reconstructed onto ML and parsimony topologies using maximum parsimony in MacClade 4.0 (Maddison & Maddison 2000), with the same coding scheme used in SIMMAP.

Results

Sequence characteristics

Mitochondrial 16S-ND1 was collected from all 82 examined specimens, whereas mtCOI was obtained from all but four individuals. Nuclear EF1-∝ was sequenced from 61 of the 82 specimens, due to PCR failure with more distantly related taxa or difficulties with sequencing heterozygous length-variable introns. GenBank Accession numbers for sequences are listed in Table 1. Protein-coding genes were conceptually translated and contained no unexpected stop codons or length variability. BLASTX (www.ncbi.nlm.nih.gov/blast/) searches of these sequences against the NCBI protein database found highest similarity to orthologues from other spiders and were appropriately conserved at the protein level. All protein-coding genes also showed greatest variation at third sites (Table 3). Thus, we found no evidence of psuedogenes among our data.

Maximal uncorrected genetic distance ('p') across all sampled taxa for the mitochondrial data was 0.172 (ML estimates in Table 3), whereas the uncorrected genetic distance EF1-∝ was 0.109. Uncorrected interspecific genetic distance among Hawaiian species ranged between 0.001 and 0.091 (average = 0.064) for the mitochondrial

Table 4 Uncorrected pairwise mitochondrial genetic distance ('p') between island populations of Hawaiian thomisid species sampled from more than one island. Minimum distance left of diagonal, maximal distance right of diagonal in cells; K, Kauai; O, Oahu; M, Maui; H, Hawaii

Species	K vs. O	K vs. M	K vs. H	O vs. M	O vs. H	M vs. H
Me. kanakana	0.027/0.033	0.027/0.032	0.023/0.029	0.005/0.006	0.012/0.021	0.010/0.019
Me. anguliventris	0.028/0.031	0.029/0.032	0.032/0.035	0.021/0.023	0.023/0.027	0.006/0.007
Me. naevigera	0.019/0.019	0.035/0.036	0.024/0.024	0.019/0.031	0.011/0.012	0.029/0.030
Me. nigrofrenata	0.028/0.028	—	0.016/0.022	—	0.015/0.017	—
Me. arida	—	—	—	—	—	0.027/0.029
Me. imbricata	—	—	—	0.021/0.025	—	—

'—' comparison unavailable because species does not occur or was not sampled from one or both islands.

data and 0.000–0.031 (average = 0.013) for the nuclear data. Mitochondrial intraspecific genetic distances between islands across Hawaiian species ranged from 0.005 (Oahu and Maui *Me. kanakana*) to 0.036 (Kauai and Maui *Me. naevigera*; Table 4). Nucleotide composition of the mitochondrial sequences was more A/T skewed (0.72), as was the intron of EF1-∝ (0.74) in comparison to its exons (0.55). Tests of nonhomogeneity of base composition across taxa (uncorrected for phylogeny) were nonsignificant for all gene partitions and at each codon position within genes (Table 3).

Phylogenetic analyses

The aligned mitochondrial data comprised 1381 nucleotides, including 517 variable sites, of which 430 were parsimony-informative (Table 3). Parsimony analyses retained six equal length trees (length = 2050, CI = 0.357, RI = 0.703). A consensus of these MPTs (Fig. S1, Supporting Information) recovered Hawaiian *Mecaphesa* as monophyletic but this node was not supported in the bootstrap consensus. The Hawaiian clade was sister to an Eastern Polynesian *Misumenops* clade (Marquesas + Society Islands). Nearly all Hawaiian species grouped into well-supported, reciprocally monophyletic clades, including species distributed across multiple Hawaiian Islands. One exception was individuals of *Me. arida* from Maui, which appeared more closely related to sympatric *Me. baltea* than to *Me. arida* from Hawaii. Intraspecific relationships among species distributed on multiple islands exhibited geographic structure largely consistent with the progression rule: {Kauai [Oahu (Maui + Hawaii)]}. Relationships between most of the Hawaiian species were weakly supported. Bayesian analyses of the mitochondrial data found support for groups that were well supported in the parsimony analysis but were less resolved at the poorly supported nodes (Fig. 2). Of the 18 Hawaiian *Mecaphesa* species, 17 formed a well-supported clade (PP = 0.91), but relationships among the Hawaiian species were largely unresolved.

The EF1-∝ data included 523 characters and 23 recoded gapped characters, with 164 variable sites, 82 of which were parsimony-informative. Parsimony analyses recovered 24 MPTs (L = 215), resulting in a partially resolved consensus (Fig. S2, Supporting Information). Despite limited variability, EF1-∝ had much less homoplasy (CI = 0.847, RI = 0.904) than the mitochondrial data. Hawaiian *Mecaphesa* were not monophyletic, as the Eastern Polynesian *Misumenops* clade was nested among the Hawaiian taxa. Strong support was found uniting the Marquesas + Society Islands *Misumenops* (BS = 94), with moderate support for monophyly of the Society Islands species (BS = 68). Some Hawaiian species that were recovered as well-supported clades by the mitochondrial data were not monophyletic (e.g. *Me. kanakana*). Bayesian analyses of the EF1-∝ data produced a consensus topology that was nearly identical to that found by the parsimony analysis (Fig. 3), with better support for various nodes.

Partition homogeneity tests comparing the collected mitochondrial fragments (16S-ND1 and COI) with EF1-∝ indicated significant incongruence in each pairwise comparison (16S-ND1 vs. COI =0.030; 16S-ND1 vs. EF1-∝ = 0.004; COI vs. EF1-∝ =0.001; all mtDNA vs. EF1-∝ = 0.012). Visual comparisons of well-supported nodes in the mt and EF1-∝ trees suggested conflicting support for relationships among a few Hawaiian species. Mitochondrial trees strongly supported *Me. imbricata* + *Mi. facundus* (BS = 100, PP = 1.00), whereas EF1-∝ united *Me. imbricata* with *Me. nigrofrenata* (BS = 64, PP = 0.87). Also, *Me. kanakana* from all islands was strongly supported as monophyletic in mt analyses (BS = 100, PP = 1.00), but were scattered in the EF1-∝ trees, uniting the Maui and Hawaii populations with different species within their respective islands.

A parsimony analysis of the combined data (1927 characters, 464 parsimony-informative) found two MPTs (L = 1937, CI = 0.432, RI = 0.696). One of the two showed all Hawaiian *Mecaphesa* as monophyletic; the other MPT differed by nesting the Eastern Polynesian *Misumenops* clade among the Hawaiian species (Fig. S3, Supporting

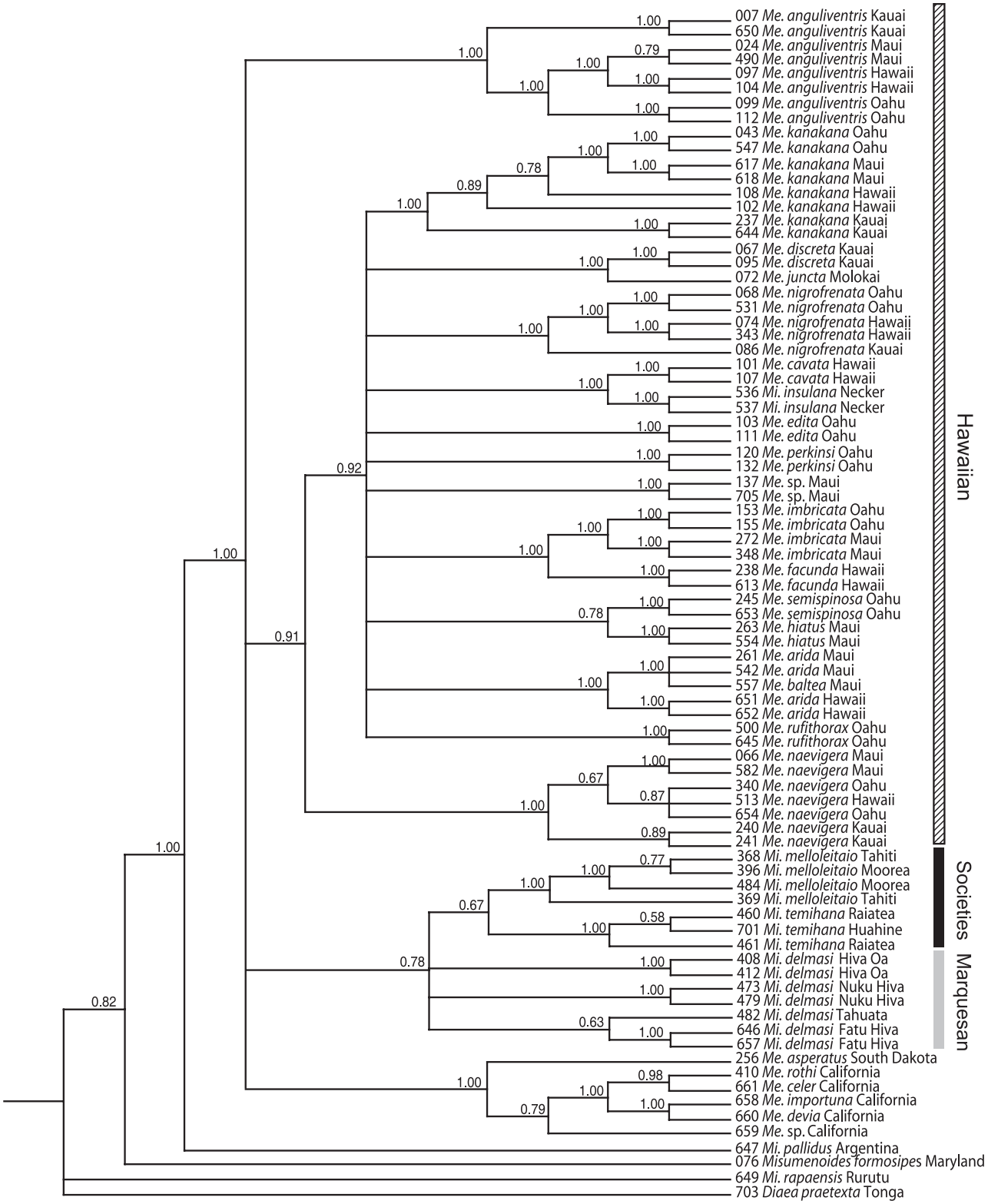

Fig. 2 Fifty per cent majority-rule consensus of the Bayesian posterior probability distribution of trees (post burn-in) for mitochondrial (16S-ND1 + COI; 1381 characters) data. Numbers above nodes indicate posterior probabilities of clades.

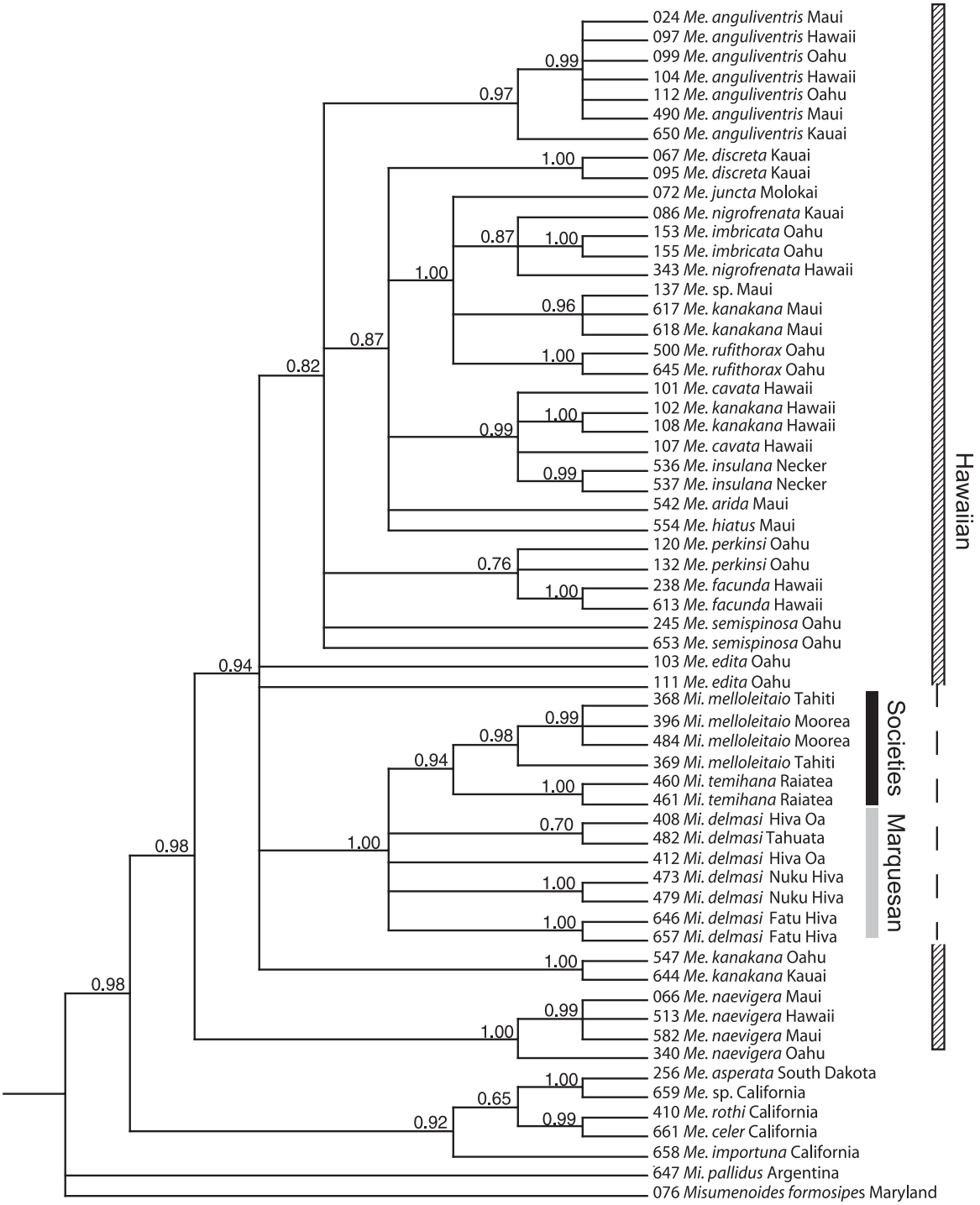

Fig. 3 Fifty per cent majority-rule consensus of the Bayesian posterior probability distribution of trees (post burn-in) for the nuclear gene EF1-∝ (523 bp + 23 gapped characters). Numbers above nodes indicate posterior probabilities of clades.

Information). Marquesan and Society Island taxa formed reciprocally monophyletic groups. Each Hawaiian species was recovered as a well-supported monophyletic group, but most relationships between these species remained weakly supported. Partitioned Bremer support (PBS) values (Table S1, Supporting Information) indicated conflicting negative support from mtCOI at the node uniting Hawaiian, Marquesan and Society Island taxa (node 2, COI PBS = –4.0)

and at the node uniting Marquesan and Society Island taxa (node 3, COI PBS = –8.7). Otherwise, PBS values were small when negative (i.e. > –2). Bayesian analyses of the combined data set yielded a consensus topology (Fig. S4, Supporting Information) that was highly congruent with the parsimony analysis showing the Marquesan + Society Islands clade nested among the Hawaiian taxa. Support was found for this Hawaiian + Marquesas + Society Islands clade (PP = 1.00) as well as for the monophyly of the Society Islands taxa (PP = 0.97). ML heuristic searches of the combined data set also found a tree that was extremely similar to the Bayesian consensus and parsimony trees (Fig. 4). Additional tree searches enforcing the constraint of Hawaiian monophyly resulted in a tree score that was only slightly greater (–ln L = 12 226.03) and not significantly different from the ML tree (–ln L = 12 225.46; P = 0.422).

Divergence time estimates

A likelihood-ratio test found the enforced clock tree to be a significantly worse fit to the data than the unconstrained ML tree (χ^2 = 96.27, d.f. = 59, P = 0.001), indicating substantial rate heterogeneity among lineages. PL analyses in r8s were applied to the ML topology using maximal age constraints on three nodes (Fig. 4, nodes A, B and C). Fleischer *et al*. (1998) outlined assumptions underlying the use of island ages as calibration points for molecular dating. Notably, topologies imply successive colonization of islands in parallel with their formation and each island population formed shortly after island emergence. Divergences between taxa distributed on older and younger islands (e.g. Kauai vs. Oahu + Maui + Hawaii) are thus not expected to exceed the maximal age of the younger islands (Fleischer *et al*. 1998; Hormiga *et al*. 2003; Jordan *et al*. 2003). Here, node 'A' of Fig. 4 was constrained so as not to exceed the maximal age of Oahu [3.7 million years (Myr); Clague 1996] because relationships among island populations of *Me. anguliventris* matched the progression rule, which assumes stepwise colonization from older to younger islands (Funk & Wagner 1995; Fleischer *et al*. 1998). Relationships of Hawaiian species to those from the Marquesas and the Society Islands allowed us to place two other maximal age constraints on the tree. The Society Islands and Marquesan Islands are estimated to be no older than 10 Myr and 6 Myr in age, respectively (White & Duncan 1996; Craig *et al*. 2001; Clouard & Bonneville 2005). Node 'B' (Fig. 4), representing the common ancestor of sister lineages restricted to these two archipelagos, would not predate 6 Myr and was constrained to this maximal age. Node 'C' preceding the Society Islands + Marquesan Islands clade was constrained to a maximal age of 10 Myr. PL cross-validation resulted in the minimal chi-square error for a smoothing value of λ = 0.025. The common ancestor of the Polynesian thomisids (node 1, Fig. 5; Table S2, Supporting Information) dated to 7.72 ± 2.43 Myr. Geologically, this ranges from shortly after the formation of Necker to a period roughly coincident with the emergence of Kauai. Node 4, corresponding to the monophyletic Hawaiian radiation (all Hawaiian species except *Me. anguliventris*), was dated to 6.08 ± 1.48 Myr. Age estimates for nodes representing Hawaiian speciation events suggest that they predominately occurred prior to the origin of Maui-Nui (originally contiguous islands of Molokai, Maui and Lanai) ~2.2 Myr. Excepting divergences within *Me. anguliventris*, the earliest estimates of intraspecific divergence events largely dated to between 1 million and 2 million years ago (Ma). The age of node 'C' was dated to 7.36 Myr, but nodes 'A' and 'B' were pushed to their maximal constraints, suggesting that the resulting ages are overestimates.

Biogeographic analysis

At basal nodes of clades including Hawaiian taxa, the SM analysis assigned highest posterior probability (PP) values to the island of Oahu (Fig. 6; Table S3, Supporting Information). Nodes 1 and 2 (Fig. 6), which also include the Eastern Polynesian *Misumenops*, were resolved to Oahu with PP values of 0.96 and 0.86, respectively. Node 4, corresponding to the monophyletic Hawaiian clade was also reconstructed as Oahu (PP = 0.99). Overall, the analysis inferred that thomisids initially colonized Oahu and experienced their early diversification on this island, which was followed by numerous colonization events to both younger and older islands (in addition to Eastern Polynesia). Reconstructions suggested several instances of colonization from Oahu to Maui-Nui and then Hawaii Island, but there were no pathways showing colonization from Kauai or Necker to younger islands. Parsimony reconstructions of island area onto the ML tree also indicated that the primary area of colonization was Oahu, whereas the parsimony trees were equivocal between Oahu and Maui (not shown).

Discussion

Hawaiian colonization, geographic origins and age

Phylogenetic analyses of both mitochondrial and nuclear data found that 17 of the 18 sampled Hawaiian *Mecaphesa* species comprise a monophyletic group. Some analyses (i.e. mt and combined data parsimony) recovered alternative phylogenies in which Hawaiian *Mecaphesa* were monophyletic. Although we did not find strong evidence for the monophyly of Hawaiian thomisids, our results clearly indicate that these spiders have undergone substantial radiation within the archipelago. Moreover, the

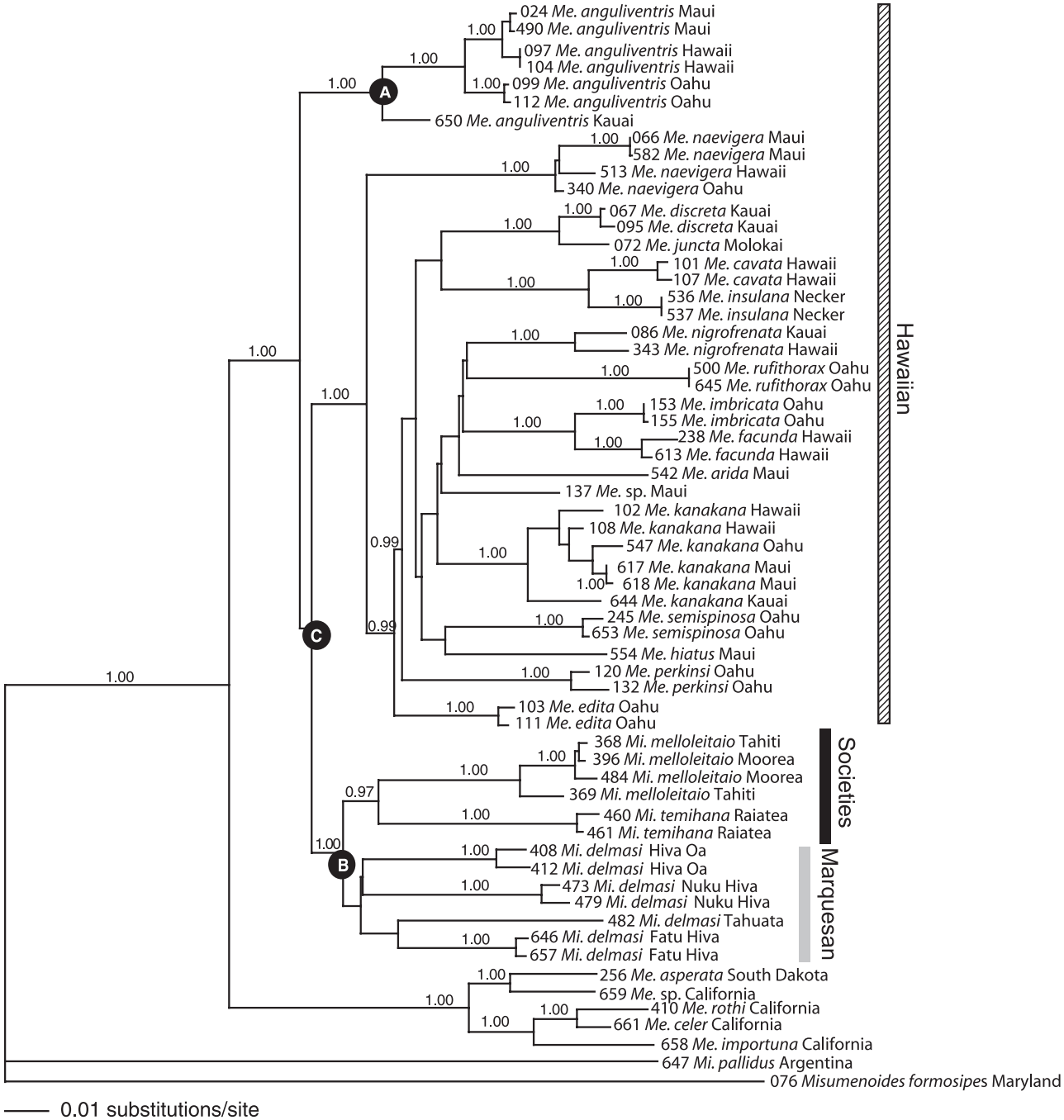

Fig. 4 Maximum-likelihood tree from combined data (1904 bp; –ln *L* = 12226.034) using GTR + I + G model. Model parameters are as follows: (A–C) = 0.7864; (A–G) = 14.8955; (A–T) = 1.9500; (C–G) = 0.8267; (C–T) = 8.0336; freqA = 0.3109; freqC = 0.1290; freqG = 0.1348; freqT = 0.4254; I = 0.5151; G = 0.5217. Numbers above nodes indicate posterior probability vales for clade from Bayesian analysis, where ≥ 0.95 (see Fig. S4). Circled node 'A' was constrained to not exceed the maximal age of Oahu island (3.7 Myr) in penalized likelihood r8s estimates of divergence dates. Node 'B' was constrained to not exceed the maximum age of the Marquesas (6 Myr), whereas node 'C' could not exceed the maximal age of the Society Islands (10 Myr).

bulk of the lineage's diversity could be traced to as few as one or two colonizing ancestors. A single colonization event would be consistent with the phylogenies in which all Hawaiian thomisids are monophyletic as well as those that nest the Society + Marquesas *Misumenops* among Hawaiian *Mecaphesa* (nuclear and combined Bayesian). From this paraphyletic arrangement, a single colonization event to Hawaii could be inferred if it was followed by

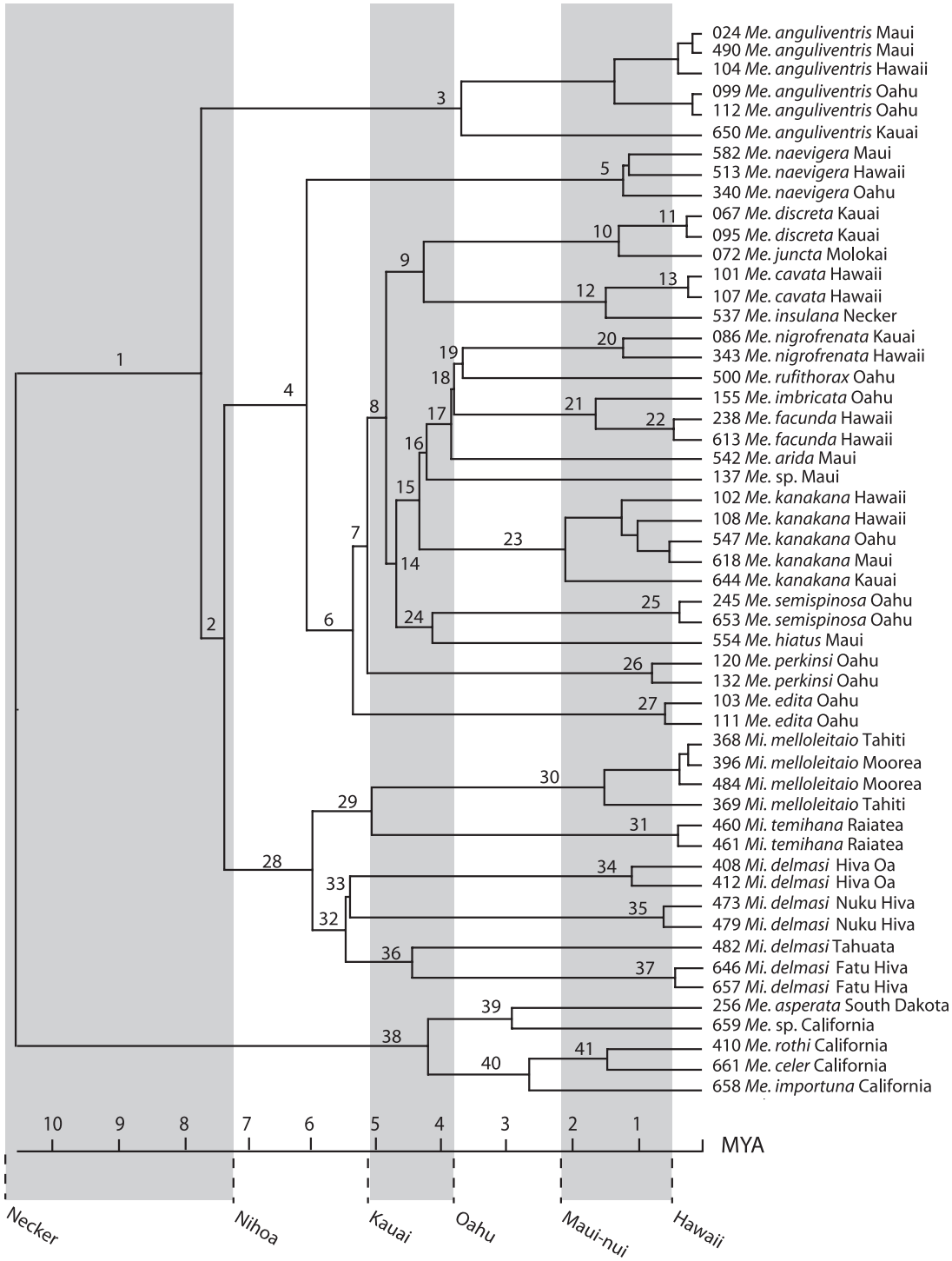

Fig. 5 Chronogram of ML tree (Fig. 4), excluding outgroups and zero-length branches. Timeline below is given in million years (Myr). Numbers on nodes correspond to those in Table S2, where age estimates, average and standard error based on bootstrap replicates are listed.

dispersal to either the Marquesas or Society Islands. Given the younger age of the Society Islands and Marquesas, it is likely that these archipelagos acquired some of their biota from the older Hawaiian archipelago (e.g. Rundell *et al*. 2004). Alternatively, paraphyly of Hawaiian thomisids could instead be explained by two colonizing ancestors, one that gave rise to *Me. anguliventris* (or *Me. naevigera*) and another that radiated into 17 or more species.

Our analyses showed that Hawaiian thomisids (genus *Mecaphesa*) are most closely related to *Misumenops* endemic

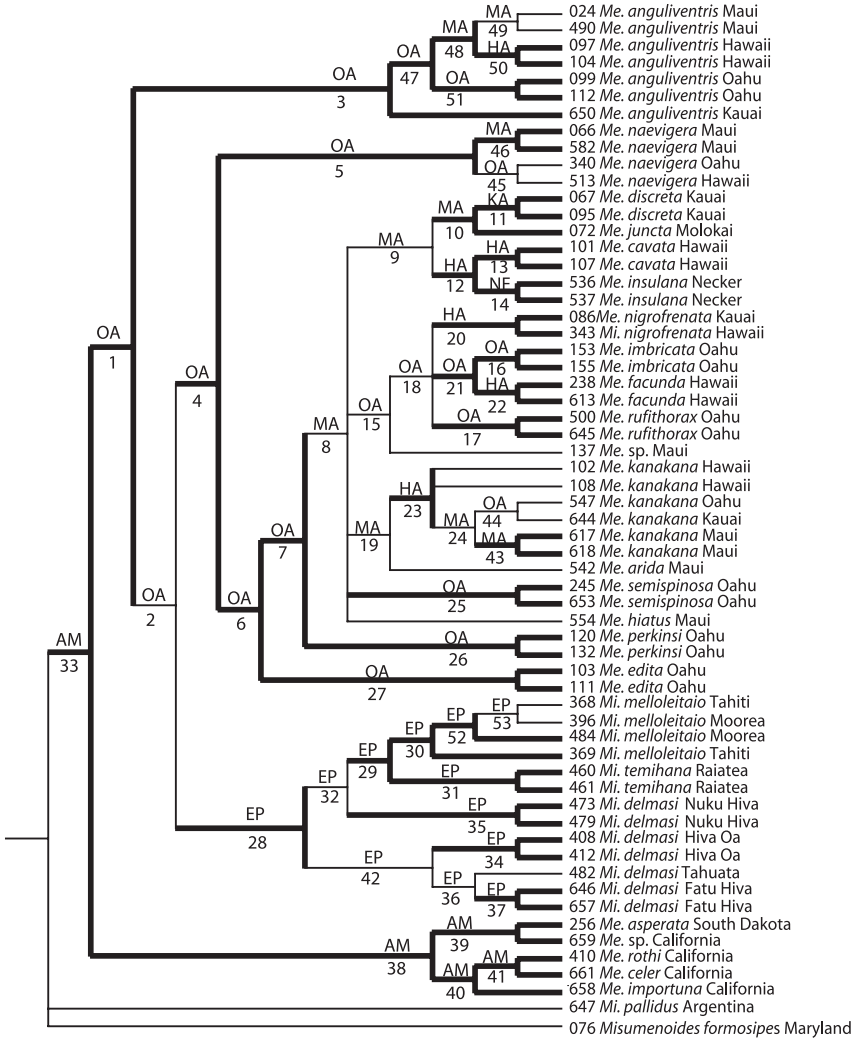

Fig. 6 Ancestral island distributions reconstructed by stochastic mapping (SM) with SIMMAP. Topology derived from Bayesian analysis of combined mitochondrial and nuclear data; thickened branches supported by posterior probability values ≥ 0.95. Labels above branches indicates range at nodes with states as follows: AM, Americas; EP, Eastern Polynesia (Marquesas and/or Society Islands); NE, Necker; KA, Kauai; OA, Oahu; MA, Maui-Nui; HA, Hawaii Island. Numbers below branches correspond to those in Table S3 where posterior probabilities of each area state are reported.

to the Marquesas (3000 km to the south) and Society Islands (4400 km to the south), two archipelagos that are most similar in distance from Hawaii to its nearest continent (3200 km). While the phylogenetic affinity between the different archipelagos of Polynesia conflicts with current taxonomy (Lehtinen & Marusik 2008), it is a biogeographic pattern shared with other organisms (e.g. *Bidens*, Ganders *et al*. 2000; *Ilex*, Cuénoud *et al*. 2000). In a previous study, we found that the Hawaiian, Society and Marquesan thomisids were more closely related to North American *Mecaphesa* than to thomisids sampled from Western Polynesia and Melanesia (Garb & Gillespie 2006), corroborating Lehtinen's (1993) hypothesis of a New World origin for Hawaiian thomisids. However, determining the precise continental or other island source of these Hawaiian spiders, as well as the sequence in which they dispersed across Polynesia, will require additional taxonomic sampling and genetic data. Additional sampling of *Mecaphesa* species, particularly those from Mexico, Central America and the West Indies (~9 spp.), the Galapagos (1 sp.) and the Juan Fernandez Islands (1 sp.), as well as additional New and Old World continental *Misumenops* species would be especially informative for addressing these biogeographic questions.

A recent review of molecular age estimates suggested that the majority of Hawaii's biota arrived after the formation of Kauai (~5 Myr), although the archipelago had land continuously available for colonization for the last 32 Myr (Price & Clague 2002). Prior to the formation of Kauai, there was a period when the islands were smaller, low, and more widely spaced, implying a reduced frequency of waif dispersal from these older islands to the current high islands and suggesting that the current high islands acquired most of their biota from sources outside of the Hawaiian chain. Given that Gardner Island (16 Myr) was at one time comparable in size to Hawaii Island (Price & Clague 2002), it is nevertheless likely that islands older than Kauai harboured some ancestors of extant Hawaiian

radiations, such as drosophilids (O'Grady & DeSalle 2008) and lobeliads (Givnish *et al*. 2009). Our relaxed molecular clock estimates suggest that *Mecaphesa* spiders may have colonized the Hawaiian archipelago as early as ~10 Ma. Because the small, older islands of Necker and Nihoa currently support thomisid populations, it is plausible that these spiders established populations long before the emergence of Kauai. However, the lower bound on their ancestor's arrival (5.29 Ma) dated to a period approximately concurrent with the emergence of Kauai (Clague 1996). This recent date is more consistent with our biogeographic analyses, which inferred an ancestral distribution restricted to the island of Oahu, followed by back-colonizations to Kauai. These conflicting results are not surprising given the strong possibility that our data may violate certain assumptions of using island ages as calibration points. For example, it is likely that colonization of Oahu by *Me. anguliventris* occurred a substantial time after its emergence, resulting in an overestimate of divergence dates. Another source of error may be associated with tree topology, which is fixed in our analysis. In some trees, all Hawaiian species were monophyletic and sister to the Eastern Polynesian clade. If the split of these two lineages coincided with colonization of either the Marquesas or Society Islands, the entire group could not exceed 10 Myr in age.

Hybridization, speciation and ecological diversification

Our mitochondrial data largely supported previous hypotheses of species boundaries for Hawaiian thomisids. An exception was found in *Me. arida* on Maui, which was more closely related to sympatric *Me. baltea* than to *Me. arida* on Hawaii. Mitochondrial sequences for *Me. baltea* and *Me. arida* from Maui were nearly identical (> 99%) raising the intriguing possibility of interspecific hybridization among sympatric species. Alternatively, the grouping of *Me. baltea* and *Me. arida* on Maui may represent an example of recent sympatric speciation (giving rise to *Me. baltea*), in which morphological divergence has preceded molecular divergence at the loci sequenced. Distinguishing between these hypotheses will require additional sampling of nuclear data from a greater number of individuals across the ranges of these two species. In contrast to the mitochondrial data, the nuclear data did not clearly support morphological species boundaries in many cases. For example, the mitochondrial data strongly united *Me. kanakana* from different islands, whereas EF1-∝ showed Maui and Hawaii populations of *Me. kanakana* as more closely related to populations of other species from their respective islands. Discordance between the mitochondrial and nuclear data could be explained by introgression among sympatric populations of different species. However, given that EF1-∝ exhibits very limited variability, it is just as likely that the placement of *Me. kanakana* populations is an artefact of retained ancestral polymorphisms that have not sorted to monophyly. Again, the collection of additional, more rapidly evolving markers is needed to address these hypotheses.

Although we found limited support for relationships among the Hawaiian species, it is nevertheless clear that crab spiders have undergone several ecological shifts within the archipelago. For example, both *Me. nigrofrenata* and *Me. arida* are found in association with filamentous lichens and are colour-camouflaged against this substrate. This is an uncommon habit for thomisid spiders and the phylogenetic placement of these two species suggests this strategy may have evolved convergently on different islands of the Hawaiian chain. Also nested within the Hawaiian radiation is the morphologically enigmatic *Me. rufithorax*, which is extremely dorso-ventrally flattened and restricted to the narrow spaces between leaves of the native vine *Freycinetia arborea* on Oahu. It is likely that a number of unknown species in the Hawaiian *Mecaphesa* radiation exhibiting other unique ecological strategies are yet to be found. Indeed, our data corroborate the genetic distinctness of a putative undescribed species from Maui (specimens 137 and 705). The rich diversity of this spider radiation, with its unique ecological specializations and complex distribution, contrasts with the widespread distribution of many of the species.

Phylogeography of widespread Hawaiian species

We proposed three hypotheses to explain the unusually widespread distribution of thomisid species across the Hawaiian Islands. First, we hypothesized that species designations may have been confounded by convergence, such that species that appear to be widespread are not. This hypothesis predicts that species designations would be inconsistent with molecular relationships because convergent morphologies can repeatedly arise in replicate environments such as an archipelago's islands (Losos *et al*. 1998; Gillespie 2004). However, our mitochondrial data identified all but one of the widespread Hawaiian species delimited by morphology (e.g. *Me. kanakana*, *Me. anguliventris*, *Me. nigrofrenata*, *Me. naevigera*, *Me. imbricata*) as belonging to genetically distinct reciprocally monophyletic clades. While our mitochondrial data strongly corroborate Suman's (1970) morphological-based taxonomy and verify the existence of multiple geographically widespread species, our nuclear data did not group all of these species into distinct clades, possibly due to its slower evolutionary rate and retention of ancestral polymorphisms.

Our second hypothesis to explain the widespread distribution of thomisid species was that these spiders have colonized the archipelago multiple times, and several of these independent lineages subsequently dispersed across

multiple islands. Multiple colonization events would account for the substantial morphological and ecological diversity found across Hawaiian thomisids, despite their ability to migrate between islands, which should otherwise limit speciation and divergence. Our results show that multiple colonizations could play some role: *Me. anguliventris* may represent an independent colonization to the archipelago and shows a progression down the island chain. However, most species belong to a single lineage that has diversified within the archipelago.

Our final hypothesis that thomisids have colonized the archipelago once, but intra-archipelago migration has been more frequent than speciation, is most consistent with our data. Extensive speciation has occurred within the archipelago, but several descendent species have subsequently dispersed between islands without developing morphologically recognizable single-island endemics. Morphological cohesion across islands may be maintained through recent (or ongoing) interisland migration and gene flow. Alternatively, interisland migration may have been more ancient such that these widespread species represent morphologically cryptic species on different islands. Intraspecific phylogeographic patterns and genetic distances can reveal whether this implied inter-island dispersal was recent or more ancient. Widespread species that have recently expanded their range through natural or human-mediated movement should exhibit limited genetic differentiation between islands and relationships that are approximately random with respect to island age. By contrast, species that have independently colonized the different islands shortly after their emergence should display substantial genetic differentiation between islands as well as relationships that mirror the progression rule. Our data mainly corroborate this latter scenario. Specifically, mitochondrial phylogenies showed that intraspecific relationships of species sampled from three or more islands largely adhere to the progression rule, implying that younger islands were colonized from older ones (e.g. *Me. anguliventris, Me. nigrofrenata*). In addition, maximal intraspecific genetic distances between islands ranged between 2.5% and 3.6% (Table 4). If these distances scale to Brower's (1994) often cited rate of arthropod mitochondrial sequence divergence (2.3% per Myr), they would suggest that intraspecific divergence began ~1 Ma and 1.5 Ma. Our estimates of divergence dates (although assuming the progression rule in *Me. anguliventris*) similarly indicate that divergence between island populations occurred as early as ~1–2 Ma. At that time, Kauai and Oahu had already emerged, Maui-Nui was in the process of forming, and Hawaii did not exist (Clague 1996). Accordingly, these dates do not support a recent spread of *Mecaphesa* species to the different Hawaiian Islands. They instead suggest that the widespread species arose on Kauai or Oahu and independently colonized Maui and Hawaii relatively soon after their emergence. The intraspecific genetic differentiation between islands further suggests that gene flow is not ongoing and that each widespread species represents a complex of morphologically cryptic species restricted to the different islands.

Insular taxa often evolve a diminished capacity for dispersal, as evidenced by wing loss in insects or increased fruit and seed size in plants (Carlquist 1974). For Hawaiian spider taxa in which it has been considered, dispersal by ballooning appears to be minimal or nonexistent (Gillespie & Oxford 1998; Hormiga 2002). The consequence of reduced dispersal is higher levels of endemism and a tight correlation between geography and genealogy, leading to the high prevalence of single-island endemics on the different islands of the Hawaiian chain (e.g. Rundell *et al*. 2004), and such a relationship has been shown in plants where decreasing dispersal ability — via wind, birds, etc. — has resulted in increasing levels of speciation, single-island endemism and net diversification (Price & Wagner 2004; Givnish *et al*. 2009). The spread of multiple thomisid lineages across the different Hawaiian Islands, coupled to their high overall species diversity, suggests that dispersal must be severely restricted in these spiders, but frequent enough to allow occasional interisland migration. Much research is still needed to understand the interplay between dispersal and adaptive diversification that have given rise to the rich diversity and complex distributions of these spiders. Hawaiian crab spiders clearly provide a valuable system for future studies exploring the relative roles of geography and ecology in adaptive divergence.

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References

Baker RH, Yu X, DeSalle R (1998) Assessing the relative contribution of molecular and morphological characters in simultaneous analysis trees. *Molecular Phylogenetics and Evolution*, **9**, 427–436.

Baldwin BG, Robichaux RH (1995) Historical biogeography and ecology of the Hawaiian silversword alliance (Asteraceae).

In: *Hawaiian Biogeography: Evolution on a Hot Spot Archipelago* (eds Wagner WL, Funk VA), pp. 259–287. Smithsonian Institution Press, Washington, D.C.

Bollback JP (2006) Stochastic character mapping of discrete traits on phylogenies. *BMC Bioinformatics*, **7**, 88.

Brower AV (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconious erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences, USA*, **91**, 6491–6495.

Carlquist S (1974) *Island Biology*. Columbia University Press, New York.

Clague DA (1996) The growth and subsidence of the Hawaiian-Emperor volcanic chain. In: *The Origin and Evolution of Pacific Island Biotas, New Guinea to Eastern Polynesia: Patterns and Processes* (eds Keast A, Miller SE), pp. 35–50. SPB Academic Publishing, Amsterdam, The Netherlands.

Clark JR, Ree RH, Alfaro ME, King MG, Wagner WL, Roalson EH (2008) A comparative study in ancestral range reconstruction methods: retracing the uncertain histories of insular lineages. *Systematic Biology*, **57**, 693–707.

Clouard V, Bonneville A (2005) Ages of seamounts, islands, and plateaus on the Pacific plate. *The Geological Society of America Bulletin*, **388**, 71–90.

Cowie RH, Holland BS (2008) Molecular biogeography and diversification of the endemic terrestrial fauna of the Hawaiian Islands. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **363**, 3363–3376.

Craig DA, Currie DC, Joy DA (2001) Geographical history of the central-western Pacific black fly subgenus *Inseliellum* (Diptera: Simuliidae: *Simulium*) based on a reconstructed phylogeny of the species, hot-spot archipelagos and hydrological considerations. *Journal of Biogeography*, **28**, 1101–1127.

Cuénoud PM, Del Pero Martinez A, Loizeau PA *et al*. (2000) Molecular phylogeny and biogeography of the genus *Ilex* L. *(Aquifoliaceae). Annals of Botany*, **85**, 111–122.

Cunningham CW (1997) Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution*, **14**, 733–740.

Danforth BN, Ji S (1998) Elongation factor-1alpha occurs as two copies in bees: Implications for phylogenetic analysis of EF-1alpha sequences in insects. *Molecular Biology and Evolution*, **15**, 225–235.

Farris JS, Kallersjo M, Kluge AG, Bult C (1994) Constructing a significance test for incongruence. *Systematic Biology*, **44**, 570–572.

Felsenstein J (2004) *PHYLIP (Phylogeny Inference Package)*, Version 3.6. Distributed by the author, WA Department of Genomic Sciences, University of Washington, Seattle, Washington.

Fleischer RC, McIntosh CE, Tarr CL (1998) Evolution on a volcanic conveyor belt: using phylogeographic reconstructions and K-Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. *Molecular Ecology*, **7**, 533–545.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.

Funk VA, Wagner WL (1995) Biogeographic patterns in the Hawaiian Islands. In: *Hawaiian Biogeography: Evolution on a Hot Spot Archipelago* (eds Wagner WL, Funk VA). Smithsonian Institution Press, Washington D.C.

Ganders FR, Berbee M, Pirseyedi M (2000) ITS base sequence phylogeny in *Bidens* (Asteraceae): evidence for the continental relatives of Hawaiian and Marquesan *Bidens*. *Systematic Botany*, **25**, 122–133.

Garb JE (1999) An adaptive radiation of Hawaiian Thomisidae: Biogeographic and genetic evidence. *Journal of Arachnology*, **27**, 71–78.

Garb JE (2003) *Evolutionary studies of Polynesian crab spiders (Araneae: Thomisidae) and* Latrodectus *spiders (Araneae: Theridiidae): phylogeny and biogeography*. PhD Thesis. University of California, Berkeley, California.

Garb JE, Gillespie RG (2006) Island hopping across the central Pacific: mitochondrial DNA detects sequential colonization of the Austral Islands by crab spiders (Araneae: Thomisidae). *Journal of Biogeography*, **33**, 201–220.

Gillespie RG (2004) Community assembly through adaptive radiation in Hawaiian spiders. *Science*, **303**, 356–359.

Gillespie RG, Howarth FG, Roderick GK (2001) Adaptive radiation. In: *Encyclopedia of Biodiversity* (ed. Levin SA). Academic Press, New York.

Gillespie RG, Oxford GS (1998) Selection on the color polymorphism in Hawaiian happy-face spiders: evidence from genetic structure and temporal fluctuations. *Evolution*, **52**, 775–783.

Gillespie RG, Rivera MJ, Garb JE (1998) Sun, surf and spiders: taxonomy and phylogeography of Hawaiian Araneae. In: *Proceeding of the 17th European Colloquium of Arachnology, Edinburgh 1997* (ed. Selden PA), pp. 41–50, British Arachnological Society, Burnham Beeches, Bucks, UK.

Gillespie RG, Roderick GK (2002) Arthropods on islands: colonization, speciation, and conservation. *Annual Review of Entomology*, **47**, 595–632.

Givnish TJ, Millam KC, Mast AR *et al*. (2009) Origin, adaptive radiation and diversification of the Hawaiian lobeliads (Asterales: Campanulaceae). *Proceedings of the Royal Society B: Biological Science*, **276**, 407–416.

Gressitt JL (1956) Some distributions patterns of Pacific island fauna. *Systematic Zoology*, **5**, 11–32.

Hedin MC (1997) Molecular phylogenetics at the population/species interface in cave spiders of the southern Appalachians (Araneae: Nesticidae*: Nesticus*). *Molecular Biology and Evolution*, **14**, 309–324.

Hedin MC, Maddison WP (2001) Phylogenetic utility and evidence for multiple copies of elongation factor-1alpha in the spider genus *Habronattus* (Araneae: Salticidae). *Molecular Biology and Evolution*, **18**, 1512–1521.

Heiling AM, Heberstein ME, Chittka L (2003) Crab spiders manipulate flower signals. *Nature*, **421**, 334.

Holland BS, Hadfield MG (2004) Origin and diversification of the endemic Hawaiian tree snails (Achatinellidae: Achatinellinae) based on molecular evidence. *Molecular Phylogenetics and Evolution*, **32**, 588–600.

Hormiga G (2002) *Orsonwelles*, a new genus of giant linyphiid spiders (Araneae) from the Hawaiian Islands. *Invertebrate Systematics*, **16**, 369–448.

Hormiga G, Arnedo M, Gillespie RG (2003) Speciation on a conveyor belt: sequential colonization of the Hawaiian Islands by *Orsonwelles* spiders (Araneae, Linyphiidae). *Systematic Biology*, **52**, 70–88.

Huelsenbeck JP, Rannala B (1997) Phylogenetic methods come of age: testing hypotheses in an evolutionary context. *Science*, **276**, 227–232.

Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754–755.

Jordan S, Simon C, Polhemus D (2003) Molecular systematics and adaptive radiation of Hawaii's endemic damselfly genus *Megalagrion* (Odonata: Coenagrionidae). *Systematic Biology*, **52**, 89–109.

Lehtinen PT (1993) Polynesian Thomisidae — a meeting of old and new world groups. *Memoirs of the Queensland Museum*, **33**, 585–591.

Lehtinen PT, Marusik YM (2008) A redefinition of *Misumenops* F. O. Pickard-Cambridge, 1900 (Araneae, Thomisidae) and review of the New World species. *Bulletin of the British Arachnological Society*, **14**, 173–198.

Losos JB, Jackman TR, Larson A, de Queiroz K, Rodriquez-Schettino L (1998) Contingency and determinism in replicated adaptive radiations of island lizards. *Science*, **279**, 2115–2118.

Maddison DR, Maddison WP (2000) *MacClade 4 Analysis of Phylogeny and Character Evolution*. Sinauer Associates, Sunderland, Massachusetts.

Magnacca KN, Danforth BN (2007) Low nuclear DNA variation supports a recent origin of Hawaiian *Hylaeus* bees (Hymenoptera: Colletidae). *Molecular Phylogenetics and Evolution*, **43**, 908–915.

Nielsen R (2002) Mapping mutations on phylogenies. *Systematic Biology*, **51**, 729–739.

O'Grady P, DeSalle R (2008) Out of Hawaii: the origin and biogeography of the genus *Scaptomyza* (Diptera: Drosophilidae). *Biology Letters*, **4**, 195–199.

Ono H (1988) *A Revisional Study of the Spider Family Thomisidae of Japan*. National Science Museum, Tokyo.

Oxford GS, Gillespie RG (1998) Evolution and ecology of spider coloration. *Annual Review of Entomology*, **43**, 619–643.

Palumbi SR, Martin A, Romano S *et al*. (1991) *The Simple Fool's Guide to PCR*. Department of Zoology, University of Hawaii, Honolulu, Hawaii.

Platnick NI (2009) *The World Spider Catalog*, Version 9.5. American Museum of Natural History, New York. Available online at http://research.amnh.org/entomology/spiders/catalog/index.html.

Posada D, Crandall KA (1998) ModelTest: testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Price JP, Clague DA (2002) How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. *Proceedings of the Royal Society B: Biological Sciences*, **269**, 2429–2435.

Price JP, Wagner WL (2004) Speciation in Hawaiian angiosperm lineages: cause, consequence, and mode. *Evolution*, **58**, 2185–2200.

Rundell RJ, Holland BS, Cowie RH (2004) Molecular phylogeny and biogeography of the endemic Hawaiian Succineidae (Gastropoda: Pulmonata). *Molecular Phylogenetics and Evolution*, **31**, 246–255.

Sanderson MJ (2003) r8s: inferring absolute rates of molecular evolution and divergence times in absence of a molecular clock. *Bioinformatics*, **19**, 301–302.

Schluter D (2000) *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford, UK.

Shimodaira H, Hasegawa M (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution*, **16**, 1114–1116.

Simmons MP, Ochoterena H (2000) Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology*, **49**, 369–381.

Simon E (1900) Arachnida. *Fauna Hawaiiensis*, **2**, 443–519.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, **87**, 651–701.

Sorenson MD, Franzosa EA (2007) *TreeRot*, Version 3. Boston University, Boston, Massachusetts.

Suman TW (1970) Spiders of the family Thomisidae in Hawaii. *Pacific Insects*, **12**, 773–864.

Swofford DL (2002) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sinauer Associates, Sunderland, Massachusetts.

Tarr CL, Fleischer RC (1995) Evolutionary relationships of the Hawaiian honeycreepers (Aves, Drepanidinae). In: *Hawaiian Biogeography: Evolution on a Hot Spot Archipelago* (eds Wagner WL, Funk VA). Smithsonian Institution Press, Washington D.C.

Vandergast AG, Gillespie RG, Roderick GK (2004) Influence of volcanic activity on the population genetic structure of Hawaiian *Tetragnatha* spiders: fragmentation, rapid population growth and the potential for accelerated evolution. *Molecular Ecology*, **13**, 1729–1743.

White WM, Duncan RA (1996) Geochemistry and geochronology of the Society Islands: new evidence for deep mantle recycling. *Geophysical Monography*, **95**, 183–206.

Whittaker RJ, Fernández-Palacios JM (2007) *Island Biogeography: Ecology, Evolution and Conservation*. Oxford University Press, Oxford, UK.

Wiens JJ (1998) Combining data sets with different phylogenetic histories. *Systematic Biology*, **47**, 568–581.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Strict consensus of the six most parsimonious trees derived from mitochondrial DNA data (16S-ND1 + COI; 1381 characters, 430 parsimony informative).

Fig. S2 Strict consensus of 24 most parsimonious trees from analysis of nuclear protein coding gene elongation factor 1-α (EF1-α; 523 bp + 23 gapped characters, 164 variable character; 82 parsimony informative).

Fig. S3 Phylogram of one of two most parsimonious trees from combined mitochondrial and nuclear data (1927 characters, 635 variable, 464 parsimony informative).

Fig. S4 Fifty per cent majority rule consensus of the Bayesian posterior probability distribution of trees (post burn-in) for the combined data analysis of nine partitions (1: 16S + tRNA leu; 2–4: mt codon positions 1,2 and 3; 5–7: EF1-α codon positions 1,2 and 3; 8: EF1-α intron; 9: intron recoded gaps).

Table S1 Bootstrap and partitioned support values for select clades in combined parsimony analysis (Fig. S3) in comparison to bootstrap and clade posterior probability values in separate analyses of mitochondrial and nuclear data

Table S2 Node age for Hawaiian thomisids in millions of years. Numbers correspond to node labels in chronogram (Fig. 5), average and standard error from 500 bootstrap replicates. Shaded rows indicate nodes with support in Bayesian analyses > 0.95

Table S3 Posterior probability (PP) values of ancestral distributions for each node in the combined Bayesian tree (Fig. S4) determined in SIMMAP 1.0. Areas with highest values are labelled on their corresponding node in Fig. 6

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