Syst. Biol. 52(1):70–88, 2003
DOI: 10.1080/10635150390132786

Speciation on a Conveyor Belt: Sequential Colonization of the Hawaiian Islands by *Orsonwelles* Spiders (Araneae, Linyphiidae)

GUSTAVO HORMIGA,[1] MIQUEL ARNEDO,[2,3] AND ROSEMARY G. GILLESPIE[2]

[1]*Department of Biological Sciences, George Washington University, Washington, D.C. 20052, USA; E-mail: hormiga@gwu.edu*
[2]*Division of Insect Biology, ESPM, 201 Wellman Hall, University of California, Berkeley, California 94720-3112, USA; E-mail (R.G.G.): gillespi@nature.berkeley.edu*

Abstract.— Spiders of the recently described linyphiid genus *Orsonwelles* (Araneae, Linyphiidae) are one of the most conspicuous groups of terrestrial arthropods of Hawaiian native forests. There are 13 known *Orsonwelles* species, and all are single-island endemics. This radiation provides an excellent example of insular gigantism. We reconstructed the cladistic relationships of *Orsonwelles* species using a combination of morphological and molecular characters (both mitochondrial and nuclear sequences) within a parsimony framework. We explored and quantified the contribution of different character partitions and their sensitivity to changes in the traditional parameters (gap, transition, and transversion costs). The character data show a strong phylogenetic signal, robust to parameter changes. The monophyly of the genus *Orsonwelles* is strongly supported. The parsimony analysis of all character evidence combined recovered a clade with of all the non-Kauai *Orsonwelles* species; the species from Kauai form a paraphyletic assemblage with respect to the latter former clade. The biogeographic pattern of the Hawaiian *Orsonwelles* species is consistent with colonization by island progression, but alternative explanations for our data exist. Although the geographic origin of the radiation remains unknown, it appears that the ancestral colonizing species arrived first on Kauai (or an older island). The ambiguity in the area cladogram (i.e., post-Oahu colonization) is not derived from conflicting or unresolved phylogenetic signal among *Orsonwelles* species but rather from the number of taxa on the youngest islands. Speciation in *Orsonwelles* occurred more often within islands (8 of the 12 cladogenic events) than between islands. A molecular clock was rejected for the sequence data. Divergence times were estimated by using the non-parametric rate smoothing method of Sanderson (1997, Mol. Biol. Evol. 14:1218–1231) and the available geological data for calibration. The results suggest that the oldest divergences of *Orsonwelles* spiders (on Kauai) go back about 4 million years. [Biogeography; cladistics; colonization; Hawaii; Linyphiidae; *Orsonwelles*; phylogenetics; speciation; spiders.]

The Hawaiian archipelago offers an unparalleled opportunity to study evolutionary patterns of species diversification because of its exceptional geographic position and geological history. The seclusion and isolation of the archipelago has resulted in a truly unique terrestrial biota, characterized by a large number of species that represent a relatively small number of species groups (Simon, 1987). The terrestrial biota of the Hawaiian Islands is the result of dispersal from many different parts of the world (Carlquist, 1980; Wagner and Funk, 1995). Some of the best-known animal radiations include the Hawaiian honeycreepers (Freed et al., 1987; Tarr and Fleischer, 1995), land snails (Cowie, 1995), and several groups of terrestrial arthropods (Howarth and Mull, 1992; Roderick and Gillespie, 1998). The radiations of Hawaiian terrestrial arthropods are particularly impressive because of the extremely high proportion of endemics (99% of the native arthropods are endemic; Eldredge and Miller, 1995). These radiations include, among others, drosophilid flies (Carson and Kaneshiro, 1976; DeSalle and Hunt, 1987; DeSalle and Grimaldi, 1992), crickets (Otte, 1994; Shaw, 1995, 1996a, 1996b), carabid beetles (Liebherr, 1995, 1997, 2000; Liebherr and Zimmerman, 1998), damselflies (Jordan et al., 2003), and tetragnathid spiders (Gillespie, 1991a, 1991b, 1992, 1993, 1994; Gillespie and Croom, 1992, 1995; Gillespie et al., 1994, 1997). Unfortunately, a large fraction of the biodiversity of the archipelago remains unknown and undocumented (Eldredge and Miller, 1995). This situation is particularly tragic given the ecological fragility of the few remaining native habitats. The Hawaiian Islands have now acquired the less salubrious reputation of being a "hotbed of extinction" (Mlot, 1995), and many species have gone and will continue to go extinct before they have been described.

The geological history of the Hawaiian archipelago is relatively well understood (Stearns, 1985; Carson and Clague, 1995), with individual islands arranged linearly by age. Niihau and Kauai are the oldest of the current high islands (ca. 5.1 million years old). Oahu is about 3.7 million years old and is located southeast of Kauai. Molokai, Maui, Lanai, and Kahoolawe (0.8–1.9 million years old) are situated on a common platform and were at some point connected above sea level forming the so-called Maui-Nui complex. Hawaii, the youngest island (<0.5 million years old), is currently located over the hot spot and still has active volcanoes. This chain continues northwest of the current eight high islands with several lower islands and atolls and a series of submerged seamounts.

The linyphiid spiders of the genus *Orsonwelles* (Fig. 1A) are some of the most conspicuous terrestrial arthropods of Hawaiian native forests. Their huge sheet webs (Fig. 1B), reaching some times up to 1 m^2 in surface area, are familiar to local biologists and naturalists because they are common and often reach high densities in native Hawaiian wet and mesic habitats. Nevertheless, *Orsonwelles* spiders are seldom seen or collected because they are nocturnal and hide in retreats during the day. At night, these large spiders can be seen walking upside-down on their webs. Not until very recently was this group identified as an endemic radiation and its taxonomic diversity evaluated. Recent work on *Orsonwelles* has revealed that the genus contains at least

[3]Present address: Department de Biologie Animal, Universitat de Barcelona, Av. Diagonal 645, E-08028 Barcelona, Spain. E-mail: marnedo@porthos.bio.ub.es

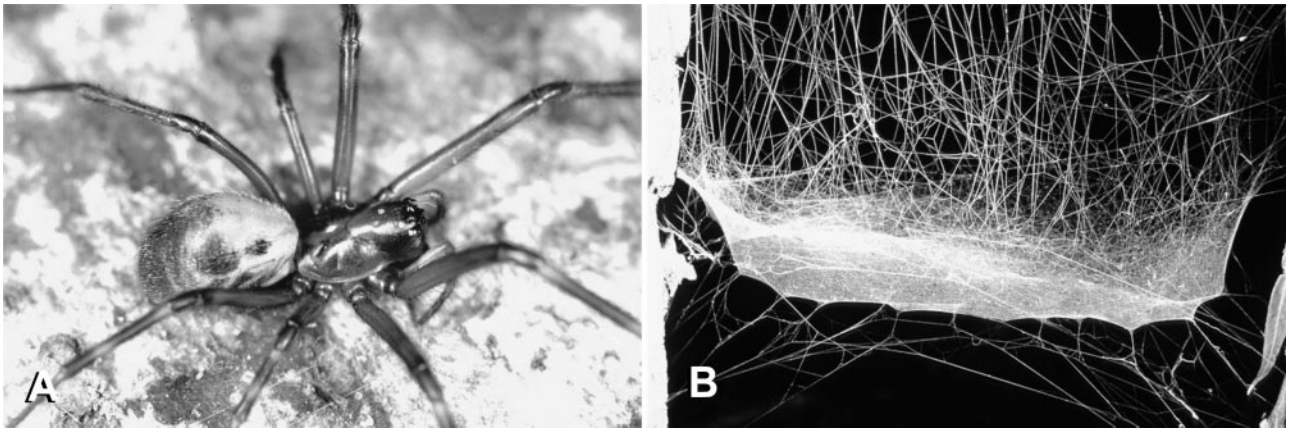

FIGURE 1. (A) *Orsonwelles graphicus* (female) from Hawaii. (B) The web of *Orsonwelles othello* from Molokai.

13 species, 11 of which were undescribed (Hormiga, 2002). All *Orsonwelles* species are single-island endemic, and most of them have very small, nonoverlapping distributions (Fig. 2). Furthermore, many *Orsonwelles* species are restricted to a single mountaintop (e.g., *O. iudicium* in Ha'upu, Kauai) or the higher elevations of a particular range (e.g., *O. bellum* on Mount Kahili, Kauai).

Until very recently, the only work on *Orsonwelles* spiders were the original taxonomic descriptions by Eugène Simon (1900). Simon's work was based on relatively few specimens, most of them juveniles (in spiders, very often only genitalic morphology can provide reliable diagnostic features for species, especially when they are closely related). He recognized only two species (*O. torosus* and *O. graphicus*) and concluded, largely based on his misidentifications of juveniles, that these species were widespread in the archipelago. Simon placed these two Hawaiian taxa in the European genus *Labulla*. Recent work on this group by Hormiga (2002) has uncovered the highly underestimated species diversity of this Hawaiian group (which includes $\geq$13 species) and highlighted the remote phylogenetic affinities of *Orsonwelles* to the type species of the genus *Labulla*, *L. thoracica*, requiring the creation of a new genus. Hormiga also detailed the highly local endemicity of these Hawaiian spiders. The monophyly of the genus *Orsonwelles* is strongly supported by numerous morphological synapomorphies, both somatic and genitalic, and by at least one web architecture character (Hormiga, 2002). Perhaps the single most striking morphological feature of *Orsonwelles* spiders is their extraordinarily large size, with some females (*O. malus*) reaching a total length of >14 mm, which makes them the largest known linyphiids (the next largest described linyphiid species seems to be *Laminacauda gigas*, an erigonine from the Chilean Juan Fernández Islands, in which the females can reach up to 9.9 mm total length, Millidge, 1991). Thus, the genus *Orsonwelles* represents a genuine case of insular gigantism. As yet, the closest ancestor to this Hawaiian radiation is unknown, a situation that has three possible explanations: (1) its geographic origin remains unknown and the highly autapomorphic nature of this lineage hinders the search for close relatives on the basis of morphological characters; (2) our knowledge of the diversity and taxonomy of the circumpacific linyphiid fauna ranges from fragmentary to very poor; and (3) the higher level cladistic structure of Linyphiidae (the second largest spider family in terms of described species and the largest in terms of genera) is only poorly understood (Hormiga, 1994a, 1994b, 2000).

The objectives of our study were to reconstruct the species-level phylogenetic relationships of *Orsonwelles* species so as to understand better the importance of the geological history of the archipelago in shaping the cladogenic events and to study the biogeographic hypotheses implied by the phylogenetic reconstructions. To reconstruct the cladistic relationships of *Orsonwelles* species, we used a combination of morphological and molecular (both mitochondrial and nuclear sequence characters) data. The nature of these different character systems allowed us to explore and contrast the contribution of these different markers in reconstructing the cladistic patterns of these Hawaiian spiders. In addition, the nucleotide sequence data, in combination with the reconstructed phylogenetic trees, allowed us to estimate the divergence times within *Orsonwelles*.

MATERIALS AND METHODS

Taxonomic Sampling

We collected specimens of 12 of the 13 *Orsonwelles* species. *Orsonwelles torosus*, known from a single museum specimen, has not been seen since R. C. L. Perkins collected the type specimen in the Waimea area (Kauai) in the 1890s. We have searched this area, now highly disturbed ecologically, and have not been able to find *O. torosus*, which we presume is extinct. For most of the remaining *Orsonwelles* species, we used multiple specimens (haplotypes) from several geographic localities

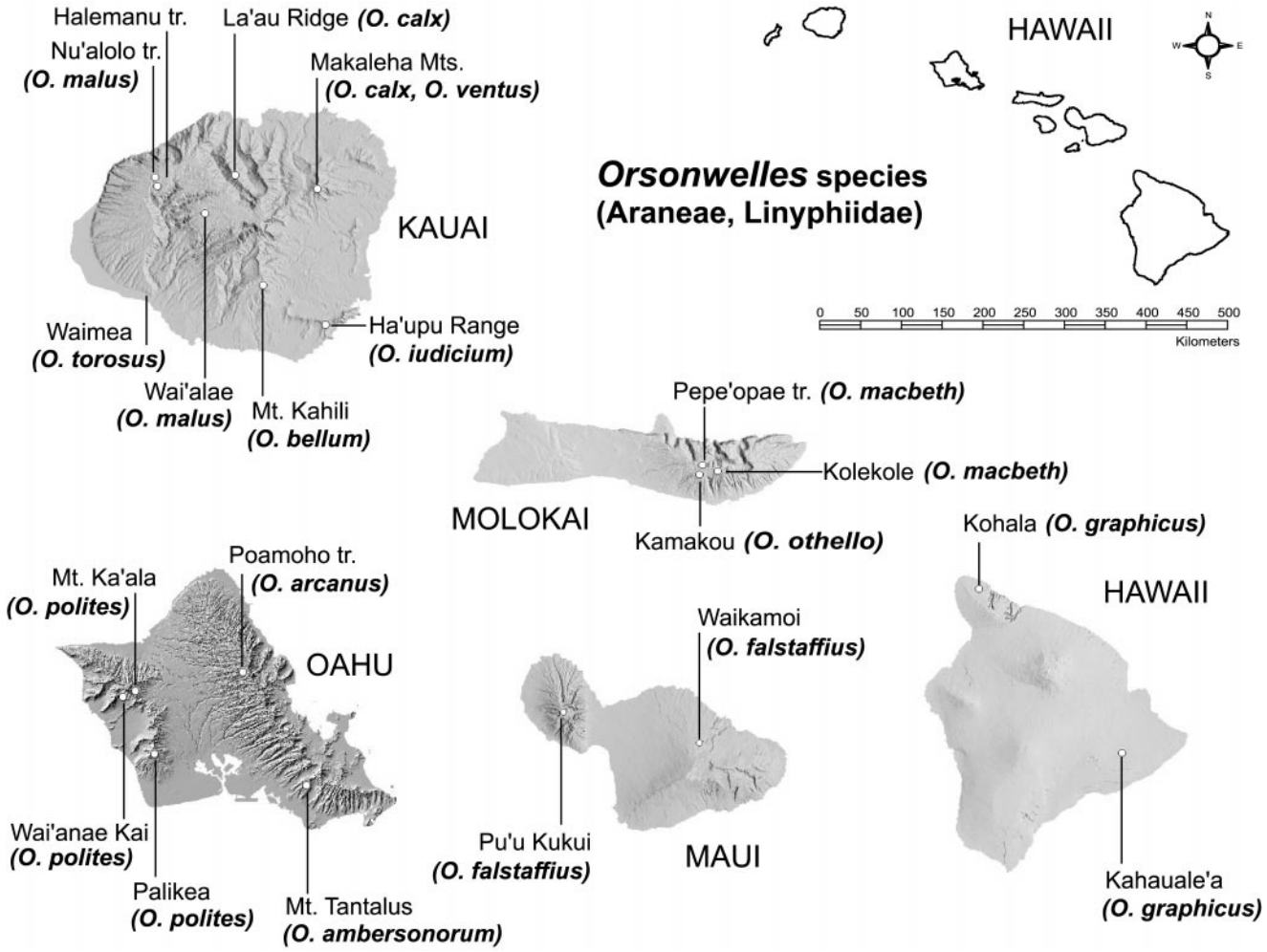

FIGURE 2. Geographic distribution of *Orsonwelles* species, with emphasis on the collecting localities of the specimens used in this study. All the species are single-island endemics. Only one case of sympatric distribution has been documented (*O. calx* and *O. ventus* in the Makaleha Mountains of Kauai). *Orsonwelles torosus*, known from a single specimen collected by R. C. L. Perkins in the late 1800s, is presumed extinct.

(Table 1) to account for intraspecific molecular variation and to test the monophyly of the species. The phylogenetic analyses include the 13 known *Orsonwelles* species and a taxonomic sample of linyphiid outgroups. *Orsonwelles* is highly unusual morphologically and to our knowledge does not closely resemble any other known linyphiid genus. The genus belongs to the Linyphiini, within the subfamily Linyphiinae (Hormiga, 2002). Although the cladistic structure of Linyphiidae is poorly understood (Hormiga, 1994b, 2000), in terms of overall morphological similarity *Orsonwelles* resembles the genus *Neriene*, but these two genera are very different in many details. The morphological character matrix includes eight outgroup terminal taxa to represent the diversity of linyphiines, including two species of *Neriene*. The data set also includes the type species of the genus *Labulla* (*L. thoracica*) because the first two described species of *Orsonwelles* had been previously placed in this Palearctic genus (Simon, 1900). The outgroup sample was based on the selection of taxa previously used for the study of the higher level phylogenetics of linyphiids based on morphological characters (Hormiga, 2000, 2002) with some variation because of availability of specimens for sequencing work.

Character Matrices

We built three different character matrices to reconstruct the phylogenetic relationships of *Orsonwelles* species. The first matrix (M1) includes the 13 known *Orsonwelles* species and 8 linyphiid outgroups scored for 71 characters (70 morphological plus one behavioral character, see Appendices 1 and 2). The second matrix (M2) included two *Orsonwelles* species (*O. malus* and *O. polites*) and eight linyphiid outgroups scored for 45 morphological characters (i.e., all the characters used in the first matrix that are phylogenetically informative within this taxonomic context) and the following five genes: the mitochondrial cytochrome oxidase I (CO1) (676 bp) and large subunit (16S) ribosomal genes (428–444 bp) and the nuclear genes large subunit (28S) ribosomal (302–317 bp), small subunit (18S) ribosomal

TABLE 1. Species, with locality and voucher data, of the *Orsonwelles* species studied. The codes refer to the taxon labels used in the cladograms of Figures 5–7.

Species	Locality	Country/ island, volcano	Haplotype	Code	Sequenced fragments
Bolyphantes alticeps	Baelum Sonderskov	Denmark			CO1, 16S, 28S, 18S, H3
Labulla thoracica	Baelum Sonderskov	Denmark			CO1, 16S, 28S, 18S, H3
Lepthyphantes minutus	Baelum Sonderskov	Denmark			CO1, 16S, 28S, 18S, H3
Linyphia triangularis	Baelum Sonderskov	Denmark			CO1, 16S, 28S, 18S, H3
Microlinyphia dana	Salt Point S.P., California	USA			CO1, 16S, 28S, 18S
Neriene radiata	Patuxent, Maryland	USA			CO1, tRNAL, 16S, ND1, ITS2, 28S, 18S, H3
N. variabilis	Patuxent, Maryland	USA			CO1, tRNAL, 16S, ND1, ITS2, 28S, 18S, H3
Pityohyphantes costatus	Patuxent, Maryland	USA			CO1, 16S, 18S
Orsonwelles ambersonorum	Mt. Tantalus	Oahu, Ko'olaus	G13	amb1	CO1, tRNAL, 16S, ND1, ITS2, 28S, 18S, H3
O. ambersonorum	Mt. Tantalus	Oahu, Ko'olaus	G18	amb2	CO1, tRNAL, 16S, ND1, ITS2
O. arcanus	Poamoho trail	Oahu, Ko'olaus	G10	arc1	CO1, tRNAL, 16S, ND1, ITS2
O. arcanus	Poamoho trail	Oahu, Ko'olaus	G14	arc2	CO1, tRNAL, 16S, ND1, ITS2
O. bellum	Mt. Kahili	Kauai	G43	bel1	CO1, tRNAL, 16S, ND1, ITS2
O. calx	Makaleha Mountains	Kauai	G3	cal1	CO1, tRNAL, 16S, ND1, ITS2
O. calx	Makaleha Mountains	Kauai	G40	cal2	CO1, tRNAL, 16S, ND1, ITS2
O. calx	La'au Ridge	Kauai	G41	cal3	CO1, 16S, ITS2
O. falstaffius	Waikamoi	East Maui	G7	fal1	CO1, tRNAL, 16S, ND1, ITS2
O. falstaffius	Pu'u Kukui	West Maui	G12	fal2	CO1, tRNAL, 16S, ND1, ITS2
O. falstaffius	Pu'u Kukui	West Maui	G15	fal3	CO1, tRNAL, 16S, ND1
O. graphicus	Kohala	Hawaii, Kohala	G1	gra1	CO1, tRNAL, 16S, ND1, ITS2
O. graphicus	Kahauale'a	Hawaii, Kilauea	G11	gra2	CO1, tRNAL, 16S, ND1, ITS2
O. iudicium	Ha'upu Ridge	Kauai	G37	iudi	CO1, tRNAL, 16S, ND1, ITS2
O. macbeth	Kolekole cabin, Kamakou TNC preserve	Molokai	G55	mac1	CO1, tRNAL, 16S, ND1, ITS2
O. macbeth	Pelekunu Lookout	Molokai	G56	mac2	CO1, tRNAL, 16S, ND1, ITS2
O. macbeth	Pepe'opae trail	Molokai	G46	mac3	CO1, tRNAL, 16S, ND1, ITS2
O. macbeth	Kolekole trail, ca. 1160 m	Molokai	G45	mac4	CO1, tRNAL, 16S, ND1, ITS2
O. macbeth	Pepe'opae trail	Molokai	G42	mac5	CO1, tRNAL, 16S, ND1, ITS2
O. malus	Wai'alae cabin	Kauai	G36	mal1	CO1, tRNAL, 16S, ND1, ITS2
O. malus	Halemanu trail	Kauai	G8	mal2	CO1, tRNAL, 16S, ND1, ITS2
O. malus	Nu'alolo trail	Kauai	G16	mal3	CO1, tRNAL, 16S, ND1, ITS2, 28S, 18S, H3
O. othello	Kolekole trail, ca. 1,080 m	Molokai	G54	oth1	CO1, tRNAL, 16S, ND1, ITS2
O. othello	Kolekole trail, ca. 1,080 m	Molokai	G39	oth2	CO1, tRNAL, 16S, ND1, ITS2
O. othello	Kamakou TNC preserve	Molokai	G21	oth3	CO1, tRNAL, 16S, ND1, ITS2
O. othello	Pepe'opae trail	Molokai	G44	oth4	CO1, tRNAL, 16S, ND1, ITS2
O. polites	Wai'anae Kai	Oahu, Wai'anaes	G9	pol1	CO1, tRNAL, 16S, ND1, ITS2
O. polites	Palikea	Oahu, Wai'anaes	G19	pol2	CO1, tRNAL, 16S, ND1, ITS2, 28S, 18S, H3
O. polites	Mt. Ka'ala	Oahu, Wai'anaes	G22	pol3	CO1, tRNAL, 16S, ND1, ITS2
O. ventus	Makaleha Mountains	Kauai	G38	ven1	CO1, tRNAL, 16S, ND1, ITS2
O. ventus	Makaleha Mountains	Kauai	G4	ven2	CO1, tRNAL, 16S, ND1, ITS2
O. ventus	Makaleha Mountains	Kauai	G5	ven3	CO1, tRNAL, 16S, ND1, ITS2

(771–775 bp), and histone H3 (H3) (328 bp). The third matrix (M3) has the 13 known *Orsonwelles* species, represented by 32 individuals, and 2 linyphiid outgroups scored for 71 morphological characters (50 of them are phylogenetically informative within this context) and the following five genes: the mitochondrial CO1 (439 bp), 16S (464–468 bp), tRNALEU$^{(CUN)}$ (tRNAL) (45 bp), and the NADH dehydrogenase subunit I (ND1) (367 bp) and the nuclear internal transcribed spacer 2 (ITS2) (353–416 bp). Each sequenced individual was considered a terminal taxon in the analyses, and the morphological characters scored for each of them were those corresponding to the species in which the individual was included. When information regarding any of the gene fragments was not available, all the characters of that data set were scored as missing. Outgroups for this matrix (*Neriene radiata* and *N. variabilis*) were chosen based on the sister taxa obtained from the analysis of the second matrix (M2). Different gene fragments have been used in the two molecular matrices because one was used to reconstruct cladogenetic events between genera (i.e., the phylogenetic structure of *Orsonwelles* outgroups) and the other was used to evaluate more recent divergences (i.e., the cladogenic events among *Orsonwelles* species).

Morphological Data

Seventy morphological and one behavioral character were scored for 21 taxa (the 13 *Orsonwelles* species plus 8 outgroup linyphiid species; matrix M1). The methods of study and most of the characters have been described and illustrated in detail by Hormiga (2002). The 15 character descriptions not provided by Hormiga (2002) are given in Appendix 1.

Molecular Data

Live specimens were collected in the field and fixed in 95% ethanol. Alternatively, when fresh material was not available specimens from museum collections (preserved in 75% ethanol) were used for extractions, with relative success mostly dependent on the length of preservation. Only one or two legs were used for extraction, except for specimens preserved in 75% ethanol, for which as many as four legs plus the carapace were used. The remainder of the specimen was kept as a voucher (deposited at the National Museum of Natural History, Smithsonian Institution, Washington, D.C.).

Total genomic DNA was extracted following the phenol/chloroform protocol of Palumbi et al. (1991) or using Qiagen DNeasy Tissue Kits. The approximate concentration and purity of the DNA obtained was evaluated through spectophotometry, and the quality was verified using electrophoresis in an agarose/Tris-borate-EDTA (TBE) (1.8%) gel. Partial fragments of four mitochondrial genes and four nuclear genes were amplified using the following primer pairs. The mitochondrial CO1 was amplified by means of C1-J-1490 and C1-N-2198 (Folmer et al., 1994) or C1-J-1751 and C1-N-2191 (Simon et al., 1994). A fragment including the 5′ half of the mitochondrial 16S ribosomal gene, the tRNAL, and the 3′ half of the ND1 was amplified with the primers LR-N-13398 (Simon et al., 1994) and N1-J-12261 (Hedin, 1997a) or N1-J-12307 (CATATTTAGAATTTGAAGCTC) (M. Rivera, pers. comm.). Alternatively, the amplification was carried out in two different fragments using the primer combinations of LR-N-13398 with LR-J-12864 (CTCCGGTTTGAACTCAGATCA) (Hsiao, pers. comm.) and LR-N-12866 with either LR-N-13398 or N1-J-12307. Nuclear markers were obtained for the following genes and primer combinations: 28S ribosomal DNA with primers 28SA and 28SB (Whiting et al., 1997), 18S ribosomal DNA with primers 5F or 18Sa2.0 and 9R (Giribet et al., 1999), H3 with H3aF and H3aR (Colgan et al., 1998), and ITS2 with ITS2-28S and ITS2-5.8S (Hedin, 1997b). The thermal cyclers, Perkin Elmer 9700, Perkin Elmer 9600, or BioRad iCycle, were used to perform either 25 (mitochondrial genes) or 40 (nuclear genes) iterations of the following cycle: 30 sec at 95°C, 45 sec at 42–48°C (depending on the primers), and 45 sec at 72°C, beginning with an additional single cycle of 2 min at 95°C and ending with another cycle of 10 min at 72°C. The PCR mix contained primers (0.48 μM each), dNTPs (0.2 mM each), and 0.6 U Perkin Elmer AmpliTaq DNA polymerase (for a 50-μl reaction) with the supplied buffer and, in some cases, an extra amount of $MgCl_2$ (0.5–1.0 mM). PCR results were visualized on an agarose/TBE (1.8%) gel. PCR products were cleaned using Geneclean II (Bio 101) or Qiagen QIAquick PCR Purification Kits following the manufacturer's specifications. DNA was directly sequenced in both directions with the cycle sequencing method using dye terminators (Sanger et al., 1977) and the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction with the AmpliTaq DNA Polymerase FS kit. Sequenced products were cleaned using Princeton Separations CentriSep columns and run out on an ABI 377 automated sequencer.

Sequence errors and ambiguities were edited using the Sequencher 3.1.1 software package (Gene Codes Corp.). Sequences were subsequently exported to the program GDE 2.2 (Genetic Data Environment) running on a Sun Enterprise 5000 Server, and manual alignments were built taking into account secondary structure information from secondary structure models available in the literature for 16S (Arnedo et al., 2001), 28S (Ajuh et al. 1991), and 18S (Guttell et al., http://www.rna.icmb.utexas.edu/). Alignment of the protein-coding genes was trivial because no length variation was observed in the sequences. Sequences have been deposited in GenBank, with the following accession numbers: 16S-tRNAleu-ND1 = AY078660–AY078666 and AY078711–AY078725; 18S = AY078667–AY078677; 28S = AY078688–AY078686; CO1 = AY078689–AY078699 and AY078744–AY078759; H3 = AY078700–AY078709; ITS2 = AY078774–AY078806.

Phylogenetic Analyses

The parsimony analyses of the morphological matrix were performed using the computer programs HENNIG86 version 1.5 (Farris, 1988) and NONA version

2.0 (Goloboff, 1993). WinClada version 0.9.99m24 (Nixon, 1999) and NDE version 0.4.9 (Page, 2001) were used to study character optimizations on the cladograms and to build and edit the character matrix, respectively. Ambiguous character optimizations were resolved using Farris optimization (ACCTRAN), which maximizes homology by favoring reversal or secondary loss over convergence. Multistate characters were treated as nonadditive (unordered or Fitch minimum mutation model; Fitch, 1971) (see Hormiga, 1994a, for justification). Successive character weighting (Farris, 1969) was performed using HENNIG86 1.5, which reweights characters by the rescaled consistency index (Farris, 1988, 1989a, 1989b). NONA (Goloboff, 1993) was used to calculate Bremer support indices (BS) (Bremer, 1988, 1994).

Cladistic analysis of the molecular data matrices was performed using direct optimization (Wheeler, 1996) as implemented in the computer program POY (Wheeler and Gladstein, 2000). This method, first suggested by Sankoff (1975), approaches the alignment problem by incorporating insertion/deletion events as additional transformations during the optimization step in tree evaluation instead of trying to reconcile sequence lengths by adding gaps as additional states. Unlike competing methods, which dissociate the process of aligning sequences from the phylogenetic analysis (i.e., search for optimal trees), in direct optimization gap assignment is an intrinsic and inseparable part of the phylogenetic inference. Nonetheless, the alignment implied by a particular tree can be recovered. Although such an alignment is particular to the tree selected, it is as much a result of the analysis as the reconstructed most-parsimonious trees. Moreover, direct optimization explores the sensitivity of the results to perturbations in the parameters (e.g., gap costs, bases transformations, morphology weights) of the analysis. Manual alignments based on secondary structure information were used to define smaller fragments of unambiguous homology. We used the secondary structure information for data management purposes and, most importantly, for identifying fragments of the different ribosomal genes that could be unambiguously considered homologous. These fragments were flanked by series of either identical nucleotides (about 10 bp), which was the case for most of the fragments into which the 18S and 28S were spliced, or recognized fully complementary stems (most of the fragments of the 16S). Once the fragments were identified, all the gaps manually inserted in them were removed and all the fragments were analyzed simultaneously by POY. Splitting the gene into smaller fragments of unambiguous homology has two advantages. It restricts the assignment of indels by the direct optimization algorithm to regions for which homology is not disputed; thus, the results are biologically more feasible. It also speeds up the analyses by reducing the computation time (Giribet, 2001).

Heuristic parsimony searches were implemented by performing 10 rounds of tree building by random addition of taxa using approximate algorithms, retaining the best round and subjecting it to sequential rounds of SPR and TBR branch swapping. This protocol was repeated until minimum-length trees were obtained in at least 3 iterations after a minimum of 10 iterations were performed up to a maximum of 100 iterations. Tree fusion and tree drifting techniques (Goloboff, 1999) were applied to the best trees retained in each iteration and to the best trees obtained overall.

Two rounds of tree fusion were applied to all pairwise combinations of the retained trees (a first round using SPR and a second round using the more thorough but computationally intensive TBR); only clades with a minimum of five taxa were fused. This strategy can optimize computation time and search efficiency (W. Wheeler, pers. comm.). Thirty branch-swapping rearrangements, one round using SPR and another round using TBR, were applied on the trees kept after fusion. Rearranged trees equal to or better than the originals under a criterion based on character fit and tree length were accepted and subsequently subjected to full SPR and TBR branch swapping, accepting only minimal trees. To cope with the effect of the heuristics of tree length calculation shortcuts, an extra TBR branch-swapping round was applied to all cladograms found within 1% of the minimum tree length. Heuristic BS values (Bremer, 1988) were estimated as a measure of clade support.

Sensitivity of the results to different parameter values (gap cost and transversion weighting) were investigated (Wheeler, 1995). Ten different parameter combinations were analyzed: equal cost of gaps, transversions, and transitions (hereinafter referred to as 111); gaps twice (211), four times (411), and eight times (811) the cost of the nucleotide transformations; and transversions twice (221, 421, 821), four times (441, 841), and eight times (881) the cost of transitions. Transversions were never given a higher cost than gaps. In all cases, morphological characters were assigned the same weight as the gap cost. The parameter combinations we explored are a small and arbitrarily chosen fraction of the almost infinite combinations available. Character and topological congruence, as measured by the incongruence length difference index (ILD; Mickevich and Farris, 1981) and the topological ILD (TILD; Wheeler, 1999) has been proposed as an objective criterion for choosing among the results of a particular parameter cost combination (but see Barker and Lutzoni, 2002, who concluded that the ILD test is a poor indicator of homogeneity and combinability and a poor indicator of congruence/incongruence). However, neither ILD nor TILD are comparable across different data matrices, their values being influenced by the actual parameter combination selected (Faith and Trueman, 2001; M. Ramirez, pers. comm.). Also, ILD and TILD values can differ depending on the actual data partition chosen, and selecting one data partition over another is usually very subjective. Because of the lack of such an objective criterion to decide among alternative weighting schemes, results from equal costs were preferred. Results under additional parameter value combinations were used to assess clade stability.

Simultaneous and partial analyses of the character matrices were performed to characterize the contribution of the different character partitions to the total

TABLE 2. Minimum and maximum calculated branch lengths and Bremer support (BS) and partitioned Bremer support (PBS) values for the different partitions defined calculated with POY through constrained searches (see Fig. 6 for clade labels). The PBS value for each clade is an average of the values for each tree obtained in the constrained searches; a maximum of 10 tree were used. Values in parentheses are the minimum and maximum PBS observed for the trees examined in each constrained search.

Clade	Branch length	Combined BS	Morphology PBS	Mitochondrial PBS	Nuclear PBS
Orsonwelles	104–174	30	11.2 (7, 18)	64.1 (49, 74)	−45.4 (−57, −37)
A	3–46	2	−0.5 (−1, 0)	3 (2, 4)	−0.5 (−1, 0)
B	16–45	1	−1.7 (−2, −1)	3.4 (2, 4)	−0.7 (−1, 0)
C	24–45	3	1.1 (0, 2)	3.7 (2, 5)	−1.9 (−4, 1)
D	4–3	1	−1.0 (−1)	2.0 (2)	0.0 (0)
E	35–60	5	3.9 (3, 6)	7.0 (7)	−5.9 (−8, −5)
F	9–32	2	0.4 (0, 2)	8.5 (7, 13)	−6.9 (−12, −5)
G	17–32	2	1.5 (1, 2)	11.5 (10, 13)	−11.0 (−12, −10)
H	7–23	4	2.0 (2)	−1.0 (−1)	3.0 (3)
I	11–16	5	4.5 (4, 5)	12.0 (11, 13)	−11.5 (−12, −11)
J	11–19	5	1.0 (1)	14.0 (14)	−10.0 (−10)

results. The following data partitions were defined: morphology versus all genes combined (genomic), and morphology versus nuclear genes combined (nuclear) versus mitochondrial genes combined (mitochondrial). In its current version, POY does not implement an incongruence test between data partitions, although theoretically it would be feasible to implement the ILD test (Farris et al., 1994) under direct optimization. The level of incongruence across the data partitions was qualitatively assessed by means of ILD and TILD indices and by assessing the increase in tree length resulting from forcing topologies resulting from complete analyses onto different characters partitions.

We also calculated partitioned BS (PBS; Baker and DeSalle, 1997) to assess the relative contribution of different data partitions of matrix M3. PBS values were calculated by diagnosing the length of a particular partition on the topologies of the most-parsimonious trees found in the simultaneous analysis of all data combined (Table 2). The partitions considered were morphology, nuclear (ITS2), and mitochondrial (CO1 + ND1 + tRNAL + 16S). Although we prefer phylogenetic analyses based on parsimony we have also explored how maximum likelihood (ML) reconstructs these cladogenetic events. ML analysis was performed using PAUP* 4.0b5 (Swofford, 2001). One of the trees obtained from direct optimization under the equal costs model was arbitrarily chosen, and the implied alignment was reconstructed after removal of *O. torosus*, the only species for which molecular data could not be sampled. The computer program Modeltest version 3.06 (Posada and Crandall, 1998) was used to select the molecular evolution model that best fit the data given the selected tree. The model of sequence evolution was assessed on the combined data (total evidence) tree, which is optimal for the morphological plus molecular observations but not necessarily for the genomic data partition alone. Topological differences between the genetic partition trees and the combined data trees should not be of great concern for choosing among alternative models of sequence evolution because tree topology does not make much difference in model estimation unless the history of the included taxa involves long internal branches (e.g., Sullivan et al., 1996).

Clade Age Estimation

The presence of a molecular clock was investigated using PAUP* by comparing the ML scores under the best model with and without enforcing the molecular clock. When the molecular clock was discarded, the data were transformed into an ultrametric tree by using the nonparametric rate smoothing method (NPRS) (Sanderson, 1997). This method replaces the constraint of constant rates of character change across a tree (i.e., the so-called molecular clock) with the assumption of autocorrelation of rates in ancestor and descendant lineages. The temporal autocorrelation limits the speed with which a rate can change from an ancestral lineage to a descendant lineage. We applied the NPRS method as implemented in the computer program TREEEDIT version 1.0 alfa 8 (Rambaut and Charleston, 2001). Branch lengths were obtained with PAUP* under parsimony, with gaps treated as a fifth state, and arbitrarily resolving ambiguous character changes using ACCTRAN. Branch lengths have been reconstructed using parsimony because ML does not account for gaps. Thus, we had to choose between a method (parsimony) that underestimates the amount of change (due to multiple hits) and a method (ML) that does not take into account one type of change (insertions-deletions). We chose parsimony.

The geographical and geological settings of the Hawaiian archipelago provide a valuable framework for calibrating genetic divergences and inferring clade ages. The oldest K-Ar dating of each island constitutes a maximum age for their harbored populations. Calibration points can be obtained by assuming that the ages of a younger island are contemporaneous with the date of split between its own species/population and the parental species/population on the older island (Fleischer et al., 1998).

RESULTS

The parsimony analysis of the morphological data set (M1) using the implicit enumeration in HENNIG86 (ie*) produced six cladograms of minimal length: length (L) = 134, consistency index (CI) = 0.65, and retention index (RI) = 0.84. After exclusion of six uninformative

characters, the new cladograms had L = 127, CI = 0.63, and RI = 0.84 (Fig. 3). The same results were also found using various heuristic search strategies in NONA. These topological results were stable under successive character weighting. In all the minimal-length trees, the six species from Kauai formed a monophyletic group, that was sister to a clade that included all the remaining species, with the three species from Oahu as the most basal lineages. The strict consensus cladogram indicated conflict in the placement of two taxa within the outgroups and one within the ingroup (Fig. 3). The monophyly of *Orsonwelles* was supported by at least 22 unambiguous synapomorphies (up to 26 under ambiguous optimizations). Within the ingroup, only the placement of *O. torosus* was ambiguously supported: half of the most-parsimonious trees had this species as sister to a clade with all the remaining five species from Kauai and the other half had it as sister to all the other *Orsonwelles* species from Kauai except *O. malus*, which was placed as the most basal taxon of this Kauai clade. In other words, *O. malus* and *O. torosus* switched positions as the most basal taxon within a clade containing all the species from Kauai. Three equally parsimonious alternatives exist for the sister group of *Orsonwelles*: *Linyphia* plus *Neriene* sister to *Microlinyphia*; *Neriene* plus *Linyphia* (with *Microlinyphia* sister to this clade plus *Orsonwelles*); or *Linyphia* plus *Microlinyphia* sister to *Neriene*. The genus *Labulla*, which had contained the first *Orsonwelles* species described, is very distantly related to *Orsonwelles* and was placed as the most basal lineage within the Linyphiini of this taxonomic sample.

The analysis of M2 resulted in a single most-parsimonious tree (Fig. 4) 1,308 steps long. The ILD and TILD were 0.05 and 0.111, respectively, for the morphology versus genomic partitions and 0.014 and 0.233, respectively, for the morphology versus mitochondrial versus nuclear partitions. This cladogram placed *Labulla* and *Pityohyphantes* as the most basal lineages within the Linyphiinae. This topology is congruent with the analysis of the exclusively morphological matrix (M1). The analysis of M2 also supports *Microlinyphia* as sister to *Linyphia*, which was also found in two of the six most-parsimonious trees resulting from the M1 matrix. The analysis of M2 supports the monophyly of *Orsonwelles* and *Neriene* species and placed *Neriene* as the single sister group to *Orsonwelles*. The sister group relationship between *Neriene* and *Orsonwelles* was not recovered in the analysis of the morphological matrix (M1), which suggested that *Linyphia* or *Microlinyphia* plus *Linyphia* is the sister group to *Neriene*. The analyses of the genomic (1 tree, 1,231 steps) and nuclear (3 trees, 398 steps) partitions were fully compatible with the tree from the total data set, except for the sister group relationship of *Labulla* and *Pityohyphantes*. The mitochondrial partition produced a polyphyletic *Neriene*, with *N. variabilis* sister to a clade consisting of a monophyletic *Orsonwelles* sister to the clade ((*N. radiata*, *Pityohyphantes*) (*Microlinyphia*, *Linyphia*)). Five of the seven nodes in the most-parsimonious tree from the M2 matrix under equal costs are robust (stable) to all the variations of morphology, gap, and transition and transversion costs that we explored (Fig. 4). Only under three combinations of high costs for the morphology, gaps, and transversions (821, 441, 881) was the monophyly of *Neriene* and the monophyly of *Neriene* plus *Orsonwelles* lost. Accordingly, *Neriene radiata* and *N. variabilis* were selected as outgroups for analyses of the matrix M3. In general, Bremer supports and clade sensitivity to parameter perturbations were closely correlated, and the weakest support was for clades most sensitive to changes in the parameter costs.

Results from the combined and partial analyses of the matrix M3 under equal parameter values are shown in Figure 5. Fifty trees of minimal length (311 steps) were recovered from the analysis of the nuclear ITS2 (Fig. 5b). The combined mitochondrial genes resulted in four trees 886 steps long (Fig. 5a), and the simultaneous analysis of all the molecular data sets combined yielded 18 trees of length 1,211 (Fig. 5c). The analysis of the complete matrix (M3, all the available character evidence) resulted in 13 trees of minimal length, 1,340 steps long (Fig. 5d). The ILD and TILD were 0.029 and 0.183, respectively, for the morphology versus genomic partitions and 0.04 and 0.16, respectively, for the morphology versus mitochondrial versus nuclear partitions. Forcing the complete (all character evidence) topology on each of the partitions resulted in an increase in length of 4 steps in the morphology partition, 1 in the genomic, 2 in the mitochondrial, and 13 in the nuclear.

BS values were estimated as a measure of clade support. POY implements a fast but very heuristic command to calculate BS consisting on additional rounds of branch swapping on the targeted tree, which can result in the gross overestimation of the actual clade support. A more accurate calculation of the BS was conducted for the simultaneous analysis of the M3 matrix by using more exhaustive constrained searches combined with the "–disagree" command implemented in POY. Both the nuclear and the mitochondrial partitions supported the monophyly of the individuals sampled for each species, except for some of the trees of the nuclear partition that showed *O. othello* paraphyletic with regard to *O. macbeth*. The complete analyses depicted only one ambiguity at the species level, the placement of *O. torosus*, which can be either sister to a clade that includes all the other species from Kauai (except for *O. malus*) or sister to a large clade that includes *O. malus* plus all the non-Kauai species. *Orsonwelles torosus*, presumably extinct, is known by from a single female and could not be coded for the male characters or the molecular characters. To test the effect of the presence of missing data, an additional analysis was conducted with *O. torosus* excluded. The 12 resulting trees (1,338 steps) supported the existence of a clade of Kauaian species, contradicting the results of the complete data matrix (in which *O. malus* was sister to a clade that included all the non-Kauai species).

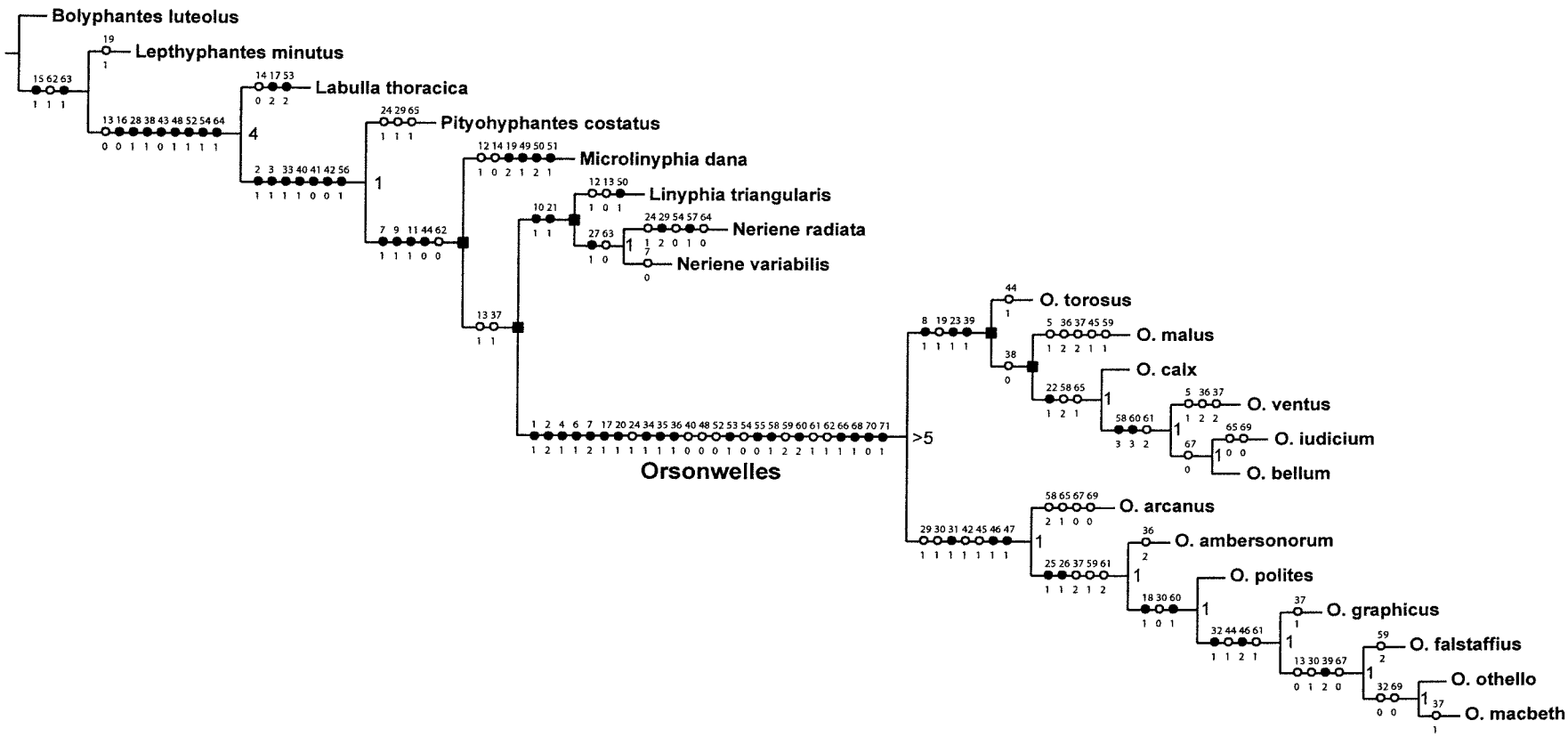

FIGURE 3. One of the six most-parsimonious trees that results from the analysis of the morphological character matrix M1 (L = 134 steps, CI = 0.65, RI = 0.84; after exclusion of six uninformative characters, L = 127 steps, CI = 0.63, RI = 0.84). The basal trichotomy is arbitrarily resolved. The nodes that collapse in the strict consensus cladogram are marked with a solid box. Bremer support values are reported under the branches; characters are optimized using Farris optimization. Open circles represent homoplastic character changes.

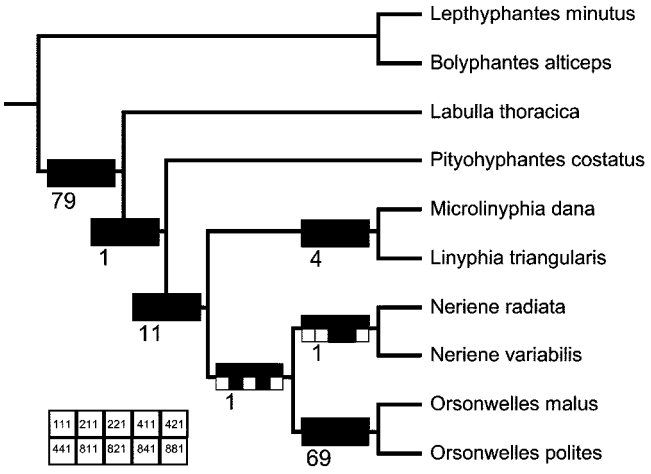

FIGURE 4. The single most-parsimonious tree (L = 1,308) resulting from the analysis of the combined morphological character and molecular matrix (M2). Bremer support values are reported under the branches. Ten different parameter combinations were analyzed and are shown in the boxes. Equal cost of gaps, transversions, and transitions is denoted 111, gaps weighted twice as 211, etc. (in all cases, morphological characters were assigned the same weight as the gap cost). Solid squares on the branches denote support from the various combinations of gap, transversion, and transition costs.

Most of the analyses of the complete data matrix under any parameter combination other than equal costs also resulted in the monophyly of all the species from Kauai. Two additional regions of the topology supported under equal costs were sensitive to changes in the parameter values (Fig. 6). *Orsonwelles polites* could replace *O. arcanus* at the base of the non-Kauai clade, the latter species moving to the base of a Molokai–Mauai–Hawaii clade, and *O. graphicus* could change position to become the sister group to a clade that contained *O. macbeth*, *O. othello*, and *O. falstaffius*. Constrained searches were performed to assess to the extent to which alternative clades recovered in the sensitivity analyses were not supported in the equal costs analysis. Forcing monophyly of Kauaian species increased the cladogram length one step, and forcing the basal position of *O. polites* in the non-Kauai clade resulted in two extra steps. Forcing *O. graphicus* to be sister group to a Maui–Molokai clade added nine extra steps. The nuclear partition strongly rejected clades F and G (with PBS values of −6.9 and −11, respectively; see Table 2) from the total evidence tree (Fig. 6), whereas the mitochondrial partition did not reject, at least strongly, any clade (all the negative values were low and could be due to the heuristics of the calculations). The fact that the morphological data agree with the mitochondrial data in the position of *O. arcanus* (as sister to a clade with all the non-Kauai species) seems to go against the possibility that the mitochondrial tree was reflecting the gene tree instead of the actual species tree.

The treatment of gaps in phylogenetic analysis has elicited much discussion in the systematics literature (see Giribet and Wheeler, 1999, and Lutzoni et al., 2000, for different views on this problem). Because coding gaps as a fifth state can potentially overweight some indels (e.g., Simmons and Ochoterena, 2000), we assessed the effect of gap coding on the parsimony reconstructions. An implied alignment was reconstructed using one of the trees, arbitrarily chosen, obtained from direct optimization under equal costs after removal of *O. torosus* (the only species for which molecular data could not be sampled); this same alignment was used for ML analyses and clade age estimation. There is no heterogeneity on base composition in the implied alignment ($\chi^2 = 26.97$; df = 3; $P = 1.0$). The implied alignment was analyzed under parsimony using PAUP* with gaps treated either as a fifth state or as missing data (?). The first treatment produces 12 most-parsimonious trees (L = 1,138) but only a single species-level topology (that of Fig. 6, after removal of *O. torosus*). Treatment of gaps as question marks resulted in 12 most-parsimonious trees (L = 1,003) and again a single species-level topology (this is the same optimal topology obtained in the ML analyses) that differs from the parsimony tree shown in Figure 6 in the position of *O. malus*, which now appears as basal to the remaining species of Kauai (thus making Kauai species monophyletic), and the placement of *O. iudicium*, which is now shown as sister of *O. ventus*. In sum, these two alternative treatments of gaps produced relatively minor changes in the species-level cladogram (some these changes occurred in regions of the cladogram that are sensitive to changes in parameter costs). These results suggest that most of the phylogenetic signal recovered by the parsimony analysis is insensitive to the characters that result from the gap coding scheme we used.

In the ML analyses, using an implied alignment, the outcome of model selection varied depending on the criterion used for assessing significance. When the likelihood ratio test was used, the TVM+I+Γ model was preferred; when the Akaike information criterion was used instead, the TIM+I+Γ model was preferred. ML heuristic searches with 20 random additions of taxa replicates were performed under both models, each yielding a single tree topology (−log likelihood = 7444.05212 for TVM+I+Γ and 7443.10947 for TIM+I+Γ). The ML trees under both models were topologically identical and differed from the parsimony tree shown in Figure 6 (after *O. torosus* had been pruned out) in the position of *O. malus*, which under ML appeared as basal to the remaining species of Kauai (thus making Kauai monophyletic), and the placement of *O. iudicium*, which was shown as sister of *O. ventus*. The same result regarding *O. malus* was obtained by direct optimization under equal costs when *O. torosus* was excluded from the analysis or when gap costs was set to a value other than 1. These results suggest that the conflict reported in the sensitivity analysis is not due to long-branch attraction problems but more likely to the negative effect of missing data during the optimization stage.

While exploring different methods of analyzing variable length DNA sequences, Belshaw and Quicke (2002) found that direct optimization using POY gave some clearly erroneous results and that those errors had a

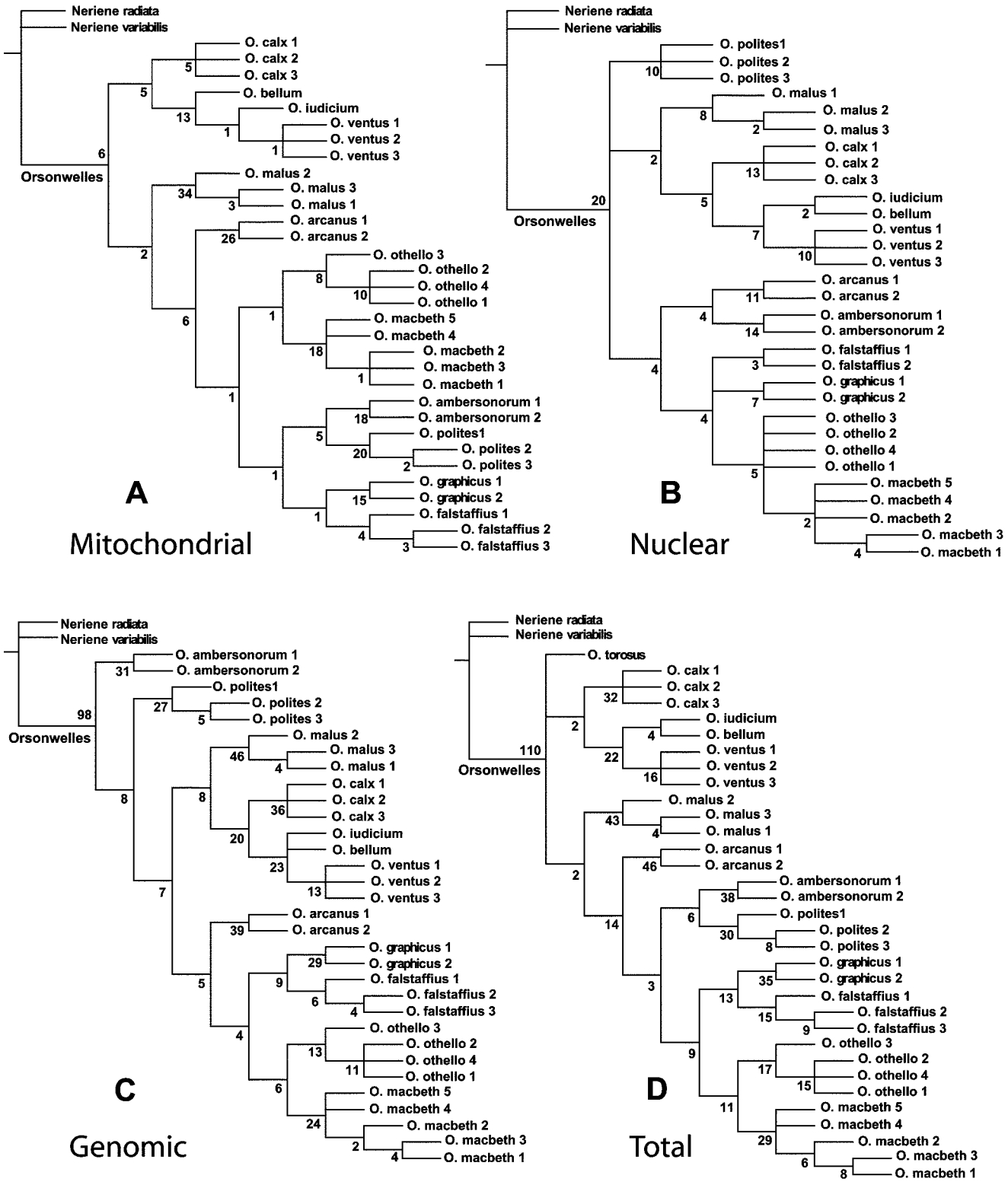

FIGURE 5. Consensus cladograms resulting from the most-parsimonious trees (MPTs) obtained in the analyses of the combined matrix (M3) and of various character partitions. Bremer support values (BS) are given below branches. (a) Mitochondrial partition of M3, strict consensus of four MPTs 886 steps long. (b) Nuclear partition of M3, strict consensus of 50 MPTs 311 steps long. (c) Genomic partition of M3, strict consensus cladogram of 18 MPTs 1,211 steps long. (d) Total evidence matrix (M3), strict consensus cladogram of 13 MPTs 1,340 steps long. BS values were calculated using the fast heuristic option in POY; most of these values differ from the more accurate BS values based on constrained searches, which are reported in Table 2, together with the partitioned Bremer support.

FIGURE 6. Strict consensus cladogram of the 13 most-parsimonious trees that result from the analysis of the M3 matrix (all character evidence combined). At the species level, there are only two most-parsimonious topologies. The 10 different parameter combinations analyzed are given in circles by with the same notation used in Figure 4. Closed circles denote clade support from the various combinations of gap, transversion, and transition costs. Shaded circles denote clades that were supported by some but not all of the most-parsimonious trees resulting from that particular parameter combination. All species were monophyletic under all the parameter combinations explored. The minimum and maximum calculated branch lengths and Bremer and partial Bremer support values for the different partitions defined are given in Table 2.

profound effect on the overall phylogenetic reconstruction. Our data do not seem to be affected by similar artifacts because the ML tree, based on an implied alignment, is topologically very similar to the POY cladograms.

The presence of a molecular clock was rejected for our data. For the TIM+I+Γ model, the likelihood scores (−L) of the preferred trees were $-L_{\text{molecular clock}} = 7477.81480$ and $-L_{\text{unconstrained}} = 7447.12089$ ($P = 0.001$, $df = 30$). For the TVM+I+Γ model the scores were $-L_{\text{molecular clock}} = 7480.93258$ and $-L_{\text{unconstrained}} = 7444.05212$ ($P = 0.001$, $df = 30$).

The ultrametric tree resulting from the NPRS transformation of parsimony-reconstructed branch lengths is shown in Figure 7. Most of the potential calibration points available for estimating clade ages had to be discarded. Although the Hawaiian Islands currently comprises eight main islands, the available geological evidence suggests that some of these islands were connected in the past. Molokai, Maui, Lanai, and Kahoolawe were all joined together forming the Maui-Nui complex, and Oahu and Molokai were linked through the Penguin bank (Carson and Clague, 1995; Fleischer et al., 1998). We

TABLE 3. Estimated divergence ages (in millions of years) of cladogenic events within the *Orsonwelles* clade. Node numbers refer to those depicted on the cladogram in Figure 7. The age of node 11 (2.6 million years) was used as the calibration point.

Clade	Estimated age (million years)
Outgroups	6.11
1	4.15
2	2.56
3	0.13
4	1.00
5	0.72
6	0.33
7	0.25
8	3.65
9	0.38
10	0.26
11	2.60
12	0.43
13	2.20
14	1.51
15	0.13
16	0.16
17	0.05
18	1.90
19	1.50
20	0.91
21	0.29
22	1.44
23	0.82
24	0.24
25	0.13
26	0.44
27	0.39

did not consider the islands of the Maui-Nui complex as calibration points to avoid the confounding effects of vicariant events. Kauai also was not used to avoid constraining the origin of the radiation, and Hawaii was rejected because only one species was present and the population sampling was far too poor to realistically assess the level of divergence. After all these considerations, we used the oldest age of the Ko'olau range in Oahu (2.6 million years), where the biogeographic reconstruction locates the origin of the non-Kauaian clade, as the calibration point. Clade ages estimations based on this calibration are given in Table 3.

DISCUSSION

Cladistic Analyses

The presence of *O. torosus* in the character matrix, despite providing only a small fraction of the morphological characters and no sequence data, influenced the direct alignment of the sequences and ultimately the rooting of the ingroup network. The morphological data alone supported the placement of *O. torosus* in a Kauai clade that also included *O. malus*. Analysis of the M3 matrix without *O. torosus* produced a single most-parsimonious species topology that recovered an all-Kauai species clade (with *O. malus* as sister to a lineage with all the remaining species from Kauai) sister to a clade that is topologically identical to clade E in Figure 6. The most-parsimonious topology of M3 without *O. torosus* differed

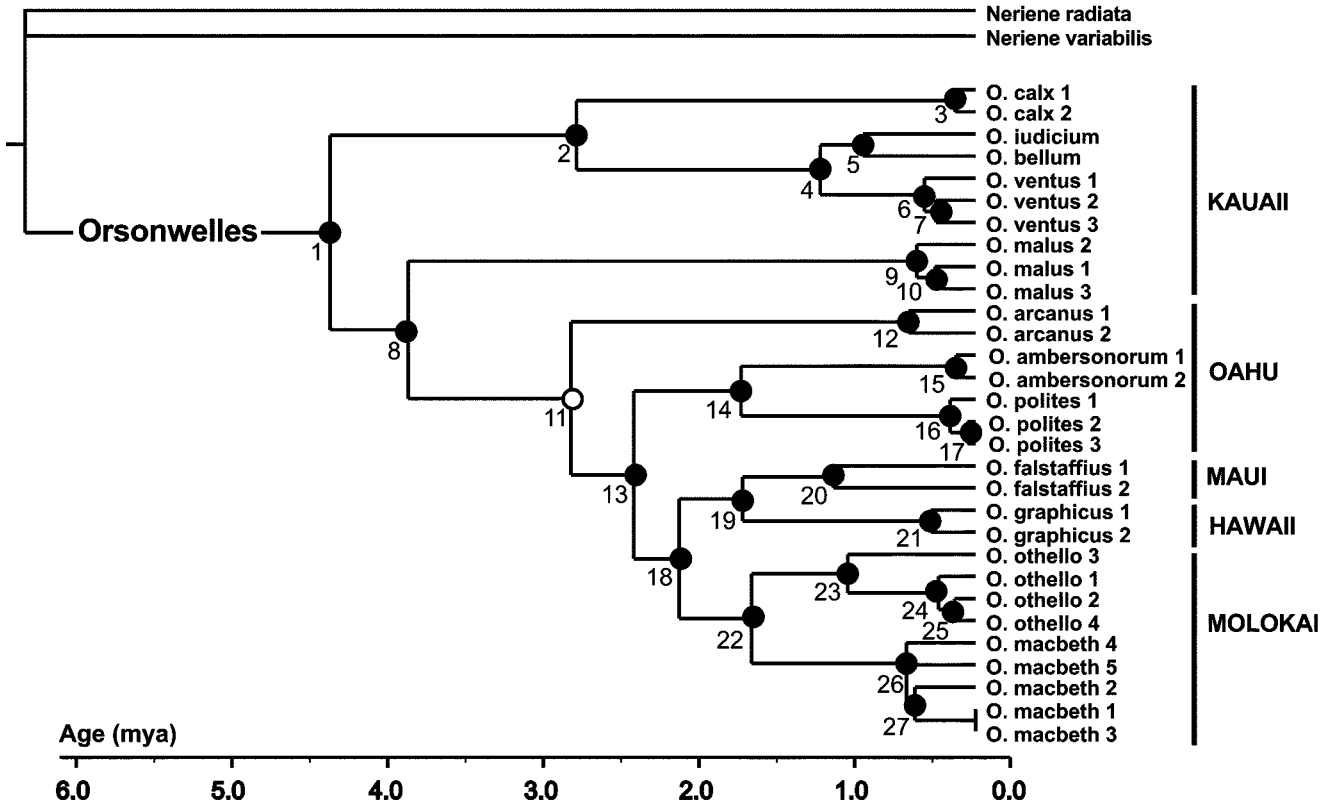

FIGURE 7. The ultrametric tree resulting from the nonparametric rate smoothing method (Sanderson, 1997) as implemented in the computer program TREEEDIT (Rambaut and Charleston, 2001). This method replaces the constraint of constant rates with the assumption of autocorrelation of rates in ancestor and descendant lineages. Branch lengths were obtained with PAUP* (Swofford, 2001) under parsimony, with gaps treated as a fifth state. Divergence ages (see Table 3) were estimated using node 11 as the calibration point (2.6 million years ago, the oldest age of the Koolau range in Oahu).

from the M3 tree (with *O. torosus* pruned) only in the placement of the root; the topology of the ingroup network was the same.

Hormiga (2002) found robust support for the monophyly of *Orsonwelles* (25 morphological and 1 behavioral synapomorphy, with BS > 10). Our sequence data corroborate the long branch length of *Orsonwelles*. In the most-parsimonious tree produced by M2, 62–81 changes supported the monophyly of the genus (18–29 base changes supported the monophyly of its putative sister genus, *Neriene*). Although the closest relatives of *Orsonwelles* have not been identified, the overall male genitalic morphology suggests that *Orsonwelles* is most similar to *Neriene*, although these two genera differ significantly in the details of the male and female genitalic morphology. *Neriene* includes 53 species from all the continents, except South America and Australia (Van Helsdingen, 1969; Platnick, 2001). Unfortunately the monophyly of *Neriene* has never been tested cladistically. If the *Orsonwelles* radiation were derived from a *Neriene* species that had colonized the Hawaiian Islands, ranking the Hawaiian species as a genus could, at least potentially, render *Neriene* paraphyletic. To address this potential problem we included in the character matrices (M1 and M2) two species of *Neriene*. In all the minimal length topologies, *Neriene* was monophyletic, and only in the analyses where gaps received the highest costs (4 or 8) did the partial mitochondrial analysis challenge this result. The long morphological branch length in *Orsonwelles* may be in part artifactual and might be shortened by addition of closer outgroups, if such were known or at least suspected. The addition of closer outgroups could improve the robustness of the results of the phylogenetic analyses, particularly for the morphological data, by adding character states more easily comparable to the features found in *Orsonwelles*. Such outgroups might also improve the instability found at the base of the *Orsonwelles* clade (i.e., the position of *O. malus* and *O. torosus*).

The use of very distant outgroup taxa in the phylogenetic analysis of molecular data may result in the assignment of the root to the longest branch of the ingroup network, irrespective of the true position of the root (Wheeler, 1990). Random outgroup effect is just a particular case of long branch attraction and is caused by spurious synapomorphies resulting from the small number of states. The position of *O. malus* in our unweighted analysis of M3 could actually be the result of a random outgroup effect; ML and differentially weighted POY searches supported the monophyly of the Kauai clade.

Biogeographic Patterns

In a review of Hawaiian lineages, Wagner and Funk (1995) found that 18 of 25 cases examined had an area cladogram that followed the progression rule either as an overall pattern or as a subpattern. Are the reconstructed cladogenetic events of *Orsonwelles* compatible with speciation through island progression? The two most-parsimonious species trees that resulted from the analysis of M3 imply the same area cladogram (Fig. 8). Eight equally parsimonious progression patterns are possible; all of them having in common an initial arrival to Kauai or some older island, dispersal from Kauai to the Ko'olaus (Oahu) and then to the Wai'anaes (Oahu). From here on, the data suggest eight alternative hypotheses for post-Oahu colonization. This ambiguity is not the result of conflicting or unresolved phylogenetic signal among *Orsonwelles* species but is the result of the monophyly of the Molokai species and the fact that both Maui and Hawaii have only one species each. With this number of terminals (three different areas in a fully resolved cladogram), the optimization of the two internal nodes is ambiguous, each with four possible areas. One of these alternative patterns fits the geological progression of volcanoes and islands, with the exception of a reversal in Oahu between the Wai'anae M and Ko'olau mountains, the former range being older than the latter. In view of the geologic evidence, the remaining seven colonization scenarios implied by the phylogenetic reconstructions, although equally parsimonious, are considered less likely. For example, one of them requires dispersal from the Ko'olaus (Oahu) to Hawaii, and the other requires dispersal from Hawaii to Molokai and then to Maui. The first scenario requires colonization of Hawaii directly from Oahu, bypassing Molokai and Maui, with these latter islands being colonized only from Hawaii. Although direct dispersal from Oahu to Hawaii is possible (and may have been documented for other groups), it does not seem very plausible to hypothesize that the Maui-Nui complex, geographically intermediate between Oahu and Hawaii, was not colonized from Oahu during the approximately 1.26 million years that the complex was available for colonization before the emergence of Hawaii, ca. 0.5 million years ago (MYA); alternatively, some Maui-Nui species may have gone extinct. Most of the parsimony analyses of the complete data matrix (M3) under any parameter combination, other than equal costs, resulted in the

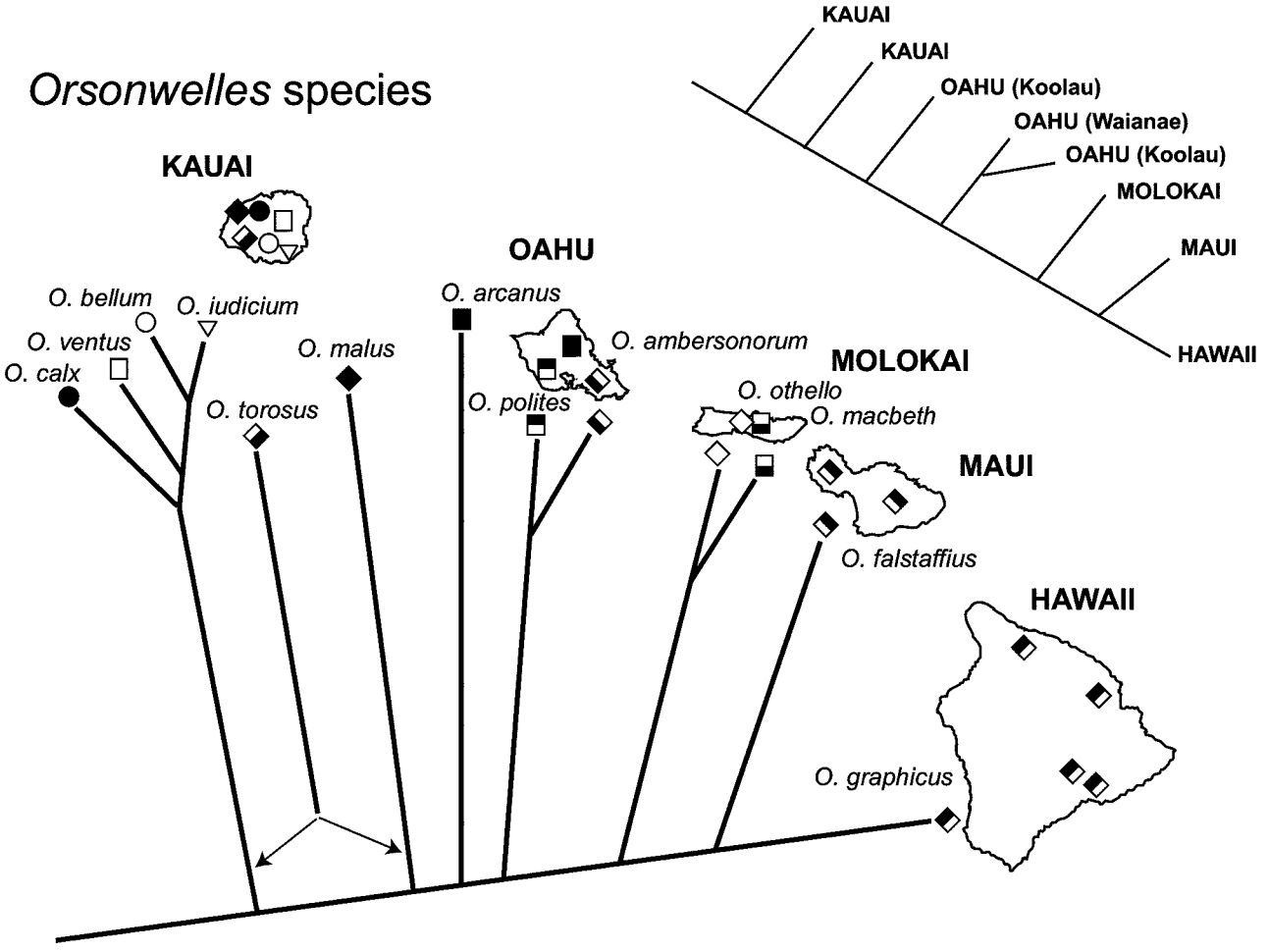

FIGURE 8. Area cladogram of *Orsonwelles* species based on the two most-parsimonious species-level trees that result from the analysis of the combined morphological and molecular data (character matrix, M3). The reduced area cladogram is given in the top of the figure.

monophyly of all the species from Kauai. This topological change would produce an area cladogram in which the optimization of the ingroup basal node would be ambiguous (as would the optimization of the distal clade including the species from Molokai, Maui, and Hawaii, because this topology remains unchanged). The ambiguity of such an area cladogram again would not be the result of conflicting or unresolved phylogenetic signal among *Orsonwelles* species but rather the result of the monophyly of the Kauai species combined with the monophyly of the Molokai species and the fact that both Maui and Hawaii have only one species each. As in the area cladogram presented in Figure 8, with this number of unique terminals (areas) the optimization of most nodes is ambiguous. Among this many equally parsimonious optimizations, there is one optimal area cladogram that is fully compatible with the progression rule. The unambiguous optimization of the ingroup basal node depends in part on the paraphyly of the species from Kauai.

The estimated ages of the cladogenic events within *Orsonwelles* (Fig. 7, Table 3) are largely congruent with the available geological evidence (Carson and Clague, 1995). Dispersal from Kauai into the Ko'olau range was used to calibrate the cladogram (2.6 MYA). The oldest divergence within the genus (node 1) was estimated to have occurred in Kauai about 4.15 MYA. Most of the cladogenesis in Kauai is estimated to have started after Oahu was colonized, around 2.56 MYA. The only species presently found in the Wai'anae Range of Oahu, *O. polites*, arrived there relatively late. The divergence between *O. polites* and *O. ambersonorum* is estimated to have occurred about 1.5 MYA, although the oldest parts of that range are ca. 3.7 million years old. This observation can be considered biogeographically odd, given the Wai'anae's older age and closer distance to Kauai, the actual source for colonizers. A reconciliation between the recovered phylogenetic pattern and the geology and geography of the islands could be achieved by suggesting the extinction of a former Waianae species or population ancestral to the current species of Oahu. Alternatively, the Ko'olau species may have covered the entire island, with the Wai'anae species arising relatively recently from this widespread stock. In some of the analyses, i.e., those under parameter combinations with higher gap cost to any of the base transformations (411, 811, 821), located the extant Waianae species, *O. polites*, as the sister group to the remaining non-Kauaian species, providing a pattern more compatible with the geological and geographical data.

The divergence between *O. macbeth* and *O. othello*, both from Molokai, is also consistent with the age of the eastern part of the island (ca. 1.76 million years). The only inconsistency between the estimated divergence ages and the geological evidence is provided by the divergence between *O. falstaffius* and *O. graphicus* (node 19, 1.50 MYA). The oldest parts of Hawaii are only about 0.5 million years old; thus, the divergence between *O. falstaffius* and *O. graphicus* implies that these species diverged before the emergence of Hawaii and that the split between the two clades was probably due to causes other than the colonization of the new island. The position of *O. graphicus* is also sensitive to changes in parameter costs. High gap costs (421, 441, 811, 821, 841, 881) favored a sister group relationship of *O. graphicus* to the Maui-Nui species. This topology would be compatible with a split of *O. graphicus* early during the colonization of Maui-Nui and its subsequent dispersal to Hawaii once the island was available for colonization. However, the reconstructed scenario would be extremely complex, requiring several extinction events.

Speciation in *Orsonwelles* occurred within islands more often (8 of the 12 cladogenic events) than between islands. This scenario seems to fit the general pattern reported for diverse Hawaiian taxa. Wagner and Funk (1995) found that speciation on the Hawaiian Islands occurred approximately one-third interisland and two-thirds intraisland. In *Orsonwelles*, more than half (five of eight) of these speciation events have occurred in Kauai.

Although allopatric speciation seems to be the dominant mode of speciation of *Orsonwelles* in the Hawaiian Islands, partially overlapping distributions of some species could be compatible with cases of sympatric or parapatric speciation. There are no obvious geographic barriers separating the distributions of the species pairs *O. arcanus* and *O ambersonorum* in Oahu's Ko'olau Mountains. Similarly, no obvious barriers separate *O. othello* and *O. macbeth* on Molokai, although on only one occasion have these two species been collected together (we collected a single female of *O. othello* along Pepe'opae trail, an area of high abundance of *O. macbeth*). In at least one locality, the higher elevations of the Makaleha Mountains of Kauai, *O. calx* and *O. ventus* are found living completely intermixed. In this case, the existence of additional localities exclusively inhabited by *O. calx* (e.g., La'au Ridge) suggests that the coexistence of these two species could be due to secondary range expansion. Localities where the two species pairs have been collected together, from the Ko'olaus and Molokai, the two members of each pair tend to differ in preferred elevation and, more importantly, in type of forest. *Orsonwelles arcanus* in Oahu and *O. macbeth* in Molokai seem to prefer higher altitude and very wet forests, whereas Oahu's *O. ambersonorum* and Molokai's *O. othello* have been largely collected in mesic forests (Hormiga, 2002). Therefore, the existence of parapatric speciation events, resulting from an ecological shift driven by adaptation to ecosystems with different humidity regimes, cannot be completely discarded, at least to explain the origin of these species pairs. More field and experimental data should be gathered to address this issue.

The prospects for determining the geographic origin of *Orsonwelles* are weak because of the highly unusual morphology of the genus and the difficulties involved in identifying its closest sister group. The widespread distribution of the genus in the islands leads to an interesting paradox. Given that *Orsonwelles* species have colonized every high Hawaiian island, why are none of the 13 species found on more than one island? Why are all *Orsonwelles* species single island endemics? The presence of these spiders throughout the archipelago

suggests that their dispersal abilities, despite their large size, are not impaired, otherwise they could not have colonized all the high islands. Most *Orsonwelles* species live in similar habitats, and some tolerate substantial habitat degradation (Hormiga, 2002). They are morphologically fairly uniform, except for genitalic differences. Their sheet webs are architecturally very similar, and they are generalist predators. All these characteristics suggest that it would be logical to find at least some species of *Orsonwelles* on more than one island, but such is not the case.

ACKNOWLEDGMENTS

Most of the *Orsonwelles* specimens studied were collected during three 1-month field trips to the Hawaiian Islands. All islands were visited at least twice except Lanai, which was visited only once. Additional specimens for study, including outgroup taxa, were made available by the following individuals and institutions: Janet Beccaloni (Natural History Museum, London), Jonathan Coddington (Smithsonian Institution, Washington, D.C.), the late Ray Forster and Anthony Harris (Otago Museum, Dunedin, New Zealand), Charles Griswold (California Academy of Sciences, San Francisco), Frank Howarth and Gordon Nishida (Bishop Museum, Honolulu), Bernar Kumashiro (Hawaii Agriculture Department, Honolulu), Norman Platnick (American Museum of Natural History, New York), and Christine Rollard (Museum National d'Histoire Naturelle, Paris). George Roderick and Dan Polhemus provided additional *Orsonwelles* specimens from Oahu and Maui and information on their webs. Adam Asquith was instrumental for the field work in Kauai; he also collected the first specimens of *O. calx* and *O. bellum* and made us aware of the importance of the Makaleha Mountains and Mt. Kahili as areas of endemism for *Orsonwelles* spiders. Nikolaj Scharff, Jonathan Coddington, and Ingi Agnarsson have accompanied us on field trips to various parts of the Hawaiian Islands and collected numerous specimens. Nikolaj Scharff also provided specimens of outgroup taxa for molecular work. The following individuals have also provided specimens or information on localities: Greta Binford, Todd Blackledge, Curtis Ewing, Jessica Garb, Laura García de Mendoza, Scott Larcher, Jim Liebherr, Geoff Oxford, Malia Rivera, Warren Wagner, and Ken Wood. Brian Farrell (Harvard University, Cambridge) hosted G.H. in his lab to test the suitability of some of the mitochondrial primers and to gather some preliminary molecular data. Gonzalo Giribet (Harvard University) ran in his computer cluster some of most computationally intensive POY analyses. The following individuals and organizations have facilitated collecting and research permits and/or access to restricted areas: David Foote (Hawaii Volcanoes National Park), Betsy Gagné (State Natural Area Reserves System), Edwin Pettys (State Department of Land and Natural Resources [DLNR]), Robin Rice (Kauai, access to Mt. Haupu), Victor Tanimoto (DLNR), and Joan Yoshioka (The Nature Conservancy). Randy Bartlett (Maui Pineapple Co.) provided access and helicopter transportation to the Puu Kukui area and collected specimens in West Maui. The Nature Conservancy has consistently provided excellent logistic support and easy access to their reserves. Alexander & Baldwin Properties and Castle & Cooke granted access to their land for fieldwork. Funding for this research has been provided by grants from the National Geographic Society (6138-98), the National Science Foundation (DEB-9712353), and the Research Enhancement Fund from George Washington University to G.H., the Ministerio de Educación y Cultura (Spain) to M.A., and the National Science Foundation (DEB-0096307) to R.G.G., with additional support from Dr. Evert Schlinger. Additional travel funds were granted by the Smithsonian Institution. M. Kuntner provided comments on an earlier draft of this manuscript. We thank Keith Crandall, Rob DeSalle, Marshal Hedin, and Chris Simon for their thorough reviews that substantially improved this manuscript.

REFERENCES

AJUH, P. M., P. A. HEENEY, AND B. E. MADEN. 1991. *Xenopus borealis* and *Xenopus laevis* 28S ribosomal DNA and the complete 40S ribosomal precursor RNA coding units of both species. Proc. R. Soc. Lond. B Biol. Sci. 245:65–71.

ARNEDO, M. A., P. OROMI, AND C. RIBERA. 2001. Radiation of the spider genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: Cladistic assessment based on multiple data sets. Cladistics 17:313–353.

BAKER, R. H., AND R. DESALLE. 1997. Multiple sources of character information and the phylogeny of Hawaiian *Drosophila*. Syst. Biol. 46:654–673.

BARKER, F. K., AND F. M. LUTZONI. 2002. The utility of the incongruence length difference test. Syst. Biol. 51:625–637.

BELSHAW, R., AND D. L. J. QUICKE. 2002. Robustness of ancestral state estimates: Evolution of life history strategy in ichneumonoid parasitoids. Syst. Biol. 51:450–477.

BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42:795–803.

BREMER, K. 1994. Branch support and tree stability. Cladistics 10:295–304.

CARLQUIST, S. 1980. Hawaii, a natural history: Geology, climate, native flora and fauna above the shoreline, 2nd edition. Pacific Tropical Botanical Gardens, Lawai, Kauai.

CARSON, H. L., AND D. A. CLAGUE. 1995. Geology and biogeography of the Hawaiian Islands. Pages 14–29 *in* Hawaiian biogeography (W. L. Wagner and V. A. Funk, eds.). Smithsonian Institution, Washington, D.C.

CARSON, H. L., AND K. Y. KANESHIRO. 1976. *Drosophila* of Hawaii: Systematics and ecological genetics. Annu. Rev. Ecol. Syst. 15:97–131.

COLGAN, D. J., A. MCLAUCHLAN, G. D. F. WILSON, S. P. LIVINGSTON, G. D. EDGECOMBE, J. MACARANAS, G. CASSIS, AND M. R. GRAY. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Aust. J. Zool. 46:419–437.

COWIE, R. H. 1995. Variation in species diversity and shell shape in Hawaiian land snails: In situ speciation and ecological relationships. Evolution 49:1191–1202.

DESALLE, R., AND D. GRIMALDI. 1992. Characters and the systematics of Drosophilidae. J. Hered. 83:182–188.

DESALLE, R., AND J. A. HUNT. 1987. Molecular evolution in Hawaiian drosophilids. Trends Ecol. Evol. 2:212–216.

ELDREDGE, L. G., AND S. E. MILLER. 1995. How many species are there in Hawaii? Bishop Mus. Occas. Pap. 41:3–18.

FAITH, D. P., AND J. W. H. TRUEMAN. 2001. Towards an inclusive philosophy for phylogenetic inference. Syst. Biol. 50:331–350.

FARRIS, J. S. 1969. A successive approximations approach to character weighting. Syst. Zool. 18:374–385.

FARRIS, J. S. 1988. HENNIG 86, version 1.5. Program and documentation. Available from D. Lipscomb, Dept. of Biological Sciences, George Washington University, Washington, DC 20052, USA.

FARRIS, J. S. 1989a. The retention index and the homoplasy excess. Syst. Zool. 38:406–407.

FARRIS, J. S. 1989b. The retention index and the rescaled consistency index. Cladistics 5:417–419.

FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1994. Testing significance of incongruence. Cladistics 10:315–320.

FITCH, W. M. 1971. Toward defining the course of evolution: Minimum change for a specific tree topology. Syst. Zool. 20:404–416.

FLEISCHER, R. C., C. E. MCINTOSH, AND C. L. TARR. 1998. Evolution on a volcanic conveyor belt: Using phylogeographic reconstructions and K-Ar-based ages of the Hawaiian Islands to estimate molecular evolutionary rates. Mol. Ecol. 7:533–545.

FOLMER, O., M. BLACK, W. HOEH, R. LUTZ, AND R. VRIJENHOEK. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3:294–299.

FREED, L. A., S. CONANT, AND R. C. FLEISCHER. 1987. Evolutionary ecology and radiation of Hawaiian *Usa* passerine birds. Trends Ecol. Evol. 2:196–203.

GILLESPIE, R. G. 1991a. Hawaiian species of the genus *Tetragnatha*: I. Spiny leg clade. J. Arachnol. 19:174–209.

GILLESPIE, R. G. 1991b. Predation through impalement of prey: The foraging behavior of *Doryonichus raptor* (Araneae, Tetragnathidae). Psyche 98:337–350.

GILLESPIE, R. G. 1992. Hawaiian spiders of the genus *Tetragnatha*: II. Species from natural areas of windward East Maui. J. Arachnol. 20:1–17.

GILLESPIE, R. G. 1993. Biogeographic patterns of phylogeny in a clade of endemic Hawaiian spiders (Araneae, Tetragnathidae). Mem. Queensl. Mus. 33:519–526.
GILLESPIE, R. G. 1994. Hawaiian spiders of the genus *Tetragnatha*: III. *Tetragnatha acuta* clade. J. Arachnol. 22:161–168.
GILLESPIE, R. G., AND H. B. CROOM. 1992. Pattern and process in speciation: A comparative approach using a Hawaiian spider radiation. Am. J. Bot. 79:127.
GILLESPIE, R. G., AND H. B. CROOM. 1995. Comparison of speciation mechanisms in web-building and non-web-building groups within a lineage of spiders. Pages 121–146 *in* Hawaiian biogeography: Evolution on a hot spot archipelago (W. L. Wagner and V. A. Funk, eds.). Smithsonian Institution, Washington, D.C.
GILLESPIE, R. G., H. B. CROOM, AND G. L. HASTY. 1997. Phylogenetic relationships and adaptive shifts among major clades of *Tetragnatha* spiders (Araneae: Tetragnathidae) in Hawai'i. Pac. Sci. 51:380–394.
GILLESPIE, R. G., H. B. CROOM, AND S. R. PALUMBI. 1994. Multiple origins of a spider radiation in Hawaii. Proc. Natl. Acad. Sci. USA 91:2290–2294.
GIRIBET, G. 2001. Exploring the behavior of POY, a program for direct optimization of molecular data. Cladistics 17:S60–S70.
GIRIBET, G., S. CARRANZA, M. RIUTORT, J. BAGUNA, AND C. RIBERA. 1999. Internal phylogeny of the Chilopoda (Myriapoda, Arthropoda) using complete 18S rDNA and partial 28S rDNA sequences. Philos. Trans. R. Soc. Lond. B Biol. Sci. 354:215–222.
GIRIBET, G., AND W. C. WHEELER. 1999. On gaps. Mol. Phylogenet. Evol. 13:132–143.
GOLOBOFF, P. A. 1993. NONA, version 2.0. Program available at http://www.cladistics.com.
GOLOBOFF, P. A. 1999. Analyzing large data sets in reasonable times: Solutions for composite optima. Cladistics 15:415–428.
HEDIN, M. C. 1997a. Molecular phylogenetics at the population/species interface in cave spiders of the southern Appalachians (Araneae: Nesticidae: *Nesticus*). Mol. Biol. Evol. 14:309–324.
HEDIN, M. C. 1997b. Speciational history in a diverse clade of habitat-specialized spiders (Araneae: Nesticidae: *Nesticus*): Inferences from geographic-based sampling. Evolution 51:1929–1945.
HORMIGA, G. 1994a. Cladistics and the comparative morphology of linyphiid spiders and their relatives (Araneae, Araneoidea, Linyphiidae). Zool. J. Linn. Soc. 111:1–71.
HORMIGA, G. 1994b. A revision and cladistic analysis of the spider family Pimoidae (Araneoidea: Araneae). Smithson. Contrib. Zool. 549:1–104.
HORMIGA, G. 2000. Higher level phylogenetics of erigonine spiders (Araneae, Linyphiidae, Erigoninae). Smithson. Contrib. Zool. 609:1–160.
HORMIGA, G. 2002. *Orsonwelles*, a new genus of giant linyphiid spiders (Araneae) from the Hawaiian Islands. Invertebr. Syst. 16:369–448.
HOWARTH, F. G., AND W. P. MULL. 1992. Hawaiian insects and their kin. Univ. Hawaii Press, Honolulu.
JORDAN, S., C. SIMON, AND D. POLHEMUS. 2003. Molecular systematics and adaptive radiation of Hawaii's endemic damselfly genus *Megalagrion* (Odonata: Coenagrionidae). Syst. Biol. 52:89–109.
LIEBHERR, J. K. 1995. Description of the larval stages of *Disenochus fractus* Sharp and the origin of the Hawaiian platynine Carabidae (Coleoptera). Syst. Entomol. 20:17–26.
LIEBHERR, J. K. 1997. Dispersal and vicariance in Hawaiian platynine carabid beetles (Coleoptera). Pac. Sci. 51:424–439.
LIEBHERR, J. K. 2000. The unity of characters: Ecological and morphological specialisation in larvae of Hawaiian platynini Carabidae (Coleoptera). Invertebr. Taxon. 14:931–940.
LIEBHERR, J. K., AND E. C. ZIMMERMAN. 1998. Cladistic analysis, phylogeny and biogeography of the Hawaiian Platynin (Coleoptera: Carabidae). Syst. Entomol. 23:137–172.
LUTZONI, F., P. WAGNER, V. REEB, AND S. ZOLLER. 2000. Integrating ambiguously aligned regions of DNA sequences in phylogenetic analyses without violating positional homology. Syst. Biol. 49:628–651.
MICKEVICH, M. F., AND J. S. FARRIS. 1981. The implications of incongruence in *Menidia*. Syst. Zool. 30:351–370.
MILLIDGE, A. F. 1991. Further linyphiid spiders (Araneae) from South America. Bull. Am. Mus. Nat. Hist. 205:1–199.
MLOT, C. 1995. Biological surveys in Hawaii, taking inventory of a biological hot spot. Science 269:322–323.
NIXON, K. C. 1999. WinClada, version 0.9.99m24. Program available at http://www.cladistics.com.
OTTE, D. 1994. The crickets of Hawaii: Origin, systematics and evolution. Orthopterists' Society, Philadelphia.
PAGE, R. D. M. 2001. NDE (Nexus data editor), version 0.4.9. Program available at http://taxonomy.zoology.gla.ac.uk/rod/NDE/nde.html.
PALUMBI, S. A., S. MARTIN, S. ROMANO, W. O. MCMILLAN, L. STICE, AND G. GRABOWSKI. 1991. The simple fool's guide to PCR, version 2.0. Department of Zoology. Univ. Hawaii, Honolulu.
PLATNICK, N. I. 2001. The world spider catalog, version 2.0. Available at http://research.amnh.org/entomology/.
POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics 14:817–818.
RAMBAUT, A., AND M. CHARLESTON. 2001. TREEEDIT, version 1.0 alfa 8. Program available at http://evolve.zoo.ox.ac.uk.
RODERICK, G. K., AND R. G. GILLESPIE. 1998. Speciation and phylogeography of Hawaiian terrestrial arthropods. Mol. Ecol. 7:519–531.
SANDERSON, M. J. 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. Mol. Biol. Evol. 14:1218–1231.
SANGER, F., S. NICKLEN, AND A. R. COULSEN. 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463–5468.
SANKOFF, D. 1975. Minimal mutation trees of sequences. Soc. Ind. Appl. Math. J. Appl. Math. 28:35–42.
SHAW, K. L. 1995. Biogeographic patterns of two independent Hawaiian cricket radiations (*Laupala* and *Prognathogryllus*). Pages 39–56 *in* Hawaiian biogeography: Evolution on a hot spot archipelago (W. L. Wagner and V. A. Funk, eds.). Smithsonian Institution Press, Washington, D.C.
SHAW, K. L. 1996a. Sequential radiations and patterns of speciation in the Hawaiian cricket genus *Laupala* inferred from DNA sequences. Evolution 50:237–255.
SHAW, K. L. 1996b. Polygenic inheritance of a behavioral phenotype: Interspecific genetics of song in the Hawaiian cricket genus *Laupala*. Evolution 50:256–266.
SIMMONS, M. P., AND H. OCHOTERENA. 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49:369–381.
SIMON, C. 1987. Hawaiian evolutionary biology: An introduction. Trends Ecol. Evol. 2:175–178.
SIMON, C., F. FRATI, A. BECKENBACH, B. CRESPI, H. LIU, AND P. FLOOK. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87:651–701.
SIMON, E. 1900. Arachnida. Pages 443–519 *in* Fauna Hawaiiensis, or the zoology of the Sandwich Isles: Being results of the explorations instituted by the Royal Society of London promoting natural knowledge and the British Association for the Advancement of Science, London. Royal Society, London.
STEARNS, H. T. 1985. Geology of the state of Hawaii, 2nd edition. Pacific Books, Palo Alto, California.
SULLIVAN, J., K. E. HOLSINGER, AND C. SIMON. 1996. The effect of topology on estimates of among-site rate variation. J. Mol. Evol. 42: 308–312.
SWOFFORD, D. L. 2001. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b5 (PPC). Sinauer, Sunderland, Massachusetts.
TARR, C. L., AND R. C. FLEISCHER. 1995. Evolutionary relationships of the Hawaiian honeycreepers (Aves, Drepanidinae). Pages 147–158 *in* Hawaiian biogeography: Evolution on a hot spot archipelago (W. L. Wagner and V. A. Funk, eds.). Smithsonian Institution Press, Washington, D.C.
VAN HELSDINGEN, P. J. 1969. A reclassification of the species of *Linyphia* Latreille, based on the functioning of the genitalia (Araneida, Linyphiidae) I. Zool. Verh. (Leiden) 105:1–303.
VAN HELSDINGEN, P. J. 1970. A reclassification of the species of Linyphia Latreille, based on the functioning of the genitalia (Araneida, Linyphiidae) II. Zool. Verh. (Leiden) 111:1–86.
WAGNER, W. L., AND V. A. FUNK (eds.). 1995. Hawaiian biogeography: Evolution on a hot spot archipelago. Smithsonian Institution Press, Washington, D.C.

WHEELER, W. C. 1990. Nucleic acid sequence phylogeny and random outgroups. Cladistics 6:363–368.
WHEELER, W. C. 1995. Sequence alignment, parameter sensitivity, and the phylogenetic analysis of molecular data. Syst. Biol. 44:321–331.
WHEELER, W. C. 1996. Optimization alignment: The end of multiple sequence alignment in phylogenetics? Cladistics 12:1–9.
WHEELER, W. C. 1999. Measuring topological congruence by extending character techniques. Cladistics 15:131–135.
WHEELER, W. C., AND D. GLADSTEIN. 2000. POY. Program and documentation, available at ftp://amnh.org/pub/molecular.
WHITING, M. F., J. C. CARPENTER, Q. D. WHEELER, AND W. C. WHEELER. 1997. The strepsiptera problem: Phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Syst. Biol. 46:1–68.
WIEHLE, H. 1956. Spinnentiere oder Arachnoidea. X. 28. Familie Linyphiidae. Tierwelt Dtsch 44:1–337.

First submitted 1 October 2001; reviews returned 25 January 2002; final acceptance 22 October 2002
Associate Editor: Keith Crandall

APPENDIX 1
MORPHOLOGICAL CHARACTERS AND CHARACTER STATES

Characters for *Orsonwelles* were described and illustrated by Hormiga (2002); additional characters are denoted with an asterisk after the character number. Illustrations refer to figures of Hormiga (2002) unless otherwise stated. All multistate characters were treated as nonadditive.

Male Genitalia

1. Male pedipalpal tibial length: 0 = short (less than twice its width); 1 = long (more than twice its width).
2. Paracymbium morphology: 0 = flat, large; 1 = small, thin; 2 = knob.
3. Paracymbium apophyses: 0 = present; 1 = absent.
4. Retromarginal cymbial apophysis: 0 = absent; 1 = present.
5*. Mesal cymbial apophysis: 0 = absent; 1 = present (Hormiga, 2002: figs. 15C, 15D).
6. Cymbial orifice: 0 = absent; 1 = present.
7. Distal end of tegulum: 0 = round; 1 = subtle to medium projection; 2 = elongated and projected.
8. Apex of tegular projection: 0 = round; 1 = pointed.
9. Suprategular base: 0 = same width; 1 = enlarged.
10. Suprategular base enlargement: 0 = flat; 1 = with depression.
11. Suprategular ectal margin: 0 = thick; 1 = thin or membranous.
12. Embolus base: 0 = broad; 1 = narrow.
13. Embolus apical half: 0 = filiform; 1 = not threadlike.
14. Embolic membrane: 0 = absent; 1 = present.
15. Column position on suprategulum: 0 = distal; 1 = proximal.
16. Fickert's gland: 0 = absent; 1 = present.
17. Terminal apophysis position: 0 = apical-ectoventral; 1 = mesal; 2 = ectal.
18*. Terminal apophysis dimension: 0 = longer than wide (Hormiga, 2002: fig. 17D); 1 = as wide as long or wider (Hormiga, 2002: fig. 29C).
19. Terminal apophysis size: 0 = small; 1 = large (similar in size to lamella characteristica); 2 = tiny.
20. Terminal apophysis shape: 0 = entire; 1 = tri (or tetra)-lobed.
21. Terminal apophysis coiling: 0 = not coiled; 1 = spirally coiled.
22*. Terminal apophysis basal process: 0 = round, straight (Hormiga, 2002: fig. 27E); 1 = angulated, twisted (Hormiga, 2002: fig. 19E).
23. Terminal apophysis apical process: 0 = pointed; 1 = round.
24. Terminal sclerite: 0 = absent; 1 = present.
25*. Terminal sclerite: 0 = membranous or bladelike (Hormiga, 2002: figs. 19B, 19G); 1 = globular (Hormiga, 2002: figs. 25C, 25G).
26*. Terminal sclerite tooth crest: 0 = absent (Hormiga, 2002: fig. 27F); 1 = present (Hormiga, 2002: figs. 25C, 25G).
27. Transversal sclerite: 0 = absent; 1 = present.
28. Lamella characteristica (LC): 0 = small; 1 = large.
29. Ectal process of lamella: 0 = flat, thin, or membranous (bladelike); 1 = thick (round section); 2 = blunt.
30*. Elongated ectal process of lamella: 0 = straight (Hormiga, 2002: figs. 21A, 21D); 1 = curved, falciform (Hormiga, 2002: fig. 27A).
31. Striated area of LC: 0 = long (at least three times longer than wider); 1 = short (less than three times longer than wider).
32*. Striated area of LC: 0 = parallel to ectal process of LC (Hormiga, 2002: figs. 17D, 39B); 1 = perpendicular to ectal process of LC (Hormiga, 2002: figs. 35C, 57C).
33. Mesal tooth of LC: 0 = absent; 1 = present.
34. Mesal tooth of LC, size: 0 = small; 1 = large.
35. Mesal tooth of LC, position: 0 = medial (open curve at LC base); 1 = basal (more closed curve).
36. Prolateral trichobothria in male pedipalpal tibia: 0 = 1; 1 = 3; 2 = 4.
37. Retrolateral trichobothria in male pedipalpal tibia: 0 = 2; 1 = 3; 2 = 4 or more.

Female Genitalia

38. Epigynal dimension (ventral view): 0 = longer than wider; 1 = as wide as long or wider.
39. Epigynum caudal region (ventral view): 0 = straight to round; 1 = V-shaped; 2 = U-shaped.
40. Dorsal plate scape: 0 = absent; 1 = present.
41. Ventral plate scape: 0 = absent; 1 = present.
42. Dorsal plate epigynal socket: 0 = present; 1 = absent.
43*. Ventral plate epigynal socket: 0 = absent (Wiehle, 1956: figs. 482, 483); 1 = present (Wiehle, 1956: figs. 263, 264).
44. Median epigynal septum: 0 = absent; 1 = present.
45*. Epigynal lateral edges: 0 = continuous (Hormiga, 2002: figs. 20D–F); 1 = interrupted in medial region (Hormiga, 2002: figs. 30A–C).
46. Epigynum lateral edge: 0 = curved; 1 = straight; 2 = sigmoid.
47. Dorsal plate incision: 0 = absent; 1 = present.
48. Atrium: 0 = absent; 1 = present.
49*. Atrium: 0 = large and conspicuous (Van Helsdingen, 1969: fig. 21); 1 = small and inconspicuous (Van Helsdingen, 1970: fig. 38).
50*. Atrium: 0 = spiral grooves (Van Helsdingen, 1969: figs. 316, 321); 1 = spiral folds (Van Helsdingen, 1969: fig. 21); 2 = smooth (Van Helsdingen, 1970: fig. 38)
51*. Copulatory duct: 0 = separate from fertilization duct (Van Helsdingen, 1969: fig. 21); 1 = spirals around fertilization duct (Van Helsdingen, 1970: fig. 38).
52. Copulatory duct turning point: 0 = absent; 1 = present.
53. Fertilization duct orientation: 0 = posterior; 1 = mesal; 2 = anterior.

Somatic Morphology

54. PME: 0 = not on black tubercles or without entire black rings; 1 = on black tubercles.
55. Male chelicerae: 0 = smooth; 1 = with stridulatory striae.
56. Cheliceral stridulatory striae: 0 = ridged; 1 = scaly; 2 = imbricated.
57*. Dorsal spur on male chelicerae: 0 = absent; 1 = present.
58. Prolateral teeth in female chelicerae: 0 = 6 or less; 1 = 9–13; 2 = 14 or 15; 3 = 16 or more.
59. Retrolateral teeth in female chelicerae: 0 = 6 or less; 1 = 7–9; 2 = 10 or more.
60. Prolateral teeth in male chelicerae: 0 = 5 or less; 1 = 9–11; 2 = 12–14; 3 = 15 or more.
61. Retrolateral teeth in male chelicerae: 0 = 5 or less; 1 = 7–9; 2 = 10 or more.
62. Retrolateral spines tibia III, female: 0 = none; 1 = 1 or more.
63. Retrolateral spines tibia IV, female: 0 = none; 1 = 1 or more.
64. Femur II–IV spines: 0 = absent; 1 = present.
65. Femur II dorsal spines, female: 0 = 3 or less; 1 = 4 or more.
66. Trichobothria femur III: 0 = absent; 1 = present.
67*. Trichobothria femur III female: 0 = 1 or 2; 1 = 3 or more.
68. Trichobothria femur IV: 0 = absent; 1 = present.
69*. Trichobothria femur IV, female: 0 = 1 or 2; 1 = 3 or more.
70. Trichobothrium metatarsus IV: 0 = present; 1 = absent.

Behavior

71. Web: 0 = without funnel: 1 = with funnel leading into a retreat.

APPENDIX 2. Matrix of morphological characters for *Orsonwelles* and outgroups.

Species	Characters
Bolyphantes luteolus	0 0 0 0 0 0 0 - 0 - 0 0 1 1 0 1 0 0 0 0 0 - - 0 - - 0 0 0 - - - 0 - - 0 0 0 - 0 1 1 1 ? - - 0 0 - - 0 0 0 0 1 0 0 0 0 0 0 0 0 0 - 0 - 0 - 1 ?
Lepthyphantes minutus	0 0 0 0 0 0 0 - 0 - 0 0 1 1 1 1 0 0 1 0 0 - - 0 - - 0 0 0 - - - 0 - - 0 0 0 - 0 1 1 1 ? - - 0 0 - - 0 0 0 0 1 0 0 0 0 0 0 1 1 0 - 0 - 0 - 1 ?
Labulla thoracica	0 0 0 0 0 0 0 - 0 - 0 0 0 0 1 0 2 0 0 0 0 - - 0 - - 0 1 0 - - - 0 - - 0 0 1 0 0 1 1 0 1 - - 0 1 0 0 0 1 2 1 1 0 0 0 0 0 0 1 1 1 0 0 - 0 - 1 0
Pityohyphantes costatus	0 1 1 0 0 0 0 - 0 - 0 0 0 1 1 0 0 0 0 0 0 - - 1 0 0 0 1 1 0 - - 1 0 0 0 0 1 0 1 0 0 - 1 - - 0 1 0 0 0 1 0 1 1 1 0 0 0 0 0 1 1 1 1 0 - 0 - 1 0
Microlinyphia dana	0 1 1 0 0 0 1 - 1 0 1 1 0 0 1 0 - 0 2 - - - - - 0 - - 0 1 0 0 - - 1 0 0 0 0 1 0 1 0 0 - 0 - - 0 1 1 2 1 1 0 1 1 1 0 0 0 0 0 0 1 1 0 0 - 0 - 1 0
Linyphia triangularis	0 1 1 0 0 0 1 - 1 1 1 1 0 1 1 0 0 0 0 0 1 - - 0 - - 0 1 0 - - - 1 0 0 0 1 1 0 1 0 0 - 0 - - 0 1 0 1 0 1 0 1 1 1 0 0 0 0 0 0 1 1 0 0 - 0 - 1 0
Neriene radiata	0 1 1 0 0 0 1 - 1 1 1 0 1 1 1 0 0 0 0 0 1 - - 1 0 0 1 1 2 - - - 1 0 0 0 1 1 0 1 0 0 - 0 - - 0 1 0 0 0 1 0 0 1 1 1 0 0 0 0 0 0 0 - 0 - 0 - 1 0
Neriene variabilis	0 1 1 0 0 0 0 - 1 1 1 0 1 1 1 0 0 0 0 0 1 - - 0 - - 1 1 0 - - - 1 0 0 0 1 1 0 1 0 0 - 0 - - 0 1 0 0 0 1 0 1 1 1 0 0 0 0 0 0 0 1 0 0 - 0 - 1 ?
O. torosus	? 1 1 0 0 0 - 1 0 0 0 0 - - 0 0 1 0 ? - ? 1 2 ? ? 1 1 1 0 1 1 1 1 0 ?
O. malus	1 2 1 1 1 1 2 1 1 0 1 0 1 1 1 0 1 0 1 1 0 0 1 1 0 0 0 1 0 - 0 0 1 1 1 2 2 0 1 0 0 0 - 0 1 0 0 0 - - 0 0 1 0 0 - 0 1 1 2 1 1 1 1 0 1 1 1 1 0 1
O. ventus	1 2 1 1 1 1 2 1 1 ? 1 0 1 1 1 0 1 0 1 1 0 1 1 1 0 0 0 1 0 - 0 0 1 1 1 2 2 0 1 0 0 0 - 0 0 0 0 0 - - 0 0 1 0 0 - 0 3 2 3 2 1 1 1 1 1 1 1 1 0 1
O. calx	1 2 1 1 0 1 2 1 ? ? 1 0 1 1 1 0 1 0 1 1 0 1 1 1 0 0 0 1 0 0 0 0 1 1 1 1 1 0 1 0 0 0 - 0 0 0 0 0 - - 0 0 1 0 0 - 0 2 2 2 1 1 1 1 1 1 1 1 1 0 1
O. iudicium	1 2 1 1 0 1 2 1 ? ? 1 0 1 1 1 0 1 0 1 1 0 1 1 1 0 0 0 1 0 0 0 0 1 1 1 1 1 0 1 0 0 0 - 0 0 0 0 0 - - 0 0 1 0 0 - 0 3 2 3 2 1 1 1 0 1 0 1 0 0 1
O. bellum	? 0 1 0 0 0 - 0 0 0 0 0 - - 0 0 1 0 0 - ? 3 2 ? ? 1 1 1 1 1 0 1 1 0 1
O. arcanus	1 2 1 1 0 1 2 0 1 0 1 0 1 1 1 0 1 0 0 1 0 0 0 1 0 0 0 1 1 1 1 0 1 1 1 1 1 1 0 0 0 1 - 0 1 1 1 0 - - 0 0 1 0 0 - 0 2 2 2 1 1 1 1 1 1 0 1 0 0 1
O. polites	1 2 1 1 0 1 2 0 1 0 1 0 1 1 1 0 1 1 0 1 0 0 0 1 1 1 0 1 1 0 1 0 1 1 1 1 2 1 0 0 0 1 - 0 1 1 1 0 - - 0 0 1 0 0 - 0 1 1 1 2 1 1 1 0 1 1 1 1 0 1
O. ambersonorum	1 2 1 1 0 1 2 0 1 0 1 0 1 1 1 0 1 0 0 1 0 0 0 1 1 1 0 1 1 1 1 0 1 1 1 2 2 1 0 0 0 1 - 0 1 1 1 0 - - 0 0 1 0 0 - 0 1 1 2 2 1 1 1 0 1 1 1 1 0 1
O. falstaffius	1 2 1 1 0 1 2 0 1 0 1 0 0 1 1 0 1 1 0 1 0 0 0 1 1 1 0 1 1 1 1 1 1 1 1 1 2 1 2 0 0 1 - 1 1 2 1 0 - - 0 0 1 0 0 - 0 1 2 1 1 1 1 1 0 1 ? 1 1 0 1
O. othello	1 2 1 1 0 1 2 0 1 0 1 0 0 1 1 0 1 1 0 1 0 0 0 1 1 1 0 1 1 1 1 0 1 1 1 1 2 1 2 0 0 1 - 1 1 2 1 0 - - 0 0 1 0 0 - 0 1 1 1 1 1 1 1 0 1 0 1 0 0 1
O. macbeth	1 2 1 1 0 1 2 0 1 0 1 0 0 1 1 0 1 1 0 1 0 0 0 1 1 1 0 1 1 1 1 0 1 1 1 1 1 1 2 0 0 1 - 1 1 2 1 0 - - 0 0 1 0 0 - 0 1 1 1 1 1 1 1 ? 1 0 1 0 0 1
O. graphicus	1 2 1 1 0 1 2 0 1 0 1 0 1 1 1 0 1 1 0 1 0 0 0 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 0 0 0 1 - 1 1 2 1 0 - - 0 0 1 0 0 - 0 1 1 1 1 1 1 1 0 1 1 1 1 0 1