

## EVOLUTION AND BIOGEOGRAPHY OF THE WOODY HAWAIIAN VIOLETS (*VIOLA*, VIOLACEAE): ARCTIC ORIGINS, HERBACEOUS ANCESTRY AND BIRD DISPERSAL

HARVEY E. BALLARD, JR.<sup>1</sup> AND KENNETH J. SYTSMA<sup>2</sup>

<sup>1</sup>Department of Environmental and Plant Biology, Ohio University, Porter Hall, Athens, Ohio 45701  
E-mail: ballardh@ohio.edu

<sup>2</sup>Department of Botany, University of Wisconsin, 132 Birge Hall, 430 Lincoln Drive, Madison, Wisconsin 53706  
E-mail: kjsytsma@facstaff.wisc.edu

**Abstract.**—Specialists studying the genus *Viola* have consistently allied the Hawaiian violets comprising section *Nosphinium*—most of which are subshrubs or treelets—with putatively primitive subshrubs in certain South American violet groups. Hawaiian violets also possess inflorescences, a floral disposition otherwise found only in other genera of the Violaceae, thus strengthening the hypothesis of a very ancient origin for the Hawaiian species. A survey of phylogenetic relationships among infrageneric groups of *Viola* worldwide using nuclear rDNA internal transcribed spacer (ITS) sequences revealed a dramatically different biogeographic origin for the Hawaiian violets: A monophyletic Hawaiian clade was placed in a close sister relationship with the amphi-Beringian tundra violet, *V. langsdorffii* s. l., in a highly derived position. This remarkable and unforeseen relationship received strong clade support values across analyses, and monophyly of the Hawaiian lineage was further indicated by a unique 26-base-pair deletion in section *Nosphinium*. The high polyploid base chromosome number ( $n \approx 40$ ) in the Hawaiian violets relates them to Alaskan and eastern Siberian populations in the polyploid *V. langsdorffii* complex. More than 50 species of the 260 allochthonous birds wintering in the Hawaiian Islands are found to breed in the Arctic, occupying habitats in which individual birds might have encountered ancestral *V. langsdorffii* populations and served as dispersers to the central Pacific region. Acquisition of derived morphological traits (e.g., arborescence and inflorescences), significance of a confirmed Arctic origin for a component of the Hawaiian flora, and the likelihood of other “cryptic” Arctic elements in the Hawaiian flora deserving independent molecular phylogenetic corroboration are discussed.

**Key words.**—Biogeography, bird dispersal, insular adaptations, internal transcribed spacers, molecular phylogeny.

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Extraordinary geographic isolation of the Hawaiian archipelago (more than 3500 km from the nearest continent), a diverse flora of nearly 1000 native angiosperm and approximately 180 pteridophyte species, and an unparalleled 86% endemism in angiosperms and about 70% in pteridophytes (Wagner et al. 1990; Sakai et al. 1995; Wagner and Funk 1995) make the Hawaiian flora one of the most tantalizing foci for evolutionary inquiry. Earlier explicit inferences on the minimum number of successful colonizations responsible for the modern-day Hawaiian vascular flora (Fosberg 1948; Carlquist 1970) have recently been updated to 291 putative events for angiosperms and an additional 115 for pteridophytes (Wagner 1991; Sakai et al. 1995). The Hawaiian flora thus offers unique opportunities for biogeographic and evolutionary studies of oceanic island systems (Carlquist 1965, 1974; Wagner and Funk 1995; Givnish 1998). Comprehensive higher-level phylogenetic studies including endemic Hawaiian plants have the potential to elucidate the biogeographic origins of those plant groups and reevaluate previous hypotheses of dispersal and character evolution. Well-established geologic dates pinpoint the time of origin of islands that formed “conveyor-belt” fashion (Carson and Clague 1995), permitting the coupling of island age with dispersal events to the islands, diversification, and interisland dispersal (e.g., Givnish et al. 1995; Baldwin 1997).

Adaptive radiation in island floras may yield dramatically different-looking sister taxa and, conversely, morphologically similar species of similar habitats on different islands that are not phylogenetic sister taxa (Givnish et al. 1995; Böhle et al. 1996; Baldwin 1997; Francisco-Ortega et al.

1997). Such misleading growth forms hamper identification of homologous structures and may misdirect efforts to infer relationships between island endemics and continental groups. The references cited above confirm this phenomenon and raise doubts about using macromorphological features alone in insular groups, especially where characters have not been investigated carefully and rigorously examined through appropriate explicit methods, that is, cladistics. Macromorphological changes resulting from an adaptive radiation may, even after rigorous scrutiny, prove problematic as indicators of phylogenetic relationship. Indeed, several morphological and ecological traits such as stem woodiness and dioecy are recurrent and well-known adaptations of island plants (Darwin 1859; Carlquist 1970, 1974; Givnish 1998). Thus, especially in insular settings, molecular data can provide an escape from the possible circularity of using morphological characters to reconstruct phylogenetic relationships and then to infer patterns of evolution with the same characters—a problem argued to be most severe in such cases of adaptive radiation (Sytsma et al. 1991; Givnish et al. 1995, 1997; Givnish and Sytsma 1997b,c). Phylogenetic analyses of molecular data provide an ideal framework for testing hypotheses of morphological and biogeographic relationship and clarifying patterns of character evolution. The use of morphological and molecular data in examining evolution in island taxa has increased in recent years (see chapters in Wagner and Funk 1995; Givnish and Sytsma 1997a; Grant 1998), and hypothesis testing of relationships and evolutionary scenarios has become more rigorous with the application of explicit (e.g., cladistic and other) analytical methods. Never-



FIG. 1. (a) *Viola langsdorffii*: herbaceous, amphi-Beringian, bog and meadow species revealed by ITS sequences as sister to Hawaiian violets; (b) *V. kauaensis*: sprawling, herbaceous, bog species on Kauai; (c) *V. chamissoniana* ssp. *tracheliifolia*: spindly, dry forest treelet on Kauai, Oahu, and Molokai; (d) *V. helenae*: subshrub of stream banks on Kauai; (e) *V. stipularis*: semishrub of Mesoamerican and Andean cloud forests, previously allied with Hawaiian violets; (f) *V. rubella*: semishrub of Chilean coastal forests, member of basal section in *Viola* based on ITS sequence data. Scale bar = 1 cm. Photo credits: (a) J. Pojar; (b–d) R. Gustafson; (e) H. Ballard; (f) T. Hashimoto.

theless, few molecular phylogenetic studies have unambiguously confirmed the biogeographic origins of insular plant groups (e.g., Baldwin et al. 1991; Kim et al. 1996; Mes and Hart 1996; Mes et al. 1996; Francisco-Ortega et al. 1997; Howarth et al. 1997).

The genus *Viola*, a moderate-sized and largely herbaceous group of about 550 species worldwide, includes approximately 10 Hawaiian species and infraspecific taxa (Wagner et al. 1990). The entirely woody stems of most Hawaiian taxa are highly unusual in the genus, and their few-flowered cymose inflorescences are unique. The genus has undergone a modest adaptive radiation (Givnish 1997; see also for review of definitions) in the Hawaiian archipelago, yielding divergent morphological phenotypes occupying different habitats at high elevations on the islands. Taxa include the decumbent, herbaceous *V. kauaensis* with reniform leaves (Fig. 1b); the tall, spindly treelet *V. chamissoniana* ssp. *tracheliifolia* with hastate leaves (Fig. 1c); and the knee-high, woody shrub *V. helenae* with lance-linear to ovate-lanceolate leaves (Fig. 1d).

Taxonomic treatments of the group vary considerably. The

most recent treatment of the Hawaiian flora recognizes seven species and three subspecies (Wagner et al. 1990), whereas a nearly simultaneously published monograph recognizes 14 species (St. John 1989). Differing taxonomic viewpoints aside, taxonomists have interpreted certain morphological traits in the Hawaiian violets as putatively primitive features shared with Latin American sections (Fig. 1e, f) and argued that Hawaiian *Viola* represent an ancient lineage in the genus. Specialists disagree, however, over whether the Hawaiian violets are monophyletic (Becker 1917; Skottsberg 1940; Wagner et al. 1990) with all taxa in section *Nosphinium* or polyphyletic with woody taxa in *Nosphinium* and herbaceous taxa in *Leptidium* (St. John 1989). Proponents of a monophyletic Hawaiian violet group do not agree on whether it is derived from or related to Latin American section *Leptidium* or section *Rubellium* (Becker 1917; Wagner et al. 1990) or represents the most basal and most isolated group in the genus (Skottsberg 1940).

A genuswide phylogenetic study of *Viola* was undertaken using nuclear rDNA internal transcribed spacer (ITS) se-

quences (Ballard 1996; Ballard et al. 1998). Availability of new molecular data for most species and subspecies of Hawaiian violets in addition to sequences for most infrageneric groups worldwide and those distributed around the Pacific basin (Ballard 1996; Ballard et al. 1998) allowed us to test the monophyly, phylogenetic position and biogeographic affinities of Hawaiian *Viola*. Our study addressed five key questions: (1) Are Hawaiian members of *Viola* monophyletic, therefore resulting from a "single" dispersal event? (2) Do they occupy a basal or near-basal phylogenetic position, therefore representing a comparatively ancient and possibly relictual group in *Viola*? (3) Are they sister to or derived from within Meso-American and Andean section *Leptidium* or section *Rubellium* (Figs. 1e, f), thus supporting a montane Neotropical biogeographic origin? (4) Are woody stems and inflorescences synapomorphies or symplesiomorphies shared between Hawaiian and Latin American violets? and (5) When did seeds of *Viola* arrive in the Hawaiian archipelago?

#### MATERIALS AND METHODS

ITS sequences were obtained for eight Hawaiian *Viola* taxa (following taxonomy of Wagner et al. [1990] with addition of *V. hosakai*) and 40 non-Hawaiian ones, as well as outgroups *Hybanthus concolor* and *Noisettia orchidiflora* based on ongoing *rbcL* studies of the Violaceae (see Appendix). At least one representative was sequenced for most morphologically and cytogenetically distinct infrageneric groups previously recognized in *Viola* (see fig. 1 in Ballard et al. 1998). Non-Hawaiian representatives encompassed nearly all caulescent and acaulescent groups, most Latin American groups including all those postulated to be related to the Hawaiians, and nearly all groups with ranges bordering on the Pacific Ocean basin. Nearly all groups with polyploid chromosome numbers and groups with an actual or potentially derived base chromosome number of  $n = 10$  as well as multiples of this, were also sampled. Thus, all potential sister groups to the Hawaiian taxa in the genus were sampled comprehensively and, in many cases, were represented by two to several species.

DNA extracts were made using a 6% CTAB protocol (Smith et al. 1991) or a modified mini-extraction protocol with SDS buffer (Edwards et al. 1991). Amplification used the polymerase chain reaction (PCR; Mullis and Faloona 1987) with primer sequence 5'-GTCCACTGAACCTTAT-CATTAG-3' ("ITS leu 1"; B. Baldwin, pers. comm.) and 5'-TCCTTCCGCTTATTGATATGC-3' ("ITS 4"; White et al. 1990) following previous protocols (Baum et al. 1994). PCR products were cleaned with a Qiagen (Valencia, CA) kit and cycle-sequenced with ABI dye-terminator chemistry using primer sequence 5'-GGAAGGAGAAGTCGTAA-CAAGG-3' ("ITS 5"; White et al. 1990) for ITS 1 and 5'-GCATCGATGAAGAACGTAGC-3' ("ITS 3B"; Baum et al. 1994) for ITS 2. Reactions were analyzed on a Perkin Elmer (Norwalk, CT) Cetus/ABI (Foster City, CA) Prism automated sequencer. The ITS 1 and ITS 2 spacer boundaries were identified against sequences for *Epilobium* (Baum et al. 1994) and *Daucus carota* (Yokota et al. 1989), and sequences were aligned visually in MacClade 3.0 (Maddison and Maddison 1992) and PAUP\* (vers. 4.01b, Swofford 1998), by inserting

gaps where needed. Data for the 18S and 26S coding regions flanking the ITS region and the 5.8S coding region between the ITS1 and ITS2 spacers were excluded prior to analysis due to the dearth of informative characters and lack of complete data in certain regions for some taxa. Initial Fitch parsimony analyses of the ITS spacers used a variety of gap treatments (Baum et al. 1994), whereas other analyses used only nucleotide variation.

Parsimony analyses in PAUP\* used a heuristic four-step procedure (Olmstead et al. 1993) with 10,000 initial random-addition sequences and subsequently varied search strategies (Maddison 1991). Internal support in the Fitch strict consensus tree was measured from 100 replicates in both a bootstrap analysis (Felsenstein 1985) and a jackknife analysis (Farris et al. 1996) with 36.8% taxon deletion (Swofford 1998), and Bremer decay values (Bremer 1988) were derived by the enforced constraints strategy (as implemented by Baum et al. 1994); consistency index (CI; Kluge and Farris 1969) and retention index (RI; Archie 1989; Farris 1989) measured internal consistency of the Fitch parsimony analysis. Weighted parsimony used empirically determined transition:transversion ratios for the two spacers separately. Jukes-Cantor (Jukes and Cantor 1969) and Kimura (1980) models were employed in neighbor-joining analyses (Saitou and Nei 1987) in PAUP\*. Maximum-likelihood analysis used a transition:transversion ratio of 2:1 and empirically calculated base frequencies in fastDNAm1 (vers. 1.1.1a, Olsen et al. 1994). Sequence divergences were obtained from PAUP\*, and mapping of morphological features and geographic distributions was done in MacClade.

#### RESULTS

##### *Sequence Characteristics*

Unaligned and aligned lengths of the ITS spacers for *Viola* and the outgroups fall within ranges reported for other angiosperm genera (Baldwin et al. 1995). However, the percentage of phylogenetically informative characters in the two spacers, 45.8%, and percent maximum sequence divergence, 28.8%, within *Viola* are higher than values for most genera and are comparable to species within *Arceuthobium* (Nickrent et al. 1994) and generic segregates of *Senecio* s. l. (Bain and Jansen 1995). ITS sequence variation in *Viola* shows uncommonly broad phylogenetic utility in resolving relationships among infrageneric groups across the genus, relationships within infrageneric groups, and even among infraspecific taxa and populations of particular species. Nucleotide divergence among the Hawaiian violets, 1.3–5.1%, is comparable to or slightly higher than ranges published for members of the silversword-greensword alliance in the Hawaiian Islands (0.0–4.5% including the 5.8S region; Baldwin et al. 1995), *Dendroseris* (0.0–4.1% using Kimura two-parameter method; Sang et al. 1994) and *Robinsonia* (0.0–6.8% using Kimura two-parameter method; Sang et al. 1995) of the Juan Fernandez Islands, and the *Sonchus* alliance of five genera in Macaronesia (0.0–4.8% using Jukes-Cantor one-parameter method; Kim et al. 1996). Hawaiian violet divergence is also equivalent to ranges observed in continental groups of *Viola*, such as the *Mexicanae*, belonging to the same *Viola-Nosophinium* clade.

### Phylogenetic Analyses

The Fitch analysis treating gaps as missing data and incorporating additional gap-code characters yielded eight equally most parsimonious trees of 980 steps based on 274 phylogenetically informative characters of 564 total, of which the strict consensus (Fig. 2) is largely resolved. Moderate to high bootstrap and decay values and jackknife values above 50% support most clades and subclades. Fitch parsimony analyses of datasets excluding gap codes resulted in some loss of resolution, increased numbers of trees, and lower CI and RI values, but retained approximately the same composition in most large and small clades. Weighted-parsimony, neighbor-joining, and maximum-likelihood analyses produced phylogenies that were concordant in most respects with the Fitch parsimony analysis including gap codes and, at least concerning the clade containing the Hawaiian violets, were essentially subsets of the Fitch analysis including gap codes. All analyses demonstrate that the Hawaiian violets are strongly monophyletic with an equally strong sister relationship to amph-Beringian *V. langsdorffii* (Fig. 1a), and they place this subclade in a terminal position within the most highly derived clade in *Viola*. Thus, a diversity of phylogenetic analyses of ITS sequence data for *Viola* confirms the previously debated monophyly of the Hawaiian violets, demonstrates a highly derived phylogenetic position for them, and reveals a remarkable and unexpected sister relationship with the amph-Beringian tundra violet, *V. langsdorffii*—a relationship never before proposed on the basis of macromorphological features.

### DISCUSSION

#### *The Hawaiian Violets are Monophyletic and Recently Derived*

The Hawaiian violets encompass substantial diversity of growth form, secondary growth, vegetative features, and reproductive structures, and, on this basis, have been segregated into two distinct sections within *Viola* by some (St. John 1989). Likewise, the affinities of the Hawaiian violets have been debated, but a unanimous consensus has emerged that they occupy a near basal position within the genus (Becker 1917; Skottsberg 1940; Wagner et al. 1990). This was recently supported by a cladistic analysis of 47 macromorphological characters for 150 of the approximately 525 species comprising *Viola*, including representatives of all infrageneric groups of *Viola*; nearly all species represented in the current ITS dataset; *V. kauaensis*, *V. maviensis*, and *V. helenae* for the Hawaiian violets; and *V. langsdorffii* (Ballard 1996). Wagner parsimony placed the Hawaiian violets in a clade with sections *Leptidium* and *Rubellium* at the base of the genus, but analysis removed *V. langsdorffii* to a derived clade consisting of caulescent and acaulescent members of section *Rostratae* sensu Clausen.

Nevertheless, the ITS data (Figs. 2, 3) confirm that the Hawaiian violets are monophyletic and reflect a geologically recent colonization event followed by an evolutionary radiation. Monophyly of Hawaiian *Viola*, its close sister relationship with *V. langsdorffii*, and low divergence among insular and continental species indicating a recent derivation, argue for submerging the Hawaiians in the *Langsdorffianae*

group in section *Viola*, to reflect our current understanding of evolutionary relationships more accurately.

The sampling of potential sister groups across *Viola* was relatively thorough, including representatives from all groups that would likely be sister to the Hawaiians by virtue of morphology, ploidy level, or proximity to the Pacific basin. Although, for space reasons, results from a reduced dataset are portrayed in Figure 2, analyses with additional taxa (not shown) of sections *Andinium* and *Leptidium*, all Hawaiian taxa plus multiple island accessions of widespread ones, and an additional *V. langsdorffii* accession from Japan (K. Inoue, unpubl. data) yielded identical phylogenetic positions and comparable or stronger levels of support. The comparative morphological homogeneity of the Latin American sections and close genetic identity of sequenced species within each indicate that these basal groups are indeed monophyletic and that their distant relations with the Hawaiian violets would remain unchanged with additions of taxa.

Such dramatic modification of growth habit to yield very different growth forms in sister taxa reinforces emerging conclusions from other studies of insular and continental groups—that features relating to growth habit alone cannot reliably be used for phylogenetic or biogeographic inference under such circumstances (Sytsma et al. 1991; Givnish et al. 1995). Molecular phylogenetic investigations of Hawaiian *Cyanea* (Campanulaceae; Givnish et al. 1994, 1995) and the Hawaiian silversword alliance (Baldwin 1997), Neotropical section *Notopleura* of *Psychotria* (Rubiaceae; Nepokroeff and Sytsma 2000), and Southern Hemisphere *Fuchsia* (Onagraceae; Sytsma et al. 1991) concur, in that derivations of a particular growth form in these groups could not be intuited merely by observation and comparison of morphological traits. Likewise, restriction-site studies of *Brocchinia* (Bromeliaceae) in the Guayana Shield tepuis of Venezuela have uncovered a remarkable and complex radiation of evolutionary specializations in closely related taxa (Givnish et al. 1997).

Molecular phylogenetic relationships within the Hawaiian violets provide a clue to the timing of successful colonization and the relative age of the radiation. Analyses with all available Hawaiian accessions included place the Oahu endemic *V. chamissoniana* ssp. *chamissoniana* sister to Kauai and Oahu accessions of *V. chamissoniana* ssp. *tracheliifolia* and position this clade basal to the rest of the Hawaiian violets. This suggests that the ancestor initially colonized Kauai and dispersed early to Oahu, the geologic age of which is approximately 3.7 million years (Carson and Clague 1995). Oahu would have become available for colonization some time after its emergence and cooling, providing a mid-Pliocene benchmark date for the initial dispersal event from the Arctic to Kauai.

#### *“Primitive” Traits in Hawaiian Violets May Be Convergent Insular Adaptations*

Island floras include a disproportionately higher incidence of origins of arborescence than expected (Carlquist 1965, 1974). Among other features, woody stems and increased stature have been viewed by some as “primitive” traits indicative of ancient age and relictual status in insular groups (Bramwell 1976; Cronk

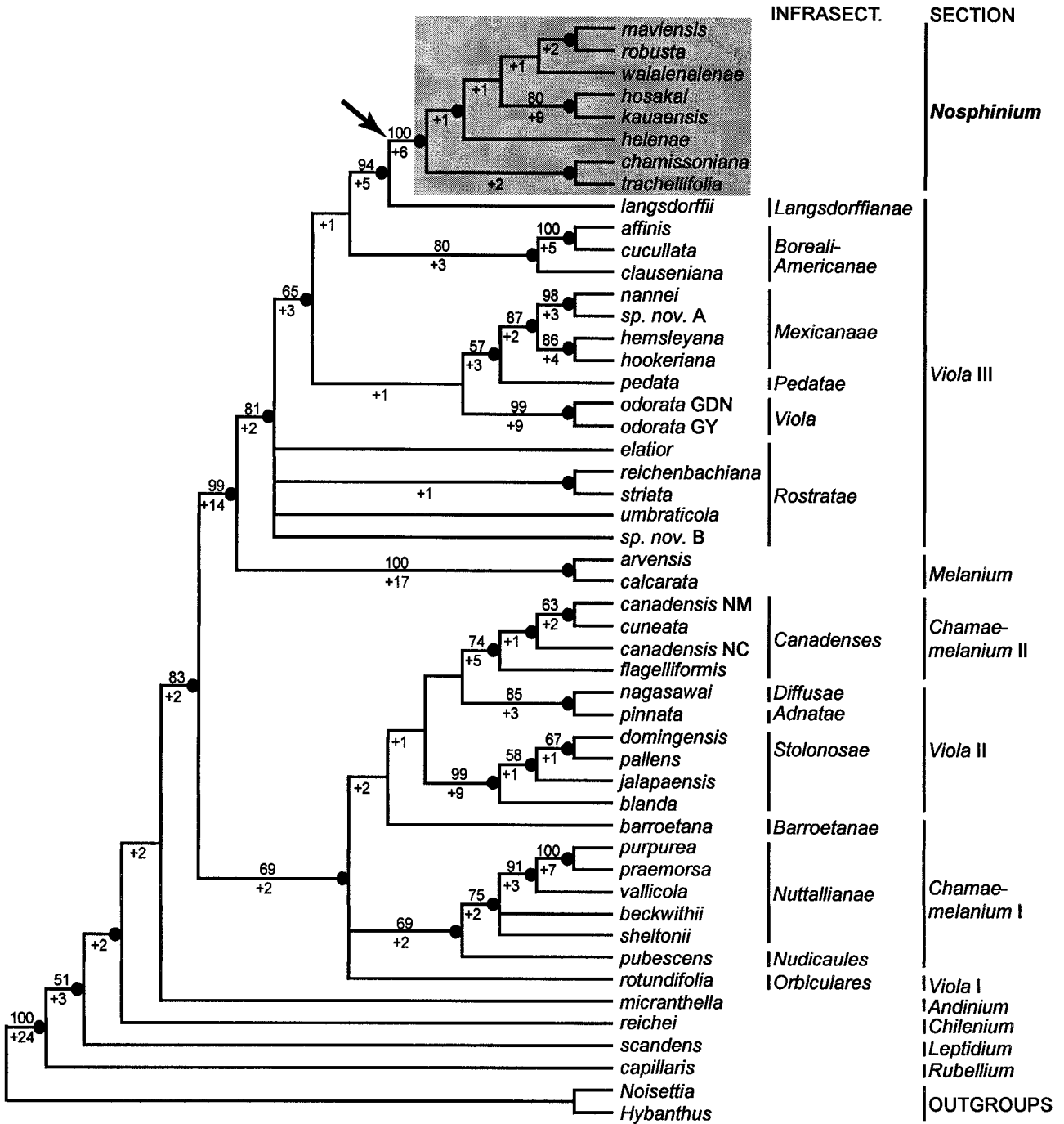


FIG. 2. Fitch strict consensus of eight most-parsimonious trees of 980 steps based on 240 phylogenetically informative nucleotide substitutions and 34 gap codes from the ITS region; CI = 0.498, CI = 0.578 with all characters, RI = 0.732. Bootstrap values > 50% noted above branches, decay values noted below branches; filled circle indicates > 50% jackknife support. Solid arrow indicates unique 26-bp deletion in Hawaiian taxa. Only subspecific epithets are used for infraspecific taxa of *V. chamissoniana*.

1992). Opponents of this “relict group” hypothesis have regarded modifications of growth form, reproductive structures, and other macromorphological features as recurrent, recent, and expected adaptations to island life (Darwin 1859; Carlquist 1965, 1970, 1974; Givnish 1998). Well-developed secondary growth is common in the Hawaiian violets and very few other groups of *Viola*, including the Latin American sections previ-

ously hypothesized to be the closest relatives to the Hawaiian species, but is all but ubiquitous in the remaining genera in the Violaceae. Multiflowered inflorescences typical of some Hawaiian species are otherwise unique in *Viola*, but are the norm in other genera of the family. These features have previously argued for a basal phylogenetic position for the Hawaiian violets in the genus.

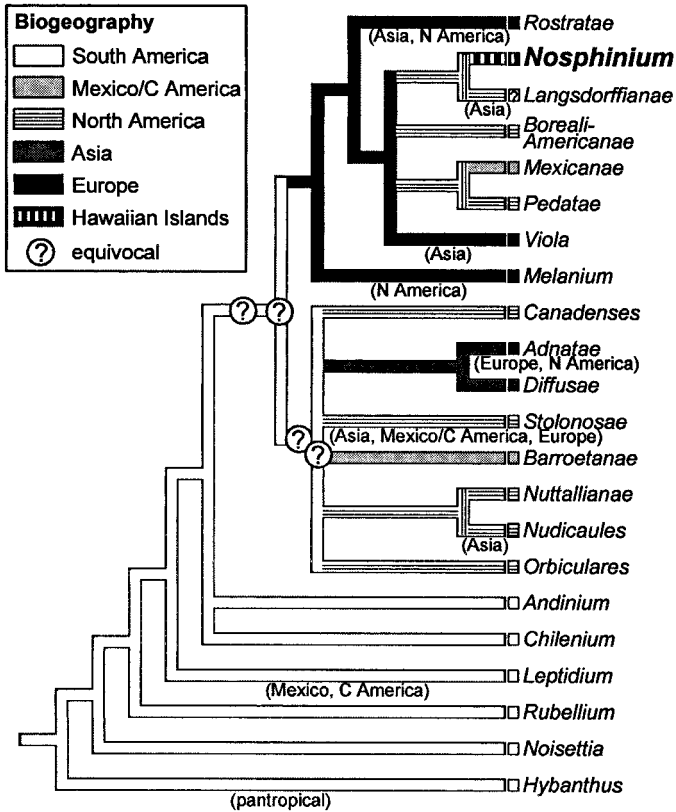


FIG. 3. Biogeographic distribution of *Viola* groups by continent, mapped using MacClade onto reduced ITS phylogeny of Figure 2; branches are collapsed to portray distinct infrageneric groups with jackknife support > 50%. Primary "center of endemism" is character state coded for groups distributed on more than one continent; continents with fewer species are noted in parentheses below the branch.

Distinguishing between the plesiomorphic or apomorphic nature of woodiness and other "island" traits is an important challenge of phylogenetic studies involving a radiation. Reliance on these features alone may be misleading and circular in reasoning (Sytsma et al. 1991; Givnish et al. 1995, 1997; Givnish 1997, 1998; Givnish and Sytsma 1997a,b,c; however, see Brooks and McLennan 1994; Luckow and Hopkins 1995; Luckow and Bruneau 1997). Molecular data often provide an independent assessment of the nature of these traits. Whereas molecular phylogenetic investigations have represented some woody groups in Hawaii and other islands as retaining secondary growth from woody ancestors (e.g., *Cyanea*, Givnish et al. 1995), they have indicated that other woody groups are derived from herbaceous ancestors (e.g., the Hawaiian silversword alliance, Baldwin et al. 1991; *Aeonium* in Macaronesia, Mes et al. 1996). In the Hawaiian violets, secondary growth (Fig. 4) and inflorescences (Fig. 5) are clearly secondarily derived in the context of an ITS phylogeny for *Viola*, with woody stems having multiple independent origins across the genus. Thus, woody stems and inflorescences in Hawaiian *Viola* are best viewed as convergent, apomorphic traits originating in a herbaceous, single-flowered ancestor flourishing under tropical oceanic island conditions.

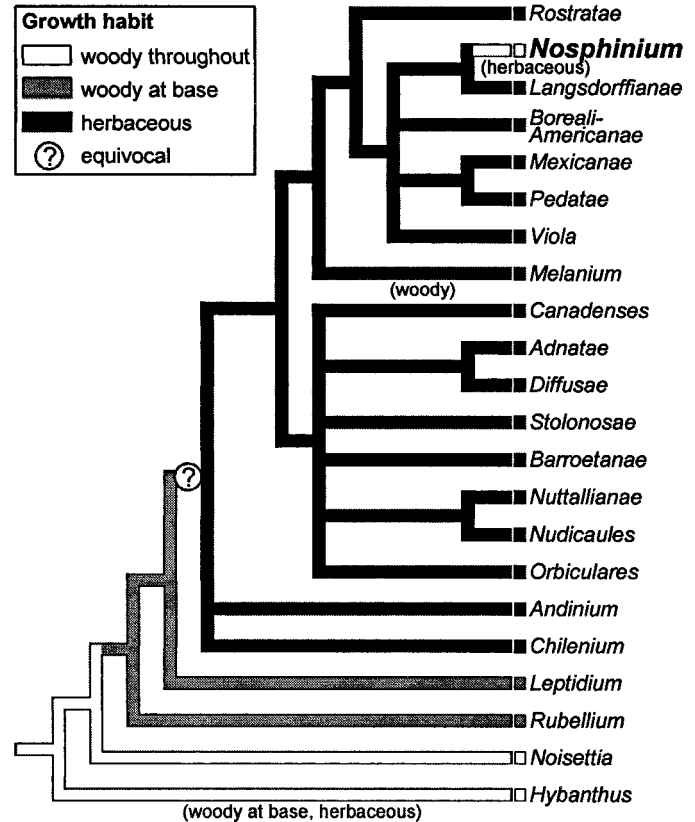


FIG. 4. Growth habit of *Viola* groups based on predominating character state in each group, mapped using MacClade onto reduced phylogeny given in Figure 3; less frequent alternative states are noted in parentheses below the branch.

Darwin (1859) first suggested woodiness as a means of avoiding competition by "overtopping", whereas Wallace (1878) and Böhle et al. (1996) later stressed the advantage of "longevity" of a woody habit in island taxa. Carlquist (1970, 1974) has argued that ideal growing conditions and availability of new ecological niches for colonists drive the repeated evolution of woodiness. Woody growth would elevate leaves above competing vegetation and improve access to sunlight for photosynthesis (Givnish 1998), whereas increased flower number and staggered maturity of flowers in an inflorescence could potentially raise the level of outcrossing (Carlquist 1965).

Both *V. langsdorffii* and *V. kauaensis* possess reclining stems, a herbaceous habit, and reniform leaf blades and both occupy bogs and boglike habitats, suggesting a close relationship. However, ITS sequence data in both the reduced and expanded datasets remove *V. kauaensis* (and Oahu *V. hosakai*) to an intermediate phylogenetic position and unite it with *V. waialalenanae* from swamp forests on Kauai. Whether the herbaceous ancestor to the clade containing *V. kauaensis* gave rise to multiple woody-stemmed lineages, but retained the herbaceous habit in the lineage leading to *V. kauaensis*, or the herbaceous habit was lost initially and secondarily regained cannot be determined with available data. Anatomical studies of stem tissue in these taxa may provide future clarification.

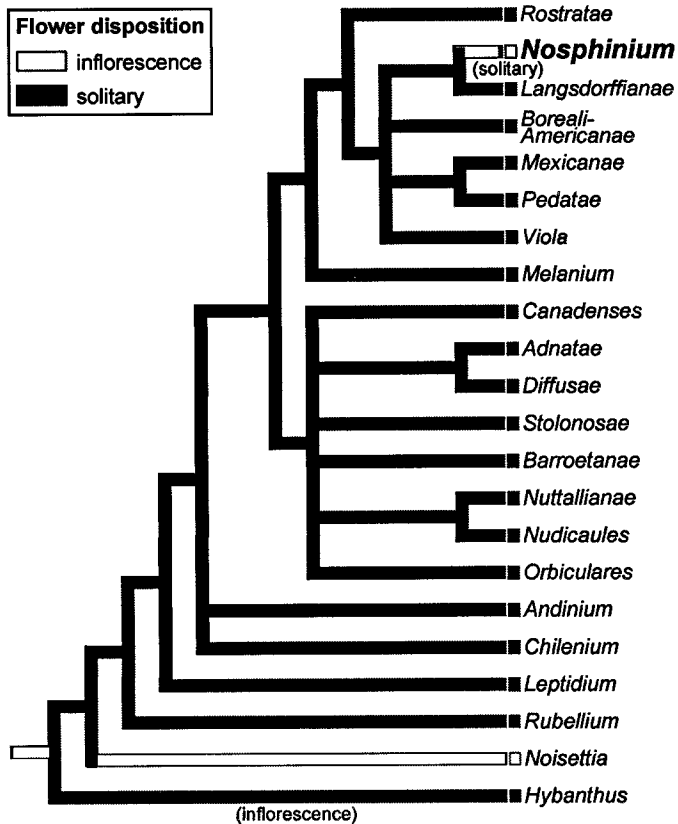


FIG. 5. Flower disposition of *Viola* groups, mapped using MacClade onto reduced phylogeny given in Figure 3; less frequent alternative states are noted in parentheses below the branch.

#### The Hawaiian Violets Originated in the Arctic

In contradiction to previous hypotheses of a South American origin, the ITS data (Fig. 2) identify the amphi-Beringian violet *V. langsdorffii* as the sister to Hawaiian *Viola*. The revelation is one of only a few published molecular phylogenetic studies (e.g., Baldwin et al. 1991; Mes et al. 1996; Howarth et al. 1997) to narrow the biogeographic origin of an oceanic island plant group to a single continental taxon from a particular geographic region. It is the first molecular phylogenetic study to discern a northern Pacific, and more specifically a probable Arctic, origin for a group of oceanic island endemics by explicit (molecular) phylogenetic analysis.

Cladistic biogeographic patterns in the genus, parphyly of *V. langsdorffii* from the American Arctic and Japan, chromosome numbers, and late Tertiary and Quaternary paleoclimatic evidence provide compelling circumstantial evidence arguing for an Arctic origin for the Hawaiian violets. Mapping of geographic distributions onto the ITS phylogeny for *Viola* groups worldwide using MacClade (Fig. 3) indicates North America as the source area. An alternative interpretation for the branch leading to the section *Melanium-Viola-Nosphinium* clade could be dispersal to Asia from North America, which would better accommodate high levels of endemism in the *Rostratae* and *Viola* groups found in Asia and Europe. This latter interpretation would represent early dispersal to the Old World, further diversification in a number

of new lineages, and back-dispersal by certain lineages into North America with subsequent speciation, thus leaving *V. langsdorffii* stranded in the Arctic and subsequently dispersing to Hawaii.

Reanalysis of the dataset was conducted with all available Hawaiian accessions and an unpublished ITS sequence temporarily provided by Ken Inoue from a Japanese population of *V. langsdorffii* (not shown). Parsimony and other analyses placed the Arctic accession sister to the Hawaiian violets in an extremely strongly supported relationship and placed the Japanese accession sister to the Arctic-Hawaiian clade, also with very strong support.

Several chromosome counts for *V. langsdorffii* sensu lato suggest three continental ploidy levels: hexaploids ( $n \approx ca. 30$ ) in Kamchatka, decaploids ( $n \approx ca. 50$ ) from Kamchatka to Japan, and dodecaploids ( $n = 60$ ) in the Queen Charlotte Islands of the Pacific Northwest (Miyaji 1929; Sokolovskaja 1963; Taylor and Mulligan 1968; Sokolovskaja and Probatova 1986). No counts have been made from American Arctic material, but would presumably include  $n = 30$  and  $n = 50$  levels. Chromosome numbers for the Hawaiian violets are  $n = 38, 40,$  and  $41-43$  (Skottsberg 1955; Carr 1978, 1985) and may constitute the ‘missing’ octoploid level of  $n \approx 40$  in *V. langsdorffii*. These counts would seem to disqualify involvement of populations in the Pacific Northwest. Most plausible is either an early hybridization event between the two Arctic cytotypes, with subsequent dispersal and stabilization of the new Hawaiian lineage, or production of an  $n = 40$  race (which may still be there or may have disappeared) early in formation of the complex. Siberian specialists recognize three to four species in the *Langsdorffianae* (Yuzepchuk and Klokov 1974). However, neither literature descriptions nor herbarium specimens reveal distinct taxa. The variability noted in flower color and plant size across the range of *V. langsdorffii* sensu lato are probably due to the combined effects of ploidy level and substrate (Ballard 1996). Until convincing evidence becomes available, *Langsdorffianae* should be viewed as consisting of one modestly variable continental taxon, *V. langsdorffii*, and its Hawaiian sisters.

Increasingly abundant and diverse paleoclimatic and geologic evidence also supports the hypothesis of a dispersal event from the Arctic region in the mid-Pliocene. From the early to mid-Pliocene—the period leading to the inferred timing of the dispersal event—the climate in the northern Pacific basin and worldwide was several degrees warmer than at present, with the temperature difference greatest in the far northern latitudes (Hultén 1963, 1964; Péwé et al. 1965; Hopkins et al. 1982; Kontrimavichus 1985; Zubakov and Borzenkova 1990; Repenning and Brouwers 1992; Graham 1999). In addition, sea levels were approximately 36 m higher than present levels and coastal areas were more heavily predominated by coniferous and temperate deciduous forest, thus further reducing available habitat for *V. langsdorffii*. Assuming that *V. langsdorffii* was already adapted to cold tundra bogs and similar ‘relictual’ microsites further south, it is probable that the geographic range of ancestral *V. langsdorffii* just prior to dispersal was confined to the Beringian Arctic region. The restriction of all Hawaiian violets to high-elevation habitats, often bogs and related wetland sites, parallels to some extent the ecology of the sister species.

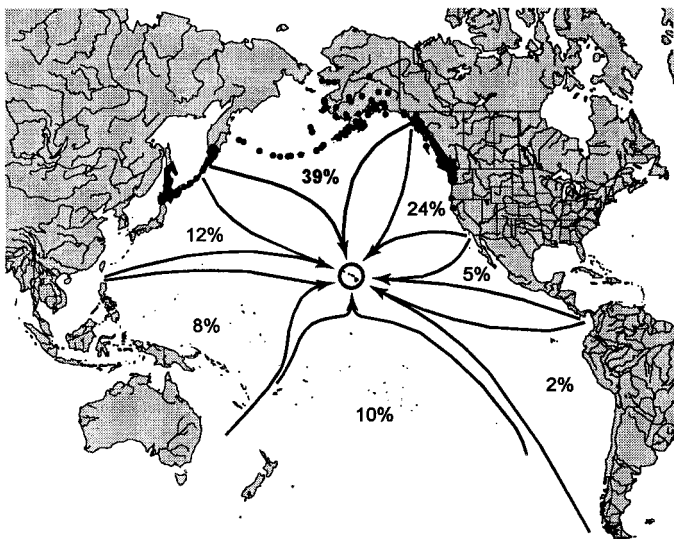


FIG. 6. Geographic subdivision of Pacific basin breeding ranges for 260 allochthonous native bird species recorded as visitors or winter-residents in the Hawaiian archipelago. Percent values are the proportion of these 260 bird species that breed in a particular region, that migrate to (and sometimes beyond) the Hawaiian Islands, and thus have been recorded there in recent times. Species whose ranges span geographic boundaries are assigned to more than one region. Black dots are known site localities for *Viola langsdorffii*, the sister species to all Hawaiian violets.

#### Arctic-Breeding Birds Carried Violet Seeds to Hawaii

Birds have been proposed as the most plausible and most common agents of dispersal for carrying seeds and fruits of colonizing angiosperms to the Hawaiian Islands (Carlquist 1970). Bird dispersal externally or internally is also a plausible dispersal mechanism for violet seeds. Experimental treatment of violet seeds, including *V. langsdorffii*, with sulfuric acid-acetic anhydride hydrolysis for 24 h leaves the "basement layer" of the testa unharmed (H. E. Ballard, unpubl. data), indicating that violet seeds could pass unharmed through bird crops during internal transport.

Present-day migration routes of birds from continental regions to the Hawaiian Islands (Flint et al. 1984; Pratt et al. 1987; Madge and Burn 1988; Kessel 1989; Peterson 1990; Paulson 1993) illustrate high visitation rates from Arctic and other northern Pacific summer breeding areas (Fig. 6). Twenty-one species of terrestrial birds, shore and wading birds, water fowl, and raptors (which could ingest rodents and other creatures that themselves had eaten seeds or fruits) migrate annually by the thousands or tens of thousands to the Hawaiian Islands. Many of these species (e.g., the bristle-thighed curlew, wandering tattler, and ruddy turnstone) have very narrow summer breeding distributions in the high Arctic. Such huge seasonal flocks of birds would theoretically provide innumerable opportunities for dispersal of fruits and seeds from the Arctic to the Hawaiian Islands.

Other evidence also suggests that northern Pacific (and Arctic) birds dispersed to Hawaii during prehistoric times. Mitochondrial studies (Quinn et al. 1991) relate the Hawaiian nene (*Nesochen sandvicensis*) most closely to small-bodied Arctic races of the Canada goose (*Branta canadensis*). The

Hawaiian duck, or koloa (*Anas wyvilliana*), and related forms on other Pacific islands are related to, or derived from, northern North American populations of the mallard (*Anas platyrhynchos*; Weller 1980). Recent discoveries of bird fossils include small-bodied waterfowl and wading birds, some of which apparently have boreal or Arctic affinities (Olson and James 1982). Arctic-to-central Pacific migratory patterns suggested by these affinities originated in the late Tertiary and the early Quaternary as a response to the unfavorable seasonal continental conditions brought on by glaciation (Selander 1965; Alerstam 1990; Elhick 1995).

Thus, extant Arctic-breeding birds form an unexpectedly large proportion of autumn visitors to the Hawaiian Islands (Fig. 3), and their ancestors may have served as frequent dispersers of propagules to the central Pacific region. The "top-heavy" pattern of potential propagule dispersal from the Arctic to the Hawaiian Islands is the reverse of the "bottom-heavy" pattern of strong Australasian and South Pacific biogeographic affinities proposed for indigenous Hawaiian vascular plants (Fosberg 1948; Carlquist 1981) based on morphological traits. This discrepancy between a theoretically high dispersal rate from the Arctic to the Hawaiian Islands and the low proportion of indigenous Hawaiian plants attributed to continental Arctic sources can be explained by two factors. Assuming frequent dispersal from the Arctic has occurred since the Hawaiian Islands became available for colonization, the primary explanation for failure of successful colonization is probably due to poor survival of most northern Pacific disseminules in the tropical oceanic environment. The secondary basis for the discrepancy, using the unexpected Arctic affinities of the Hawaiian violets as an example, involves "phytogeographic suggestion" (Iltis and Shaughnessy 1960), that is, misinterpretation of tropical affinities for an indigenous plant group due to influence of the prevailing phytogeographic viewpoint (i.e., tropical ancestry for most of the flora).

#### Other Native Hawaiian Angiosperm Taxa Need Biogeographic Reevaluation

If morphological convergence has obscured a comparatively recent derivation and Arctic origin in the endemic Hawaiian violets, as revealed by ITS sequence data, then misinterpretation of biogeographic affinities and phylogenetic relationships is likely with other Hawaiian endemics. The Hawaiian flora includes 15 endemic taxa other than *Viola* section *Nosphinium* that include Arctic relatives (Hultén 1929, 1968; Yuzepchuk and Klovov 1974; Wagner et al. 1990). Particularly worthy of molecular phylogenetic investigation are *Alsinidendron* (four species), *Schiedea* (22 species), *Artemisia* (three species), *Calamagrostis* (one species), *Carex* (four species), *Deschampsia* (one species), *Festuca* (one species), *Luzula* (one species), *Platanthera* (one species), *Poa* (three species), *Ranunculus* (two species), *Silene* (seven species), *Sisyrinchium* (one species), *Trisetum* (two species), and *Vaccinium* (three species). Molecular phylogenetic analyses of *Alsinidendron* and *Schiedea* (Sakai et al. 1997) and of *Platanthera* (Hapeman and Inoue 1997) are close to uncovering the ancestral stock of these two Hawaiian groups. In addition, several species are apparently conspecific



to species of northern latitudes on the mainland, including *Carex echinata*, *Carex macloviana*, and *Drosera anglica*. These demand population-level molecular systematic approaches to identify likely continental source populations.

In summary, ITS sequence data provide several remarkable new insights into the biogeographic origin and evolution of the Hawaiian violets and urge molecular phylogenetic re-evaluation of other native Hawaiian taxa as well. Genuswide phylogenetic studies of *Viola* reveal that the Hawaiian violets are monophyletic and sister to the amphi-Beringian *V. langsdorffii*. Molecular data refute previous hypotheses of a basal phylogenetic position and a South American origin for the group. A basal Kauai-Oahu split provides a mid-Pliocene upper limit of 3.7 million years for successful colonization on Kauai and the subsequent radiation leading to present-day taxa. "Primitive" woody stems and inflorescences are re-interpreted as convergent insular traits with plausible adaptive advantages. A biogeographic hypothesis invokes long-distance dispersal of ancestral *V. langsdorffii* from the Arctic to Kauai by migrating birds, with subsequent diversification following establishment. Arctic-breeding bird species form an unexpectedly high proportion of the visiting or winter-resident Hawaiian avifauna and may have frequently dispersed Arctic propagules to the Hawaiian Islands. However, the discrepancy between this heavy potential Arctic floristic input and the large percentage of Hawaii's flora attributed to tropical Pacific sources predominantly reflects poor establishment success (rather than infrequency of dispersal) of northern propagules in tropical oceanic island conditions and perhaps erroneous attribution of tropical biogeographic affinities to Arctic-derived groups based on morphological convergences. In this latter regard, several native taxa require molecular phylogenetic investigation as potentially overlooked examples of Hawaiian plant groups with Arctic origins.

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#### LITERATURE CITED

- Alerstam, T. 1990. Bird migration. Cambridge Univ. Press, Cambridge, U.K.
- Archie, J. W. 1989. Homoplasy excess ratios: new indices for measuring levels of homoplasy in phylogenetic systematics and a critique of the consistency index. *Syst. Zool.* 38:253–269.
- Bain, J. F., and R. K. Jansen. 1995. A phylogenetic analysis of the aureoid *Senecio* (Asteraceae) complex based on ITS sequence data. *Plant Syst. Evol.* 195:209–219.
- Baldwin, B. G. 1997. Adaptive radiation of the Hawaiian silversword alliance: congruence and conflict of phylogenetic evidence from molecular and non-molecular investigations. Pp. 103–128 in T. J. Givnish and K. J. Sytsma, eds. *Molecular evolution and adaptive radiation*. Cambridge Univ. Press, New York.
- Baldwin, B. G., K. W. Kyhos, J. Dvorak, and G. D. Carr. 1991. Chloroplast DNA evidence for a North American origin of the Hawaiian silversword alliance (Asteraceae). *Proc. Natl. Acad. Sci. USA* 88:1840–1843.
- Baldwin, B. G., C. S. Campbell, J. M. Porter, M. J. Sanderson, M. F. Wojciechowski, and M. J. Donoghue. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Ann. Mo. Bot. Gard.* 82:247–277.
- Ballard, H. E., Jr. 1996. Phylogenetic relationships and infrageneric groups in *Viola* (Violaceae) based on morphology, chromosome numbers, natural hybridization and internal transcribed spacer (ITS) sequences. Ph.D. diss., University of Wisconsin, Madison, WI.
- Ballard, H. E., K. J. Sytsma, and R. R. Kowal. 1998. Shrinking the violets: phylogenetic relationships of infrageneric groups in *Viola* (Violaceae) based on internal transcribed spacer DNA sequences. *Syst. Bot.* 23:439–458.
- Baum, D. A., K. J. Sytsma, and P. C. Hoch. 1994. A phylogenetic analysis of *Epilobium* (Onagraceae) based on nuclear ribosomal DNA sequences. *Syst. Bot.* 19:363–388.
- Becker, W. 1917. *Violae Asiaticae et Australenses*. I. Bearbeitung der Gruppe "Sandvicenses" W. Bckr. ined. *Beih. Bot. Centralbl.* 34, Abt. Teil 2:209–216.
- Böhle, U.-R., H. H. Hilger, and W. F. Martin. 1996. Island colonization and evolution of the insular woody habit in *Echium* L. (Boraginaceae). *Proc. Nat. Acad. Sci. USA* 93:11740–11745.
- Bramwell, D. 1976. The endemic flora of the Canary Islands: distribution, relationships and phytogeography. Pp. 207–240 in G. Kunkel, ed. *Biogeography and ecology in the Canary Islands*. Dr. W. Junk Publishers, Hague, The Netherlands.
- Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42:795–803.
- Brooks, D. R., and D. A. McLennan. 1994. Historical ecology as a research programme: scope, limitations and the future. Pp. 1–27 in P. Eggleton and R. Vane-Wright, eds. *Phylogenetics and ecology*. Academic Press, London.
- Carlquist, S. 1965. *Island life*. Natural History Press, Garden City, CA.
- . 1970. *Hawaii, a natural history: geology, climate, native flora and fauna above the shoreline*. Pacific Tropical Botanical Garden, Lawai, HI.
- . 1974. *Island biology*. Columbia Univ. Press, New York.
- . 1981. Chance dispersal. *Am. Sci.* 69:509–516.
- Carr, G. D. 1978. Chromosome numbers of Hawaiian flowering plants and the significance of cytology in selected taxa. *Amer. J. Bot.* 65:236–242.
- . 1985. Additional chromosome numbers of Hawaiian flowering plants. *Pac. Sci.* 39:302–304.

- Carson, H. L., and D. L. Clague. 1995. Geology and biogeography of the Hawaiian Islands. Pp. 14–29 in W. L. Wagner and V. A. Funk. *Hawaiian biogeography: evolution on a hot spot archipelago*. Smithsonian Institution Press, Washington, DC.
- Cronk, Q. C. B. 1992. Relict floras of Atlantic islands: patterns assessed. *Biol. J. Linn. Soc.* 46:91–103.
- Darwin, C. 1859. *The origin of species by means of natural selection*. John Murray, London.
- Edwards, K., C. Johnstone, and C. Thompson. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Res.* 19:1349.
- Elhick, J. 1995. *The atlas of bird migration*. Random House, New York.
- Farris, J. S. 1989. The retention index and homoplasy excess. *Syst. Zool.* 38:406–407.
- Farris, J. S., V. A. Albert, M. Källersjö, D. Lipscomb, and A. G. Kluge. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12:99–124.
- Felsenstein, J. 1985. Confidence limits of phylogenies: an approach using the bootstrap. *Evolution* 39:783–791.
- Flint, V. E., R. L. Boehme, Y. V. Kostin, and A. A. Kuznetsov. 1984. *A field guide to birds of the USSR including eastern Europe and central Asia*. Princeton Univ. Press, Princeton, NJ.
- Fosberg, F. R. 1948. Derivation of the flora of the Hawaiian Islands. Pp. 107–119 in E. C. Zimmerman, ed. *Insects of Hawaii*. Vol. 1. Univ. of Hawaii Press, Honolulu.
- Francisco-Ortega, J., D. J. Crawford, A. Santo-Guerra, and R. K. Jansen. 1997. Origin and evolution of *Argyranthemum* (Asteraceae: Anthemideae) in Macaronesia. Pp. 407–431 in T. J. Givnish and K. J. Sytsma, eds. *Molecular evolution and adaptive radiation*. Cambridge Univ. Press, New York.
- Givnish, T. J. 1997. Adaptive radiation and molecular systematics: aims and conceptual issues. Pp. 1–54 in T. J. Givnish and K. J. Sytsma, eds. *Molecular evolution and adaptive radiation*. Cambridge Univ. Press, New York.
- . 1998. Adaptive radiation of plants on oceanic islands: classical patterns, molecular data, new insights. Pp. 281–304 in P. Grant, ed. *Evolution on islands*. Oxford Univ. Press, Oxford, U.K.
- Givnish, T. J., and K. J. Sytsma, eds. 1997a. *Molecular evolution and adaptive radiation*. Cambridge Univ. Press, New York.
- . 1997b. Homoplasy in molecular vs. morphological data: the likelihood of correct phylogenetic inference. Pp. 55–101 in T. J. Givnish and K. J. Sytsma, eds. *Molecular evolution and adaptive radiation*. Cambridge Univ. Press, New York.
- . 1997c. Consistency, characters, and the likelihood of correct phylogenetic inference. *Mol. Phyl. Evol.* 7:320–330.
- Givnish, T. J., K. J. Sytsma, J. F. Smith, and W. J. Hahn. 1994. Thorn-like prickles and heterophylly in *Cyanea*: adaptations to extinct avian browsers on Hawaii? *Proc. Nat. Acad. Sci. USA* 91:2810–2814.
- . 1995. Molecular evolution, adaptive radiation, and geographic speciation in *Cyanea* (Campanulaceae, Lobelioideae). Pp. 259–287 in W. L. Wagner and V. A. Funk. *Hawaiian biogeography: evolution on a hot spot archipelago*. Smithsonian Institution Press, Washington, DC.
- Givnish, T. J., K. J. Sytsma, J. F. Smith, W. J. Hahn, D. H. Benzing, and E. M. Burkhardt. 1997. Molecular evolution and adaptive radiation in *Brocchinia* (Bromeliaceae: Pitcairnioideae) atop tepuis of the Guayana Shield. Pp. 259–312 in T. J. Givnish and K. J. Sytsma, eds. *Molecular evolution and adaptive radiation*. Cambridge Univ. Press, New York.
- Graham, A. 1999. *Late Cretaceous and Cenozoic history of North American vegetation*. Oxford Univ. Press, New York.
- Grant, P., ed. 1998. *Evolution on islands*. Oxford Univ. Press, Oxford, U.K.
- Hapeman, J. R., and K. Inoue. 1997. Plant-pollinator interactions and floral radiation in *Platanthera* (Orchidaceae). Pp. 433–454 in T. J. Givnish and K. J. Sytsma, eds. *Molecular evolution and adaptive radiation*. Cambridge Univ. Press, New York.
- Hopkins, D. M., J. V. Matthews Jr., C. E. Schweger, and S. B. Young. 1982. *Paleoecology of Beringia*. Academic Press, New York.
- Howarth, D. G., D. E. Gardner, and C. W. Morden. 1997. Phylogeny of *Rubus* subgenus *Idaeobatus* (Rosaceae) and its implications toward colonization of the Hawaiian Islands. *Syst. Bot.* 22:433–441.
- Hultén, E. 1929. *Flora of Kamtchatka and the adjacent islands*. III. Dicotyledoneae Droseraceae-Cornaceae. Almqvist and Wiksell Förlag, Stockholm, Sweden.
- . 1963. The distributional conditions of the flora of Beringia. Pp. 7–22 in J. L. Gressitt, ed. *Pacific Basin biogeography*. Bishop Museum Press, Honolulu, HI.
- . 1964. *The circumpolar plants*. II. Dicotyledons. Almqvist and Wiksell Förlag, Stockholm, Sweden.
- . 1968. *Flora of Alaska and neighboring territories, a manual of the vascular plants*. Stanford Univ. Press, Stanford, CA.
- Iltis, H. H., and W. M. Shaughnessy. 1960. Primulaceae: Primrose family. Preliminary reports on the flora of Wisconsin no. 43. *Trans. Wis. Acad. Sci. Arts Lett.* 49:119.
- Jukes, T. H., and C. R. Cantor. 1969. Evolution of protein molecules. Pp. 21–123 in N. H. Munro, ed. *Mammalian protein metabolism*. Academic Press, New York.
- Kessel, B. 1989. *Birds of the Seward Peninsula, Alaska*. Univ. of Alaska Press, Fairbanks.
- Kim, S.-C., D. J. Crawford, J. Francisco-Ortega, and A. Santos-Guerra. 1996. A common origin for woody *Sonchus* and five related genera in the Macaronesian islands: molecular evidence for extensive radiation. *Proc. Nat. Acad. Sci. U.S.A.* 93:7743–7748.
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16:111–120.
- Kluge, A. G., and J. S. Farris. 1969. Quantitative phyletics and the evolution of anurans. *Syst. Zool.* 18:1–32.
- Kontrimavichus, V. L., ed. 1985. *Beringia in the Cenozoic Era*. A. A. Balkema, Rotterdam, The Netherlands.
- Luckow, M., and A. Bruneau. 1997. Circularity and independence in phylogenetic tests of ecological hypotheses. *Cladistics* 13:145–151.
- Luckow, M., and H. C. F. Hopkins. 1995. A cladistic analysis of *Parkia* (Leguminosae, Mimosoideae). *Am. J. Bot.* 82:1300–1320.
- Maddison, D. R. 1991. The discovery and importance of multiple islands of most-parsimonious trees. *Syst. Zool.* 40:315–328.
- Maddison, W. P., and D. R. Maddison. 1992. *MacClade 3.0: analysis of phylogeny and character evolution*. Sinauer Associates, Sunderland, MA.
- Madge, S., and H. Burn. 1988. *Waterfowl: an identification guide to the ducks, geese and swans of the world*. Houghton Mifflin Co., Boston, MA.
- Mes, T. H. M., and H. 't Hart. 1996. The evolution of growth forms in the Macaronesian genus *Aeonium* (Crassulaceae) inferred from chloroplast DNA RFLPs and morphology. *Mol. Ecol.* 5:351–363.
- Mes, T. H. M., J. vanBrederode, and H. 't Hart. 1996. Origin of the woody Macaronesian Sempervivoideae and the phylogenetic position of the east African species of *Aeonium*. *Bot. Acta* 109:477–491.
- Miyaji, Y. 1929. Studien über die Zahlen Verhältnisse der Chromosomen bei der Gattung *Viola*. *Cytologia* 1:28–58.
- Mullis, K. B., and F. A. Faloon. 1987. Specific synthesis of DNA

- in vitro via a polymerase catalyzed chain reaction. *Methods Enzymol.* 155:335–350.
- Nepokroeff, M., and K. J. Sytsma. 2000. Evolution in cloud forest *Psychotria* section *Notopleura* via growth form diversification. Pp. 75–78 in N. M. Nadkarni and N. T. Wheelwright, eds. *Monteverde: ecology and conservation of a tropical cloud forest*. Oxford Univ. Press, New York.
- Nickrent, D. L., K. P. Schuette, and E. M. Starr. 1994. A molecular phylogeny of *Arceuthobium* (Viscaceae) based on nuclear ribosomal DNA internal transcribed spacer sequences. *Am. J. Bot.* 81:1149–1160.
- Olmstead, R. G., B. Bremer, K. M. Scott, and J. D. Palmer. 1993. A parsimony analysis of the Asteridae sensu lato based on *rbcL* sequences. *Ann. Mo. Bot. Gard.* 80:700–722.
- Olsen, G. J., H. Matsuda, R. Hagstrom, and R. Overbeek. 1994. fastDNAm1: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. *Comput. Appl. Biosci.* 10:41–48.
- Olson, S., and H. F. James. 1982. *Prodromus of the fossil avifauna of the Hawaiian Islands*. Smithsonian Contributions in Zoology no. 365. Smithsonian Institution, Washington, DC.
- Paulson, D. 1993. *Shorebirds of the Pacific Northwest*. Univ. of Washington Press, Seattle.
- Peterson, R. T. 1990. *A field guide to western birds*. 3rd ed. Houghton Mifflin Co., Boston, MA.
- Péwé, T. L., D. M. Hopkins, and J. L. Giddings. 1965. The Quaternary geology and archaeology of Alaska. Pp. 355–374 in H. E. Wright Jr. and D. G. Frey, eds. *The Quaternary of the United States*. Princeton Univ. Press, Princeton, NJ.
- Pratt, H. D., P. L. Bruner, and D. G. Berrett. 1987. *A field guide to the birds of Hawaii and the tropical Pacific*. Princeton Univ. Press, Princeton, NJ.
- Quinn, T. W., G. F. Shields, and A. C. Wilson. 1991. Affinities of the Hawaiian goose based on two types of mitochondrial DNA data. *Auk* 108:585–593.
- Repenning, C. A., and E. M. Brouwers. 1992. Late Pliocene–Early Pleistocene ecologic changes in the Arctic Ocean borderland. *U.S. Geol. Survey Bull.* 2036:1–37.
- Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing evolutionary trees. *Mol. Biol. Evol.* 4:406–425.
- Sakai, A. K., W. L. Wagner, D. M. Ferguson, and D. R. Herbst. 1995. Origins of dioecy in the flowering plants of the Hawaiian Islands. *Ecology* 76:2517–2529.
- Sakai, A. K., S. G. Weller, W. L. Wagner, P. S. Soltis, and D. E. Soltis. 1997. Phylogenetic perspectives on the evolution of dioecy: adaptive radiation in the endemic Hawaiian genera *Schiedea* and *Alsinodendron* (Caryophyllaceae: Alsinoideae). Pp. 455–473 in T. J. Givnish and K. J. Sytsma, eds. *Molecular evolution and adaptive radiation*. Cambridge Univ. Press, New York.
- Sang, T., D. J. Crawford, S.-C. Kim, and T. F. Stuessy. 1994. Radiation of the endemic genus *Dendroseris* (Asteraceae) on the Juan Fernandez Islands: evidence from sequences of the ITS regions of nuclear ribosomal DNA. *Am. J. Bot.* 81:1494–1501.
- Sang, T., D. J. Crawford, and T. F. Stuessy. 1995. ITS sequences and the phylogeny of the genus *Robinsonia* (Asteraceae). *Syst. Bot.* 20:55–64.
- Selander, R. K. 1965. Avian speciation in the Quaternary. Pp. 527–542 in H. E. Wright Jr. and D. G. Frey, eds. *The Quaternary of the United States*. Princeton Univ. Press, Princeton, NJ.
- Skottsberg, C. 1940. Observations on Hawaiian violets. *Meddeland. Göteborgs Bot. Trädgård.* 13:451–528.
- . 1955. Chromosome numbers in Hawaiian flowering plants. *Ark. Bot.* 3:63–70.
- Smith, J. F., K. S. Sytsma, J. S. Shoemaker, and R. L. Smith. 1991. A qualitative comparison of total cellular DNA extraction protocols. *Phytochem. Bull.* 23:2–9.
- Sokolovskaja, A. P. 1963. Geographical distribution of polyploidy in plants: investigation of the flora of the Kamchatka Peninsula. *Vestn. Leningradsk. Univ. Ser. Biol.* 15:38–52.
- Sokolovskaja, A. P., and N. S. Probatova. 1986. Chromosome numbers in some representatives of the Asteraceae, Iridaceae, Poaceae, Primulaceae, Violaceae from the far east of the USSR. *Bot. Zh. (Leningr.)* 71:1423–1425.
- St. John, H. 1989. Revision of the Hawaiian species of *Viola* (Violaceae). *Hawaiian plant studies* no. 135. *Bot. Jahrb. Syst. Pflanzengesch. Pflanzengeogr.* 111:165–204.
- Swofford, D. 1998. *PAUP\*: phylogenetic analysis using parsimony (\*and other methods)*. Vers. 4. Sinauer Associates, Sunderland, MA.
- Sytsma, K. J., J. F. Smith, and P. E. Berry. 1991. The use of chloroplast DNA to assess biogeography and evolution of morphology, breeding systems, and flavonoids in *Fuchsia* sect. *Skinnera* (Onagraceae). *Syst. Bot.* 16:257–269.
- Taylor, R. L., and G. A. Mulligan. 1968. *Flora of the Queen Charlotte Islands. 2. Cytological aspects of the vascular plants*. Plant Research Institute, Central Experimental Farm, Ottawa, Ontario, Canada.
- Wagner, W. L. 1991. Evolution of waif floras: a comparison of the Hawaiian and Marquesan archipelagos. Pp. 267–284 in E. C. Dudley, ed. *The unity of evolutionary biology: the proceedings of the fourth International Congress of Systematics and Evolutionary Biology*. Dioscorides Press, Portland, OR.
- Wagner, W. L., and V. A. Funk, eds. 1995. *Hawaiian biogeography: evolution on a hot spot archipelago*. Smithsonian Institution Press, Washington, D.C.
- Wagner, W. L., D. R. Herbst, and S. H. Sohmer. 1990. *Manual of the flowering plants of Hawai'i*. Univ. of Hawaii Press and Bishop Museum Press, Honolulu, HI.
- Wallace, A. R. 1878. *Tropical nature and other essays*. Macmillan, London.
- Weller, M. W. 1980. *The island waterfowl*. Iowa State Univ. Press, Ames, IA.
- White, T. J., T. Birns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in M. Innis, D. Gelfand, J. Sninsky, and T. White, eds. *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, CA.
- Yokota, Y., T. Kawata, Y. Iida, A. Kato, and S. Tanifuji. 1989. Nucleotide sequences of the 5.8S rRNA gene and internal transcribed spacer regions in carrot and broad bean ribosomal DNA. *J. Mol. Evol.* 29:294–301.
- Yuzepchuk, S. V., and M. V. Klokov. 1974. Violaceae. Pp. 262–360 in B. K. Shishkin, ed. *Flora of the U.S.S.R.* Israel Program for Scientific Translations, Jerusalem.
- Zubakov, V. A., and I. I. Borzenkova. 1990. *Global palaeoclimate of the Late Cenozoic*. Elsevier, Amsterdam, The Netherlands.

## APPENDIX

GenBank accession numbers for ITS sequences of taxa of *Viola* and outgroups. See Ballard et al. (1998) for voucher information for non-Hawaiian taxa and older Hawaiian accessions.

Taxon	GenBank accession numbers (ITS1, ITS2)
<b>Outgroups</b>	
<i>Hybanthus concolor</i> (T. F. Forster) Spreng.	AF097218, AF097264
<i>Noisetia orchidiflora</i> (Rudge) Ging.	AF097219, AF097265
<b>Viola</b>	
Sect. <i>Chilenium</i> W. Becker	
<i>V. reichei</i> Skottsberg	AF097223, AF097269
Sect. <i>Andinium</i> W. Becker	
<i>V. micranthella</i> Wedd.	AF097222, AF097268
Sect. <i>Rubellium</i> W. Becker	
<i>V. capillaris</i> Pers.	AF09722, AF097266
Sect. <i>Nosphinium</i> W. Becker	
<i>V. chamissoniana</i> Ging. ssp. <i>chamissoniana</i>	USA, HI, Oahu, Makua Keaau Forest Reserve, Wood 1833, Perlman & Obata (US); AF115955, AF115959
<i>V. chamissoniana</i> ssp. <i>robusta</i> (Hillebr.) W. L. Wagner, Herbst & Sohmer	USA, HI, Molokai, Pepe' Opae Bog, Kamakao Preserve, Ne-pokroeff 910 (WIS); AF115956, AF115960
<i>V. chamissoniana</i> ssp. <i>tracheliifolia</i> (Ging.) W. L. Wagner, Herbst & Sohmer	AF09726, AF097307
<i>V. helena</i> C. Forbes & Lydgate	AF097260, AF097306
<i>V. hosakai</i> St. John	USA, HI, Oahu, Koolau Mts., Puu Pauao, Perlman & Garnett 14704 (NTBG); AF115957, AF115961
<i>V. kauaensis</i> A. Gray	AF097262, AF097308
<i>V. maviensis</i> H. Mann	AF097263, AF097309
<i>V. waialenalena</i> (Rock) Skottsbg.	USA, HI, Kauai, Waimea District, Alaka'i Swamp, Warshauer 3383 & McEldowney (BISH); AF115958, AF115962
Sect. <i>Leptidium</i> Ging.	
<i>V. scandens</i> Willd. Ex Roem. & Schult.	AF097221, AF097267
Sect. <i>Melanium</i> Ging.	
<i>V. arvensis</i> Murray	AF097242, AF097288
<i>V. calcarata</i> L.	AF097243, AF097289
Sect. <i>Chamaemelianum</i> Ging.	
<i>V. barroetana</i> Hemsley	AF097224, AF097270
<i>V. beckwithii</i> T. & G.	AF097227, AF097273
<i>V. canadensis</i> L. ("NC")	AF097231, AF097277
<i>V. canadensis</i> L. ("NM")	AF097232, AF097278
<i>V. cuneata</i> S. Watson	AF097234, AF097280
<i>V. flagelliformis</i> Hemsley	AF097233, AF097279
<i>V. praemorsa</i> Kellogg	AF097228, AF097274
<i>V. pubescens</i> Aiton	AF097225, AF097271
<i>V. purpurea</i> Kellogg	AF097229, AF09727
<i>V. sheltonii</i> Torr.	AF097226, AF097272
<i>V. vallicola</i> A. Nelson	AF097230, AF097276

## APPENDIX

Continued.

Taxon	GenBank accession numbers (ITS1, ITS2)
<b>Sect. <i>Viola</i></b>	
<i>V. affinis</i> Leconte	AF097251, AF097297
<i>V. blanda</i> Willd. var. <i>palustriformis</i> A. Gray	AF097238, AF097284
<i>V. clauseniana</i> M. S. Baker	AF097254, AF097300
<i>V. cucullata</i> Aiton	AF097252, AF097298
<i>V. domingensis</i> Urban	AF097237, AF097283
<i>V. elatior</i> Fries	AF09726, AF097292
<i>V. hemsleyana</i> Calderón	AF097258, AF097304
<i>V. hookeriana</i> HBK.	AF097257, AF097303
<i>V. jalapaensis</i> W. Becker	AF097235, AF097281
<i>V. langsdorffii</i> Ging.	AF097259, AF097305
<i>V. macloskeyi</i> Lloyd ssp. <i>pal-lens</i> (Ging.) M. S. Baker	AF097236, AF097282
<i>V. nagasawai</i> Makino & Hayata	AF097239, AF097285
<i>V. nannei</i> Polak.	AF097255, AF097301
<i>V. odorata</i> L. ("GDN")	AF097249, AF097295
<i>V. odorata</i> L. ("GY")	AF027250, AF097296
<i>V. pedata</i> L.	AF097253, AF097299
<i>V. pinnata</i> L.	AF097240, AF097286
<i>V. reichenbachiana</i> Boreau	AF097248, AF097294
<i>V. rotundifolia</i> Michx.	AF097241, AF097287
<i>V. sp. nov. A</i>	AF097256, AF097302
<i>V. sp. nov. B</i>	AF097245, AF097291
<i>V. striata</i> Aiton	AF097247, AF097293
<i>V. umbraticola</i> HBK.	AF097244, AF097290