

THE ORIGIN AND NUMBER OF INTRODUCTIONS OF THE HAWAIIAN ENDEMIC *SILENE* SPECIES (CARYOPHYLLACEAE)¹

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The Hawaiian endemic *Silene* are a small group of woody or semiwoody representatives from a large, predominantly herbaceous, species-rich genus. We here investigated the origin and number of introductions of the endemic Hawaiian *Silene* based on phylogenetic relationships inferred from DNA sequences from both the plastid (the *rps16* intron) and the nuclear (ribosomal internal transcribed sequences, ITS, and intron 23 of the *RPB2* gene) genomes. *Silene antirrhina*, a widespread weedy American annual, is strongly supported as sister to a monophyletic group consisting of the Hawaiian *Silene*, indicating a single colonization event. There are no obvious morphological similarities between *S. antirrhina* and any of the species of Hawaiian *Silene*. Our results suggest an American origin for the Hawaiian endemics because that would require only a single trans-ocean dispersal. Two of the Hawaiian endemics (*S. struthioloides* and *S. hawaiiensis*) that form a subclade in the analyses have evolved woodiness after introduction to the Hawaiian Islands. Our results contribute to other recent results based on molecular phylogenetics that emphasize the American continent as a source area for the Hawaiian flora and support a striking morphological radiation and evolution of woodiness from a single introduction to the archipelago.

Key words: Caryophyllaceae; Hawaiian *Silene*; ITS; phylogeny; *RPB2*; *rps16*; *Silene antirrhina*; woodiness.

Although islands have been forming over the Hawaiian hot spot for the past 85 million years (My) (Clague, 1996), most colonization of the current high islands has occurred within the past 5 My because dispersal between islands was previously limited by an extended period when only small, low, widely spaced islands were formed (Price and Clague, 2002). The oldest and westernmost of the main Hawaiian Islands is Kauai (ca. 5 My old). The island of Hawaii is the easternmost and youngest island, with an age of ca. 500 000 years (Carson and Clague, 1995). The Hawaiian Islands are positioned in extreme isolation in the middle of the Pacific Ocean. They belong to the Hawaiian-Emperor volcanic chain that extends northward to Kamchatka and the Aleutian Islands. There are no islands in the eastward direction between Hawaii and the American continents, so the closest islands are the Polynesian and Micronesian islands in the south and southwest.

There are 1030 native species of flowering plants on Hawaii (Wagner et al., 1990, 2005a). Because of the islands are isolated volcanic in origin, and have never been attached to the continental mainland, plant colonists could only have arrived by long-distance dispersal, followed by speciation in situ. Fosberg (1948) hypothesized that there were 272 original

immigrant seed plants, with most originating from the Indo-Pacific area (40%) or from America (mainly North America, excluding the northernmost arctic parts) (18%). Sakai et al. (1995) updated (partly based on Wagner et al., 1990) the number of introductions of seed plants to 291 original immigrants. In 45 genera, *Silene* being one of them, the species are suggested to be the result of more than one colonization. Price and Wagner (2004) mentioned a further updated, slightly lower, number of 263 introductions, which excluded ca. 15 questionably indigenous species. A number of recent studies have used molecular phylogenies to infer the origin of Hawaiian angiosperms; a few examples summarized in Table 1 (for more examples, see Price and Wagner, 2004).

Silene L. is a large (ca. 650 species) genus belonging to the tribe *Sileneae* DC. ex Ser. in the family Caryophyllaceae. It is distributed mainly in the northern hemisphere, but native species can be found on all continents except Australia. There are two major centers of diversity: one in the Mediterranean/Middle East and one in Central Asia. The genus consists mainly of herbaceous plants and, more rarely, small shrubs or subshrubs. The flowers have free petals, with each petal consisting of a usually visible limb that can be divided or entire, and a claw that is included within the synsepalous calyx. *Silene* is an important model system for genetic and plant breeding system studies, gynodioecy (e.g., McCauley et al., 2005), dioecy (e.g., Negrutiu et al., 2001), and polyploidy (e.g., Popp et al., 2005). There are also a number of cultivated species and widespread weeds.

The genus *Silene* has been shown in previous molecular phylogenetic studies to consist of two major clades of approximately equal size, which are tentatively classified as *Silene* subgenus *Silene* and *Silene* subgenus *Behen* (Moench) Bunge (Oxelman et al., 1997; Popp and Oxelman, 2004). In the most recent taxonomic revision covering the entire genus, *Silene* is divided into 44 sections, without any rank above that

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TABLE 1. Origin for a number of taxa as inferred by published phylogenies and previous hypothesized origin as presented in Fosberg (1948).

Group	Family	Origin	Reference	Fosberg
Silverswords	Asteraceae	N America	Baldwin et al. (1991)	Obscure
Hawaiian violets	Violaceae	N America/Arctic	Ballard and Sytsma (2000)	Obscure
Sanicles	Apiaceae	N America	Vargas et al. (1998)	Obscure
<i>Geranium</i>	Geraniaceae	N America	Pax et al. (1997)	Obscure
<i>Stachys</i>	Lamiaceae	N America	Lindqvist and Albert (2002)	Austral
<i>Labordia</i>	Loganiaceae	New Zealand/South Pacific	Motley and Cross (1999)	Indo-pacific
<i>Peperomia</i>	Piperaceae	New Zealand/South Pacific	Cross and Motley (2000)	Indo-pacific, American, obscure
<i>Psychotria</i>	Rubiaceae	New Zealand/South Pacific	Nepokroeff et al. (1999, 2003)	Indo-pacific, American
<i>Tetramolopium</i>	Asteraceae	New Zealand/South Pacific	Lowrey et al. (2001)	Indo-pacific
<i>Tetraplasandra</i> group	Araliaceae	New Zealand/South Pacific	Plunkett et al. (1997)	Obscure
<i>Pittosporum</i>	Pittosporaceae	South Pacific	Gemmill et al. (2002)	Indo-pacific
<i>Hesperomannia</i>	Asteraceae	Africa	Kim et al. (1998)	American
<i>Kokia</i>	Malvaceae	E Africa	Seelanan et al. (1997)	Obscure
<i>Schiedea</i>	Caryophyllaceae	Undefined circumboreal source	Nepokroeff et al. (2005)	Obscure
<i>Rubus</i>	Rosaceae	Two colonizations; North American	Howarth et al. (1997); Alice and Campbell (1999)	American
<i>Scaevola</i>	Goodeniaceae	Three colonizations, South Pacific	Howarth et al. (2003)	Indo-pacific
<i>Vaccinium</i>	Ericaceae	Uncertain, boreal-arctic	Powell and Kron (2002)	Austral
<i>Gunnera</i>	Gunneraceae	N American	Wanntorp and Wanntorp (2003)	American

(Chowdhuri, 1957). This classification has been followed by most authors but needs to be revised because molecular studies have indicated that many of the sections of Chowdhuri are polyphyletic (e.g., Oxelman and Lidén, 1995; Desfeux and Lejeune, 1996; Oxelman et al., 1997).

On the Hawaiian Islands, there are seven endemic species of *Silene* (Wagner et al., 1990) that can be divided into two groups based on morphology and, in particular, growth form. *Silene alexandri* Hillebr., *S. cryptopetala* Hillebr., *S. degeneri* Sherff, *S. lanceolata* A. Gray, and *S. perlmanii* W. L. Wagner, Herbst, and Sohmer are subshrubs, with more or less lanceolate to oblanceolate leaves, that are at least a few millimeters wide and a few centimeters long. The flowers are white with notched petals. Within this group, *S. cryptopetala* is odd in having very small petals that lack a claw (Wagner et al., 1990), an unusual condition in the genus. A second group consists of xeromorphic shrubs and comprises the two taxa *S. struthioloides* A. Gray and *S. hawaiiensis* Sherff. Members of the xeromorphic group have slender, subulate, and sometimes recurved leaves that are less than 1 mm wide. They have flowers that are greenish or yellowish white fading to maroon with deeply cleft petals, and they both have a prominent enlarged root. Wagner et al. (1990) suggest that the two groups of native Hawaiian *Silene* are the result of two independent colonizations.

Previous classifications (e.g., Chowdhuri, 1957) have implicitly suggested a Japanese origin for the Hawaiian *Silene*. Of the Hawaiian endemics, Chowdhuri (1957) recognized *S. alexandri*, *S. lanceolata*, and *S. struthioloides*, which he placed in *Silene* sect. *Paniculatae* Boiss. subsect. *Sclerophyllae* Chowdhuri. He also included two Japanese *Silene*, *S. japonica* Rohrb. and *S. tanakae* Maxim, in the same subsection. The taxonomic standing of these two species is dubious, and their placing in subsection *Sclerophyllae* is questionable. *Silene japonica* is described from a single fragmentary specimen, and *S. tanakae* is described from a single specimen raised from seeds in the Botanical Garden in St. Petersburg. *Silene japonica* is described from a locality on the east side of Honshu, an area now with a dense human population. *Silene tanakae* is described from the Ryukyu Islands, between the Japanese main islands and Taiwan. It is mentioned in *Flora of the*

Ryukyus (Hatusima, 1971), but it is noted that the type specimen is the only known collection. Walker (1976) says that its presence on the Ryukyus is dubious. Neither *S. tanakae* nor *S. japonica* are mentioned in *Flora of Japan* (Ohwi, 1965).

In the present study, molecular phylogenies are used to examine the origin of *Silene* in Hawaii in terms of the number of independent colonization events that is, whether there have been two independent origins as suggested by Wagner et al. (1990) or a single event as described for many Hawaiian plant and animal groups recently. We will also address the geographical origin of *Silene* on Hawaii and in particular investigate the theory, based on the classification of Chowdhuri (1957), of a Japanese origin for the Hawaiian *Silene*.

MATERIALS AND METHODS

Selection of taxa for analysis—The *Silene* species from Hawaii are here represented by *S. alexandri*, *S. lanceolata*, *S. perlmanii*, *S. struthioloides*, and *S. hawaiiensis*. The first three represent one of the putative colonizations, whereas the last two represent the other putative colonization. *Silene degeneri* and *S. cryptopetala* are probably extinct, and very few herbarium sheets exist. We did not have access to any material of *S. cryptopetala*, and we failed to extract DNA from the little material we had from *S. degeneri*. We could therefore not include them in the present analysis. The Japanese species *S. tanakae* is included for the test of the classification of Chowdhuri, but *S. japonica* is not included because we have not been able to find any material. It is probably only known from the type material, which likely was destroyed during the World War II bombings of Berlin. The Hawaiian *Silene* belong to *Silene* subgenus *Silene*, and we have included, based on Oxelman and Lidén (1995), Desfeux and Lejeune (1996), and Oxelman et al. (1997, 2001, unpublished data), species representing different lineages from this subgenus and additional representatives from *Silene* subgenus *Behen* and more distant outgroups (*Lychnis coronaria* (L.) Desr. and *Viscaria vulgaris* Bernh.). Materials used in this study are presented in the Appendix.

DNA regions—Three DNA regions were used for this study: two from the nuclear genome and one from the chloroplast genome. The chloroplast gene *rps16* intron (Oxelman et al., 1997) was used. Nuclear sequences included the internal transcribed spacer regions with the intervening 5.8S gene (ITS) from the nuclear ribosomal arrays and intron number 23 (as of the *Arabidopsis* gene; Larkin and Guilfoyle, 1993) of the nuclear *RPB2* gene. The latter is a region that has been successfully used in several previous studies in the tribe *Sileneae*

TABLE 2. Primers used for PCR and sequencing.

Primer	Primer sequence (5' → 3')
ITS:	
P17 ^c	CTACCGATTGAATGGTCCGGTGAA
P16 ^c	TCACTGAACCTTATCATTAGAGGA
P25 ^a	GGGTAGTCCCGCCTGACCTG
26S-82R ^c	TCCCGGTTGCTCGCCGTTACTA
<i>rps16</i> :	
rpsF ^b	GTGGTAGAAAGCAACGTGCGACTT
rpsR2R ^b	TCCGGATCGAACATCAATTGCAAC
rpsR3R ^d	CGATAGACGGCTCATTGGGATA
rpsF2a ^d	CTTGAAGGACATGATCTGTTGTGGA
<i>RPB2</i> :	
10PoppF ^e	CNCAGGAGGATATGCCATGGAC
F7327 ^c	CCATCYCGTATGACAATCGGYCAGCTT
F7381 ^c	AGCGTCTCCTTCTTACCACATGAGC
R7555 ^c	CCACGCATCTGATACCCACATTTCTG
R7586 ^c	CCCMGTGTGACCATTGTACATTGTCT
11aBOx2R ^e	GTGAATCTTGTTCATCMACCATRGTCT

^a Lidén et al. (1995).

^b Oxelman et al. (1997).

^c Popp and Oxelman (2001).

^d Popp et al. (2005)

^e Unpublished.

(Popp and Oxelman, 2001, 2004; Popp et al., 2005). Using low-copy regions is beneficial in that they are not affected by the different processes (reviewed by Alvarez and Wendel, 2003) that can affect tandemly repeated regions such as the ribosomal cistrons. Low-copy genes like *RPB2* have rarely been used in studies of Hawaiian plants; none of the studies presented in Table 1 use low-copy genes.

DNA extraction, amplification, cloning, and sequencing—Total genomic DNA was extracted from herbarium material using the method described by Oxelman et al. (1997). The DNA was purified after precipitation using the QIAquick PCR Purification Kit (QiaGen, Crawley, West Sussex, UK) protocol. For some extractions, the material was disintegrated using a Mini-Beadbeater (BioSpec Products, Bartlesville, Oklahoma, USA). The plant tissue was put in a tube with the lysis buffer and beads (2.5 mm zirconia/silica beads), and the Mini-Beadbeater was operated at 4800 rpm for 40 s. DNA from *S. perlmannii* was extracted using the DNEasy Plant Mini Extraction Kit (QiaGen, Valencia, California, USA) at the University of South Dakota.

The polymerase chain reaction (PCR) was prepared in 25- μ l reactions consisting of buffer (supplied with the enzyme), 0.2 mM of each dNTP, 1.5–2.5 mM MgCl₂, 0.5 μ M of each primer, 0.005% bovine serum albumin, and 0.025 U *Taq* polymerase (Advanced Biotechnologies, Epsom, Surrey, UK). For samples that were difficult to amplify, Q-solution (QiaGen, Crawley, West Sussex, UK) or 1% dimethyl sulfoxide (DMSO) was added. These reactions were run on an Eppendorf Mastercycler Gradient (Eppendorf, Hamburg, Germany) with an initial denaturation at 95°C for 5–10 minutes followed by 34–40 cycles at 95°C for 30 s, at 55–58°C for 1 min, and at 72°C for 2–2.5 min. The cycling was terminated at 72°C for 10–15 min. Phusion High-Fidelity DNA Polymerase (FINNZYMES, Espoo, Finland) was used for some PCR reactions, which proved difficult to amplify with *Taq* polymerase. These reactions were prepared in 50- μ l reactions consisting of buffer (supplied by the manufacturer), 0.2 mM of each dNTP, 0.4 mM of each primer, and 0.02 U Phusion polymerase and run with an initial denaturation at 98°C for 2.5 min followed by 34 cycles of 98°C for 15 s, 63–66°C for 25 s, and 72°C for 3 min. The cycling was terminated at 72°C for 10 min.

The PCR products were purified with either QIAquick PCR Purification Kit or with Multiscreen PCR (Millipore, Solna, Sweden) following the manufacturer's protocol. Primers used for PCR and sequencing are listed in Table 2. The ITS region for *S. perlmannii* was amplified and sequenced for both strands using primers LEU1 and ITS4 following the protocol detailed in Nepokroeff et al. (2003).

The *RPB2* sequences of *S. corinthiaca* and *S. echinospermoides* appeared polymorphic, so cloning of the PCR products was performed with the TOPO

TA Cloning kit (Invitrogen, Paisley, UK) following the manufacturer's protocol. For *S. echinospermoides*, two different sequences from 10 clones were found. They form a monophyletic group, and a consensus sequence was created from them and is used in the analyses. Only one sequence from seven clones was found for *S. corinthiaca*.

The sequencing reactions were prepared using the DYEnamic ET Terminator Cycle Sequencing Premix Kit (Amersham Pharmacia Biotech/GE Healthcare Bio-Sciences AB, Uppsala, Sweden) following the protocol from the manufacturer, with the exception that the reaction volumes were set to 10 μ l. Visualization was performed on a MegaBACE 1000 DNA Analysis System (Amersham Pharmacia Biotech/GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Some reactions were prepared using the ABI PRISM BigDye Terminator Cycle Sequencing Kit (Applied Biosystem, Foster City, California, USA), and the samples were sent to the Karolinska Institutet (Sweden) or the Rudbeck Laboratory (Sweden) and visualized on an ABI PRISM 377 Sequencer (Perkin-Elmer, Wellesley, Massachusetts, USA).

Alignment and phylogenetic analyses—All sequences were edited using Sequencher 3.1.1 (Gene Codes Corp., Ann Arbor, Michigan, USA) and aligned manually in Se-Al v2.0a11 (Rambaut, 1996). Gaps were introduced and coded as separate binary characters manually following the "simple gap coding" of Simmons and Ochoterena (2000) for those gaps with overlapping regions.

Parsimony analyses were performed with PAUP* version 4.0b10 (Swofford, 2003). Heuristic searches employed 1000 random addition sequences and the TBR (tree-bisection-reconnection) branch-swapping algorithm, with MulTrees on. The analyses of the *rps16* and *RPB2* data sets created very large numbers of saved trees, so to reduce this number branches were allowed to collapse if it was possible for them to have zero length (amb-). Maximum parsimony bootstrap (MPB) analysis was carried out with 1000 replicates, four random addition sequences, TBR branch-swapping algorithm, with MulTrees off.

RESULTS

The separate analyses of the three regions resulted in trees with largely congruent topologies, at least for clades closely related to the Hawaiian clade (see Figs. 1–3). The *rps16* tree and the *RPB2* tree have congruent patterns for the clades closely related to the Hawaiian clade, and the ITS tree is slightly conflicting but with very low support (MPB < 50%). Therefore, we performed an analysis on the combined data set consisting of the sequences from all three regions (Fig. 4). Table 3 summarizes the characteristics of the matrices and the resulting trees. We arbitrarily named the clades A through G in the following text and assigned membership of the individual sequences from the combined data tree (Fig. 4). The geographic distributions of the species included in the seven clades are presented in Table 4.

Clade A—The Hawaiian *Silene* comprises a monophyletic group together with *S. antirrhina*, a weedy annual species widely distributed over the American continents. The support for this group is strong in all trees. *Silene antirrhina* is sister to the Hawaiian endemics in the combined and ITS trees, with moderate and weak support, respectively. The two morphologically distinct groups, previously thought to represent two introductions, are seen as two monophyletic groups in the combined analysis. *Silene perlmannii* and *S. alexandri* are strongly supported as sisters, with *S. lanceolata* as a weakly supported sister to them. The other morphologically recognized group, with *S. hawaiiensis* and *S. struthioloides*, receives moderate support. These clades are also seen in the ITS tree, with the exception that the relationship of *S. lanceolata* is unresolved.

Clades B and C—Clade B comprises a few species from the western Mediterranean area, and clade C consists of species

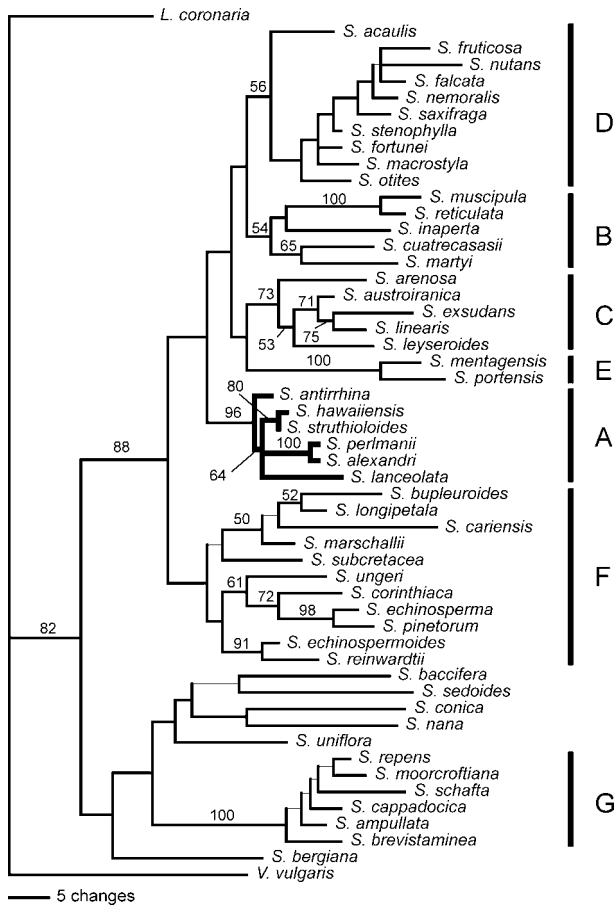


Fig. 1. One of 2420 most parsimonious (MP) trees found in the analysis of the ITS data set. Branches marked with thin lines are collapsed in the strict consensus tree. Numbers associated with nodes (above or pointing at branches) indicate MP bootstrap frequencies. Branch lengths are proportional to the number of changes. The clade with the endemic Hawaiian *Silene* and *S. antirrhina* (clade A) is marked with thick lines.

from the Middle East. These two clades together (i.e., clade BC) are strongly supported as sister to the Hawaiian endemics and *S. antirrhina*.

The association between clades A, B, and C is seen in the *rps16* and the *RPB2* trees as well, although with less support (MPB 72–87%) and less resolution within the clade in the latter case. In the ITS tree, clade A is sister to a much more inclusive clade, but the support here is very low (MPB < 50%). This more inclusive clade includes, besides clade A and B, clades D and E, presented next.

Clade D—Clade D is a very diverse clade comprising perennial species from the Arctic, Europe, and northern Asia, the Mediterranean, and northern, central, and/or eastern Asia and probably represents a large number of species, most of which were classified by Chowdhuri in the sections *Paniculatae* Boiss., *Siphonomorpha* Otth, *Tataricae* Chowdhuri, *Otites* Otth, *Macranthae* (Rohrb.) Chowdhuri, and *Graminifoliae* Chowdhuri. The support for clade D is very low in the combined, ITS and *rps16* trees (MPB < 56%). Clade D is polyphyletic in the *RPB2* tree, and parts of it are unresolved within a moderately supported clade (MPB 85%) together with

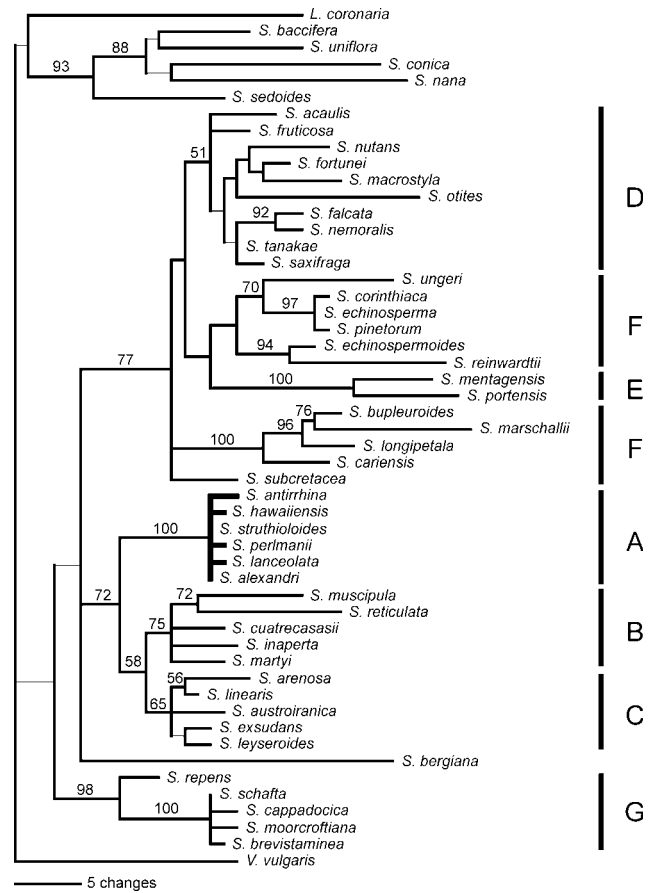


Fig. 2. One of the 160 most parsimonious (MP) trees found in the analysis of the *rps16* data set. Branches marked with thin lines are collapsed in the strict consensus tree. Numbers associated with nodes (above or pointing at branches) indicate MP bootstrap frequencies. Branch lengths are proportional to the number of changes. The clade with the endemic Hawaiian *Silene* and *S. antirrhina* (clade A) is marked with thick lines.

the clade comprised of clades A, B, C and E. The support for clade ABCDE comes mainly from two deletions: one 13 bp long and the other 14 bp long. If the two characters coded for these are removed from the matrix, the MPB support for the clade becomes < 50%. The Japanese *S. tanakae* is not closely related to the Hawaiian *Silene* but is found within clade D, as sister to *S. fortunei*, in a moderately supported clade (MPB 93%) including two other Far East species, *S. stenophylla* and *S. macrostyla*.

Clades E and F—Both these clades consist of species from the Mediterranean area; clade E is from the western part and clade F from the eastern part. They are not further discussed here because they are not resolved as close to the Hawaiian endemics or to *S. tanakae*. Eggen and Oxelman (unpublished data) have examined the phylogenetic relationships of these groups in detail.

Clade G—This clade comprises a geographically diverse group, with species from Anatolia, the Caucasus, the Himalayan region, and most of the eastern parts of Asia, extending into the northwest part of North America. The group

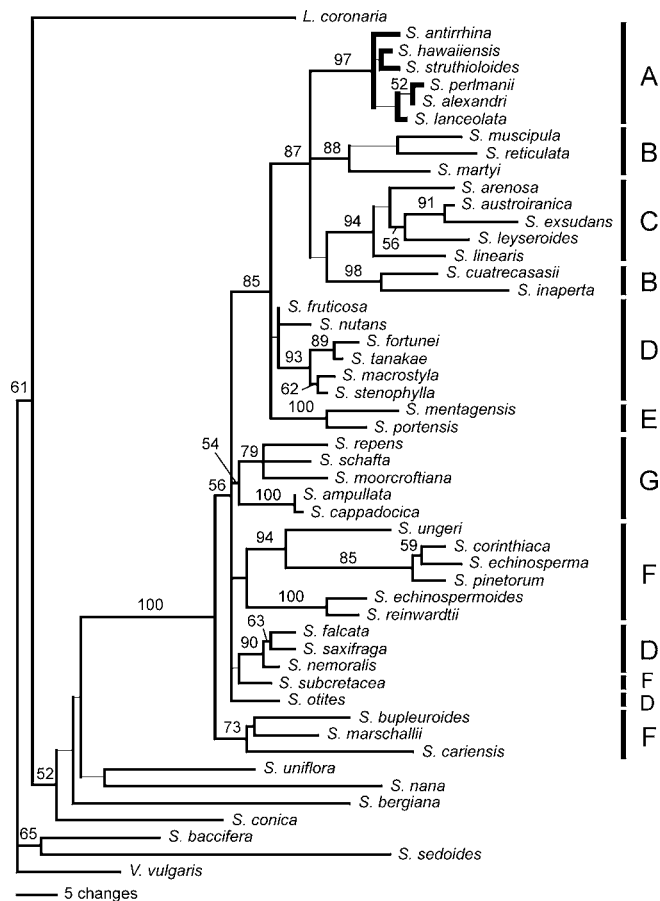


Fig. 3. One of the 300 most parsimonious (MP) trees found in the analysis of the *RPB2* data set. Branches marked with thin lines are collapsed in the strict consensus tree. Numbers associated with nodes (above or pointing at branches) indicate MP bootstrap frequencies. Branch lengths are proportional to the number of changes. The clade with the endemic Hawaiian *Silene* and *S. antirrhina* (clade A) is marked with thick lines.

has not previously been recognized but receives strong support in all trees except the *RPB2* tree, where it receives low support. It includes the odd *S. ampullata*, a species that has fruits that are one-seeded nuts rather than the many-seeded capsules that all other *Silene* species have.

Silene bergiana, that is here sister to the other representatives of *Silene* subgenus *Silene*, is the only included representative of a group (“*Cinnosilene*”) previously recognized by Oxelman and Lidén (1995) and Oxelman et al. (1997).

DISCUSSION

Our phylogenetic analyses suggest that the endemic Hawaiian *Silene* species are the result of a single colonization of the Hawaiian Islands, and the hypothesis of two independent introductions put forward by Wagner et al. (1990) can be rejected. The two lineages are instead the result of a morphological radiation. A number of Hawaiian plant and animal groups formerly believed to have resulted from multiple

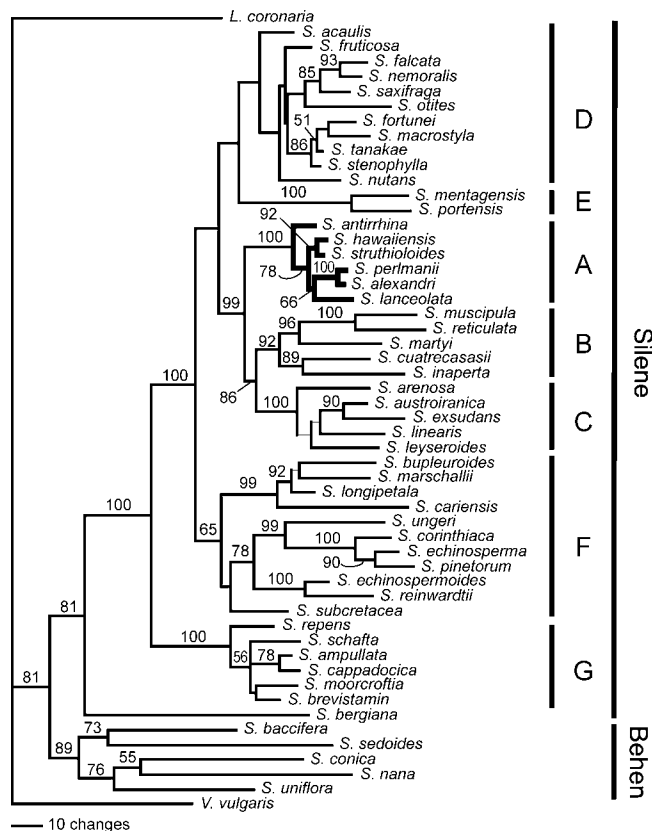


Fig. 4. One of the 18 most parsimonious (MP) trees found in the analysis of the combined data set (ITS, *rps16*, *RPB2*). Branches marked with thin lines are collapsed in the strict consensus tree. Numbers associated with nodes (above or pointing at branches) indicate MP bootstrap frequencies. Branch lengths are proportional to the number of changes. The clade with the endemic Hawaiian *Silene* and *S. antirrhina* (clade A) is marked with thick lines.

introductions (Fosberg, 1948) have been shown to have had a single origin e.g., Hawaiian species of *Psychotria* (Rubiaceae; Nepokroeff et al., 2003), Hawaiian species of *Peperomia* (Piperaceae; Cross and Motley, 2000) and *Labordia* (Loganiaceae; Motley and Cross, 1999), but see also Howarth et al. (2003), showing that *Scaevola* has colonized the Hawaiian Islands three separate times. For ferns, multiple introductions have been shown for *Dryopteris* by Geiger and Ranker (2005).

Within the Hawaiian lineage, the position of *S. lanceolata* is uncertain, with only weak support as sister to the *S. perlmanii*/*S. alexandri* clade. *Silene lanceolata* is the most widespread of the endemic Hawaiian *Silene*. It occurs (or has occurred in the past) on almost all of the main Hawaiian Islands, including the oldest one, Kauai. Almost all other species are found on just a single island. The close relationship between *S. struthioloides* and *S. hawaiiensis* is consistent with their morphological similarity, differing in minor characters such as the degree of hairiness and rigidity of the leaves. They have also been shown to be close genetically by Westerbergh and Saura (1994). *Silene perlmanii* and *S. alexandri* are also similar morphologically, differing in the degree of hairiness on the calyx and pedicels and in pedicel length.

There is no obvious morphological evidence for the close relationship between the American species *S. antirrhina* and

TABLE 3. Characteristics of the matrices and the resulting trees.

	Positions	No. MPT found	Length	CI	RI
Combined	3230	18	1699	0.4833	0.6979
ITS	655	2420	631	0.3978	0.6482
<i>rps16</i>	1221	160	397	0.5598	0.8042
<i>RPB2</i>	1348	300	613	0.6266	0.7701

Note: CI = consistency index excluding uninformative characters, MPT = most parsimonious trees, RI = retention index.

the Hawaiian *Silene* (Fig. 4). *Silene antirrhina* is a weedy, diploid, annual species that is distributed throughout North and South America. It is herbaceous, erect, and fairly large (up to 50 cm) and grows from a taproot. The flowers are inconspicuous, with petals almost or totally included within the calyx. *Silene antirrhina* is not closely related to other native American *Silene*, most of which are polyploid and belong to two clades in *Silene* subgenus *Behen* (cf. Fig. 4; Oxelman and Lidén, 1995; Oxelman et al., 1997; Popp and Oxelman, 2004, in press). *Silene antirrhina* has previously been classified together with some of the Mediterranean *Silene* species in *Silene* section *Behenantha* Otth (Chowdhuri, 1957), a section that has been shown to be polyphyletic (Oxelman et al., 1997). The clade containing *S. antirrhina*, and the Hawaiian *Silene* species is strongly supported as sister (MPB 99%, Fig. 4) to a clade comprised of species from the Mediterranean and the Middle East.

The relationship between the Hawaiian *Silene* and *S. antirrhina* suggests the possibility of an American origin for the Hawaiian endemics. A number of recent studies have shown an American origin for Hawaiian plant groups; some of these studies are mentioned in Table 1. Several of these were noted as having an “obscure” origin by Fosberg (1948), suggesting that he might have underestimated the American continents as a source area for Hawaiian immigrants. Fosberg suggested examples with an American origin that have been shown to have their source area elsewhere, e.g., *Psychotria* and *Hesperomannia* (see Table 1), but there are fewer of these. It should be noted that the clade with *S. antirrhina* and the Hawaiian *Silene* is sister to the clade with the Mediterranean and Middle East species (clades B and C in Figs. 1–4) but that the closest relative to the combined clade (clades A, B, and C together) cannot be inferred with confidence from these phylogenies. Clade D has a large Eurasian geographical distribution, and the entire subgenus *Silene* has an Old World distribution except for the *S. antirrhina*/Hawaiian *Silene* clade. One easily assumes, given the combined tree presented here, that the ancestor of *S. antirrhina* and the Hawaiian endemics originated in the Mediterranean area and was dispersed across the Atlantic Ocean to the American continents, and then the ancestor of the Hawaiian endemics was dispersed from the American continents to the Hawaiian Islands. However, what can be inferred from the analyses is that the ancestor of the Hawaiian *Silene*/*S. antirrhina* clade probably originated in the Old World given that clade A is nested within a clade with non-American distribution (i.e., *Silene* subgenus *Silene*). Parsimony optimizations of the ancestral area to clade A and the more inclusive ABC clade are ambiguous. It is possible that the ancestor of the endemic Hawaiian *Silene* arrived to the Hawaiian Islands from an unknown source area and that a representative from this lineage was dispersed to the American continents. This scenario does, however, require two long-

TABLE 4. Geographical distribution of the species included in clades A–G presented in Fig. 4.

Clade	Species	Distribution	
Clade A	<i>Silene alexandri</i>	Hawaiian Islands	
	<i>Silene antirrhina</i>	American	
	<i>Silene hawaiiensis</i>	Hawaiian Islands	
	<i>Silene lanceolata</i>	Hawaiian Islands	
	<i>Silene perlmanii</i>	Hawaiian Islands	
Clade B	<i>Silene struthioloides</i>	Hawaiian Islands	
	<i>Silene cuatrecasasii</i>	SW Mediterranean	
	<i>Silene inaperta</i>	W Mediterranean	
	<i>Silene martyi</i>	SW Mediterranean	
	<i>Silene muscipula</i>	Mediterranean	
Clade C	<i>Silene reticulata</i>	SW Mediterranean	
	<i>Silene arenosa</i>	Middle East	
	<i>Silene austroiranica</i>	Middle East	
	<i>Silene exsudans</i>	Middle East	
	<i>Silene leyserooides</i>	Middle East	
Clade D	<i>Silene linearis</i>	Middle East	
	<i>Silene acaulis</i>	Circumpolar	
	<i>Silene falcata</i>	NE Mediterranean	
	<i>Silene fortunei</i>	E Asia	
	<i>Silene fruticosa</i>	Greece/Sicily	
Clade E	<i>Silene macrostyla</i>	E Asia	
	<i>Silene nemoralis</i>	C, S Europe	
	<i>Silene nutans</i>	Europe, N Asia	
	<i>Silene otites</i>	SC, W Europe	
	<i>Silene saxifraga</i>	S Europe	
	<i>Silene stenophylla</i>	E Russia, Siberia	
	<i>Silene tanakae</i>	Japan	
	<i>Silene mentagensis</i>	W Mediterranean	
	<i>Silene portensis</i>	W Mediterranean	
	Clade F	<i>Silene bupleuroides</i>	SE Europe, NE Mediterranean, Middle East
<i>Silene cariensis</i>		NE Mediterranean	
<i>Silene corinthiaca</i>		Greece/Aegea	
<i>Silene echinosperma</i>		Greece/Aegea	
<i>Silene echinospermoides</i>		NE Mediterranean	
<i>Silene longipetala</i>		NE, E, SE Mediterranean, Middle East	
<i>Silene marschallii</i>		NE, E Mediterranean, Middle East	
<i>Silene pinetorum</i>		Greece/Aegea	
<i>Silene reinwardtii</i>		NE Mediterranean	
<i>Silene subcretacea</i>		Tibet	
<i>Silene ungeri</i>		Greece/Aegea	
Clade G		<i>Silene ampullata</i>	NE Mediterranean, Middle East
		<i>Silene brevistaminea</i>	Afghanistan
		<i>Silene cappadocica</i>	NE Mediterranean, Middle East
		<i>Silene moorcroftiana</i>	Himalaya
	<i>Silene repens</i>	NE, E Asia, N America	
<i>Silene schafta</i>	Caucasus		

distance dispersals over the Pacific Ocean and can therefore be considered as a less parsimonious explanation.

The Japanese *S. tanakae* is, in the combined analysis (Fig. 4), a member of a clade that includes other species from East Asia. The classification of Chowdhuri (1957), which includes *S. tanakae* in the same subsection as the Hawaiian endemic *Silene*, is not congruent with the phylogenetic patterns found here. We do not know if this is true for *S. japonica* as well because we have not been able to see any type material for this species, which probably is the only existing material. Williams (1896) placed *S. japonica* in *Silene* series *Brachypodae* Boiss., whereas *S. tanakae* was placed in *Silene* series *Italicae* Rohrb., the latter together with, e.g., *S. fortunei* and several, but not all, of the Hawaiian *Silene*. He considered the placement of *S.*

japonica as “doubtful and tentative” and suggested that *S. japonica* is highly distinct from other species in the series *Brachypodae*. *Silene japonica* is described as having leathery leaves and purple flowers, but is otherwise very generally described.

The phylogenetic position of the woody or semiwoody Hawaiian *Silene* as sister to the annual herbaceous species *S. antirrhina* and the *S. antirrhina*/Hawaiian *Silene* clade as sister to the annual and herbaceous Middle East and Mediterranean *Silene* clade is consistent with the evolution of the perennial, woody habit after dispersal to the Hawaiian Islands. Many species of plants that are typically herbaceous on the mainland have evolved woodiness and arborescence on islands (Carlquist, 1974). A number of examples of this growth form evolution have been documented for Hawaiian plants, e.g., the silverswords (Baldwin et al., 1991), *Schiedea* (Wagner et al., 1995, 2005b), the lobelioids (Givnish et al., 1995), the violets (Ballard and Sytsma, 2000), and the mints (Lindqvist and Albert, 2002); see also Price and Wagner (2004) for discussion and more examples. The evolution of woodiness has also been seen on other oceanic islands, e.g., Macaronesia, where it has been shown for genera such as *Sonchus* (Kim et al., 1996), *Echium* (Böhle et al., 1996), and *Convolvulus* (Carine et al., 2004). However, the endemic *Silene* species from the Canary Islands are woody-based perennials and seem to have originated from a group of woody-based perennials (M. Lundberg et al., Uppsala University, unpublished manuscript).

The evolution of woody habit in insular plants likely has complex underpinnings, but has been proposed as the result of the ecological release of herbaceous, mainland colonists to fill the large woody plant niche (Carlquist, 1974). Givnish (1998) suggested that the evolution of woodiness has involved the initial invasion of open or partially open habitats followed by increased specialization and adaptation for more competitive environments. Birds are the most likely dispersal agents to distant oceanic islands, at least for plants of inland habitats with relatively large seeds, with seeds from fleshy fruits transported inside the animal and other seeds stuck on their feathers or skin or in mud on their feet. Birds are likely to visit open or partially open habitats and to carry with them seeds from similar environments on the mainland, thereby establishing the colonizer in a suitable environment (Givnish, 1998).

Seeds of *Silene* have no adaptations for long-distance dispersal. They have no wings of reasonable size (i.e., that might aid in flying) and the fruit is a dry capsule, but the seeds can probably stick to feathers or to mud on feet. It is possible that the ancestor of the Hawaiian *Silene* and *S. antirrhina* was a weedy herbaceous annual, much like *S. antirrhina*, and that it was dispersed to the Hawaiian Islands on birds visiting open habitats.

Conclusions—The probable American origin of the Hawaiian *Silene* lineage is yet another example of the importance of the American continents as a source area for Hawaiian plant groups and the significance of birds as dispersal agents. But even though the American origin seems like the most likely explanation, one must keep in mind the difficulties, like the ones seen here, in unambiguously inferring the direction of dispersal.

Hawaiian *Silene* is yet another instance in which a morphologically heterogeneous group turns out to be descended from a single common introduction. We show, in spite of the little molecular differentiation of the Hawaiian *Silene* and

its continental sister-taxon, morphological radiation has resulted in two distinct morphological groups and that the woody habit has been derived in situ in Hawaii from herbaceous ancestors.

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APPENDIX. Material used for phylogenetic analyses. Herbarium abbreviations according to Holmgren et al. (1990). Missing data are indicated with a —.

Taxon—Voucher; GenBank accession nos.: ITS; *rps16*; *RPB2*.

Lychnis coronaria (L.) Desr.—Bengt Oxelman 2278 GB; X86891; Z83165.1; —. *Lychnis coronaria* (L.) Desr.—Magnus Popp 1050 UPS; —; —; AJ634069.1. *Silene acaulis* (L.) Jacq.—Bengt Oxelman 2243 GB; X86860; Z83189.1; —. *Silene alexandri* Hillebr.—Wood 6036 US; EF060222; EF061382; EF061344. *Silene ampullata* Boiss.—Al-Dabbagh and Jasim 48874 LE; EF060223; —; EF061345. *Silene antirrhina* L.—M. A. Wincent and T. G. Lammers 3137 GB; DQ908631; Z83193; EF061318. *Silene arenosa* K. Koch—Grossheim 24.vii.1919 B; EF060203; EF061363; EF061323. *Silene austroiranica* Rech. f., Aell. & Esfand—Rechinger et al. 3261 B; EF060204; EF061364; EF061324. *Silene baccifera* (L.) Roth—Bengt Oxelman 2287 GB; X86889; Z83169.1; AJ296139.1. *Silene bergiana* Lindman—Holmdahl 1182 GB; X86835; Z83191; AJ634076.1. *Silene brevistaminea* Gilliin—Hedge and Wendelbo W 9534 GB; EF060235; EF061395; —. *Silene bupleuroides* L.—Bengt Oxelman 2266 GB; X86864; —; —. *Silene bupleuroides* L.—Bengt Oxelman 2392 UPS; —; EF061360; EF061319. *Silene cappadocica* Boiss. & Heldr.—Wendelbo and Assadi 28000 GB; EF060224; EF061383; EF061346. *Silene cariensis* Boiss.—Bengt Oxelman 1681 GB; EF060205; EF061365; EF061325. *Silene conica* L.—Bengt Oxelman 1944 GB; X86832; Z83170.1; —. *Silene conica* L.—Bengt Oxelman 1898 GB; —; —; AJ634077.1. *Silene corinthiaca* Boiss. & Heldr.—Bengt Oxelman 1934 GB; EF060206; EF061366; EF061326. *Silene cuatrecasasii* Pau & Font Quer—OPTIMA ITER V 1553 RNG; EF060207; EF061367; EF061327. *Silene echinosperma* Boiss. & Heldr.—Bengt Oxelman 2225 GB; X86845.1; Z83196; EF061330. *Silene echinospermoides* Huber-Mor.—Bengt Oxelman 2202 GB; EF060208; EF061368; EF061328/EF061329. *Silene exsudans* Boiss. & Heldr.—Bengt Oxelman 1660 GB; EF060209; EF061369; EF061331. *Silene falcata* Sibth. & Sm.—Bengt Oxelman 2373 UPS; EF060225; EF061384; EF061347. *Silene fortunei* Vis.—Zheng-Yu Li 20954 PE; EF060226; —; —. *Silene fortunei* Vis.—Harry Smith 6631 UPS; —; EF061385; EF061348. *Silene fruticosa* L.—Bengt Oxelman 934 GB; X86865; Z83188.1; AJ634078.1. *Silene hawaiiensis* Sherff.—Degener, Greenwell and Murashige 19828 GB; EF060218; EF061378; EF061340. *Silene inaperta* L.—Bengt Oxelman 1724 GB; EF060210; EF061370; EF061332. *Silene lanceolata* A. Gray—Perlman and Castilio 14329 US; EF060221; EF061381; EF061343. *Silene leyseroides* Boiss.—Kramer 75-156 BSB; EF060211; EF061371; EF061333. *Silene linearis* Decne—M. Bierkamp and P. Zinth 177 BSB; EF060212; EF061372; EF061334.

Silene longipetala Vent.—Grey-Wilson and Hewer 899 GB; EF060232; EF061392; —. *Silene macrostyla* Maxim.—Smith 6883 UPS; EF060227; EF061386; EF061349. *Silene marschallii* C. A. Mey.—Wendelbo and Assadi 27674 GB; EF060228; EF061387; EF061350. *Silene martyi* Emb. et Maire—F. Jacquemoud and D. Jeanmonod MAR1168 G; EF060213; EF061373; EF061335. *Silene mentagensis* Coss.—Jahandiez 329 LD; EF060236; EF061396; EF061358. *Silene moorcroftiana* Wall. ex Benth—B. Dickoré 17783 GOET; EF060229; EF061388; EF061351. *Silene muscipula* L.—Bengt Oxelman 1780 GB; EF060201; Z83197.1; EF061320. *Silene nana* Kar. et Kir.—Kereverzova and Mekeda 1976.V.5 LEGB; EF060217; EF061377; EF061339. *Silene nemoralis* Waldst. & Kit.—Bengt Oxelman 2259 GB; EF060230; EF061389; EF061352. *Silene nutans* L.—; U30962/U30989; —; —. *Silene nutans* L.—Magnus Popp 1045 UPS; —; EF061361; EF061321. *Silene otites* (L.) Wibel—Anja Rautenberg 83 UPS; EF060233; EF061393; EF061356. *Silene perlmanii* W. L. Wagner, D. R. Herbst & Sohmer—Perlman & Obata 5401 BISH; EF060220; EF061380; EF061342. *Silene pinetorum* Boiss. & Heldr.—Greuter S4408 B; EF060214; EF061374; EF061336. *Silene portensis* L.—Sanchez Garcia 17.6.1974 C; EF060237; EF061397; EF061359. *Silene reinwardtii* Roth—Bengt Oxelman 2391 UPS; EF060215; EF061375; EF061337. *Silene repens* Patr.—Egger 431 WTU; DQ908662; DQ908842; —. *Silene repens* Patr.—Argus 1068 UPS; —; —; DQ908755. *Silene reticulata* Desf.—Davis 53457 BM; EF060216; EF061376; EF061338. *Silene saxifraga* L.—Anja Rautenberg 213 UPS; EF060234; EF061394; EF061357. *Silene schafta* S. G. Gmel. ex Hohen.—Bengt Oxelman 2264 GB; X86852; Z83194.2; —. *Silene schafta* S. G. Gmel. ex Hohen.—Magnus Popp 1053 UPS; —; —; AJ634088.1. *Silene sedoides* Poir.—Magnus Popp 1011 UPS; AJ299814; AJ294962.1; AJ296106.1. *Silene stenophylla* Ledeb.—Molau and Raszhivin 4092 GB; X86872.2; —; EF061353. *Silene struthioloides* A. Gray—Henrickson 3854 US; EF060219; EF061379; EF061341. *Silene subcretacea* F. N. Williams—Hu and Chuang 6 UPS; EF060231; EF061390; EF061354. *Silene tanakae* Maxim.—Maxim. 24.IX.1887 LE; —; EF061391; EF061355. *Silene ungeri* Fenzl—Bengt Oxelman and Lars Tollsten 1431 GB; EF060202; EF061362; EF061322. *Silene uniflora* Roth—Bengt Oxelman 2197 GB; X86849; Z83173.1; DQ908807. *Viscaria vulgaris* Röhl.—Magnus Popp 1051 UPS; AJ629911; AJ629912; AJ634090.1