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Phylogenetic Relationships of Silene multinervia and Silene Section Conoimorpha (Caryophyllaceae)

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Abstract—The Californian Silene multinervia (Caryophyllaceae) and Eurasian members of section Conoimorpha in subgenus Behenantha are the only Silene species that have calyces with 15 or more prominent, unbranched veins. We show that S. multinervia, which has been considered a recent introduction of the Asian S. coniflora (section Conoimorpha) to North America, is clearly not synonymous with the latter species based on morphological or molecular data. We present a chromosome count of S. multinervia (2n = 24), which is different from the base number x = 10, a putative synapomorphy for section Conoimorpha. Gene trees based on sequences from three different genomes fail to group S. multinervia with the European section Conoimorpha species. The S. multinervia sequences form a monophyletic group placed in an unresolved position within subgenus Behenantha.

Keywords—BEAST, cpDNA, chromosome count, coalescent, mitochondrial DNA, RNA polymerase genes.

Intercontinental disjunctions of plant species or species-pairs have received considerable interest from biogeographers (e.g. Raven 1972; Thorne 1972; Lee et al. 1996; Wen 1999; Milne 2006). Classical explanations often include vicariance or anthropogenic introduction. However, recent studies based on molecular data suggest that the most likely explanation for some Eurasia-North America disjunctions is pre-human dispersal events [e.g. Plantago occa Forsk. (Meyers and Liston 2008), Oligomeris linifolia (Vahl) J. F. Macbr. (Martín-Bravo et al. 2009), and Senecio malacensis A. Gray (Coleman et al. 2003)]. In other cases, species which previously have been regarded by some botanists as native to North America have been found to have been introduced by humans [e.g. Caikle edentula (Bigelow) Hook. (Raven and Axelrod 1978; Sauer 1988, p. 34) and Vulpia myuros (L.) C. C. Gmel. (Raven and Axelrod 1978)].

Silene L. (Caryophyllaceae) is a genus of approximately 700 species, most of which have their natural distribution in Eurasia (Oxelman et al. 2001). There are, however, also native Silene species in North and South America as well as in Africa, and species that have spread as weeds throughout the world. Silene is divided into the subgenera Silene and Behenantha (Otth) Endl. [syn. S. subgenus Behen (Dumort.) Rohrb.] (Popp and Oxelman 2004). Silene subgenus Silene includes the well-known species S. acaulis L. and S. gallica L., whereas major groups in Silene subgenus Behenantha include section Melandrion (Röhl.) R. K. Rabeler (containing the familiar S. latifolia Poir.), sections Phylsochynis (Bentham) Bocquet and Conoimorpha Otth, the Silene vulgaris group, and S. noctiflora L. (with the closely related S. turkestanica Regel, Sloan et al. 2009). The Flora of North America lists 52 native and 18 introduced or naturalized North American Silene species (Morton 2005). Most of the North American species belong to subgenus Behenantha, either to the section Phylsochynis s. 1. (Popp et al. 2005; Popp and Oxelman 2007) or to the S. menziesii group (Popp and Oxelman 2007), while S. antirrhina L. and S. repens Patrin belong to Silene subgenus Silene (Eggens et al. 2007; Popp and Oxelman 2007).

Considerable attention has been given to the phylogenetic position of the section Melandrion to facilitate understanding of the evolution of dioecy (e.g. Atanassov et al. 2001; Filatov and Charlesworth 2002; Filatov 2005; Nicolas et al. 2005; Rautenberg et al. 2010), and in several cases section Conoimorpha has been suggested to be the sister group to these dioecious species (e.g. Desfeux and Lejeune 1996; Erixon and Oxelman 2008a).

Common morphological features for Silene, as circumscribed by Oxelman et al. (2001), are flowers with 10 stamens and three or five styles, five free petals, a synsepalous calyx, and a capsule that usually splits open into twice as many teeth as the number of styles. Two important characters in identification of Silene species are anthophore length and the coronal scales. The anthophore is a structure that separates the attachment of the calyx and corolla. The coronal scales are present as small appendages on the border between the petal limb and the petal claw (the part of the petal that is hidden in the calyx).

Silene multinervia S.Watson (Caryophyllaceae) is a Californian taxon (Hitchcock and Maguire 1947) that always has been placed into the otherwise Eurasian section Conoimorpha (e.g. Watson 1890; Hitchcock and Maguire 1947; Šourková 1971, as the separate genus Pleconax Raf.). Silene multinervia and section Conoimorpha share a distinctive morphological feature: all species have several (15–60) unbranched prominent parallel veins on the calyx (all other Silene species have 10 principal veins and/or branching nervature). Silene section Conoimorpha also has a base chromosome number of x = 10 (Greuter 1995), whereas all other Silene have x = 12 with one known exception (S. fortunei Vis., 2n = 30; Bari 1973). Members of section Conoimorpha have elevated nucleotide substitution rates in chloroplast (Erixon and Oxelman 2008b) and mitochondrial DNA (Sloan et al. 2009), compared to other members of the genus. The circumscription of the group (e.g., Rohrbach 1868; Chowdhuri 1957) has been controversial since its first appearance in the taxonomic literature (Otth 1824). The species currently recognized in the group (Silene amentophila Boiss. & Heldr., S. conica L., S. coniflora Nees ex Otth, S. conoidea L., S. lydia Boiss., S. macrodonta Boiss., S. subconica Friv., S. gisebachii (Davidov) B. Pirker & Greuter, and S. sartorii Boiss. & Heldr.; Pirker and Greuter 1997) have their native distribution in Europe and southwest to central Asia (Table 1), although S. conica and S. conoidea are introduced as weeds around the
world (e.g. Rozefelds et al. 1999; Morton 2005; Global Compendium of Weeds 2007). *Silene multinervia* has recently been put into synonymy with the southwest/central Asian species *S. coniflora* (Morton 2005; followed by Hartman and Rabeler 2008). On the other hand, Popp and Oxelman (2007) and Rautenberg et al. (2010) showed, based on cpDNA and nrDNA ITS sequence data, that *S. multinervia* does not form a monophyletic group with Eurasian samples from the section Conoimorpha. However, the sampling in either of these two studies was not focused on *S. multinervia* or section Conoimorpha.

Using DNA sequences from samples of *S. multinervia*, *S. coniflora*, and six other taxa from *Silene section Conoimorpha*, a chromosome count of *S. multinervia*, and sequence data from several outgroup species with emphasis on potentially closely related species in *Silene* subgenus Behenantha, we address the following questions: Is there any morphological or molecular support for the synonymization of *S. multinervia* with *S. coniflora*? Is there any morphological or molecular support for the inclusion of *S. multinervia* in *Silene section Conoimorpha*? Does *S. multinervia* represent a recent introduction to the Californian flora? What is the phylogenetic position of section Conoimorpha?

### MATERIALS AND METHODS

#### Study Species—The present study includes *Silene multinervia* and seven of the nine species from *Silene section Conoimorpha* (Table 1), as well as a large outgroup sampling, with special emphasis on potentially closely related species in *Silene* subgenus Behenantha.

The members of section Conoimorpha are briefly characterized in Table 1, but a few of them deserve mention here. *Silene multinervia* grows in California and Mexico on burnt open ground, after forest fires, and is recognized by 20 calyx veins and no coronal scales (Watson 1890; Jepson 1914; Hartman and Rabeler 2008). *Silene coniflora* grows from southwest to central Asia and has 20 calyx veins and oblong coronal scales (Schischkin 1970). *Silene lydia* is a species sharing some of the of features section Conoimorpha (more than 10 unbranched parallel veins), but also having enough features to be placed in a section of its own (5 section Lydace Greuter) for Greuter (1995). *Silene lydia* has a chromosome number of 2n = 20, or possibly 2n = 22 (preliminary data by B. Pirker, discussed in Greuter 1995), long glandular hairs on the calyx, and no anthophore (Greuter 1995). It is distributed in the southeastern Balkans and western Anatolia (Greuter 1995). The Greek endemics *S. griesebachii* and *S. sartorii* were not included in the molecular analysis. They are similar to *S. subgenus*, but differ in petal shape and venation, and the former has distinct seeds and longer anthophore (Pirker and Greuter 1997). Rautenberg et al. (2008) found some indications of a close relationship between *S. nocticola* and section Conoimorpha. Previous molecular phylogenetic studies (e.g. Oxelman and Lidén 1995; Oxelman et al. 2001; Popp and Oxelman 2001, 2004, 2007; Rautenberg et al. 2010) have revealed that section Conoimorpha is confidently embedded in subgenus Behenantha, which has a poorly resolved basal relationships, but also having some features some six to seven million years ago (Erixon and Oxelman 2008a; Frajman et al. 2009). We therefore sampled outgroup taxa primarily to represent major lineages from subgenus Behenantha.

#### Chromosome Count—A chromosome count was determined for *Silene multinervia* based on a plant grown from seeds collected in Napa County, California (Appendix 1). Prior to fixation in Carnoy I solution (3 volumes absolute alcohol and 1 volume glacial acetic acid), growing roots were pretreated with equal parts 0.1% colchicine and 0.002M 8-hydroxyquinoline for 2 hrs. After fixation and hydrolysis in 1N HCl at 60°C for 2 mins, root-tip meristems were prepared. Flower buds were fixed and hydrolyzed in Carnoy I solution. All tissues were stained with aceto-orcein on clean slides and squashed under a coverslip.

#### Morphology—Herbarium specimens from CAS, G, GB, LE, MW, S, UPS, and WU (abbreviations according to Holmgren and Holmgren 1998), and Arne Strid’s private herbarium (in Orbæk, Denmark), of *Silene multinervia*, *S. coniflora*, and other representatives of *Silene section Conoimorpha* were studied as physical specimens or as images deposited in the Sileneae database (http://www.sileneae.info). Specimens were compared to keys, descriptions, and illustrations in the literature (Ottl 1824; Boissier 1867; Roehrbach 1868; Watson 1890; Williams 1896; Jepson 1914; Post 1932; Hitchcock and Maguire 1947; Blakelock 1957; Khoshoo and Bhatia 1963; Mouterde 1966; Zohary 1966; Bajtenov 1969; Schischkin 1970; Ghanzanfar and Nasir 1986; Melzheimer 1988; El-Oqlah and Karim 1990; Hosny et al. 1992; Chater et al. 1993; Greuter et al. 1997; Boulos 1999; Morton 2005; Hartman and Rabeler 2008; Calìf Mayor 2009).

#### DNA Extraction, Amplification, and Sequencing—DNA was extracted from living or herbarium material using a modified Carlson/Yoon method (Oxelman and Lidén 1995). Voucher details and GenBank accession numbers are listed in Appendix 1. Three cpDNA regions (the matK gene, the atp1 intron, and the trnl-trnsf intergenic space), three mitochondrial DNA (mtDNA) regions [the protein-encoding ATP synthase subunit 1 (atp1), cytochrome c oxidase subunit 3 (cox3), and NADH dehydrogenase subunit 9 (nad9)], ITS from nuclear ribosomal DNA, and four low-copy nuclear regions (parts of the RNA polymerase genes RP2, RP3, RP24, and RP22) were amplified. The PCR products were either purified using MilliPore multiscreen PCR plates in a vacuum manifold (Millipore, Billerica, Massachusetts) and sequenced by Macrogen Inc. in Seoul, South Korea or purified with Exonuclease I and shrimp alkaline phosphatase (USB Corporation, Cleveland, Ohio), cycle sequenced with BigDye v3.1 (Applied Biosystems, Foster City, California), and analyzed on an ABI 3130xl capillary sequencer. In addition to already published PCR and sequencing primers for matK (Fior et al. 2006; Mower et al. 2007; Slooam 2009; B. Biber et al. 2009; Greuter et al. 2009; Popp et al. 2004, 2007; Oxelman et al. 1997; trnLF (Oxelman et al. 2005), RP2 (Popp and Oxelman 2004), RP2 (Popp and Oxelman 2001), RP2 (Popp and Oxelman 2004), cox3 (Duminil et al. 2002), nad9 (Duminil et al. 2002), ITS (Popp and Oxelman 2001), the following primers were used for amplification and sequencing: atp1_Coni_F (GCKGCGACAAATGKYKATTG), atp1_Coni_R (TATTCCTTCTACAGCA), trnL_Coni_F (CCWACATTATTACGCGCCTA), trnL_Coni_R (CCCAAATGCTACATAAACAC), atp1_Coni_R3 (CGGCTCTTTCTACAGCA), cox3_Coni_F (GaAAAAGAAATCTAGCCTAAC), cox3_Coni_R (TACTTTCTACAGCA).

### Table 1. Native distribution and number of calyx veins of *Silene multinervia* and the members of *Silene section Conoimorpha*. *Silene griesebachii* and *S. sartorii* were not included in the molecular analyses.

<table>
<thead>
<tr>
<th>Species</th>
<th>Native distribution</th>
<th>Number of calyx veins</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Silene ammophila</em> Boiss. &amp; Heldr.</td>
<td>Greece (Crete and Karpathos)</td>
<td>15-20</td>
<td>Pirker and Greuter 1997</td>
</tr>
<tr>
<td><em>Silene conica</em> L.</td>
<td>Europe to Central Asia</td>
<td>30</td>
<td>Schischkin 1970</td>
</tr>
<tr>
<td><em>Silene coniflora</em> Boiss.</td>
<td>Southwest to Central Asia</td>
<td>20</td>
<td>Schischkin 1970</td>
</tr>
<tr>
<td><em>Silene griesebachii</em> (Davidov)</td>
<td>Mediterranean to Southwest and Central Asia</td>
<td>30</td>
<td>Greuter 1995</td>
</tr>
<tr>
<td><em>Silene lydia</em> Boiss.</td>
<td>Mediterranean to Southwest and Western Anatolia</td>
<td>30</td>
<td>Greuter 1995</td>
</tr>
<tr>
<td><em>Silene macrodonta</em> Boiss.</td>
<td>Eastern Mediterranean</td>
<td>20</td>
<td>Watson 1890; Jepson 1914; Hartman and Rabeler 2008</td>
</tr>
<tr>
<td><em>Silene multinervia</em> S. Watson</td>
<td>California and Mexico</td>
<td>20</td>
<td>Watson 1890; Jepson 1914; Hartman and Rabeler 2008</td>
</tr>
<tr>
<td><em>Silene sartorii</em> Boiss. &amp; Heldr.</td>
<td>Mediterranean</td>
<td>30</td>
<td>Pirker and Greuter 1997</td>
</tr>
<tr>
<td><em>Silene subconica</em> Friv.</td>
<td>Mediterranean</td>
<td>30</td>
<td>Pirker and Greuter 1997</td>
</tr>
</tbody>
</table>
(GBGGTGAAATMTGCTGCTAG), nad9 Cono1 F (ACACNCGTTTCT GGATC), nad9 Cono1 R (CAAGAARTGGTCAAAGAACGT). Eighty sequences were new to this study, and additional sequences were obtained from GenBank Appendix 1).

**Sequence Alignment and Analysis**—Sequence reads were assembled into contigs and edited using the Staden package version 1.6.0 for Mac OS X (Staden 1996) with phred version 0.020425 (Ewing and Green 1998) and phrap version 0.990319 (http://www.phrap.org) or using Sequencher v.4.5 (Gene Codes, Ann Arbor, Michigan). Base polymorphisms were coded using the NC-IUPAC ambiguity codes. Sequence alignment was performed manually in QuickAlign (Müller and Müller 2003), following the criteria of Popp and Oxelman (2004). The alignments of the three cpDNA regions were analyzed separately and checked for strongly supported conflicts (see definition below). As such conflicts were not found, the alignments were concatenated into a cpDNA data set. The mtDNA regions were analyzed both separately and concatenated into a single data set. The nuclear regions were analyzed separately.

Simple indel coding (Simmons and Ochoterena 2000) was applied to the alignments using SeqState version 1.36, build 19.10.2007 (Müller 2005) and the alignments using PAUP* v.4.0b10 (Swofford 2002). Maximum parsimony analyses were carried out using heuristic searches with TBR branch swapping, the mtDNA tree on (but a limit of maxtrees set to 5,000), and 10 random addition sequences. For bootstrap support, 1,000 replications were performed, with the mtDNA option off.

Bayesian phylogenetic analysis was performed using MrBayes 3.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) with nucleotide models as proposed by MrModeltest version 2.2 (Nylander 2004), using the Akaike information criterion. Four MCMC chains were run for five million generations with trees and parameter values saved every 1,000th generation, in two parallel runs. Convergence of MrBayes analyses was checked using the split frequency diagnostic (runs with average standard deviations of < 0.01 were considered as converged), Tracer v1.5 (Rambaut and Drummond 2007), and AWTY (Wilgenbusch et al. 2004; Nylander et al. 2008). The first 25% of the trees were discarded as burn-in.

The *BEAST* (starbeast) mode in BEAST v1.5.4 (Drummond and Rambaut 2007) infers gene trees and, at the same time, estimates a species tree. Differences in effective population size will influence the tree topologies. Data matrices and phylogenetic trees are available on TreeBASE (study number S11178).

**Results**

Statistics for the alignments and phylogenetic analyses, as well as the model of evolution proposed for MrModeltest for the DNA regions are presented in Table 2.

**Chloroplast Genes**—In the concatenated chloroplast data set, *Silene* section *Conoimorpha* is a well-supported monophyletic group containing all species reported to belong to the section except *S. multinervia* (Fig. 1a). All *S. multinervia* accessions form a monophyletic group placed in an unresolved position in subgenus *Behenantha*, outside the rest of section *Conoimorpha*. *Silene lydia* is placed as sister to the rest of section *Conoimorpha*. All species relationships within section *Conoimorpha* are strongly supported. The pattern is congruent between all included cpDNA regions (data not shown), and between phylogenetic methods (Fig. 1a).

**Mitochondrial DNA**—As in the cpDNA tree, the European and Asian members of section *Conoimorpha* form a strongly supported monophyletic group in the mtDNA tree (Fig. 1b). In the concatenated mtDNA data set, section *Conoimorpha* groups with *S. noctiflora* + *S. turkestanica* with strong support and *S. multinervia* is weakly to moderately supported as sister

<table>
<thead>
<tr>
<th>Region</th>
<th>Number of terminals in PAUP* and MrBayes</th>
<th>Number of included characters (nucleotides/indels)</th>
<th>Number % of parsimony informative characters</th>
<th>Percentage of missing data</th>
<th>CI (R/L)</th>
<th>Substitution model</th>
<th>Average SD of split frequencies (MrBayes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPA2</td>
<td>58/(54 + 1)</td>
<td>2,560 (2,483/77)</td>
<td>246/9.6%</td>
<td>2.7%</td>
<td>0.804 (0.860)</td>
<td>GTR + Γ</td>
<td>0.003984</td>
</tr>
<tr>
<td>RPB2</td>
<td>50</td>
<td>1,100 (984/116)</td>
<td>295/26.8%</td>
<td>4.4%</td>
<td>0.759 (0.833)</td>
<td>GTR + Γ</td>
<td>0.003924</td>
</tr>
<tr>
<td>RPD2a</td>
<td>43/(38 + 10)</td>
<td>2,148 (2,013/135)</td>
<td>242/11.3%</td>
<td>14.9%</td>
<td>0.831 (0.869)</td>
<td>GTR + Γ</td>
<td>0.003065</td>
</tr>
<tr>
<td>RPD2b</td>
<td>45/(42 + 12)</td>
<td>838 (743/95)</td>
<td>216/25.8%</td>
<td>6.1%</td>
<td>0.815 (0.890)</td>
<td>GTR + Γ</td>
<td>0.003385</td>
</tr>
<tr>
<td>ITS</td>
<td>52</td>
<td>888 (853/35)</td>
<td>164/18.4%</td>
<td>9.3%</td>
<td>0.537 (0.729)</td>
<td>GTR + Γ + K</td>
<td>0.006320</td>
</tr>
<tr>
<td>mtDNA</td>
<td>49</td>
<td>2,099 (2,099/0)</td>
<td>434/20.7%</td>
<td>3.5%</td>
<td>0.693 (0.848)</td>
<td>GTR + Γ</td>
<td>0.009918</td>
</tr>
<tr>
<td>atp1</td>
<td>44</td>
<td>960 (960/0)</td>
<td>195/20.3%</td>
<td>0.5%</td>
<td>0.680 (0.823)</td>
<td>GTR + Γ + K</td>
<td>0.005406</td>
</tr>
<tr>
<td>cox3</td>
<td>44</td>
<td>674 (674/0)</td>
<td>127/18.8%</td>
<td>5.2%</td>
<td>0.735 (0.897)</td>
<td>GTR + Γ + K</td>
<td>0.006995</td>
</tr>
<tr>
<td>nad9</td>
<td>43</td>
<td>464 (464/0)</td>
<td>112/24.1%</td>
<td>5.0%</td>
<td>0.771 (0.894)</td>
<td>GTR + Γ</td>
<td>0.007122</td>
</tr>
<tr>
<td>cpDNA</td>
<td>55/(51 + 0)</td>
<td>4,182 (3,944/238)</td>
<td>513/12.2%</td>
<td>32.3%</td>
<td>0.782 (0.819)</td>
<td>GTR + Γ + K</td>
<td>0.005501</td>
</tr>
<tr>
<td>matK</td>
<td>45</td>
<td>1,722 (1,708/14)</td>
<td>157/9.1%</td>
<td>33.8%</td>
<td>0.820 (0.849)</td>
<td>GTR + Γ</td>
<td>0.003329</td>
</tr>
<tr>
<td>rps16</td>
<td>40</td>
<td>1,048 (966/82)</td>
<td>172/16.4%</td>
<td>4.7%</td>
<td>0.753 (0.848)</td>
<td>GTR + Γ</td>
<td>0.004645</td>
</tr>
<tr>
<td>trnL.F</td>
<td>40</td>
<td>1,405 (1,272/133)</td>
<td>176/12.5%</td>
<td>8.1%</td>
<td>0.796 (0.784)</td>
<td>GTR + Γ</td>
<td>0.005577</td>
</tr>
</tbody>
</table>
Fig. 1. Phylogram obtained from MrBayes for the concatenated chloroplast DNA (cpDNA) sequences (a), and for the concatenated mitochondrial DNA (mtDNA) sequences (b). Values associated with nodes are Bayesian posterior probabilities/parsimony bootstrap values. Posterior probabilities/ bootstrap values lower than 0.70/50 are not indicated. Branch lengths represent estimated number of changes per site. The gray boxes indicate the key groups: C = section Conoimorpha, M = S. multinervia, and N = S. noctiflora + S. turkestanica. Numbers and letters after species names indicate different specimens (Appendix 1). Genera are represented as follows: Ag = Agrostemma, At = Atocon, B = Beta, E = Eudanthe, H = Heliosperma, L = Lychnis, P = Petrocoptis, S = Silene, and V = Viscaria.
Fig. 2. Phylogram obtained from MrBayes for the low-copy nuclear RNA polymerase genes RPA2 (a), RPB2 (b), RPD2a (c), and RPD2b (d). Values associated with nodes are Bayesian posterior probabilities/parsimony bootstrap values. Posterior probabilities/boostrap values lower than 0.70/50 are not indicated. Branch lengths represent estimated number of changes per site. Dashed lines represent parts of the tree where the maximum parsimony bootstrap consensus tree has a differing topology (bootstrap support lower than 60%). The gray boxes indicate the key groups C = section Conoimorpha, M = S. multinervia, and N = S. noctiflora + S. turkestanica. Numbers and letters after species names indicate different specimens (Appendix 1). GenBank accession numbers are used to identify different sequences from the same specimen. All species belong to Silene except At = Atocion, E = Eudianthe, L = Lychnis, and P = Petrocoptis.
There is no support for S. multinervia S. noctiflora + S. turkestanica relationships between section Conoimorpha (Fig. 1b). The different mtDNA gene trees show different patterns in terms of branch length variation (supplemental data S1). Silene multinervia occupies a branch that is somewhat longer than the majority of other Silene branches, but still much shorter than the extreme lineages (Fig. 1b). The position of S. multinervia is more or less ambiguously resolved in all three mtDNA gene trees (supplemental data S1). The internal relationships within Eurasian Conoimorpha are strongly supported and agree with the cpDNA tree (Fig. 1b).

**Nuclear Genes**—In all nuclear gene trees, the members of section Conoimorpha, with the exception of S. multinervia, form a strongly supported monophyletic group (Figs. 2–3). The relationships within Conoimorpha are generally well resolved, strongly supported, and congruent with other regions (Figs. 2–3). Generally, the topological relationships in Behenantha outside of section Conoimorpha are unresolved, or conflicting between different nuclear genes (Figs. 2–3). In the ITS tree the relationships between section Conoimorpha, S. multinervia, and S. noctiflora + S. turkestanica are unresolved (Fig. 3). In the RPD2a and RPD2b trees, S. multinervia is placed as a close relative of the Physolychnis group with moderate (RPD2a) or strong (RPD2b) support, while S. noctiflora + S. turkestanica form a moderately to strongly supported sister group to the members of section Conoimorpha (Fig. 2c–d). In RPB2, RPD2a, and RPD2b, S. multinervia and the rest of section Conoimorpha are separated by at least one moderately to strongly supported node (Fig. 2b–d).

*BEAST Analysis*—In the species tree obtained by the *BEAST* analysis based on cpDNA and data from the RNA polymerase genes RPA2, RPD2a, and RPD2b, the topological relationships between section Conoimorpha, S. multinervia, and S. noctiflora + S. turkestanica are poorly resolved (Fig. 4). There is no support for S. multinervia as the sister group to section Conoimorpha. In the RPD2a and RPD2b gene trees, S. noctiflora + S. turkestanica form a monophyletic group with section Conoimorpha (PP = 0.97 and 0.78, respectively; supplemental data Fig. S2), but in the species tree the PP for this grouping is 0.63 (Fig. 4).

**Dating**—The 95% HPD (highest posterior density) ages of the MRCA (most recent common ancestor) of the S. multinervia sequences vary in the different gene trees, between 0.0021 (RPD2a) and 0.64 million years (RPA2). In the combined species tree in the *BEAST* analysis, the 95% HPD ages of the MRCA of section Conoimorpha are 1.6–5.7 million years (Fig. 4). The age of the MRCA of S. multinervia and its closest sister group (section Physolychnis) has a 95% HPD interval of 1.9–7.1 million years in the combined species tree, although this node has a posterior probability of only 0.60 (Fig. 4).

**Chromosome Count**—Twenty-four chromosomes could readily be counted from several metaphase plates prepared from root-tips of Silene multinervia, and also from mitotic metaphase plates prepared from flower buds.

**Morphology**—There are several phenotypic differences between the allegedly synonymous S. multinervia and S. coniflora: S. multinervia lacks coronal scales and has basal leaves that are oblanceolate and cauline leaves that are lanceolate-linear (Fig. 5A). Silene coniflora has coronal scales and grass-like linear leaves (Fig. 5B). The number of calyx veins is 20 in both S. multinervia and S. coniflora. Although the protologue by Otth, citing the original author Nees, states the number of calyx veins to be 30 (Otth 1824), the examined S. coniflora specimens have 20 calyx veins, a number that is also supported by previously published reports (Boissier 1867; Rohrbach 1868; Williams 1896; Post 1932; Blakelock 1957; Zohary 1966; Bajtenov 1969; Schischkin 1970; Hosny et al. 1992; Boulos.

**Discussion**

**Is There any Morphological or Molecular Support for the Synonymization of *S. multinervia* to *S. coniflora***?—*Silene coniflora* is the representative of section *Conoimorpha* that most resembles the superficial appearance of *S. multinervia*, with the similarity mainly based on the number of calyx veins. Careful study of plant material, however, reveals that the North American and southwest/central Asian species are two distinct entities that easily can be distinguished morphologically based on leaf morphology and presence/absence of coronal scales. None of the gene phylogenies show any support for the synonymy of *S. multinervia* and *S. coniflora*.

**Is There any Morphological Support for the Inclusion of *S. multinervia* in *Silene* Section *Conoimorpha***?—The common characteristic nervature of *Silene* section *Conoimorpha* and *S. multinervia*, with 15 or more densely packed, prominent parallel calyx veins, is not present in any other members of the genus. Other *Silene* species have calyces with 10 veins,

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**Fig. 4.** Species tree obtained from *BEAST* for *Silene* and *Lychnis*, based on cpDNA, *RPA2*, *RPD2a*, and *RPD2b* sequences. Values associated with nodes are Bayesian posterior probabilities (above branches) and median node ages in million years (under branches). The horizontal bars represent 95% HPD (highest posterior density) intervals of node ages. The gray boxes indicate the key groups C = section *Conoimorpha*, M = *S. multinervia*, and N = *S. noctiflora* + *S. turkestanica*. All species belong to *Silene* except *L. = Lychnis*.
or with a different distribution of the veins. Among the
close relatives in Silene subgenus Behenantha, the dioecious
members of section Melandrium have female flowers with
20 branching veins and male flowers with 10 veins, S. vulgaris
and its close relatives have an anastomosing pattern on the
calyx, whereas the members of section Physolychnis have
10 veins. We have not found any synapomorphies for
S. multiviria and section Conoimorpha other than the nervature.
Our chromosome count of S. multiviria (2n = 24) is the same
as for most other diploid Silene species, but differs from what
Morton (2005) reports for Asian S. coniflora material (2n = 20).
We have, however, not been able to find any original chro-
mosome counts of S. coniflora in Bari (1973), the IPCN data-
base (Goldblatt and Johnson 1979), the S. coniflora literature
listed in Material and Methods, or other literature on chro-
mosome counts in Silene. Several reports show that other mem-
bers of section Conoimorpha have 2n = 20 (e.g. Khoshoo 1960;
Khoshoo and Bhata 1963; Greuter 1995), or possibly 2n = 22
in S. lydia (Greuter 1995). Thus, cytological evidence do not
support the inclusion of S. multiviria in section Conoimorpha,
whereas the presence of many densely packed calyx veins is
a potential synapomorphy.

Is There any Molecular Support for the Inclusion of
S. multiviria in Silene Section Conoimorpha?—The present
study, based on a more thorough sampling of specimens and
taxa, supports previous studies indicating that S. multiviria
does not form a monophyletic group with the Eurasian
species of section Conoimorpha (Popp and Oxelman 2007;
Rautenberg et al. 2010). The relationships between the dif-
f erent groups from Silene subgenus Behenantha are largely
unresolved, and hence it is difficult to pinpoint the phyl-
genetic position of S. multiviria. Although the *BEAST
trees and the gene phylogenies of RPA2, RPB2, RPD2a,
and RPD2b are somewhat incongruent regarding the rela-
tionships within subgenus Behenantha, they all indicate that
S. multiviria is not the closest relative of section Conoimorpha.
Other molecular studies also have had problems resolving the
positions of several groups in subgenus Behenantha (e.g. Popp
and Oxelman 2007; Rautenberg et al. 2010), and Erixon and
Oxelman (2008a) suggested that an ancient radiation is respon-
sible for the pattern seen in the cpDNA data.

If S. multiviria and S. section Conoimorpha are sister line-
ages, the non-monophyly of the groups could potentially be
explained by incomplete lineage sorting effects, which would
be reasonable if the branching events leading to the radiation
of subgenus Behenantha were separated by short time spans
and/or large effective population sizes. If S. multiviria
and section Conoimorpha are not each other’s closest relatives,
the apparent morphological synapomorphy (many densely
packed unbranched calyx veins) could be caused by conver-
genent evolution or by a deep coalescent event of the gene(s)
responsible for this feature. The chronograms indicate that
the split between S. multiviria and section Conoimorpha line-
eges must be several million years old, so even if S. multiviria
and section Conoimorpha are sister groups, the hypothesis
of human-mediated dispersal of S. multiviria from Eurasia to
America can be safely rejected.

The species from section Conoimorpha included in our
species tree analyses (S. ammolitha, S. conica, S. coniflora,
S. conoides, S. lydia, S. macrodonta, and S. subconica) form
a strongly supported monophyletic group. Silene grisebachii
and S. sartorii could unfortunately not be sampled for the
present study, but given their great morphological, eco-
logical, and geographical resemblance (Pirker and Greuter 1997)
to the rest of the species, it is sound to hypothesize that
they also belong to the section Conoimorpha clade.

Silene Section Conoimorpha and S. noctiflора—In accor-
dance with Sloan et al. (2009), the members of Silene section
Conoimorpha and the monophyletic group S. noctiflora +
S. turkestanica both have extremely high substitution rates
in the mitochondrial genes atp1, cox3, and nad9. Silene
multiviria has slightly elevated rates, as compared to the rest
of the genus. Due to the extreme variations in substitution
rates between the sampled taxa, it is difficult to use the
mtDNA phylogeny to draw conclusions on the relationships
between different lineages. In the RPD2a and RPD2b phylo-
genies, S. noctiflora + S. turkestanica form a monophyletic group
with section Conoimorpha. This topology is partly supported
by a recent study, where the 3' part of the SIX1/SIY1 gene
indicates monophyly of S. noctiflora and section Conoimorpha,
although with low support (Rautenberg et al. 2008). If this
sister group relationship reflects the species phylogeny, it
would support a single origin of the elevated substitution
rates in section Conoimorpha and S. noctiflora + S. turkestanica.
However, the incongruence of the tree topologies inferred
from other nuclear and chloroplast genes (Figs. 1a, 2a–b, 3–,4;
and the 5' part of SIX1/SIY1 gene in Rautenberg et al. 2008)
makes this relationship remain ambiguous.

Congruence Between Organellar Phylogenies—Recent studies
in Silene vulgaris have found evidence of paternal transmis-
sion and recombination in organelle genomes, resulting in
incongruence between cpDNA and mtDNA gene trees within
the species (McCauley at al. 2005; Houliston and Olson 2006;
McCauley et al. 2007; McCauley and Ellis 2008). These results
raise the possibility of phylogenetic conflicts between cpDNA
and mtDNA at the interspecific level. Plant mtDNA sequences
are often uninformative at local phylogenetic scales, because
substitution rates in plant mtDNA are generally low compared to those in plant chloroplast and nuclear genomes and compared to mtDNA of other organisms (Wolfe et al. 1987; Palmer and Herbon 1988). In our dataset, the extreme differences in branch lengths in the Silene mtDNA phylogenies preclude using mtDNA to infer relationships among the major lineages. On the other hand, the rate acceleration provides the rare opportunity to use plant mtDNA to resolve the relationships at a local phylogenetic scale within section Conoimorpha. Within section Conoimorpha, we found that the different mitochondrial regions are congruent with each other, with the cpDNA regions, and with the nuclear regions, except for a few weakly supported deviations. Therefore, if paternal leakage and recombination have occurred within section Conoimorpha, they do not appear to have generated significant phylogenetic conflicts between chloroplast and mitochondrial genomes.

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Literature Cited


index letters to match voucher specimens to sequences in the gene trees
(Figs. 1–4) in those species where more than one specimen was produced sequences.

Agrostemma githago L.—Sloan 001 (VPJ): FJ589241 (atpl), FJ589564 (cox3), FJ589432 (nad9). Atocion lechenfeldiianum (Bauhmg.) M. Popp—Snedaker et al. (CAS), FJ589724 (atpl), FJ589628 (cox3), FJ589834 (nad9). A. rupestris (L) Osmund—Schlosser & Fairm 11439 (LJU) 5, FJ587682 (matK), FJ588399 (rps16), FJ584040 (ITS), FJ589313 (RAP2), FJ583944 (RPD2a), FJ583961 (RPD2b), F. Braun 02-Aug-2006 (LJU) 5, FJ587687 (RPB2). Beta vulgaris L.—BA100024 (atpl, cox3, nad9). Eulindaethe lactea (Aiton) Rchb. ex Willd—Sendrille et al. 690 (GB) 5, FJ589243 (atpl), FJ589066 (cox3), FJ589434 (nad9), FN821109 (matK), Oxelman 1876 (GB) 5, ZB096548 (matK), X869916 (atpl), XC162138 (rnfl), FN823256 (RPD2b), FJ589244 (atpl), FJ589246 (RPB2), FJ583942 (RPD2a), FJ583946 (RPD2b). Heliosoma psilobium (Walst. & Kit.) Rchb.—Zog ZH 11348 (Z): FJ589244 (atpl), FJ589567 (cox3), FJ589435 (nad9). Lycmenis calceolica L.—Osmund 1227 (GB) 5, ZB1361 (rps16), X86984 (ITS), JAE2926 (RAP2), JAE34142 (RPB2), JAE34141 (RPB2).—Exon 68 (UPS) 5, EU308012 (rnfl), L. coronaria (L.) Des.—N/A. Collected by D. Sloan: Charlotteville, Virginia, U. S. A. 5, FJ587685 (atpl), FJ589245 (cox3), FJ589436 (matK), FN821108 (matK), Oxelman 1876 (GB) 5, ZB096549 (matK), X869917 (atpl), XC162139 (rnfl), FN823257 (RPD2b), FJ589245 (atpl), FJ583942 (RPD2a), FJ583946 (RPD2b). L. flo-cuculli L.—Osmund 2200 (GB): ZB3163 (exon16), EU216218 (rnfl), FN823928 (ITS), JAE34070, JAE34071 (RPB2), JAE34141, JAE34145 (RPD2a), JAE34113 (RPB2).—F. l. flo-jois (L.) Des.—Osmund ITS-FLO 3160: EU216219 (rnfl), JAE34072 (RPB2), Osmund 2297 (GB): X86982 (ITS), JAE34122, JAE34124 (RPB2), JAE34123 (RPB2).—Petroscoptys pyrenica A. Br.—Schweinf. et al. (KGB), FJ589725 (atpl), FJ589668 (cox3), FJ589958 (matK), FHJCS94964 (rps16), FHJCS94018 (ITS), FHJCS94388 (RPB2), FHJCS7911 (RPB2), FHJCS79611 (RPB2).—RPS16 HRP 2629 (GB): ZB3166 (exon16), EU216218 (rnfl), FN823928 (ITS), JAE34070, JAE34071 (RPB2), JAE34141, JAE34145 (RPD2a), JAE34113 (RPB2).—R. l. silene (L.) Jacq.—Schweinf. 5315 (WU): FHJCS94246 (atpl), FHJCS94091 (cox3), FHJCS94937 (matK), FHJCS9435 (nad9), F. koikfierii Schmal.—Porterius 3814 (LE): FHJCS9428 (atpl), FHJCS9429 (cox3), FHJCS9430 (matK), FHJCS9431 (nad9), FHJCS9432 (RPB2), FHJCS9433 (RPD2b).—R. a. auriculata (L.) Horn.—Rautenberg 32 (UPS): FN823122 (rnfl), FHJCS9528 (RPB2).—R. a. conica.—Exon 70 (UPS) 5, FHJCS9528 (atpl), FHJCS9530 (atpl), FHJCS9549 (nad9), FHJCS9550 (matK), FN821129 (exon16), EU221624 (rnfl), FN821100 (ITS), FHJCS95197 (RPB2), FHJCS95198 (RPB2), FHJCS95199 (RPB2), FHJCS93490 (RPB2), FHJCS93491 (RPD2a), FHJCS93492 (RPD2b).—R. a. conica.—Exon 70 (UPS): FN823122 (rnfl), FHJCS9528 (RPB2).—R. a. conica.—Exon 70 (UPS): FN823122 (rnfl), FHJCS9528 (RPB2).—R. a. conica.—Exon 70 (UPS): FN823122 (rnfl), FHJCS9528 (RPB2).—R. a. conica.—Exon 70 (UPS): FN823122 (rnfl), FHJCS9528 (RPB2).—R. a. conica.—Exon 70 (UPS): FN823122 (rnfl), FHJCS9528 (RPB2).—R. a. conica.—Exon 70 (UPS): FN823122 (rnfl), FHJCS9528 (RPB2).—R. a. conica.—Exon 70 (UPS): FN823122 (rnfl), FHJCS9528 (RPB2).—R. a. conica.—Exon 70 (UPS): FN823122 (rnfl), FHJCS9528 (RPB2).—R. a. conica.—Exon 70 (UPS): FN823122 (rnfl), FHJCS9528 (RPB2).—R. a. conica.—Exon 70 (UPS): FN823122 (rnfl), FHJCS9528 (RPB2).—R. a. conica.—Exon 70 (UPS): FN823122 (rnfl), FHJCS9528 (RPB2).—R. a. conica.—Exon 70 (UPS): FN823122 (rnfl), FHJCS9528 (RPB2).
S. turkestanica Regel—Kiseleva 20. VI. 1970 (MW), FJ89404 (atp1), FJ89425 (cox3), FJ89494 (nad9), FN821195 (matK), FN821315 (rps16), FN821371 (trnLF), FN821147 (ITS), HM595192 (RPB2), HQ334957 (RPD2a), HM595313 (RPD2b), S. uniflora Roth—Erixon 73 (UPS) E: FJ589304 (atp1), FJ89426 (cox3), FJ89495 (nad9), FJ89545 (matK), EU221620 (trnLF); Oxelman 2197 (GB) 34; Z83173 (rps16), X86849 (ITS), DQ988710 (RPB2), DQ988719 (RPB2), DQ988707 (RPD2a), DQ988780 (RPD2b), S. viscosa (L.) Pers.—Rautenberg 104 (UPS) B: FN821200 (matK), FN821316 (rps16), FN821372 (trnLF), FN821148 (ITS), HM595194 (RPB2), HM595251 (RPB2), HQ334958 (RPD2a), HM595316 (RPD2b); Oxelman 2288 (GB) 34; X86831 (ITS), HM595249, HM595250 (RPB2), S. vulgaris (Moench) Garcke—EF1394601, EF139465, EF139471, EF139480, EF139482 (atp1), EF139560, EF139565, EF139571, EF139580, EF139582 (cox3), EF139610, EF139615, EF139621, EF139630, EF139632 (nad9), S. vulgaris subsp. angustifolia (DC.) Hayek—Thulin 5717 (UPS): FJ376828 (matK), FN821317 (rps16), FN821374 (trnLF), FN821149 (ITS), HM595195 (RPB2), HM595252 (RPB2), S. zawadzki Herbich—Oxelman 2241 (GB): FJ89307 (atp1), FJ89429 (cox3), FJ89498 (nad9), FN821201 (matK), Z83177 (rps16), EU221621 (trnLF), X86893 (ITS), AJ634108 (RPB2), AJ634109 (RPD2a), AJ634108 (RPD2a). Viscaria alpina (L.) G. Don—Frajman & Schönswetter 11415 (LJU): FJ89308 (atp1), FJ89430 (cox3), FJ89499 (nad9), V. vulgaris Bernh.—Schönswetter & Frajman 11097 (LJU): FJ89309 (atp1), FJ89431 (cox3), FJ89500 (nad9).