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East Asian Lychnis

Phylogeny and systematics

Maria Ullbors

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Department of Systematic Botany
Supervisor: Bengt Oxelman

Abstract

Phylogenetic analysis of *Lychnis* ssp. were performed by sequencing three chloroplast regions: the *rps*16 intron, the *trn*L intron and the *trn*L-*trn*F spacer, the *psb*B-*pet*G spacer and the nuclear ITS region. The datasets were analysed through maximum parsimony, maximum likelihood and Bayesian inference. The hypothesis that all cultivated East Asian *Lychnis* ssp. should be ordered under the name of *Silene banksia* is here opposed by the differences in the genomes of these taxa. Closely related taxa are *L. wilfordii*, *L. fulgens*, *L. sieboldii* and *L. cognata*, which forms clades in all of the trees. *Lychnis coronata* and *L. chalcedonica* form successive sister groups to the *L. wilfordii* clade. The trees are largely congruent which indicates substantial support. Morphological studies of *Lychnis* resulted in a short determination key to the East Asian *Lychnis* taxa.

Contents

Introduction

The genus *Lychnis* L. belongs to the subfamily *Caryophylloideae* of the family Caryophyllaceae. *Lychnis* L. contains about 25 species that grow in N and E Africa, Europe and C and E Asia (Oxelman *et al.* 2001). The name *Lychnis* comes from the Greek word for lamp: *lychnos,* and refers to the use of grey-felted leaves of *L. coronaria* as lamp-wicks. The name was used by Theophrastus already in 370-285 BC according to the new Royal Horticultural Society Dictionary of Gardening (Huxley 1992). The taxonomy of *Lychnis* is interesting from a horticultural perspective because some of them are extensively cultivated as ornamentals. Well known decorative cultivars among others are Maltese Cross, Rose Campion and Flower-of-Jove.

The taxonomic history of *Lychnis* is complicated. Greuter (1995) included *Lychnis* as well as all other taxa of the tribe *Sileneae*, except *Agrostemma*, within *Silene*. Molecular phylogenetic studies have shown, that *Lychnis,* including its type species *L. chalcedonica*, *L. coronaria*, *L. flos-jovis*, *L. flos-cuculi*, the rare North African taxon *L. lagrangei* and the East African group previously recognized as *Uebelinia*, constitutes a monophyletic group within the tribe *Sileneae* and could be kept as its own genus aside *Silene* (Oxelman *et al.* 2001).

In the 1850´s a cultivated hybrid called *Lychnis* ×*haageana* was brought about with the parental plants *L. fulgens*, assumed from Siberia, and *L. sieboldii* from Japan. Selfing of the hybrid give rise to a variation of forms looking more or less like the *´fulgens´* type or the *´sieboldii´* type (Mabberley 1999). Several times during the history, closely related *Lychnis* plants, has been brought to Europe from Asia and been described as different species.

It is difficult to determine among some of the *Lychnis* taxa, which ones are wild types and which ones are cultivars. These disputed plants could have been cultivated during a long time before they were brought to Europe. For example, *L. coronata* is depicted in the Five Dynasties drawn by Xu Xi in the tenth century. *Lychnis coronata* could be of a hybrid origin itself (Mabberley 1999). Mabberly suggested that the East Asian *Lychnis* material should be organized under one name: *Silene banksia,* and then ordered into three informal cultivar groups.

The '*Senno*' group is regarded as the group nearest to a putative parental wild type. Here belongs, among others, *Lychnis fulgens*, *Agrostemma bungeana* and *Lychnis senno*. In the '*Coronata*' group, *Lychnis coronata*, *L. grandiflora*, *Hedona sinensis*, *L. speciosa* and *L. sieboldii* are put. In the third group, the '*Haageana*' group, *Lychnis* ×*haageana* and other putative hybrid cultivars.

Although it may be hypothesized from a biogeographical and a morphological point of view, that the East Asian *Lychnis* are closely related to *L. chalcedonica*, this has not been tested using DNA sequence data before.

In the Flora of Japan (Akiyama 2006) *L. sieboldii* is described as a wild type that grows in Japan, North East China and Korea. In Flora of China (Zhou *et al.* 2001), *L. coronata* is treated as a species growing in China and Japan, but Akiyama (2006) does not mention *L. coronata* at all. Mabberley (1999) states that he has never seen any wild material of *L. coronata* in Japan. In the Chinese and Japanese flora, *L. fulgens* and *L. wilfordii* are considered as widespread in East Asia as wild types. *Lychnis chalcedonica* grows in parts of Mongolia and Russia according to the flora of China (Zhou *et al.* 2001).

The use of phylogenetics in botany can answer many different questions. It can give an idea of evolutionary relationships, describe the rate of evolutionary change, when and how diversification of lineages occurred etc. (Judd *et al.* 2002).

Molecular techniques are of great help within phylogenetic studies. Through sequencing and comparison between DNA sequences of different species, the obtained gene phylogenies can be used to deduce species relationships. Since the *Sileneae* tribe expresses homoplasy morphologically, a molecular approach is well suited (Oxelman & Lidén 1995). During hybridization the chloroplastic DNA is inherited from the mother plant and nuclear DNA is inherited both from the mother- and the father plant. To examine a hybridization hypothesis it is necessary to examine nuclear DNA. Cytoplasmic DNA may complement and give indications on which is the maternal line.

The material in this project was examined morphologically and phylogenetically. Three chloroplast regions: the *rps*16 intron, the *trn*L intron and the *trn*L-*trn*F spacer, and *psb*B-*pet*G spacer, and the nuclear ITS region was used.

The aim of this study is to investigate the genus *Lychnis*. Taxonomy and phylogeny will be examined, especially with the consideration of the East Asian species used in cultivation. In particular, possible hybridization origins of these species, as hypothesized by Mabberley (1999), are considered.

Materials and methods

Morphological study

For morphological studies, comparisons between available herbarium specimens were performed among the different taxa, but with a concentration towards the specimen used for DNA extraction. Herbarium material from UPS, S, GB and KYO was studied (abbreviations according to Holmgren *et al.*). For recognition and comparison of the studied species, the Flora of China (Zhou *et al.* 2001) and the Flora of Japan (Akiyama 2006) were used for reference. The herbarium material was studied by means of a stereomicroscope. A key of the studied *Lychnis* taxa was compiled through the results in the morphological descriptions and with the help of the key in the Flora of China, the Flora of Japan and a key for cultivated species of *Lychnis* (Lawrence 1953).

Plant material used for PCR and phylogenetic analysis

Plant material of *Lychnis* for PCR and phylogenetic analysis, was collected from herbarium material. Additional sequences were collected from the BOxTax database (http://boxtax.ebc.uu.se) or GenBank. Material for DNA extraction was chosen with consideration of the possible amount of DNA present and also with respect of the condition of each herbarium sheet. Either seeds, leaves, buds or flowers were used for extraction. The *Lychnis* specimens used for DNA extractions, and the BOxTax/GenBank sequences used for alignments and phylogenetic analysis, are listed in Table 1.

Table 1. Plant material, BOxTax sequence ID (4-digit number) or GenBank accession nr. (Z or AJ nr.), and BOxTax specimen ID for DNA sequences used for phylogenetic analysis.

Taxon	B.T. sequence ID or GenBank accession nr.				B.T.spec. ID
	*trn*LF	*psb*E-*pet*G	*rps*16	ITS	
Agrostemma githago L. [2]	2995	2534	Z83154		7030
Atocion rupestre L.	3001	2539	Z83160		2404
Eudianthe laeta (Ait.) Reichb. Ex. Willk.	2998	2535	Z83155		1341
Heliosperma alpestre (Jacq.) Griseb.				1254	7091
Heliosperma alpestre (Jacq.) Griseb.	3002	3072	1337		7696
Lychnis chalcedonica L. [1]	2814				13725
Lychnis chalcedonica L. [1]		2817			13724
Lychnis chalcedonica L.	3007	3078			7242
Lychnis chalcedonica L. [2]			Z83164	1883	2487
Lychnis cognata Maxim. [1]				2809	13448
Lychnis coronaria (L.) Desr. [2]				1882	2488
Lychnis coronata Thunb. [1]		2816	2796	2792	13718
Lychnis flos-cuculi L.	2997	2536	Z83163	1880	2406
Lychnis flos-cuculi L. [3]				1927	12807
Lychnis flos-jovis (L.) Desr.	2996	2537	Z83166	1881	12761
Lychnis fulgens Fisch. ex Spreng. [1]				2807	13566
Lychnis kiusiana Makino [1]			2800	2791	13710
Lychnis lagrangei Coss.			86	8	7243
Lychnis miqueliana Rohrb. ex Franch.&Sav. *				2799	13707
Lychnis miqueliana Rohrb. ex Franch.&Sav.				2786	2523
Lychnis senno Siebold&Zucc. [1]			2803		13728
Lychnis sieboldii Van Houtte [1, 2]	2813		2794	2798	13704
Lychnis wilfordii (Regel) Maxim. [1]	2787		2793	2789	13701
Lychnis wilfordii (Regel) Maxim. [1]				2811	13567
Petrocoptis pyrenaica [2] (Bergeret) A. Br. Ex Walp.	2999	2538	1201	1879	2486
Silene aegyptiaca (L.) L.f.	3004	3063	1749		3446
Silene atocioides Boiss.	1752	3064	3003	1742	1176

Silene atocioides Boiss.				1741	3301
Silene atocioides Boiss.				939	1114
Silene conica L.			Z83170	1865	1964
Silene conica L.	3011	3066			7825
Silene cryptoneura Stapf.	3009	3067	1753	1739	1115
Silene fruticosa L.	3006	3070	Z83188		1064
Silene integripetala Bory&Chaub.			AJ294973	50	1056
Silene integripetala Bory&Chaub.	3012	3071			1920
Silene latifolia Poir.	3010	3073			7823
Silene latifolia Poir.			Z83171		2518
Silene littorea Brot. [2]	3016	3074		2533	13501
Silene littorea Brot.			88		2395
Silene pseudoatocion Desf.				1835	1317
Silene pseudoatocion Desf.	3005	1506	2532		7821
Silene samia Melzh.&Christodoulakis			Z83168	1829	2413
Silene samia Melzh.&Christodoulakis	3029	2648			7822
Silene schafta S.G.Gmel. ex. Hohen. [2]			1160	1827	2474
Silene schafta S.G.Gmel. ex. Hohen. [2]	2527	2647		1788	7615
Silene sordida Hub. Mor.&Reese	3008	3075			7824
Silene sordida Hub.-Mor.&Reese			1123		2411
Silene sorensenis(B.Boivin) Bocquet	3013	3076	767	760	7702
Silene uniflora Roth	3015	3077			7827
Silene uniflora Roth			Z83173		2403
Silene zawadzkii Herbich [2]	3014	3041	92		2447
Uebelinia abyssinica				44	6182
Viscaria vulgaris Bernh. [2]				45	7613
Viscaria vulgaris Bernh.	3000	2540	Z83157		2405

[1] Sequences extracted for this project

[2] Garden origin

[3] Published in Systematics of Euromediterranean Silene (Caryophyllaceae): evidence from aphylogenetic analysis using ITS sequences. C.R. Acad. Sci. Paris, Sciences de la vie/Life sciences, Evolution, 1996, 319: 351-358.

DNA extraction

A Mini-BeadBeater (BioSpec Products, USA) was used to crush the dried herbarium material. Silica beads and plant material was added to tubes. A mix of 750 µl Carlson buffer and 20 µl mercaptoethanol for each tube was preheated for 10 minutes in 60 ºC, and then added. The tubes were then put into the Mini-BeadBeater and shaken for 40 seconds at 5000 rpm. The samples were then incubated for 60 minutes and mixed every 15 minutes by turning them by hand. Following this, a one-minute centrifugation was performed and the samples were then moved to new tubes. One volume of chloroform/isoamylalcohol 24:1 was added to each tube that was put on a slow shake for 30 minutes at 100 rpm. The tubes were then centrifuged for 10 minutes at 10000 rpm. The upper, DNA containing, aqueous layer were then transferred to new tubes and mixed again with one volume of chloroform/isoamylalcohol 24:1. The tubes was inverted a couple of times then centrifuged at 5 minutes at 10000 rpm. The aqueous layers were then transferred to new tubes, 0.1 volume of 3M sodium acetate and 2 volumes of ice cold 95% ethanol were added to each tube. The tubes were then placed in a freezer overnight to precipitate.

Next day, the tubes were centrifuged for 10 minutes at 14,000 rpm to obtain the DNA pellets. The ethanol was poured off and 750 µl wash buffer of 76% ethanol and 10 mM ammonium acetate was added. After 20 minutes the tubes were centrifuged one more time for 10 minutes at 14,000 rpm. The wash buffer was then poured off. The pellets were left to dry for about 30 minutes in a fume hood and kept away from light to avoid degradation of the DNA. The pellets were then dissolved in 100 µl 10 mM Tris HCl with a pH of 8.5. Purification of each sample were the performed with GFX DNA purification kit (Amersham Biosciences).

In case any herbarium material was very old or in bad condition, several steps in the DNA extraction course were modified. For example, the tubes could be left on the slow shake with chloroform/isoamylalcohol 24:1 for about four hours instead of 30 minutes. The tubes were then exchanged after a couple of hours to avoid melting of the plastic. Also the overnight DNA precipitation was sometimes prolonged.

Amplification and sequencing

The PCR (polymerase chain reaction) were performed with 50 µl reactions. Each reaction tube contained: 1 µl DNA, 5 µl 10 X buffer supplied by the polymerase manufacturer, 3 µl (25 mM) $MgCl_2$, 1 µl (10 mM) dNTP´s, 0.3 µl TAQ-Polymerase (ABgene), 0.3 µl bovine serum albumin (Roche diagnosis) and 12.5 µl primer.

The primers used to amplify the chloroplastic DNA was *rps*F and *rps*R2R (Oxelman *et al.* 1997) for the *rps*16 intron, *trn*LA_PE and *trn*L-Sara_R for the *trn*L/F region (Erixon & Oxelman, in review) and *psb*E-RF and *pet*G-R for the *psb*E/*pet*G spacer (Erixon & Oxelman, in review). Primers used to amplify the nuclear ITS region were P17 and 26S-82R for ITS, (Oxelman & Lidén 1995, Bolmgren & Oxelman 2004).

All PCR reactions were run on an Eppendorf Mastercycler Gradient Thermal Cycler. The *rps16*- and ITS-regions were amplified with a denaturation temperature of 97 ºC for 3 minutes, followed by 34 cycles (97 ºC for 20 seconds, 55 ºC for 1 minute and 72 ºC for 2 minutes). The cycling ended with 72 ºC for 10 minutes. The *trnL*- and *petG*-region were amplified with a denaturation temperature of 98 ºC for 4 minutes followed by 34 cycles (98 ºC for 15 seconds, 58 ºC for 25 seconds, and 72 ºC for 3 minutes). The cycling ended with 72 ºC for 10 minutes.

Gel electrophoresis was performed with ethidium bromide staining on a 1% agarose gel. The PCR product was run on the gel for 30 minutes at 110 V. UV photographing of the PCR product followed to check the products.

The PCR products were purified with a Millipore cleaner. The PCR products were then sent to Macrogen, Korea, who performed the sequencing reactions.

Editing and alignment

The obtained sequences were edited and aligned, both automatically and by hand, in Geneious Pro 3.0.6 (Drummond *et al*. 2007). Multiple sequence alignment was performed in Geneious by using the automatic alignment for the complements and then manually adjust them. Simple gap coding was performed with SeqState 1.32 according to the principles of Simmons & Ochoterena (2000).

Phylogenetic analyses

Maximum parsimonious trees and bootstrap values were estimated with PAUP*, version 4.0b10 (Swofford 2000), with 100 replicates of random sequence addition, TBR branch swapping, Multrees option in effect and gaps treated as missing.

MrModeltest 2.2 (Nylander 2004) was used for choosing model parameters for maximum likelihood (parameter values fixed) and Bayesian inference analyses, for the *rps16*, *psbE-pet*G and ITS sequences data sets, using hLRTs (hierarchical Likelihood Ratio Tests). For *trnL*, Modeltest 3.7 (Posada & Crandall) was used instead of using the Akaike Information Criterion (AIC). Maximum likelihood trees were estimated with PAUP* with 100 replicates of random sequence addition, TBR branch swapping and Multrees option in effect. Bayesian inferences were conducted with MrBayes version 3.1.2 (Huelsenbeck & Ronquist 2003). To examine the parameters from the MrBayes output, Tracer 1.3 (Rambaut & Drummond 2003) was used.

Results

Morphological study

The *Lychnis* ssp. are biennial or perennial herbs that are subglaborous to pubescent. The stems are erect. Leafs are lanceolate to ovate-lanceolate and have an acute apex. The inflorescence is a dichasium or a solitary flower. The calyx is tubular to narrowly funnel-shaped or narrowly campanulate, usually not inflated. The calyx is 10-veined and has five teeth. The flowers have five petals that are long-clawed. The limb of the petals is white, pink or red and is entire, bifid, 4-fid or fimbriate with coronal scales at the base. The androgynophore is more or less conspicuous, glabrous and there are 10 stamens. The ovary is 1-loculed and there are numerous of ovules. Five styles are present opposite of the calyx teeth and their bases are persistent in the fruit. Dehiscing of the capsule is septicidal with five teeth. There are numerous of seeds and they are reniform, minute and tubercular. A key to *Lychnis* follows on the next page. Specific information about each single taxa examined morphologically is described in table 2.

Key to East Asian *Lychnis*

1. Inflorescence a dense, corymblike dichasium: 1. *L. chalcedonica*
- Inflorescence not corymblike: 2

2. Petals white: 10. *L. gracillima*
- Petals red, orange or pink: 3

3. Petal limb unlobed, apex nearly entire, petal limb orange: 2. *L. miqueliana*
- Petal limb lobed or dentate: 4

4. Petal apex dentate, unlobed, limb orange: 3. *L. coronata*
- Petal apex lobed, limb dentate or entire, orange or red: 5

5. Petal limb 2-lobed, sometimes with lateral subulate teeth, apex obtuse: 6
- Petal limb with several acute irregular fimbriate lobes: 8

6. - Petal limb orange, shallowly two-cleft, limb obovate, obscurely dentate: 5. *L. cognata*
- Petal limb red: 7

7. - Petal limb deeply two-cleft, lanceolate, bracts and calyx densely villous: 4. *L. fulgens*
- Petal limb two-cleft, limb apex clearly dentate, leaf base rounded: 9. *L. sieboldii*

8. Petal limb pink, calyx narrowly cylindrical: 6. *L. kiusiana*
- Petal limb red, calyx narrowly elliptic: 9

9. Androgynophore ca. 10 mm or more: 7. *L. senno*
- Androgynophore ca. 5 mm or less: 8. *L. wilfordii*

Table 2. Morphological description of East Asian *Lychnis*

1. ***Lychnis chalcedonica*** Linnaeus, Sp. Pl. 1: 436. 1753. *Silene chalcedonica* (Linnaeus) E.H.L.Krause.

Leaves	Ovate or ovate-lanceolate, 5-12 × 2-5 cm, both surfaces sparsely pilose.
Inflorescence	Inflorescence a terminal, dense, corymblike, 10-50-flowered dichasium. Pedicel much shorter than calyx, slender; bracts lanceolate. Flowers 1.5-2 cm in diam. Calyx tubular or tubular-clavate, 1.2-1.5(-1.7) × ca. 3 mm, pilose at veins; teeth triangular-lanceolate, ca. 3 mm.
Petals	Petal limb orange-red, broadly obovate, 7-9 mm, bifid to 1/3; lobes obovate, each with a subulate lateral tooth; claw oblanceolate, base ciliate; coronal scales linear, apex acute.
Stem	Hispid with multicellular eglandular hairs. Stems simple or rarely branched. 50-100 cm

2. ***Lychnis miqueliana*** Rohrb. Linnaea 36:677 (1870). Silene miqueliana (Rohrb.,) H. Ohashi et H. Nakai, J. Jap.Bot. 71:269 (1996)

Leaves	Nearly sessile, ovate to narrowly elliptic-lanceolate, 5-14 cm long, 2.5-5 cm wide, base attenuate, petiole like, apex acuminate, veins on both surfaces, margins pubescent.
Inflorescence	Sparsely arranged in terminal or axillary dischasia, ca. 4-5 cm across. Calyx narrowly cylindrical, 2.5-3 cm long, glabrous, apex shallowly 5-lobed; scarious, ciliate.
Petals	Petal limb cinnabar-red, widely obovate, 2.5-3 cm long, margins nearly entire.
Stem	Stems erect, upwardly branched sparsely pubescent. 50-80 cm tall.

3. ***Lychnis coronata*** Thunberg, Syst. Nat., ed. 14. 435. 1784. *Agrostemma banksia* Meerburgh; *Hedona sinensis* Loureiro; *Lychnis grandiflora* Jacquin; *Silene banksia* (Meerburgh) Mabberley; *S. grandiflora* (Jacquin) H. Ohashi & H. Nakai.

Leaves	Ovate-lanceolate, (5-)8-15 × (1-)2.5 cm, both surfaces subglabrous, ciliate at margin, base cuneate, apex acuminate.
Inflorescence	Dichasium several flowered. Pedicel very short, sparsely pubescent; bracts lanceolate, herbaceous, ciliate at margin. Flowers 4-5 cm in diam. Calyx tubular, (2.5-)3-3.5 cm × 3.5-5 mm, veins prominent, glabrous, teeth 8-10 mm.
Petals	Petal limb salmon-pink, obovate, (1.5-)2-2.5 cm, unlobed, apex irregularly incised-dentate; claw not exceeding calyx. Stamens included.
Stem	Stem solitary, rarely sparsely caespitose. 50-90 cm tall.

4. ***Lychnis fulgens*** Fischer ex Sprengel, Nov. Prevent. 26. 1818. *Silene fulgens* (Sprengel) E. H. L. Krause, Sturm, Deutschl. Fl. Ed.2, 5:96 (1901).

Leaves	Ovate or ovate-lanceolate, 3.5-10 × 2-4 cm, both surfaces and margin pilose, base rounded, rarely broadly cuneate, slightly clasping, apex acute.
Inflorescence	Dichasium several flowered, dense, rarely corymblike. Pedicel 3-12 mm; bracts lanceolate, herbaceous, densely villous, ciliate at margin. Flowers 3.5-5 cm in diam. Calyx narrowly campanulate, 1.5-2.8 cm × 4-8 mm, villous, densely so at veins, slightly inflated in fruit; teeth triangular.
Petals	Petal limb crimson-red, 2-lobed to 1/2; lobes linear, apex obtuse, sometimes obscurely denticulate, each lobe with a subulate, lateral tooth; claw narrowly lanceolate, not exceeding calyx; coronal scales dark red, narrowly elliptic.
Stem	Stems simple or branched above. 50-85 cm tall.

5. ***Lychnis cognata*** Maximowicz, Prim. Fl. Amur. 55. 1859. *Lychnis fulgens* Fischer var. cognata (Maximowicz) Regel; *Silene cognata* (Maximowicz) H. Ohashi & H. Nakai.

Leaves	Ovate-lanceolate, 5-11 × 1-4 cm, more densely pilose at veins, base broadly cuneate, apex acute.
Inflorescence	Dichasium several-flowered, or sometimes flowers solitary in leaf axils. Pedicel 3-12 mm; bracts laxly villous. Flowers 3.5-5 cm in diam. Calyx narrowly campanulate, 2-2.5 cm × 3.5-5 mm, laxly villous at veins, slightly inflated in fruit; teeth triangular, ca. 3 mm.
Petals	Petal limb orange-red or reddish, bifid, apically obtuse, each with a subulate lateral tooth, main lobes obovate, margin entire or obscurely denticulate; claw slightly protruding from calyx, glabrous, base narrowly cuneate; coronal scales dark red, apex dentate.
Stem	Stems simple or branched above. 30-90 cm tall.

6. ***Lychnis kiusiana*** Makino, Bot. Mag. Tokyo 17:57(1903). *Silene kiusiana* Makino, H. Ohashi & H. Nakai, J. Jap. Bot. 71:269 (1996).

Leaves	Sessile, linear-lanceolate, 4.5-11 cm long, 0.6-1.2 cm wide, base cuneate to rounded, both surfaces and margins sparsely pubescent.
Inflorescence	Flowers in dichasia, 1.5-2.5 cm across. Pedicel of terminal flower 0.6-1.6 cm long. Calyx narrowly cylindrical, 2-2.5 cm long, pubescent.
Petals	Petal clawed; limb pink, obovate, ca. 1 cm long. Apex fimbriate.
Stem	Stems 0.6-1 m tall, reflexed hairs.

7. ***Lychnis senno*** Siebold & Zuccarini, Fl. Jap. 1:98, 1839. *Agrostemma bungeana* D. Don; *Lychnis bungeana* (D. Don) Fischer ex Lindley; *Silene bungeana* (D. Don) H. Ohashi & H. Nakai.

Leaves	Elliptic-lanceolate, (4-)8-12 × 2-3 cm, both surfaces pubescent, ciliate at margin, base cuneate, apex acuminate.
Inflorescence	Dichasium many flowered. Pedicel 2-5(-15) mm; bracts lanceolate, pubescent. Flowers 3.5-5 cm in diam. Calyx tubular to narrowly funnel-shaped, (2-)2.5-3 cm × 2.5-3.5 mm, slightly inflated in fruit, pilose at veins; teeth triangular, 2-4 mm.
Petals	Petal limb deep red, triangular-obovate, irregularly parted into numerous incised-dentate lobes; claw often exceeding calyx, narrowly cuneate, glabrous.
Stem	Stem solitary, simple or branched above. 50-100 cm tall.

8. ***Lychnis wilfordii*** Regel Maximowicz, Bull. Acad. Imp. Sci. Saint-Petersburg. sér. 3, 17: 178. 1872. *Lychnis fulgens* Fischer var. wilfordii Regel, Bull. Soc. Imp. Naturalistes Moscou 34(4): 576. 1861; *Silene wilfordii* (Regel) H. Ohashi & H. Nakai, J. jap. Bot.

Leaves	Sessile, ovate-lanceolate or narrowly lanceolate, 3-12 × 1-2.5 cm, glabrous, margin thickly ciliate.
Inflorescence	Dichasium rather dense, many flowered. Pedicel 3-20 mm; bracts linear-lanceolate. Flowers 2.5-3 cm in diam. Calyx narrowly funnel-shaped, 1.5-2 cm × 4-5 mm, veins prominent, teeth ca. 3 mm.
Petals	Petal limb bright red, 4-lobed; lobes subulate to broadly filiform, apex acute; claw narrowly cuneate; coronal scales dark red, oblong.
Stem	Stems simple or branched above. 45-100 cm tall.

9. ***Lychnis sieboldii*** Van Houtte, Fl. Serres 10:31 (1855). *Lychnis coronata* Thunb. var. *sieboldii* (Van Houtte) L. H. Bailey, Stand. Cycl. Hort. 4: 1929 (1916). *Silene sieboldii* (Van houtte) H. Ohashi & H. Nakai, J. jap. Bot. 71:270 (1996).

Leaves	Sessile, narrowly ovate to widely ovate, 5-8 cm long, 2.5-4.5 cm wide, base rounded, apex acuminate, both surfaces pubescent.
Inflorescence	Densly arranged in dichasia, 2.5-3.5 cm across, short pedicle. Calyx cylindrical, 2.5-3 cm long, 5-lobed, veins near apex with long soft hairs.
Petals	Petals deep red, rarely white, 2-2.5 cm long, clawed, limb ovate, shallowly bilobed, margins of lobes near apex denticulate.
Stem	Stems erect, 40-90 cm tall, with reflexed hairs.

10. ***Lychnis gracillima*** (Rohrb.) Makino, in Jap. Bot. 2:19 (1921). Rohrb. In Linnaea 36:679 (1870).

Leaves	Sessile, lanceolate to widely lanceolate, 5-14 cm long, 1-3.5 cm wide, base cuneate to rounded, apex acuminate, upper face glabrous, lower surface nearly glabrous.
Inflorescence	Dichasia ca. 2 cm across. Pedicle slender, 2-6 cm long. Calyx cylindrical at flowering and campanulate in fruit, 0.7-1.2 cm long, apex shallowly 5-lobed.
Petals	Petals white, shallowly bilobed, margins denticulate.
Stem	Stems erect, 0.4-1 m tall, upwardly branched with soft hairs.

Phylogenetic analysis

The "*Lychnis coronaria*" sequence in the ITS tree, had a phylogenetic position which was at odds to previous published ITS data based on other material determined as *L. coronaria* (Oxelman & Lidén 1995, Desfeux *et al*. 1996, Popp & Oxelman 2004). The sequence had highest BLAST similarity to a *Silene gallica* sequence off those deposited at GenBank per January 29, 2008. There has likely been some kind of error with regards to this DNA sample. The specimen voucher of this sequence is from Kew (Kew ID: 8851) according to accession number AY857966 from GenBank.

Most parsimonious trees (MPTs), maximum likelihood trees (MLTs) and MrBayes trees were formed for each sequences of ITS, *rps*16, *trn*L and *pet*G. For ITS, the search for MPTs resulted in 531 trees with an consistency index (CI) of 0.5800 and retention index (RI) of 0.7361. For *rps*16, the search for MPTs resulted in 368 trees with an CI of 0.5808 and RI of 0.6949. A MP strict consensus tree of *rps*16, with branch lengths and bootstrap values for the ingroup, is shown in fig. 1. One of the ITS MLTs with branch lengths and bootstrap values is shown in fig. 2. Bayesian inference analysis resulted in trees supporting the MPTs and MLTs.

The bootstrap analysis showed strong support for a monophyletic *Lychnis* clade that was separated from *Silene* in all the trees. In the *trn*L and *rps*16 MPTs, *L. wilfordii* and *L. sieboldii* grouped together with over 95% bootstrap support in both of the trees. This grouping was also true for the *rps*16 and *trn*L MLTs. *Lychnis chalcedonica* and *L. coronata* were close sistergroups in the trees with an 80% bootstrap support in the *pet*G MPT. *Lychnis fulgens*, *L. wilfordii*, *L. cognata* and *L. sieboldii* formed a monophyletic group with 69% bootstrap support.

The chloroplast DNA trees supported each other. The *trn*L trees supported the clade of *L. wilfordii* and *L. sieboldii* in the *rps16* trees. The *petG* trees only consisted of two interesting *Lychnis* taxa, and that was *L. chalcedonica* and *L. coronata*, which formed a clade with an over 80% bootstrap support in the MPT.

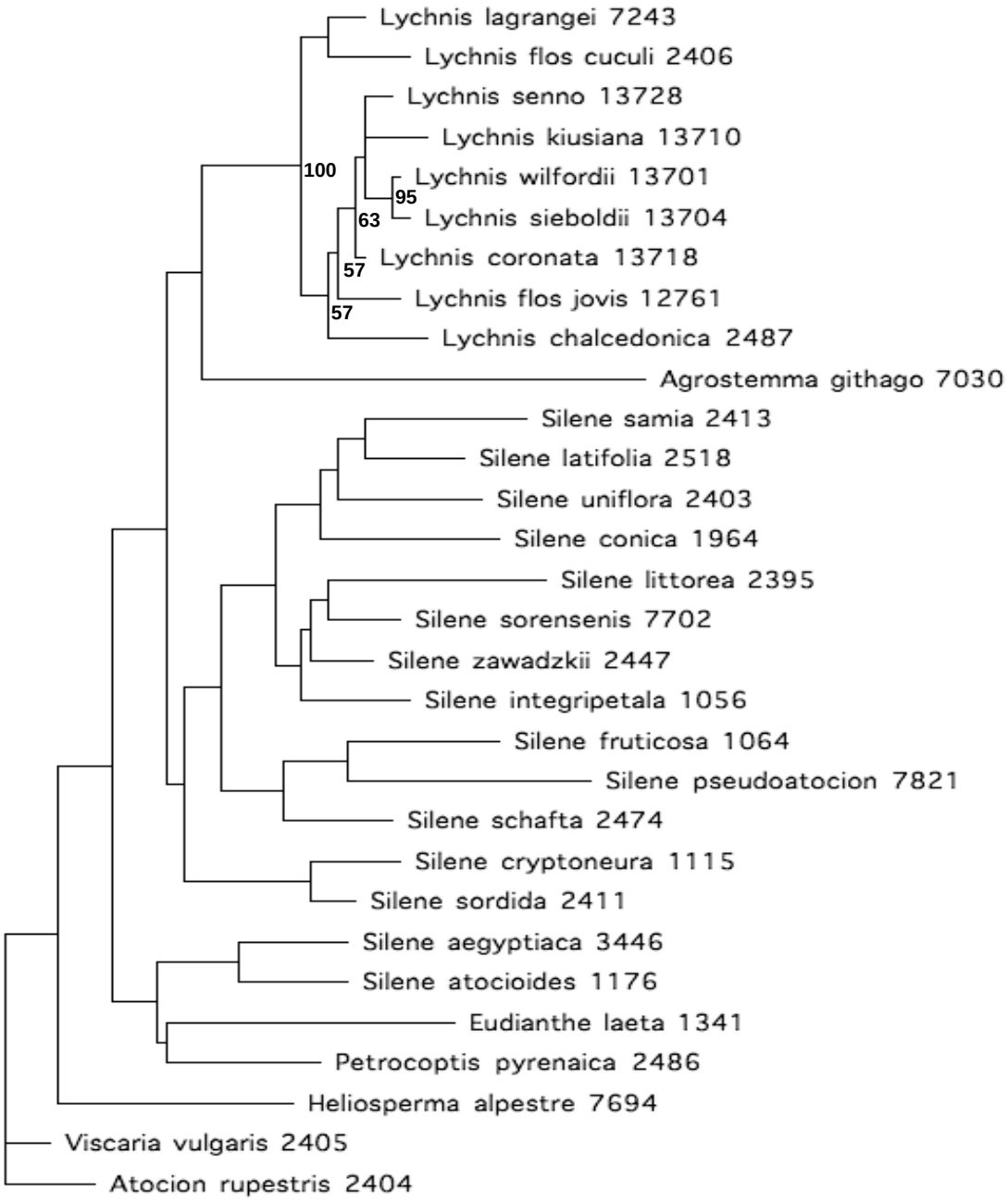

Fig. 1 One of 368 most parsimonious trees recovered from the heuristic searches of the *rps*16 dataset. Branch lengths and significant bootstrap values for the ingroup are shown. The numbers indicate BOxTax specimen ID (see Table 1 for details).

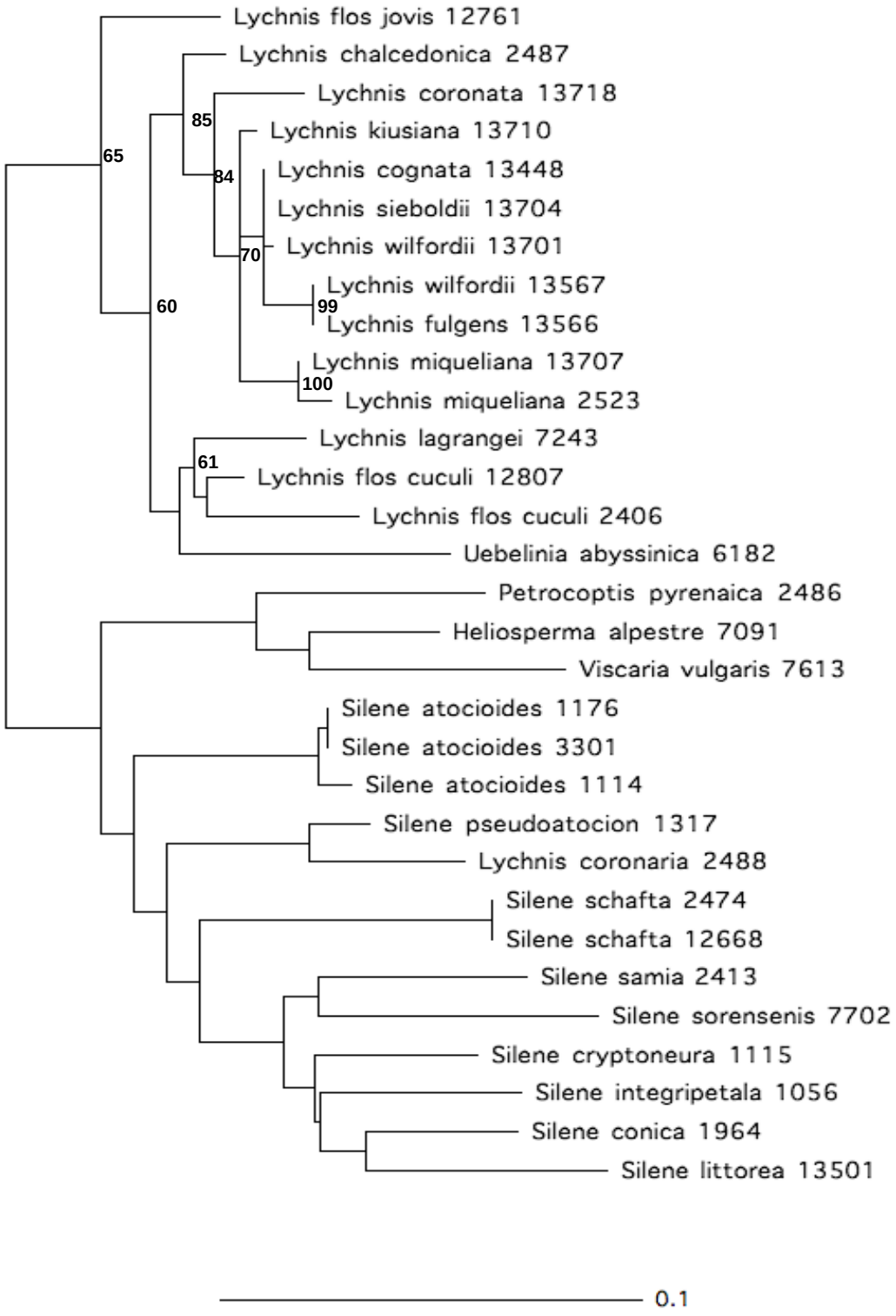

Fig. 2 A most likely phylogram of the ITS region resulting from maximum likelihood analysis. Branch lengths and bootstrap values for the ingroup are shown. The numbers indicate BOxTax specimen ID (see Table 1 for details).

Discussion

According to Mabberley (1999), selfing of the garden hybrid *Lychnis ×haageana* results in forms looking more or less like *L. sieboldii* and *L. fulgens*. These two taxa are closely related in the phylogenetic trees (fig. 1, fig. 2) and are in their turn closely related to *L. wilfordii, L. cognata* and *L. kiusiana*.

Mabberley (1999) suggested three informal groups under the name of *Silene banksia*. One of these includes *Lychnis coronata* and *L. sieboldii*, but there is no support for this from the molecular data in the phylogenetic trees (fig. 1, fig. 2). Since *L. wilfordii* and *L. sieboldii* form clades together that exclude *L. coronata*, there is no need for synonymization of *L. coronata* and *L. sieboldii* as Mabberley suggests.

Mabberley (1999) argues that if *L. coronata* has a hybrid origin, then *L. fulgens*, which is morphologically similar to *L. wilfordii*, should be examined as a putative parent. He also thinks *L. senno* could be closely related to *L. fulgens* and this seems to be the case according to the *rps*16 tree (fig. 1).

The literature agrees about the geographical distribution of *L. fulgens* and *L. wilfordii* and the phylogenetic relationship between these two are close according to the trees. They are distributed both in Japan and China as wild types. The East Asian *Lychnis* species are closely related to *L. chalcedonica* that grows in parts of Mongolia and Russia. *Lychnis coronata* is mentioned as a plant used medicinally in the flora of China (Zhou *et al.* 2001), which could indicate wild origin, but Mabberley states that he hasn't seen any wild *L. coronata*. *Lychnis coronata* is morphologically similar to *L. cognata* and *L. sieboldii*. If *L. coronata* is a garden hybrid, a difference in the placement in the *rps*16 and ITS trees would support this, but the trees are congruent. More data is needed to elucidate the origin of *L. coronata* and the certain garden hybrid *Lychnis ×haageana* should be included in further experiments for comparison.

Mabberley (1999) proposed that all cultivated East Asian *Lychnis* material should be named *Silene banksia*. The results presented here, does demonstrate considerable sequence variation that is phylogenetically congruent between the nuclear and chloroplast regions studied. The ITS and rps16 sequence variation is shown as branch lengths in fig. 1 and fig. 2. The grouping under the name of *Silene banksia* is not supported by my study.

It would be a good idea to perform hybridization experiments with wild-collected material in the future, as suggested by Mabberley (1999). These experiments should be combined with further molecular analyses. Garden hybrids should be sequenced and compared with wild collected material to identify which *Lychnis* taxa are wild and not.

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