UPPSALA
UNIVERSITET

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 328

Taxonomy and Reticulate Phylogeny of *Heliosperma* and Related Genera (*Sileneae*, Caryophyllaceae)

BOŽO FRAJMAN

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2007

ISSN 1651-6214
ISBN 978-91-554-6946-7
urn:nbn:se:uu:diva-8171

Dissertation presented at Uppsala University to be publicly examined in Lindahlsalen, EBC, Norbyvägeb 18A, Uppsala, Thursday, September 27, 2007 at 10:00 for the degree of Doctor of Philosophy. The examination will be conducted in English.

Abstract

Frajman, B. 2007. Taxonomy and Reticulate Phylogeny of *Heliosperma* and Related Genera (*Sileneae*, Caryophyllaceae). Acta Universitatis Upsaliensis. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 328. 34 pp. Uppsala. ISBN 978-91-554-6946-7.

Heliosperma (nom. cons prop.) comprises 15—20 taxa, most of them endemic to the Balkan Peninsula. DNA sequences from the chloroplast (*rps16* intron, *psbE-petG* spacer) and the nuclear genome (ITS and four putatively unlinked RNA polymerase genes) are used to elucidate phylogenetic relationships within *Heliosperma*, and its position within *Sileneae*. Three main lineages are found within *Heliosperma*: *Heliosperma alpestre, H. macranthum* and the *H. pusillum*-clade. The relationships among the lineages differ between the plastid and the nuclear trees. Relative dates are used to discriminate among inter- and intralineage processes causing such incongruences, and ancient homoploid hybridisation is the most likely explanation.

The chloroplast data strongly support two, geographically correlated clades in the *H. pusillum*-group, whereas the relationships appear poorly resolved by the ITS data, when analysed under a phylogenetic tree model. However, a network analysis finds a geographic structuring similar to that in the chloroplast data. Ancient vicariant divergence followed by hybridisation events best explains the observed pattern. The morphological and taxonomical diversity in the *H. pusillum*-group is possibly ecology-induced, and is not correlated with the molecular data.

Phylogenetic patterns regarding the origin of *Heliosperma* are complicated, probably influenced by reticulate and sorting events. At least two ancient lineages have been involved in its evolution, one most closely related to *Viscaria*/*Atocion* and the other to *Eudianthe*/*Petrocoptis*.

Atocion and *Viscaria* are sister genera, most species-rich on the Balkans, and including six/three species. Phylogenies do not support their traditional classification, and provide a framework for a taxonomic revision. *Atocion compactum* is found in three different positions in the chloroplast tree, and in a single clade in the nuclear gene trees. Using relative dates we demonstrate that hybridisation with subsequent chloroplast capture is a feasible explanation for the pattern observed. This, and other observed reticulate patterns, highlights the importance of hybridisation in plant evolution.

Keywords: Sileneae, *Silene*, *Heliosperma*, *Atocion*, *Viscaria*, taxonomy, phylogenetics, relative dating, reticulate evolution, chloroplast capture, homoploid hybridisation, BEAST, r8s, PATHd8, Prometheus, *RPA2, RPB2, RPD2a, RPD2b*, Balkan Peninsula, endemics

Božo Frajman, Department of Evolution, Genomics and Systematics, Norbyv. 18C, Uppsala University, SE-75236 Uppsala, Sweden

ISSN 1651-6214
ISBN 978-91-554-6946-7
urn:nbn:se:uu:diva-8171 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-8171)

Distributor: Uppsala University Library, Box 510, SE-751 20 Uppsala
www.uu.se, acta@ub.uu.se

List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I Frajman, B., and R. K. Rabeler. 2006. Proposal to conserve the name *Heliosperma* against *Ixoca* (Caryophyllaceae, *Sileneae*). Taxon 55: 807-808.

II Frajman, B. 2007. Proposal to reject the name *Cucubalus quadrifidus* (*Heliosperma quadrifidum*, *Silene quadrifida*) (Caryophyllaceae, *Sileneae*). Taxon 56: 260-261.

III Frajman, B., and B. Oxelman. 2007. Reticulate phylogenetics and phytogeographical structure of *Heliosperma* (*Sileneae*, Caryophyllaceae) inferred from chloroplast and nuclear DNA sequences. Mol. Phylogen. Evol. 43: 140-155.

IV Frajman, B., Eggens, F., and B. Oxelman. Hybrid origins and homoploid reticulate evolution within *Heliosperma* (*Sileneae*, Caryophyllaceae) – a multigene phylogenetic approach with relative dating. Manuscript.

V Frajman, B., Heidari, N., and B. Oxelman. Phylogenetic relationships of *Atocion* and *Viscaria* (*Sileneae*, Caryophyllaceae) inferred from chloroplast, nuclear ribosomal, and low-copy gene DNA sequences. Manuscript.

VI Frajman, B., Thollesson, M., and B. Oxelman. Taxonomic revision of *Atocion* and *Viscaria* (*Sileneae*, Caryophyllaceae). Manuscript.

Papers I-III are reproduced in this thesis with the kind permission of the publishers.

In all papers BF had major responsibility for writing the text, with comments and suggestions given by the co-authors, mainly BO. BF was responsible for most of the lab work, as well as for the phylogenetic analyses. BO was responsible for the age estimation in paper III. FE was responsible for dating performed with PATHd8 and r8s in Paper IV. NH was responsible for DNA extraction from c. ten herbarium specimens, amplification of sequences from these, and some additional sequences used in Paper V. MT was responsible for the development of the Prometheus ontology for the *Sileneae* database, which was used in Paper VI. The studies were planned and conducted in cooperation between BF and BO. Sequences not yet submitted to GenBank are replaced with the accession numbers from the *Sileneae* database (http://boxtax.ebc.uu.se).

Important note. In Paper V new nomenclatural combinations are made, and in Paper VI *A. reuterianum* is lectotypified. These papers will be submitted for publication elsewhere. In order to make clear that the new names are not validly published in this thesis, the references to the basionyms, necessary according to the International Code of Botanical Nomenclature, are omitted. The same is done by omitting the statement "hic designatus", needed for a lectotypification to be valid.

Contents

Abbreviations

cpDNA	chloroplast DNA
nrDNA	nuclear ribosomal DNA
ITS	Internal transcribed spacer
RNAP	RNA polymerase
MPB	Maximum Parsimony Bootstrap
PP	Posterior Probability
Myr	Milion years

Introduction

Molecular phylogenetics, the study of evolutionary relationships among organisms based on molecular, mostly DNA sequence data, has revolutionised systematics during the last 15 years. New insights, often contradicting the traditional, primarily morphology-based hypotheses of relationships among taxa, have led to improved classifications that, allegedly, better reflect "true" evolutionary relationships. Much information about phylogenetic relationships is still lacking, but some groups have been well studied. Among them is the tribe *Sileneae* DC. ex Ser. (Caryophyllaceae), where a considerable amount of molecular data has been accumulated and several studies have focused either on overall phylogeny of the tribe, i.e. relationships among different genera (e.g. Oxelman and Lidén, 1995; Oxelman et al., 1997; Popp and Oxelman, 2004; Erixon and Oxelman, 2006), or on the interspecific relationships within smaller groups, mostly in *Silene* L. (e.g. Popp and Oxelman, 2001; Popp et al., 2005; Eggens et al., 2007).

The tribe *Sileneae* comprises c. 700, mostly herbaceous species, distributed mainly in the Northern Hemisphere. The most recent generic classification of the tribe recognised eight genera (Oxelman et al., 2001), among which *Silene* is the most diverse and species-rich, having a world-wide distribution (not native in Australia). Other, smaller genera of the tribe, such as *Heliosperma* (Rchb.) Rchb., *Atocion* Adans. and *Viscaria* Bernh. have predominantly European distributions.

Heliosperma

Heliosperma, nom. cons. prop., (≡ *Ixoca* Raf.), comprises approximately 15 to 20 taxa. Most of them are endemic to relatively small areas of the Balkan Peninsula. However, *H. alpestre* (Jacq.) Griseb. is endemic in the Eastern Alps (Fig. 1), and *H. pusillum* (Waldst. & Kit.) Rchb. is more widespread, distributed in the southern European mountains from the Sierra Cantabrica to the Carpathians (Chater et al., 1993; Jalas and Suominen, 1986). The *Heliosperma* species mostly grow in damp, open habitats, often on calcareous rocks. Plants belonging to *Heliosperma* are caespitose, perennial herbs with slender branched stems up to 30 cm. The calyx is funnel-shaped, the petals are white, or pink, with a 4(-6)-lobed limb. The gynoecium is composed of three (rarely four or five) carpels, with a corresponding number of styles, and a capsule dehiscing with six (rarely eight or ten) teeth. The variation in habit, leaf-shape and hairiness shows some geographical and ecological correlation, which has been treated variously by taxonomists in the past.

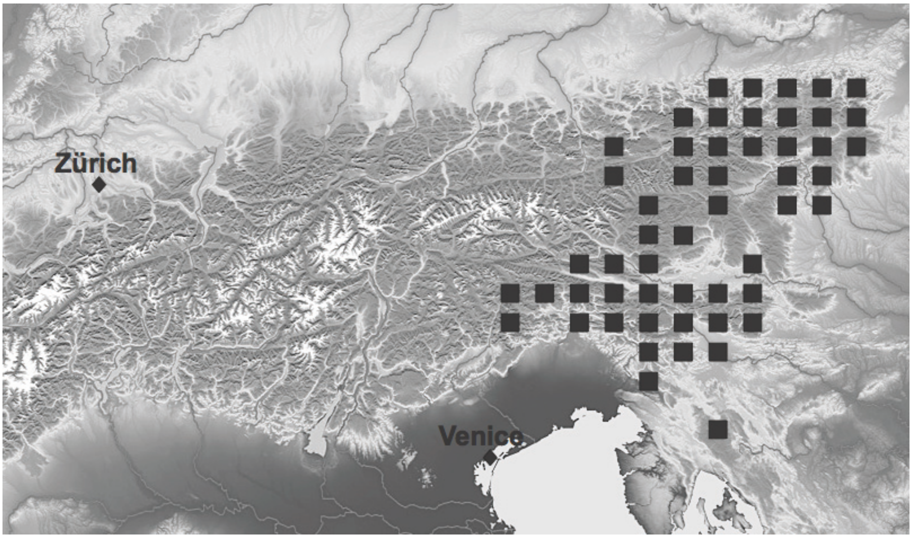

Figure 1. Distribution of *Heliosperma alpestre*, based on data compiled for the project IntraBioDiv (http://intrabiodiv.vitamib.com/). Only confirmed records were included. Distribution data provided by H. Niklfeld, Department of Biogeography and Botanical Garden, University of Vienna. Background reproduced from http://upload.wikimedia.org.

The circumscription of *Heliosperma* is traditionally based on the characteristic seeds, which are winged with a dorsal crest consisting of two or more rows of long papillae, and the quadrifid petals. The position of *Heliosperma* within *Sileneae* has been unstable throughout the taxonomic history. The only species of *Heliosperma* described by Linnaeus (1753) was *H. quadrifidum* (L.) Griseb., as *Cucubalus quadrifidus* L. (the name now being proposed for rejection). It was soon transferred to the genus *Silene* L. (Linnaeus, 1759) but treated as *Lychnis* L. by Scopoli (1772) and Linnaeus (in Murray, 1774). Later, most botanists recognised these plants as *Silene*, until Reichenbach (1832, 1841) described *Heliosperma*, first as a part of *Silene* and later as an independent genus. A similar approach was taken by Rafinesque (1840), who applied the generic name *Ixoca* Raf. to these plants, but this name was not used again until recently.

Most authors of the 19th century recognised *Heliosperma* as an independent genus within *Sileneae* (Table 1), with the exception of Fenzl (in Endlicher 1839), who treated *Heliosperma* as a part of *Saponaria* L. (including also *Melandrium* Röhl. and some other *Silene* species). Braun (1843) argued that *Saponaria* sensu Fenzl cannot exist as a natural group, due to too few similarities among *Heliosperma, Saponaria* sensu stricto and *Melandrium*. He thought *Heliosperma* might be related to *Melandrium* due to its paracarpous ovary, or to some *Silene*, like *S. rupestris* L. (≡ *Atocion rupestre* (L.) Oxelman), that it resembles in the overall appearance. In Rohrbach's (1868) opinion, *Heliosperma* is most closely related to *Silene* sensu stricto, but shares some characteristics with *Melandrium* and *Petrocoptis* A. Braun. Williams (1896) recognised the similarity in habit between *Heliosperma* and some *Silene*. However, he primarily used fruit characters in his classification and treated *Heliosperma* as closely related to *Melandrium*, both having a capsule dehiscing by twice as many teeth as the number of styles. According to Williams (1896), their closest relatives are *Lychnis* and *Petrocoptis*, all having the carpels opposite the calyx teeth, a conspicuous anthophore and an apparently unilocular capsule. Neumayer (1923a, 1923b) argued in favour of lumping *Heliosperma, Lychnis, Viscaria, Uebelinia* Hochst. and some *Melandrium* species with *Silene*. Ascherson and Graebner (1920), and Pax and Hoffman (1934), as well as most of the botanists during that period, recognised *Heliosperma* as an independent genus. Chowdhuri (1957) thought that the seed shape is not an adequate character to recognise *Heliosperma* at the generic level. He treated it as a section within *Silene*, »closely related to (and probably derived from) sect. *Rupifraga* Otth.« the latter including *Atocion rupestre*. Since then, most authors have followed Chowdhuri and treated *Heliosperma* as a section of *Silene*, without discussing its relations to other sections or genera (e.g. Chater and Walters, 1964; Meusel and Mühlberg, 1978-1979; Chater et al. 1993, Bittrich, 1993; Greuter 1995).

Table 1. Classification of *Heliosperma* in taxonomic history and number of species (taxa down to subspecific rank) recognised.

Reference	**Classification**	**Number of species (taxa)**
Reichenbach (1832)	*S.* [unranked] *Heliosperma*	3
Fenzl (1839)	*Saponaria*	2
Rafinesque (1840)	*Ixoca*	1
Reichenbach (1842)	*Heliosperma*	3
Braun (1843)	*Heliosperma*	2
Rohrbach (1869-70)	*Heliosperma*	5
Williams (1896)	*Heliosperma*	-
Neumayer (1923, 1927)	*S.* sect. *Heliosperma*	3 (17)
Ascherson and Graebner (1929)	*Heliosperma*	7 (c. 15)
Chowduri (1957)	*S.* sect. *Heliosperma*	4
Chater and Walters (1964)	*S.* sect. *Heliosperma*	9 (11)
Sojak (1972)	*Ixoca*	7
Trinajstić (1979)	*Heliosperma*	13 (15)
Greuter et al. (1984)	*Silene*	11 (16)
Ikonnikov (1984)	*Ixoca*	18
Chater et al. (1993)	*Silene*	8 (10)
Greuter (1995)	*S.* sect. *Heliosperma*	-
Oxelman et al. (2001)	*Ixoca*	c. 8

Oxelman and Lidén (1995) and Oxelman et al. (1997, 2001), on the basis of molecular phylogenies inferred from plastid *rps*16 intron sequences and nuclear ribosomal internal transcribed spacer (ITS) sequences, argued for recognition of *Heliosperma* at the generic level, clearly separated from the core of *Silene* and *Lychnis*. The exact position of *Heliosperma* within *Sileneae* remained uncertain.

Figure 2. Heliosperma oliverae, the most recently described species of *Heliosperma*. It is only known from the Prokletije Mts (Albania/Montenegro/Serbia) from only a few localities, where it grows in northerly exposed siliceous rock crevices. Here at Maja Šćapica Mt. (*locus classicus*), Prokletije Mts., Montenegro (photo B. Frajman, July 2005).

As far as the interspecific relationships are concerned, no recent revisionary work has been done on *Heliosperma*. The most comprehensive study was performed by Neumayer (1923b, 1927), who recognised three species: *H. macranthum* Pančić, *H. alpestre* and *H. pusillum* (as *Silene quadridentata* (L.) Pers.), the latter including 16 infraspecific taxa. Ascherson and Graebner (1920) thoroughly revised Central European *Heliosperma*, recognising seven species, with many infraspecific taxa. Chowdhuri (1957) listed only four species, whereas Chater and Walters (1964) recognised seven species. Trinajstić (1979), in his revision of Yugoslavian *Heliosperma* taxa (almost all taxa in the genus grow in the territory), recognised 13 species (plus two additional subspecies), Greuter et al. (1984) 11 species (and five subspecies) and Chater et al. (1993) eight species. Only Ikonnikov (1984), who made several new combinations in *Ixoca*, dealt with interspecific relationships and described two sections, *Ixoca* sect. *Ixoca* Ikonn. and *Ixoca* sect. *Pusilla* Ikonn., the former including two species (*H. arcanum* Zapał. and *H. alpestre*) and the latter all other species (16 recognised by Ikonnikov). The number of taxa (species) recognised by botanists in the past is presented in Table 1. Recently, one further species has been described, *H. oliverae* Niketić & Stevanović (Niketić and Stevanović, 2007; Fig. 2).

Atocion and *Viscaria*

Compared to *Heliosperma*, the taxonomic position of *Atocion* and *Viscaria*, since the beginning of the Linnaean classification in 1753, has been more stable. Until the recent phylogenetic studies by Oxelman and Lidén (1995) and Oxelman et al. (1997), *Atocion* taxa have been classified into two sections of *Silene*, sect. *Rupifraga* Otth, and sect. *Compactae* (Boiss.) Schischk., which were thought to be closely related (e.g. Otth, 1824; Rohrbach, 1868; Williams, 1896; Chowdhuri, 1957). Rafinesque (1840), who split *Silene* into several smaller genera, classified *S. armeria* L. (Fig. 3) as an independent genus *Atocion* (*A. armeria* (L.) Raf.). This was followed by Oxelman et al. (2001), who, based on molecular studies, transferred some species related to *A. armeria* from the sections *Rupifraga* and *Compactae* to *Atocion*, which now contains six species. They are annuals to short lived perennials with Eurasian distribution, from North-West Europe to Iran.

Viscaria has often been classified as an independent genus, or alternatively, lumped with *Lychnis*, to which it has been thought to be closely related based on the five-styled ovary and capsule dehiscing by five teeth (Otth, 1824; Rohrbach, 1868; Williams, 1896; Chowdhuri, 1957). Molecular studies (Oxelman and Lidén, 1995; Oxelman et al., 1997) have shown that *Viscaria* is sister to *Atocion* and not related to *Lychnis*. The distribution range of *Viscaria* covers Europe, Greenland and northern North America.

Figure 3. The inflorescence of *Atocion armeria* (L.) Raf. is a compound dichasium. Due to its purple-pink flowers, this species is often grown in gardens as an ornamental. Photo from a natural population in west Serbia (photo B. Frajman, July 2005).

Reticulate evolution

Ideas about reticulate relationships in plants, often explained by hybridisation, are old. The ability of different species to hybridise has often been used as a proof of their relatedness, also in *Sileneae* (Gärtner, 1849; Nägeli, 1865; Rohrbach, 1868). Molecular phylogenetics, however, has shown, that the ability of hybridisation does not necessarily imply that the hybridising taxa are most closely related (e.g. Popp and Oxelman 2001). The long-lasting controversies and conflicting ideas about relationships among different groups of *Sileneae* (e.g. Otth, 1824; Fenzl in Endlicher, 1839; Braun, 1843; Rohrbach, 1868; Williams, 1896; Neumayer, 1923; Chowdhuri, 1957) may suggest that reticulate events could have been involved in the evolution of *Sileneae*. On the basis of conflicting morphological characteristics Rohrbach (1868) drew a network (Fig. 4) representing his ideas about complex relationships within *Sileneae*. Although *Sileneae* phylogenies based on DNA sequences have not corroborated Rohrbach's ideas in many aspects, the notion that reticulate relationships are prevalent in *Sileneae*, still remains.

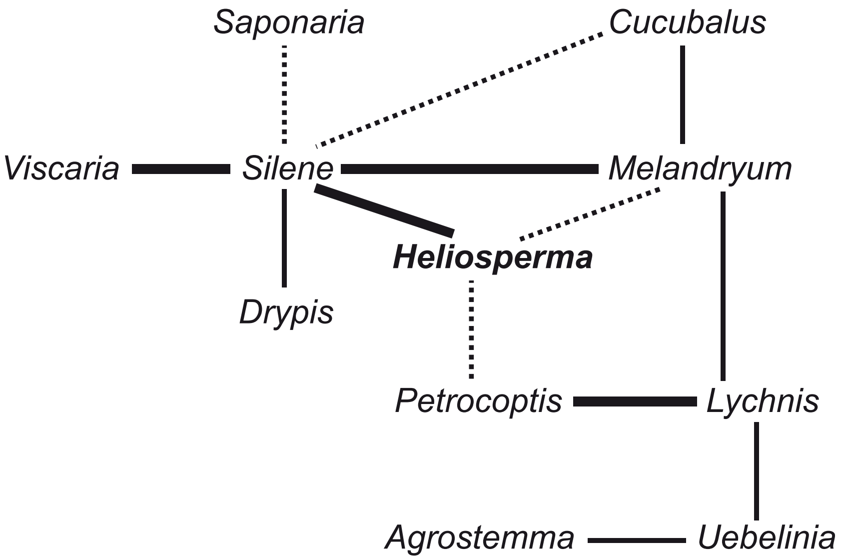

Figure 4. Reticulate relationships within *Sileneae* as drawn by Rohrbach (1868). The line width represents the degree of relatedness.

Since its beginning some 20 years ago, molecular phylogenetics has become a powerful tool to test hypotheses on relationships among taxa, and to detect possible reticulation events in the evolutionary history of organisms. Hybridisation between different lineages can be reflected in discordant gene phylogenies. However, caution is needed when such incongruences are detected, since several other processes can lead to similar patterns (reviewed by Wendel and Doyle, 1998). These processes can be interlineage (hybridisation, lateral gene transfer between organismal lineages) or intralineage (incomplete lineage sorting, orthology/paralogy conflation, which may be further complicated by recombination between alleles or genes). Moreover, phylogenetic trees inferred from different genes may disagree due to systematic (the phylogenetic models and methods applied fail to converge to the correct phylogeny) or stochastic errors. Even if it is difficult to discriminate among those processes, data from several unlinked regions, and estimations of the relative ages of conflicting groups in different gene phylogenies, can be used to potentially distinguish among these events (Holder et al., 2001; Rosenberg and Nordborg, 2002). The application of low-copy nuclear gene trees has improved the understanding of reticulate evolution in polyploids, to lesser extent also in diploid taxa. However, the extent of homoploid hybridisation in nature, leading to the formation of new organismal lineages at the same ploidy level as their progenitors, remains unclear (Rieseberg, 1997).

Aims

The studies presented here deal primarily with three genera in the tribe *Sileneae*: *Heliosperma*, *Atocion* and *Viscaria*. The first three papers are exclusively dedicated to *Heliosperma*, the fourth paper to *Sileneae* with emphasis on *Heliosperma*, whereas the last two papers deal with *Atocion* and *Viscaria*.

Papers I and II address nomenclatural issues. The aim of the first of them is to present arguments for rejecting the senior name *Ixoca* and conserving its junior synonym *Heliosperma*, which has been used more extensively in the taxonomic literature and is better known to botanists from the major part of the distribution area of the genus. The second paper presents arguments for rejecting the Linnaean name *Cucubalus quadrifidus* L. (= *H. alpestre*), which was, soon after its introduction, misapplied and commonly used for another species of *Heliosperma*.

The aim of **Paper III** is to examine phylogenetic and biogeographic patterns within *Heliosperma* and to investigate whether the differentiation of *Heliosperma pusillum* sensu lato into low and high elevation taxa is due to phylogenetic or ecological differentiation.

In **Paper IV** we explore the potential of multiple gene phylogenies to address the question of the origin of *Heliosperma*, and the relationships among its three major lineages, the *H. alpestre*, the *H. macranthum*, and the *H. pusillum* group. We use the relative ages of well supported groups to address conflicting gene phylogenies concerning the relationships between the *Heliosperma* lineages and their implications for the hypothesised hybridisation history within the genus, and the relationships to other *Sileneae* taxa.

The last two papers focus on *Atocion* and *Viscaria*, two sister genera that are closely related to *Heliosperma*. In **Paper V** we explore phylogenetic relationships among the taxa belonging to *Atocion* and *Viscaria*, using several nuclear and chloroplast genes. In particular, we test the monophyly of both genera, using a near-complete taxon sampling. The phylogenetic study from paper V is used as a framework for a taxonomic revision in **Paper VI**. Descriptions of the species belonging to *Atocion* and *Viscaria* are presented.

Materials and Methods

Materials and methods are described in detail and with appropriate references in the papers. Here only a brief summary, mostly without references, is given.

Papers I and II are based on extensive reviews of the taxonomic and floristic literature as well as of herbarium (type) material.

Papers III, IV and V are based on analyses of DNA sequences obtained from the plant material listed with voucher data in the respective papers. DNA sequences from the outgroup taxa were mostly available previously, whereas sequences from *Heliosperma*, *Atocion* and *Viscaria* were produced for these studies. DNA was in most cases extracted from silica-gel dried plant parts that were collected during excursions to Pyrenees, Alps, Dinaric Mountains and Carpathians, where also the morphology and ecology of different taxa were observed. Alternatively, DNA was extracted from herbarium material. Specific DNA regions were amplified in PCR reactions, using region-specific primers that were available previously or specifically designed for our purposes. All primers are listed in the respective papers. DNA regions used were the chloroplast *psbE-petG* spacer and the *rps16* intron, as well as nuclear ribosomal ITS sequences and four presumably unlinked low-copy DNA regions from the RNA polymerase (RNAP) gene family. These were *RPA2*, *RPB2*, *RPD2a* and *RPD2b*, encoding the second largest subunit of RNAP I, II, and IV (both *RPD2* genes), respectively. The amplified regions were sequenced, contigs were edited and sequences aligned. Matrices of aligned sequences were used for phylogenetic analyses. Parsimony analyses were performed using PAUP* version 4.0b10 for Windows (Swofford, 2002), and Bayesian analyses using MrBayes (Huelsenbeck and Ronquist, 2001) or BEAST (Drummond and Rambaut, 2006). In Papers IV and V we also estimated the absolute ages of branching events in the gene trees, using three different programmes, r8s (Sanderson, 2003), PATHd8 (Britton et al., in press) and BEAST. To calibrate our chronograms, 33 Myr was used as the age of *Sileneae* in BEAST, and 27 Myr as the age of the ingroup (i.e. *Sileneae* without *Agrostemma*) in r8s and PATHd8 analyses. The calibration ages were estimated with PATHd8, using the chloroplast *matK* gene sequence data of Caryophyllaceae presented by Fior et al. (2006). For calibration of the *matK* tree, we used the inflorescence fossil (*Caryophylloflora paleogenica* G. J. Jord. & Macphail). The extremes of the age interval 40-55 Mya of the fossil were used as fixage for the “higher Caryophyllaceae” node when dating the *matK*-tree. The estimated age interval for the tribe *Sileneae* was 24 to 33 Myr, and 33 Myr-age was arbitrarily chosen as root age for all gene trees, in order to enable relative date comparisons.

Paper VI is based on literature review (original descriptions, taxonomic monographs, national and regional floras), review of herbarium material from different herbaria, and field observations. Species descriptions were produced based on detailed morphological studies (Fig. 5) and literature review, using the standard angiosperm terminology and ontology implemented in the Prometheus Description Model (Pullan et al., 2005).

Figure 5. For the taxonomic revision of *Atocion* and *Viscaria*, flowers from herbarium specimens were boiled, dissected, and the character states observed under the stereomicroscope. Here the flower of *Viscaria alpina*, with removed calyx. Quantitative floral characters were measured using a digital image analysis system. The measures are indicated here as originally displayed (photo B. Frajman, July 2007).

Papers I and II – Nomenclatural issues

Papers I and II are nomenclatural proposals, whose main aim is to provide nomenclatural stability and clear up the confusion connected with the use of certain names in the past.

The first proposal aims to conserve the name *Heliosperma* against *Ixoca* (Table 2).

Table 2. The proposal to conserve the name *Heliosperma* and reject the name *Ixoca*, in its standard formulation, as presented in *Taxon* (Paper I).

(1737) ***Heliosperma* (Rchb.) Rchb.**, Deut. Bot. Herb.- Buch 206. 1841 (*Silene* [unranked] *Heliosperma* Rchb., Fl. Germ. Excurs. 817. 1832), **nom. cons. prop.** Typus: *Silene quadrifida* (L.) L. (*Cucubalus quadrifidus* L., *H. quadrifidum* (L.) Griseb.).

(≡) ***Ixoca* Raf.**, Autik. Bot. 25. 1840, **nom. rej. prop.** Typus: *I. tenella* Raf., nom. illeg. (*Cucubalus quadrifidus* L).

The name *Ixoca* was introduced one year earlier than *Heliosperma* for the same group of plants, and has therefore priority according to the rules of botanical nomenclature (McNeill et al., 2007). However, it has been extremely rarely used in botanical literature. The prevailing and better known name is *Heliosperma*, which is used in most of the countries where the plants belonging to this genus are growing, and where the diversity of taxa is highest. The name *Ixoca* has only been in use in a few east European countries, where only one species occurs. For those who consider these plants to form *Silene* sect. *Heliosperma*, there is no nomenclatural problem, but now that phylogenetic research has shown the desirability of restoration of generic rank, this proposal becomes necessary to make possible the continued use of the much better known generic name *Heliosperma* for this group.

The second proposal aims at rejecting the name *Cucubalus quadrifidus* L. (and its later nomenclatural synonyms), which has been ambiguously used in the taxonomic history. The name was first introduced by Linnaeus (1753) for the plants nowadays known as *Heliosperma alpestre*. Linnaeus (1759) himself started the confusion, by using this name for two species, the original *H. alpestre*, as well as for the plant nowadays known as *Heliosperma pusillum* (Waldst. & Kit.) Rchb. Jacquin (1774), another important and well known botanist contemporary with Linnaeus, applied the Linnaean name to the latter species, and coined the name *Silene alpestris* Jacq., which then actually is a junior synonym of *Cucubalus quadrifidus*. Jacquin's approach was followed by most of botanists, who for almost two centuries used the specific epithet "*quadrifidus*" for the plants nowadays known as *H. pusillum*. Recently, however, the name "*quadrifidus*" has been used a few times in its original sense. The proposal, if accepted by the Nomenclature Committee of the International Association of Plant Taxonomists, and approved by the International Botanical Congress, will avoid the confusion surrounding the name *C. quadrifidus* and make possible a continued use of the name *H. alpestre*.

Paper III – *Heliosperma* phylogeny

Nuclear ITS and chloroplast *rps16* intron sequences both support the monophyly of *Heliosperma*, but the data are not conclusive with regard to its position within the tribe *Sileneae*. Three strongly supported clades are found in both data sets, corresponding to *Heliosperma alpestre*, *H. macranthum* (Fig. 6) and the *H. pusillum* clade, including all other taxa. The relationships among these three lineages differ between the nuclear and the plastid data sets: nuclear data support a sister relationship of *H. alpestre* and *H. macranthum*, whereas in the chloroplast tree *H. macranthum* is sister to the *H. pusillum* lineage. Possible explanations for such incongruence are either lineage sorting (Pamilo and Nei, 1988) or ancient hybridisation (e.g. Rieseberg and Wendel, 1993; Linder and Rieseberg, 2004), but the data are not conclusive in this respect and these hypotheses are further tested in Paper IV.

Figure 6. *Heliosperma macranthum* represents one of the three main evolutionary lineages in *Heliosperma*. It is endemic to Prokletije and Komovi Mts (Albania, Montenegro, Serbia). Unlike other taxa of *Heliosperma*, it has large pink flowers, which are often zygomorphic and pollinated by butterflies (photo B. Frajman, August 2006, Komovi Mts).

Hierarchical relationships within the *H. pusillum* clade are poorly resolved by the ITS data, whereas the *rps16* intron sequences form two well-supported clades which are geographically, rather than taxonomically, correlated (Fig. 7). The borderline between the *rps16* clades is the border region between Serbia and Bosnia/Herzegovina. A similar geographical structure is found in the ITS data, when analyzed with the NeighborNet method (Bryant and Moulton, 2004), which also suggests further geographical partitions (Fig. 7).

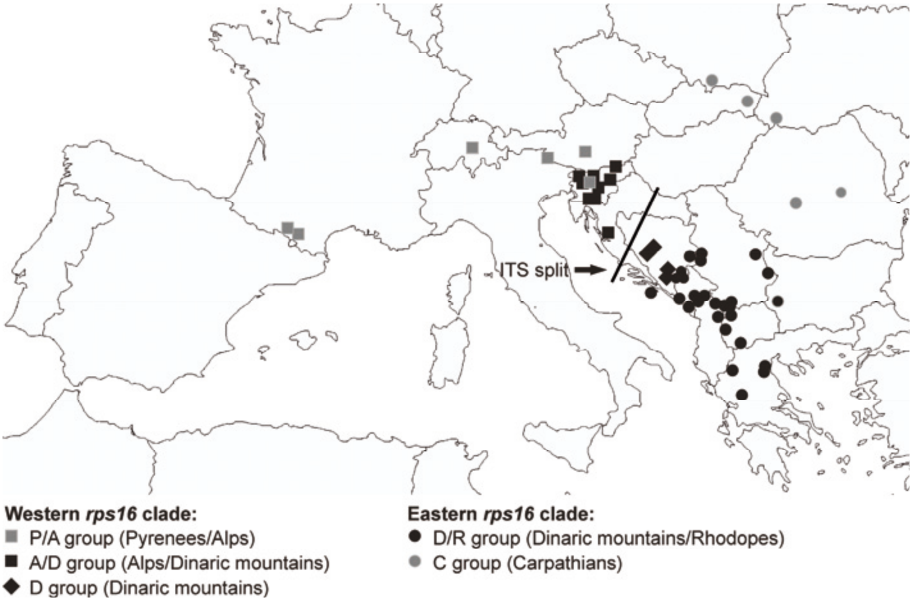

Figure 7. Distribution of the accessions of the *H. pusillum* clade as revealed by the ITS and *rps16* data. Dots correspond to the eastern *rps16* clade, squares and diamonds to the western clade. Further subgrouping is based on the ITS split network and geographic criteria: populations from the Carpathians form the C group and all remaining populations from the eastern *rps16* clade form the D/R group. The accessions from the area between the borderlines of the *rps16* clades and the ITS main split form the D group, populations from NW outskirts of the Dinaric mountains and the SE Alps form the A/D group and more divergent populations from the Alps and the Pyrenees form the P/A group.

Possible explanations for the observed geographical pattern are ancient divergence of the two *H. pusillum* lineages, followed by more recent hybridisation events, or incomplete lineage sorting of ancestors polymorphic for the ITS region together with incomplete concerted evolution. Sharing of the same chloroplast types among different species from the same geographical areas has been observed in other plant groups (e.g. McKinnon et al., 2001; Okuyama et al., 2005), and is often explained as a result of chloroplast capture, i.e. hybridisation and fixation of an introgressed chloroplast haplotype. An alternative hypothesis, which appears relevant in our study, is ancient divergence and isolation of the two *H. pusillum* lineages, followed by more recent hybridisation. Chloroplast capture would likely result in a mosaic geographic pattern.

Hybrids between distantly related taxa can seriously distort an otherwise hierarchical tree structure (e.g. McDade, 1992), and incomplete concerted evolution between distantly related rDNA repeats could cause similar problems (Alvarez and Wendel, 2003). We favour hybridisation as the most likely explanation for two reasons. First, the apparent rate of change within *Heliosperma* is slightly higher for *rps16* as compared to ITS. In contrast, in the *Sileneae* outgroup, ITS substitution rates are more than twice as high as those for *rps16*, a situation more in agreement with what has been observed in other rate comparisons of noncoding cpDNA and ITS. Hybridisation between previously isolated ITS lineages could potentially homogenise them, and the reduced sequence diversity due to non-random extinction of ribotypes in different hybrids would appear as a reduced substitution rate, as observed in the ITS sequences of *Heliosperma*. Second, unlike most other *Sileneae* ITS sequences, the *H. pusillum* group sequences display extensive polymorphisms (additive polymorphic sites, APS), indicating extensive processes that are likely to be much younger than the split between the cpDNA groups. Hybridisation and gene flow could be responsible for at least some of the polymorphic sequence patterns. A detailed analysis of the distribution of less common bases (either indicated by APS, or fixed) at different nucleotide positions among different geographic groups indicates gene flow along the Dinaric mountains, i.e. between the eastern and the western *rps16* clades. Interestingly, there is also no obvious morphological differentiation between the eastern- and western-group populations of the most widespread taxon, *H. pusillum*. There is rather a continuous morphological variability across its distribution range.

The phylogenetic analyses of the two data sets do not support the taxonomic diversity of the *H. pusillum* group. The morphological differentiation into more widespread high elevation taxa, and low elevation narrow endemics, is not correlated with the molecular data, and is possibly a result of ecological differentiation.

In Paper IV we further explore the relationships among the three *Heliosperma* lineages, whereas the detailed relationships within the *H. pusillum*-group are still awaiting further phylogenetic investigations and taxonomic revision, which are beyond the scope of this thesis.

Paper IV – Reticulate origin of *Heliosperma*

We have used a framework, where relative divergence times are used to discriminate among inter- and intralineage processes causing topological conflicts among gene trees, to evaluate different phylogenetic explanations for conflicting gene trees in the tribe *Sileneae* (Fig. 8).

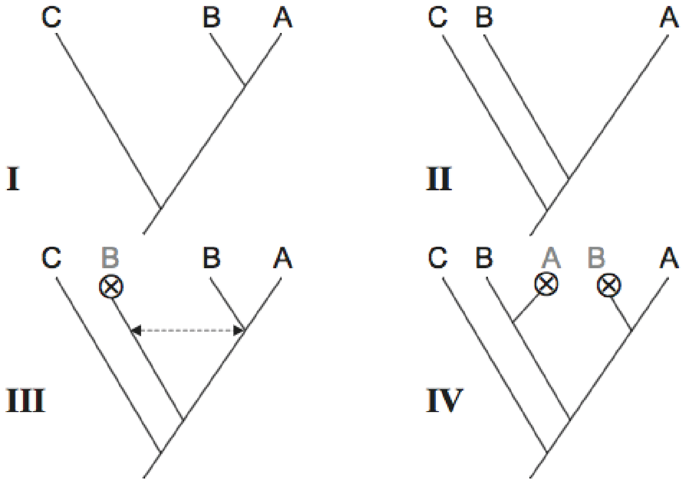

Figure 8. Two trees (I and II) with identical topologies, but different divergence times between lineages A and B, and hypothetical explanations of their history. If tree I represents the organismal lineage phylogeny, then the gene phylogeny in tree II is best explained by graph IV, where a duplication of the gene preceded the split between A and B, both of which have lost a copy afterwards. Conversely, if tree II reflects the lineage phylogeny, then tree I is best explained by graph III, which depicts an old split between A and B. Hybridisation/lateral gene transfer between species A and B after their divergence have caused the age of the copy present in B to reflect the age of this event, rather than the age of lineage split. The original copy of the B lineage has gone extinct. A, B and C represent species lineages. Crossed circles represent extinction (or non-sampled gene copies).

Conservative coalescence times for the bifurcations observed in our gene trees were calculated using unrealistically large populations sizes (100,000) and generation times (5 years) for *Heliosperma* (see Maddison and Knowles, 2006), enabling stringent discrimination between incomplete lineage sorting and other causes of gene tree incongruence. In absence of other dating errors, we regard it highly improbable that differences in divergence times in different gene trees larger than 2 million years are attributable to incomplete lineage sorting of nuclear alleles. For organelle DNA, the corresponding interval is 1 million years.

The incongruences between the chloroplast DNA and the nuclear regions regarding the relationships among the three major lineages of *Heliosperma* (*H. alpestre*, *H. macranthum*, and the *H. pusillum* lineage) are best explained by homoploid hybridisation. Incomplete lineage sorting can be rejected as a plausible explanation, because of the large differences between the divergence times. A difference of c. 5 Myr between the age estimations of *Heliosperma* from the nuclear and the chloroplast data is well outside of the estimated conservative coalescence time of 2 Myr for nuclear genes. Chloroplast capture via hybridisation, i.e. introgression of chloroplasts from one species into another, is often a preferred explanation for incongruence between chloroplast and nuclear gene trees. However, the relatively younger age of the *H. macranthum* stem lineage in the nuclear genes, compared to the chloroplast genes (Fig. 9), rejects the explanation that *H. macranthum* has captured plastids of the *H. pusillum* lineage (or vice versa). The data support the explanation that there has been hybridisation going on between the *H. alpestre* and *H. macranthum* lineages, with subsequent extinction of the "original" *H. alpestre* nuclear copies.

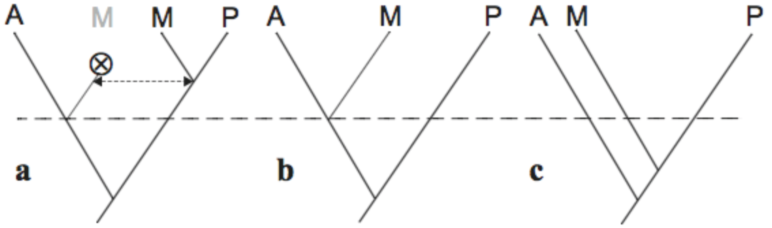

Figure 9. Hypothetical explanation of chloroplast capture within *Heliosperma* (a). The arrow indicates hypothetical hybridisation and capture of cpDNA. (b) and (c) summarise relationships inferred by nuclear and chloroplast DNA in *Heliosperma*, respectively. The dashed line represents the age of the *H. macranthum* stem lineage inferred by nuclear DNA. Crossed circles represent extinction (or non-sampled gene copies). A–*H. alpestre*, M–*H. macranthum*, P–*H. pusillum*.

The origin of *Heliosperma* itself is complicated and is likely to include both reticulate and sorting events. At least two different lineages have been involved, one most closely related to *Viscaria* and *Atocion*, and another related to *Eudianthe* and/or *Petrocoptis*.

We compared three different dating methods to infer divergence times in the gene trees: a Bayesian approach (implemented in BEAST; Drummond and Rambaut, 2006) that takes topological (and other parameters') uncertainties into account, as opposed to the penalized likelihood approach (implemented in r8s; Sanderson, 2003) and the mean path length method (MPL, implemented in PATHd8; Britton et al., in press), which both perform age estimations on a single tree. The ages inferred with the three dating methods are largely correlated, with some differences due to different calibration strategies.

Our estimations of the absolute ages show that *Heliosperma* probably originated during the Middle to Late Miocene. In Paper III we suggested that it is reasonable to assume a pre-Pleistocene origin of the *H. pusillum* group from a strict molecular clock and the range of published substitution rates in non-coding plastid DNA. Our results in Paper IV, using relaxed molecular clock methods, support this hypothesis.

Papers V and VI – *Atocion* and *Viscaria*

Chloroplast (*rps16* intron, *psbE-petG* spacer region) and nuclear (ITS and RNA polymerase gene family) DNA sequences were used to infer phylogenetic relationships among the subordinate taxa of *Atocion* and *Viscaria* in Paper V. Relative dating was used to discriminate among intralineage and interlineage processes that cause incongruences among different gene-tree topologies (as in paper IV).

Silene tatarinowii Regel and *S. hoefftiana* Fisch. are not related to *Atocion*, as often classified in the past, but rather positioned within *Silene*. *Atocion asterias* (Griseb.) Tzvelev is positioned within *Viscaria*, and a new nomenclatural combination is therefore made (*Viscaria asterias* (Griseb.) Frajman). Both, *Atocion* and *Viscaria*, form monophyletic groups, except in the *RPA2* tree, where *V. alpina* G. Don is sister to the rest of *Atocion*/*Viscaria* with strong support. However, it is difficult to conclude which processes (lineage sorting, hybridisation or duplication/extinction) were responsible for the incongruences observed.

In *Viscaria*, *V. alpina* forms a basal clade (with the exception of *RPA2*), and *V. asterias* is sister to *V. vulgaris* Bernh. (incl. *V. atropurpurea* Griseb.). Infraspecific differentiation of *V. alpina* is not supported by sequence data.

The traditional sectional delimitation of *Atocion* taxa is not supported phylogenetically. *A. armeria* and *A. lerchenfeldianum* (Baumg.) M. Popp are most closely related, and *A. rupestre* is their closest relative. *Atocion compactum* (Fisch. ex Hornem.) Tzvelev and *A. reuterianum* (Boiss. et Blanche) Frajman form a sister clade to the other *Atocion* taxa in the ITS and *RPD2b* trees. In the cpDNA tree the accessions of *A. compactum* are found in three different positions compared to the nuclear gene trees. The accessions in the three groups are geographically correlated (Ukraine/Georgia, Turkey and Macedonia, respectively). The observed pattern can possibly be attributed to hybridisation of *A. compactum* with the *A. armeria* lineage as maternal lineage, and capture of *A. armeria* plastids, at least twice in the evolutionary history of *A. compactum*.

Results of the phylogenetic analyses are used as basis for the taxonomic revision of *Atocion* and *Viscaria* in Paper VI. It is difficult to find morphological synapomorphies that differentiate *Atocion* and *Viscaria* from other *Sileneae*, mostly due to a high morphological heterogeneity and diversity within *Silene*. One of the main characters that differentiate the two genera, is the glaucous coating present on the surface of all above-ground vegetative parts in *Atocion*, but not in *Viscaria*. *Viscaria* taxa are perennials with creeping rhizomes, whereas *Atocion* comprises annuals to short-lived perennials. Moreover, *Viscaria* has thyrsoid inflorescences, i.e. panicle-like clusters with cymose lateral and terminal partial inflorescences (mostly dichasia), whereas the inflorescence in *Atocion* is a terminal compound dichasium. It is difficult to classify the basic inflorescence type of *Viscaria asterias*, as it has a very dense, head-like inflorescence.

Species descriptions of *Atocion* and *Viscaria* are presented, and information on their ecology (habitat) and distribution are provided. Also taxonomic issues are thoroughly addressed. *Viscaria atropurpurea* is lumped with *V. vulgaris*, due to a lack of correlation of other morphological characters with the anthophore length, which is used as the only constant character to differentiate between these two taxa.

Sammanfattning (Swedish summary)

Det ligger i den mänskliga naturen att vilja organisera saker och ting, att skapa ordning i kaos – med andra ord, att vara systematisk. Ordning är syftet med systematisk biologi, ett ämnesområde inom vilket man studerar organismernas mångfald, som utvecklats i vad som kallas ”livets träd”. Taxonomins huvudsyfte är att organisera och klassificera organismer i olika grupper som reflekterar denna fylogenetiska släktskap.

På Linnés tid klassificerades växter mestadels med hjälp av likheter i blommans struktur. Detta är basen i till exempel Linnés sexualsystem, men dessa likheter reflekterar ofta inte de evolutionära släktskapsförhållandena. Darwin bidrog till systematisk biologi genom sina idéer om evolutionära släktskapsförhållanden mellan organismer, där alla har utvecklats från en gemensam anfader. Sedan Darwins tid har målet för systematiken varit att producera ett system som speglar dessa naturliga, evolutionära släktskapsförhållanden. Molekylär fylogeni, dvs. studier av evolutionära släktförhållanden mellan organismer baserat på molekylära data (mestadels från DNA-sekvenser), har revolutionerat systematiken de senaste 15 åren. Nya insikter som ofta strider mot de traditionella hypoteserna, vilka är mestadels morfologibaserade antaganden om olika gruppers inbördes släktskap, har lett till förbättrade klassifikationer som antagligen bättre speglar ”sanna” evolutionära förhållanden. Det är visserligen fortfarande mycket information som saknas, men vissa grupper har blivit relativt väl studerade. En av dessa är tribusen *Sileneae* i familjen Caryophyllaceae.

Tribusen *Sileneae* omfattar ca 700 mestadels örtartade arter med en huvudsaklig utbredning på norra halvklotet. Den senaste klassifikationen på släktesnivå erkände åtta släkten, av vilka *Silene* är det mest mångformiga och artrika. Detta släkte har en världsomfattande utbredning. Andra, mindre släkten i tribusen, såsom *Heliosperma*, *Atocion* och *Viscaria* har mestadels europeiska utbredningar och det är på dessa släkten det huvudsakliga fokus ligger i denna avhandling. Tre av artiklarna är inriktade på *Heliosperma*, ett släkte som har sin utbredning i Sydeuropas berg, från Sierra Cantabrica till Karpaterna men med störst diversitet på Balkanhalvön. Syftet i artikel I och II är att bringa klarhet i den nomenklaturiska förvirring som råder, orsakad av att olika namn har använts för samma växt. En speciell gren inom systematiken kallas nomenklatur och syftar till att stabilisera organismernas namn, en ofta besvärlig uppgift. Botaniker har internationellt kommit överens om regler om hur namn korrekt ska användas. Det är vanligt att en och samma växt har fått olika namn av olika botanister men i ett stabilt system ska varje organismgrupp ha endast ett korrekt namn. I normalfallet ska det först publicerade namnet för organismgruppen användas. För släktet *Heliosperma* är egentligen namnet *Ixoca* publicerat ett år tidigare än *Heliosperma*, och *Ixoca* borde därmed vara det giltiga namnet. *Ixoca* har dock nästan aldrig använts medan namnet *Heliosperma* istället nyttjats flitigt. I sådana fall kan man dock för den internationella nomenklaturkommittén (The Nomenclature Committee of the International Association of Plant Taxonomists) föreslå att det äldre namnet (i detta fall *Ixoca*) ska förkastas och att det yngre namnet (här *Heliosperma*) ska bevaras. Detta är gjort i det här fallet. Vi har även gjort ett liknande förslag för namnparet *Heliosperma quadrifidum – H. alpestre*.

I artikel III studerar vi de fylogenetiska släktskapsförhållandena mellan arterna i *Heliosperma*. För undersökningen använder vi två DNA-regioner, en från kloroplasterna och en från det nukleära genomet. Två arter, *H. alpestre* och *H. macranthum*, bildar självständiga evolutionära linjer medan alla andra arter bildar en gemensam linje, som vi har valt att kalla *H. pusillum*-gruppen efter den mest vittspridda *Heliosperma*-arten. Våra resultat tyder på att släktet *Heliospermas* evolutionära historia är komplex och involverar nätverkslika släktskapsförhållanden, orsakade av hybridiseringar både mellan de tre linjerna såväl som inom *H. pusillum*-gruppen, där en överraskande gammal uppdelning upptäcktes i kloroplasternas DNA, som normalt bara nedärvs via moderslinjen. Denna uppdelning är tydligt geografiskt korrelerad, men knappast morfologiskt. När vi studerar cellkärnans DNA, som nedärvs genom både moders- och faderslinjen, försvinner uppdelningen helt då vi försöker att rekonstruera fylogenin enligt en trädmodell. Den geografiska uppdelningen är dock synlig om vi låter de olika populationerna vara besläktade i ett nätverk. Vi framkastar hypotesen att den gamla geografiska uppdelningen delvis har suddats ut i kärngenomet genom hybridisering mellan tidigare isolerade populationer. Resultaten tyder också på att den mångformighet som finns hos arterna i *H. pusillum*-gruppen beror på ekologiska skillnader då den saknar stöd i de fylogenetiska mönster som DNA-sekvenserna uppvisar.

I artikel IV fortsätter vi att studera det evolutionära släktskapet mellan de tre *Heliosperma*-linjerna och undersöker dessutom *Heliospermas* position inom *Sileneae*, dvs. de evolutionära släktskapsförhållandena mellan *Heliosperma* och närstående släkten. Vi har använt DNA-sekvenser från fem olika regioner (en från kloroplast-DNA och fyra från kärngenomet) och jämfört såväl position som relativ ålder för olika grupper i de fylogenetiska träd som vi skapat. Resultaten visar att släktskapsförhållandena är komplicerade och att olika genträd uppvisar olika mönster. Vi utvecklar i artikeln ett ramverk som använder relativa åldrar på olika grupper för att särskilja mellan olika evolutionära processer som kan orsaka sådana inkongruenser. Exempel på sådana processer är hybridisering och genduplicering följt av utdöende av någon av de duplicerade genkopiorna. Våra resultat tyder på att minst en, förmodligen flera mycket gamla (upp till ett tiotal miljoner år) hybridiseringar har orsakat åtminstone några av olikheterna som vi ser.

I artikel V och VI studerar vi de fylogenetiska släktskapsförhållandena mellan arterna av *Atocion* och *Viscaria*. Släktet *Atocion* omfattar fem arter som är annueller eller kortlivade perenner och som har eurasisk utbredning, från Europa till Iran. *Viscaria* omfattar tre arter med utbredning i Europa – en art finns också på Grönland och nordligaste Nordamerika. Även i detta fall noteras olikheter mellan genträden och de relativa åldrarna på olika grupper i dem. *Atocion compactum* verkar till exempel ha hybridiserat med närbesläktade arter och på så sätt ”lånat” deras kloroplaster. I artikel VI presenteras en taxonomisk revision av den här gruppen som är baserad på den fylogenetiska studien i artikel V.

Sammanfattningsvis visar våra resultat att den trädmodell som använts flitigt för att reda ut evolutionära släktskapsförhållanden mellan olika arter inte är tillräcklig inom gruppen *Sileneae*. Genom att studera enskilda gener från olika delar av genomet kan nätverkslika släktskapsförhållanden rekonstrueras. Det här angreppssättet har än så länge använts på endast ett fåtal växtsläkten. Eftersom botanister länge har ansett att hybridisering är en viktig evolutionär process hos växter, finns det dock anledning att tro att liknande nätverklika evolutionära släktskapsförhållanden kommer att upptäckas i många fler växtgrupper.

Acknowledgements – Tack – Zahvala

There are so many persons I should thank: friends, family, colleagues, … that I will for sure forget someone. To prevent this, I want to thank all who have helped and supported me during my PhD studies.

The main responsibility that you can read these lines now, has Bengt, my supervisor, who has supported me since I first came to Uppsala and showed me the path to follow in the sometimes confusing world of science. Bengt, thanks for always taking your time, for all the discussions we had, for answering my questions,… You are a person one can just wish to have as a mentor!

Many thanks to other teachers at "Systematisk botanik", who were involved in my PhD progress, Isabel, Katarina (thanks for reading the "kappa" and Paper V!), Leif, and Niklas. Special thanks to Mats for showing me some, for me still unknown, plants during the "floristikkurs" in 2003, for always being ready to give a nomenclatural advise, and for carefully reading and commenting all my manuscripts as well as the "kappa".

Of course, my, sometimes long days at the EBC would have been dull without all fellow PhD students: Andreas, Anja (thanks for always solving some BoxTax and other computer-related problems!), Anneleen, Annika, Björn-Axel, Cajsa (thanks for reading the ”kappa”!), Catarina, Christina, Dick, Elisabeth, Frida (thanks for translating my sammanfattning!), Hege, Heidi (from Oslo), Henrik, Hugo, Johannes, Karolina, Kristina, Magnus, Maria, Mattias, Per, Rikke, Sanja, Sunniva, Yonas,… Sanja, hvala za pogovore v materinščini in nasploh za prijetno druženje! Big thank especially to Anelinčka and Hugi, for all your crazy ideas, great pizzas from Backen, borrowing bikes and other stuff,… Thanks, guys! And of course, Per and Magnus, thanks for helpful advises in the lab and elsewhere! In the lab, Nahid was of great help, and when she was not around, Afsaneh answered the questions. Inga was the first to show me around in the lab. Tack! Many thanks also to Ulla, who handled the the financial matters with great care.

My life in Uppsala would be rather lonely without friends outside EBC. First, thanks to Josefin for taking me as a roommate during my longest stay in Uppsala in 2003! It was for sure crazier than it would have been otherwise! You, Alex, Karin, Erik, Elias, Åsa, Henrik, Anna, Karin,… were there when it was a fun-time! Thanks Elias, for always being ready to accommodate me during my shorter stays in Uppsala. And Erik for making great dinners! Peter accompanied me during long runs in winter-Uppsala; thanks also for letting me grow vegetables in your little garden!

Sweden is not only Uppsala, and it was always nice to meet friends from Stockholm and elsewhere – you made my stays here much nicer: Anna & Mats, Maria, Urška, Maria and Maria. So, what's the most common women's name in Sweden?! ;) Še posebna zahvala Urški, ki je vedno razumela, kadar se mi je kaj na Švedskem zdelo nenavadno…

Večji del časa podiplomskega študija sem preživel v Sloveniji. Včasih so bila potovanja med Ljubljano in Uppsalo naporna, tudi krmiljenje med delom, povezanim z doktoratom, in drugimi obveznostmi v službi. Nejc, hvala, da si se strinjal, da doktorski študij opravim v Uppsali. Gotovo sem veliko odnesel od tega in mi bo v pomoč na nadaljnji znanstveni poti! Hvala tudi za pomoč pri izbiri ene od treh ponujenih doktorskih tem – izkazala se je za zelo zanimivo. Marina, tudi tebi hvala za vso podporo na moji podiplomski poti! Vsekakor bi bil brez Tinke moj študij na Švedskem nemogoč. Hvala za vse izmenjave na vajah, dvojne obremenitve v času moje odsotnosti, poslušanje in bodrenje v času prisotnosti – si res super cimra! V času mojega prvega obiska Uppsale je "vskočila" tudi Simona – hvala! Tjaša, hvala za pomoč pri prepariranju cvetov, prelaganju herbarijskih pol, izpisovanju etiket… In Alešu za pomoč pri oblikovanju naslovnice, zaganjanju BEASTa in vse majhne in velike nasvete računalniških mojstrov! Seveda bi življenje na Katedri brez Jasne, Sabine, Barbare, Tomaža (kdaj greva spet laufat?!), Tatjane, Katje in Irene ne bilo enako. Hvala vsem! Posebna zahvala tudi Tonetu, ki si s svojim bogatim znanjem, izkušnjami in literaturo o slanozorjih vedno priskočil na pomoč. Hvala tudi vsem, ki ste nabirali slanozorje zame!

Puno hvala svim kolegima sa Balkana, koji ste mi pomogli sakupljati "Heliosperme" ili ste me pratili na terenu: Faruk, Danka, Sneža, Marjan, Goga, Bojan, Vlada i prof. Matevski. Hvala tudi Martinu za družbo in pomoč na ekskurziji po Balkanu, ter Boštjanu za nabiranje slanozorjev. Many thanks are due to Michal from Krakow and Alexander from Lviv for collecting *Heliosperma* in the Carpathians, as well as to the curators from different herbaria for the loans, and permission to take samples for DNA extraction. Č. Šilić and A. Strid kindly provided locality-information from Bosnia and Greece, respectively. Many thanks to H. Niklfeld for the distribution map of *H. alpestre*. For the financial support I owe my gratitude to Erasmus-Socrates, EMBO, and Helge Ax:son Johnsons Stiftelse.

Brez družine in prijateljev bi vse skupaj ne bilo mogoče. Hvala mami za vso vzpodbudo. Polonci za svetle trenutke. Petru, ker si vedno tam. Polonci, Lidiji, Andreji, Roku, Pimu, Silviji za prijateljstvo. In seveda vsem drugim prijateljem v Sloveniji in izven nje!

References

Alvarez, I., & J. F. Wendel, 2003. Ribosomal ITS sequences and plant phylogenetic inference. Molec. Phylogen. Evol. 29: 417-434.

Ascherson, P., & P. Graebner, 1920. Caryophyllaceae-Lychnidae. Synopsis der Mitteleuropäischen Flora 5(2). Gebrüder Borntraeger, Leipzig.

Bittrich, V., 1993. Caryophyllaceae. In: Kubitzki, K., Rohwer, J. G. & V. Bittrich, The Families and Genera of Vascular Plants 2. Springer Verlag, Berlin. Pp. 206-236.

Braun, A., 1843. Beitrag zur Feststellung natürlicher Gattungen unter den Sileneen. Flora 26: 349-388.

Britton, T., Anderson, C. L., Jacquet, D., Lundqvist, S., & K. Bremer. In press. Estimating divergence times in large phylogenetic trees. Accepted in Syst. Biol.

Bryant, D., & V. Moulton, 2004. Neighbor-Net: An agglomerative method for the construction of phylogenetic networks. Molec. Biol. Evol. 21: 255-265.

Chater, A. O., & S. M. Walters, 1964. *Silene* L. In: Tutin, T. G., Heywood, V. H., Burges, N. A., Valentine, D. H., Walters, S. M., & D. A. Webb (eds.), Flora Europaea 1. Cambridge University Press, Cambridge. Pp. 158-181.

Chater, A. O., Walters, S. M., & J. R. Akeroyd, 1993. *Silene* L. In: Tutin, T. G., Burges, N. A., Chater A. O., Edmondson, J. R., Heywood, V. H., Moore, D. M., Valentine, D. H., Walters, S. M., & D. A. Webb (eds.): Flora Europaea. Cambridge University Press, Cambridge. Pp. 191-211.

Chowdhuri, P. K., 1957. Studies in the genus *Silene*. Notes Roy. Bot. Gard. Edinburg 22: 221-278.

Drummond, A. J., & A. Rambaut, 2006. BEAST v1.4. Available from http://beast.bio.ed.ac.uk/

Eggens, F., Popp, M., Nepokroeff, M., Wagner, W. L., & B. Oxelman, 2007. The origin and number of introductions of the Hawaiian endemic *Silene* species (Caryophyllaceae). Amer. J. Bot. 94: 210-218.

Endlicher, S., 1839. Genera plantarum secundum ordines naturales. Apud Fr. Beck Universitatis Bibliopolam, Vindobonae.

Erixon, P., & B. Oxelman, 2006. Reticulate or treelike chloroplast DNA evolution in *Sileneae* (Caryophyllaceae)? In: Erixon, P., Phylogenetic support and Chloroplast evolution in *Sileneae* (Caryophyllaceae). Acta Universitatis Upsaliensis, Uppsala.

Fior, S., Karis, P. O., Casazza, G., Minuto, L., & F. Sala, 2006. Molecular phylogeny of the Caryophyllaceae (Caryophyllales) inferred from chloroplast *matK* and nuclear rDNA ITS sequences. Amer. J. Bot. 93: 399-411.

Gärtner, K. F., 1849. Versuche und Beobachtungen über die Bastarderzeugung im Pflanzenreiche. K. F. Herring, Stuttgart.

Greuter, W., 1995. *Silene* (*Caryophyllaceae*) in Greece: a subgeneric and sectional classification. Taxon 44: 543-581.

Greuter, W., Burdet, H. M., & G. Long (eds.), 1984: Med – Checklist. Geneve. Pp. 270-272

Holder, M. T., Anderson, J. A., & A. K. Holloway, 2001. Difficulties in detecting hybridization. Syst. Biol. 50: 978-982.

Huelsenbeck, J. P., & F. Ronquist. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754-755.

Ikonnikov, S., 1984. Notae de Caryophyllaceis, 7. Novosti Sist. Vyssh. Rast. (Petrograd) 21: 61-67.

Jacquin, N. J., 1774. Florae austriacae, sive plantarum selectarum in Austriae Archiducatu sponte crescentium Icones ad vivum coloratae, et descriptonibus ac synonymis illustratae 2. Leopoldi Joannis Kaliwoda, Viennae.

Jalas, J., & J. Suominen (eds.), 1986. Atlas Florae Europaeae 7, Caryophyllaceae (Silenoideae). Committee for mapping the flora of Europe and Societas Biologica Fennica Vanamo, Helsinki.

Linder, C. R., & L. H. Rieseberg, 2004. Reconstructing patterns of reticulate evolution in plants. Amer. J. Bot. 91: 1700-1708.

Linnaeus, C., 1753. Species Plantarum. Impensis Laurentii Salvii, Holmiae.

Linnaues, C., 1759. Systema Naturae II: Vegetabilia, ed. 10. Holmiae.

Maddison, W. P., & L. L. Knowles, 2006. Inferring phylogeny despite incomplete lineage sorting. Syst. Biol. 55: 21-30.

Mallet, J., 2007. Hybrid speciation. Nature 446: 279-283.

McDade, L. A., 1992. Hybrids and phylogenetic systematics. II. The impact of hybrids on cladistic analysis. Evolution 46: 1329-1346.

McKinnon, G. E., Vaillancourt, R., Jackson, H. D. & B. M. Potts, 2001. Chloroplast sharing in the Tasmanian eucalypts. Evolution 55: 703-711.

McNeill, J., Barrie, F. R., Burdet, H. M., Demoulin, V., Hawksworth, D. L., Marhold, K., Nicolson, D. H., Prado, J., Silva, P. C., Skog, J. E., Wiersema, J. H., & N. J. Turland (eds.), 2007. International Code of Botanical Nomenclature 2006 (Vienna Code). Regnum Vegetabile 146: 1-568.

Meusel, H., & H. Mühlberg, 1978-1979. *Silene* L. In: Hegi, G., Illustrierte Flora von Mitteleuropa 3 (2), Verlag Paul Parey, Berlin & Hamburg. Pp. 1043-1172.

Murray, I. A. (ed.)., 1774. Systema Vegetabilium. Gottingae & Gothae.

Nägeli von, C., 1865. Die Bastardbildung im Pflanzenreiche. Bot. Mitth. 2: 187-235.

Neumayer, H., 1923a. Die Frage der Gattungsabgrenzung innerhalb der Silenoideen. Verh. Zool. - Bot. Ges. Wien 72: 53-59.

Neumayer, H., 1923b. Einige Fragen der speziellen Systematik, erläutert an einer Gruppe der Gattung *Silene*. Österr. Bot. Zeitschr. 72: 276-287.

Neumayer, H., 1927. *Silene*. In: Hayek, A., Prodromus Florae peninsulae Balcanicae 1. V. Des Repertoriums, Dahlem b. Berlin.

Niketić, M., & V. Stevanović, 2007. A new species of *Heliosperma* (Caryophyllaceae) from Serbia and Montenegro. Bot. J. Linn. Soc. 154: 55-63.

Okuyama, Y., Fujii, N., Wakabayashi, M., Kawakita, A., Ito, M., Watanabe, M., Murakami, N., & K. Makoto, 2005. Nonuniform concerted evolution and chloroplast capture: heterogeneity of observed introgression patterns in three molecular data partition phylogenies of Asian *Mitella* (Saxifragaceae). Molec. Biol. Evol. 22: 285-296.

Otth, A., 1824. *Silene* L. In: Candolle de, A.-P. (ed.), Prodromus systematis naturalis Regni Vegetabilis 1, Paris, Strasbourg & London. Pp. 367-385.

Oxelman, B., & M. Lidén, 1995. Generic boundaries in the tribe *Sileneae* (*Caryophyllaceae*) as inferred from nuclear rDNA sequences. Taxon 44: 525-542.

Oxelman, B., Lidén, M., Rabeler, R. K., & M. Popp, 2001. A revised generic classification of the tribe *Sileneae* (Caryophyllaceae). Nordic J. Bot. 20: 743-748.

Oxelman, B., Lidén, M., & D. Berglund, 1997. Chloroplast *rps16* intron phylogeny of the tribe *Sileneae* (*Caryophyllaceae*). Pl. Syst. Evol. 206: 393-410.

Pamilo, P., & M. Nei, 1988. Relationships between gene trees and species trees. Molec. Biol. Evol. 5: 568-583.

Pax, F., & K. Hoffmann, 1934. Caryophyllaceae. In: Engler, A., & H. Harms (eds.), Die natürlichen Pflanzenfamilien. Wilhelm Enhelmann, Leipzig. Pp. 275-363.

Popp, M., & B. Oxelman, 2001. Inferring the history of the polyploid *Silene aegaea* (Caryophyllaceae) using plastid and homoeologous nuclear DNA sequences. Molec. Phylogen. Evol. 20: 474-481.

Popp, M., & B. Oxelman, 2004. Evolution of a RNA polymerase gene family in *Silene* (Caryophyllaceae) — incomplete concerted evolution and topological congruence among paralogues. Syst. Biol. 53: 914-932.

Popp, M., Erixon, P., Eggens, F., & B. Oxelman, 2005. Origin and evolution of a circumpolar polyploid species complex in *Silene* (Caryophyllaceae) inferred from low-copy nuclear RNA polymerase introns, rDNA, and chloroplast DNA. Syst. Bot. 30: 302-313.

Pullan, M. R., Armstrong, K. E., Paterson, T., Cannon, A., Kennedy, J. B., Watson, M. F., McDonald, S., & C. Raguenaud, 2005, The Prometheus Description Model: an examination of the taxonomic description-building process and its representation. Taxon 54: 751-765.

Rafinesque, C. S., 1840. Autikon Botanikon. Philadelphia.

Reichenbach, L., 1832. Flora Germanica Excursoria. Carolum Cnobloch, Lipsiae.

Reichenbach, L., 1841. Der deutsche Botaniker - Das Herbarienbuch. Arnoldischen Buchhandlung, Dresden & Leipzig.

Rieseberg, L. H., 1997. Hybrid origins of plant species. Annual Rev. Ecol. Syst. 28: 359-89.

Rieseberg, L. H., & J. F. Wendel, 1993. Introgression and its consequences in plants. In: Harrison, R. G. (Ed.), Hybrid zones and the evolutionary process. Oxford University Press, New York. Pp. 70-109.

Rohrbach, P., 1868. Monographie der Gattung *Silene*. Wilhelm Engelmann, Leipzig.

Rohrbach, P., 1869-70. Synopsis der Lychnideen. Linnaea 36: 170-270.

Rosenberg, N. A., & M. Nordborg, 2002. Genealogical trees, coalescent theory and the analysis of genetic polymorphisms. Nature Rev. Genetics 3: 380-390.

Sanderson, M. J., 2003. r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. Bioinformatics 19: 301-302.

Scopoli, J. A., 1772. Flora Carniolica 1, ed. 2. Impensis Ioannis Pavli Kravss, Bibliopolae Vindobonensis.

Sojak, J., 1972. Nomenklatoricke poznamky (Phanerogamae). Čas. Nár. Muz., Řada Přír. 140: 127-128.

Swofford, D. L., 2002. PAUP*. Phylogenetic analysis using parsymony (*and other methods), version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Trinajstić, I., 1979. *Heliosperma* (Reichenb.) Reichenb. In: Trinajstić, I. (Ed.), Flora Analytica Jugoslaviae 1(5). Šumarski fakultet Sveučilišta u Zagrebu, Zagreb. Pp. 627-636.

Wendel, J. F., & J. J. Doyle, 1998. Phylogenetic incongruence: Window into genome history and molecular evolution. In: Soltis, P., Soltis, D., & J. Doyle. Molecular systematics of plants II. Kluwer Academic Press, Dodrecht. Pp. 265-296

Williams, F. N., 1896. A revision of the Genus *Silene*. Bot. J. Linn. Soc. 32: 1-196.

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 328

Editor: The Dean of the Faculty of Science and Technology

Distribution: publications.uu.se
urn:nbn:se:uu:diva-8171

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2007