Evolutionary patterns and processes in the genus *Potentilla* L. (Rosaceae)

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to my family

Contents

Summary

Firstly, a reconstruction of phylogenetic relationships based on three chloroplast (cp) DNA markers comprising 98 species of the genus *Potentilla* and 15 additional genera from the tribe *Potentilleae* (Rosaceae) is presented. The phylogeny supported the current generic concept of two subtribes (*Fragariinae* and *Potentillinae*), and resolved major lineages within the subtribe *Potentillinae*, comprising also taxonomically highly diverse but molecularly little diverged core group of *Potentilla*. Age estimates of phylogenetic splits resolved in the *Potentilleae* using Bayesian inference, suggested a diversification of the tribe in the Eocene and radiation of two major evolutionary lineages (subtribes) at approximately comparable times. Ancestral area reconstructions based on the recent distribution ranges suggested an Asian origin for *Potentilla* s.str., and explained its arrival in Europe and particularly in North America by multiple dispersal events. The combination of the phylogenetic, geographic and fossil record data with inferred time estimates and taxonomy revealed strongly contrasting evolutionary patterns: rapid speciation on a continental and worldwide scale accompanied by multiple intercontinental dispersals opposing to the largely diverged lineages of limited taxonomic diversity and vicariant geographic distribution. Furthermore, hybridisation and polyploidisation as drivers of speciation were identified in two case studies of restricted taxonomical and geographical coverage. Combined analysis of AFLPs, cpDNA sequences and ploidy levels, used in a case study of *P. argentea* group in Europe, identified four main lineages within the *Potentilla argentea* group, revealing two ploidy levels. Allopolyploid origin was confirmed for the hexaploid *P. argentea*, which apears to be apomictic. The diploid *P. argentea* is a self-pollinator with a highly reduced genetic variability and *P. calabra* is reproducing sexually. A Late Quaternary migration route from Iberian Peninsula throughout the western Europe to Scandinavia and probably also farther to the Baltic region was suggested for the diploid *P. argentea* and no clear geographical patterns were detected for the hexaploid *P. argentea*, most probably due to independent immigration of genetically divergent lineages, which resulted in an overlap of several immigration routes. Finally, *P. alpicola* and *P. collina* populations in the South Tyrol were examined. On one hand, *P. argentea* and *P. pusilla* have been identified as parental taxa for the apomictic *P. alpicola*. On the other hand, apomictic *P. collina* populations are regarded rather as recent derivatives of the hexaploid *P. argentea*. Studied populations seem to evolve multiply, at each locality separately, however some populations share similar evolutionary history.

Zusammenfassung

Die Rekonstruktion der phylogenetischen Verhältnissen, basierend auf drei Chloroplast-DNA-Marker (cpDNA), von 98 Arten der Gattung *Potentilla* und 15 weiteren Gattungen aus dem Tribus *Potentilleae* (Rosaceae) wird vorgestellt. Die Phylogenie unterstützt die aktuelle Tribus-Teilung in zwei Subtriben (*Fragariinae* und *Potentillinae*) und definiert mehrere Linien innerhalb des Subtribus *Potentillinae*, einschließlich der taxonomisch sehr unterschiedlichen, aber molekular wenig differenzierten *Potentilla*-Kerngruppe. Die Altersschätzungen der phylogenetischen Spaltungen wurden in den *Potentilleae* mit Hilfe der Bayesischen Inferenz gelöst und schlugen eine Diversifizierung des Stammes im Eozän und Radiation von zwei großen evolutionären Linien (Subtriben) zu ungefähr gleicher Zeit vor. Die Rekonstruktionen der historischen Verbreitung anhand bisheriger Verbreitungsgebiete schlugen asiatische Herkunft für *Potentilla* s.str. vor und erklärten ihre Ankunft in Europa und insbesondere in Nordamerika durch mehrere Einwanderungsereignisse. Die Kombination von phylogenetischen, geographischen und Fossilien-Daten zusammen mit Altersabschätzungen und Taxonomie zeigten stark kontrastierende Entwicklungsmuster: einerseits schnelle Artbildung auf kontinentaler und weltweiter Ebene, begleitet von mehreren interkontinentalen Ausbreitungen, andererseits getrennte Linien von begrenzter taxonomischer Vielfalt mit vikariierender geographischer Verbreitung. Weiterhin wurden Hybridisierung und Polyploidisierung als wichtige Prozesse der Artbildung in zwei Fallstudien von beschränkter taxonomischer und geographischer Abdeckung identifiziert. Kombinierte Analysen von AFLPs, cpDNA Sequenzen und Ploidie wurden in einer Fallstudie der *P. argentea*-Gruppe in Europa verwendet. Es wurden vier Hauptlinien innerhalb der *Potentilla argentea*-Gruppe identifiziert, die zwei Ploidiestufen zeigten. Die allopolyploide Herkunft wurde für die hexaploide *P. argentea* bestätigt, welche eine apomiktische Vermehrung aufweist. Die diploide *P. argentea* ist höchstwahrscheinlich ein Selbstbestäuber mit einer stark reduzierten genetischen Variabilität, und *P. calabra* vermehrt sich sexuell auskreuzend. Eine spätquartäre Wanderungsroute, ausgehend von der Iberischen Halbinsel, nach Westeuropa, Skandinavien und wahrscheinlich auch weiter in die Baltische Region wurde für die diploide *P. argentea* vorgeschlagen. Im Gegensatz dazu konnten keine klaren geografischen Muster für die hexaploide *P. argentea* identifiziert werden, höchstwahrscheinlich aufgrund der unabhängigen Zuwanderung von genetisch unterschiedlichen Abstammungslinien, die in eine Überlappung von mehreren Einwanderungsrouten führten. Schließlich wurden *P. alpicola*- und *P. collina*-Populationen aus Südtirol untersucht. Einerseits wurden *P. argentea* und *P. pusilla* als Eltern für die apomiktische *P. alpicola* identifiziert. Auf der anderen Seite können die apomiktischen *P. collina* Populationen eher als Abkömmlinge der bisherigen hexaploiden *P. argentea* betrachtet werden. Die Populationen scheinen sich an jedem Ort getrennt entwickelt zu haben. Trotzdem teilen einige Populationen ähnliche Evolutionsgeschichte.

Chapter 1

Introduction

1.1 Sources of plant variation and evolution

The colorful variety of life-forms, their complexity and diversity have been attracting scientists since the ancient times. However, only since mid-19th century, when Darwin's complex theory (Darwin 1859) revolutionised biology, it has generally been accepted that this variety is a dynamic system, able to react to the changing surrounding environment. This ability was referred to as **"evolution"**.

Evolution is a change of the genetic composition or inherited traits of populations over time through successive generations (Futuyama 2005). The processes resulting in altered genetic configuration, thus generating variability and shifting the balance within populations have diverse mechanisms and are active on different levels. The major ones are: mutation and recombination, genetic drift, natural selection and gene flow.

Mutations act mostly on the lowest level by chemically modifying the primary structure of the deoxyribonucleic acid (DNA) and thus resulting in new variants of the genes – alleles (e.g. Fondon et al. 2004, Hanzawa et al. 2005). Processes of recombination during the reductional division also create new combinations of genes (e.g. Akhunov et al. 2003), which are consequently transferred to new zygotes (also referred to as "sex"). In plants, this happens either between genes coming from different parents (outcrossing) or within one individual (self-fertilisation). Stochastic events caused, for instance, by sudden environmental changes (so called population bottlenecks; Young et al. 1996, Linhart & Grant 1996) or realised by splitting of the subpopulations from the original population (founder effects; Ladizinsky 1985, Eckert et al. 1996) are evolutionary highly significant as well. Such events may radically change the allelic ratios, enabling rare alleles to dominate within newly established populations and hence promoting so far marginal traits. Environmental factors also drive the processes of natural selection. Favourable fitness-related alleles make more likely for an organism to survive and successfully reproduce in its current environment, thus becoming more common in the following generations of a population (e.g. Agrawal et al. 2005, Gómez et al. 2006). Individuals with the lower fitness (i.e. low viability and poor reproduction) became on the other hand rare together with the alleles they carry.

Gene flow (allele migration) is perceived as a transfer of alleles from one population to another by means of seed, pollen dispersal or by clonal reproduction. Pollen dispersal is often accomplished by interpopulational or interspecific hybridisation, which is generally considered to have two opposing impacts (Seehausen 2004). On one hand, hybridisation between populations or species may have a homogenizing effect that counteracts the differentiation. On the other hand it is often considered for its creative potential, as a

mechanism which promotes single alleles or even their combinations, which are an advantage (Barton 2001, Arnold 2006) or by combining previously isolated gene pools. Furthermore, geneflow may also be accomplished via horizontal gene transfer as observed in bacteria (Barlow 2009) or in yeast (Hall et al 2005).

1.2 Hybridisation, polyploidisation and apomixis

Hybridisation was recognised already by Linné (1762) as a potentially innovative evolutionary force, playing an important role in speciation and phenotypic diversification. His view was not adopted in his era, but became more of an issue in the late 19th century and thereafter (e.g. Naudin 1863, Kerner 1894–1895, Lotsy 1916, Anderson 1949, Stebbins 1959, Barton & Hewit 1985, Arnold 1997, Soltis & Soltis 2009). If successful, hybridisation facilitates the rise of new genotypes by means of combining previously isolated gene pools: at the populational level it results in significant shifts of allele frequencies and, as already mentioned, it may also promote advantageous mutations and their combinations. In general, character intermediacy in the progeny is usually expected, but hybrids often exhibit extreme phenotypes or novel characters, referred to as either "heterosis" (in first generation hybrids) or "transgressive segregation" (Rieseberg et al. 1999).

Nevertheless, hybridisation often results in sterile offspring, mainly because the precise pairing of chromosomes during the meiosis is not fulfilled. Parents with different chromosome numbers or chromosomes that differ in length or order of genes may cause insufficient homology resulting in malfunctioning gametes. Furthermore, other factors such as individual non-matching genes (nucleo-cytoplasmic interactions, Levin 2003) or hybrid necrosis (Bomblies & Weigel 2007) enhance the sterility.

There are two principal mechanisms for overcoming possible hybrid sterility. Firstly, recombination may result in enhanced homology between chromosomes of different parental origin. Resulting hybrids have then the same chromosome numbers as their parents (Müntzing 1930, Rieseberg et al. 1995). Secondly, genome doubling or polyploidisation may take place. Very closely related to hybridisation is allopolyploidy, following mainly heteroploid crosses (Wingë 1917, Soltis & Soltis 2000). After hybridisation between two (or more) distantly related species, doubling of the genome may occur (however, not necessarily in this order), thus overcoming the common sterility in hybrids by providing each chromosome with a pairing partner. It usually results in instantaneous speciation, because the backcrossing with the parents produces usually unviable or sterile offspring (Seehausen 2004). Polyploidisation may also occur without hybridisation, which is referred to as "autopolyploidy". However, allopolyploidy seems to be more prevalent in nature (Soltis & Soltis 2000).

Polyploidisations have a major effect on the evolution of plants and as much as 70% of angiosperms are thought to be of polyploid origin (Masterson 1994). Furthermore, the frequency of polyploid speciation is estimated by Otto & Whitton (2000) as 2–4%. As summarised by Raymond et al. (2002) the inimitableness of the polyploidisation lies in the fact that the entire genomes become duplicated within a common nucleus, yielding whole-

genome equivalents of redundant genetic information. When compared to their diploid parents, polyploids experience a phase of lower mutational load, due to genetic buffering and they can evolve faster, at least when the beneficial mutations are partially dominant and when their population size is small to moderate (Otto & Whitton 2000).

The hybridisation and polyploidisation is also believed to be fundamental to the occurrence of apomixis (asexual reproduction through seeds). Apomixis is found almost exclusively in polyploids and highly heterozygous species (Savidan 2000). In sterile amphiploids (allopolyploids), it may serve as another mechanism for "escaping the sterility" (De Wet et al. 1974), but beyond that it provides an additional selective advantage for overcoming any minority-related disadvantage in sympatry with parents (Levin 1975). Moreover, apomixis also preserves favourable characters and features related to the fixed heterozygosity or hybrid vigour (Richards 2003). It has been shown that plants reproducing via apomixis tend to grow at higher altitudes and latitudes and are generally considered more effective colonisers, which is referred to as "geographical parthenogenesis" (Vandel 1928, Hörandl 2006). If facultative apomixis is considered, genetically persistent evolutionary lines mix with constantly renewed and unique genotypes resulting from outcrossing. In this way, although the apomicts are practically almost clonal, they can step inside the evolutionary processes such as recombination and gene flow as well as provide the outcrossers with possibly advantageous alleles from the era of their establishment.

During the last decades, several new tools and techniques have been utilised in order to study the above mentioned phenomena. The fast and precise data acquisition based on flow cytometry is an ideal tool to collect ploidy related information (Kron et al. 2007). Furthermore, flow cytometric seed screen can be utilised in order to assess whether the progeny has arisen through apomixis or not (Matzk et al. 2000). Flow cytometry is often combined with molecular markers, which have greatly facilitated the detection of hybridisation and the recognition of allopolyploids in many plant groups. Amplified fragment length polymorphisms (AFLPs), microsatellites and chloroplast DNA (cpDNA) sequences are becoming a standard toolset in plant systematics, evolutionary and population biology (e.g. Schönswetter et al. 2007, Tremetsberger et al. 2009) and in combination with appropriate statistical analysis they are able to provide reliable answers for evolution-related questions.

1.3 Potentilla *as a research target*

The Rosaceae is a large plant family containing several economically important edible fruit species as well as ornamental plants (e.g. subfamily Maloideae, raspberry, strawberry, roses). Not only because of that it was intensively studied in many aspects, including also the past and ongoing evolutionary processes (Potter et al. 2007). Consequently, in several subgroups and genera phenomena like interspecific hybridisation, polyploidy and apomixis (e.g. Lo et al. 2009) have been detected.

Potentilla is one of the largest genera in the Rosaceae, containing about 500 species of annual, biennial and perennial herbs (Soják 2008). They are generally Holarctic in distribution, though some are also found in the montane biomes of the New Guinea Highlands. Its

elements show a preference for open habitats of mountainous to alpine or arctic regions as well as for xeric communities. Hybridisation, often followed by polyploidisation or introgression, is suggested to be an important mechanism in the speciation and evolution of this highly polymorphic genus (Müntzing & Müntzing 1941; Rutishauser 1943). Furthermore, botanists have also recognised that the *Potentilla* complex contain distinct elements that could be distinguished as separate genera (Rydberg 1898, Wolf 1908). This taxonomic uncertainity was further supported by the discovery of the viable intergeneric hybrids between *Potentilla* and *Fragaria* (Ellis 1962). In order to solve this controversy, molecular marker-based phylogenetic analyses have been conducted (Eriksson et al. 1998, Eriksson et al. 2003). However, although these molecular studies provided a phylogenetic framework for the intratribal classification of the *Potentilleae*, they were still based on a limited number of species.

Possible hybrid origin of several taxa, morphological variability, intermediacy and consequent taxonomic complexity, mainly among the taxa from grex/series *Argenteae* T. Wolf and *Aureae* T. Wolf, have been a major issue already in the 19th and at the beginning of the 20th century (e.g. Krašan 1867, Čelakovský 1889, Domin 1908). Later on, the presence of apomixis (Gentscheff 1938, Hunziker 1954) and extensive intraspecific ploidy variation (Müntzing & Müntzing 1941, Ehrendorfer 1970) supported this view. *Potentilla collina* group, from the series *Argenteae* Wolf., seems to be a suitable model system for studying the influence and contribution of the above mentioned phenomena (hybridisation, polyploidisation, apomixis) to the evolution of the genus. The observed morphological variability and exclusive polyploidy, with occasional observation of chromosome aberrations (Müntzing 1958) are explained by the hybrid origin of the group (Wolf 1908, Asker & Fröst 1970). Furthermore, within the group, the development of both female and male gametophytes was reported to be absent or disturbed. Obligate or close to obligate apomixis by means of apospory and pseudogamy (Håkansson 1946) and full or partial male sterility have been detected (Gentscheff & Gustafsson 1940).

Although there are several studies demonstrating the presence of sympatric apomictic and sexual types, the possibility of their genetic interaction (e.g. through hybridisation) and subsequent cytotype diversity, they are rather rare (e.g. Paun et al. 2006) or do not focus on the underlying evolutionary mechanisms. Therefore, I strongly believe that the presented thesis will contribute to a better understanding of these phenomena as well as of their evolutionary impact in the genus *Potentilla*.

1.4 Overview of the chapters

The presented thesis is composed of four chapters (including the Introdcution – Chapter 1), each of them dealing with a different aspect of the evolution within the genus *Potentilla*. The first step (Chapter 2) describes the inference of the phylogenetic position of the majority of *Potentilla* species, including the taxa analysed in the later chapters in detail. Furthermore, age estimates of phylogenetic splits were inferred with the aid of the available fossil record. The ancestral area reconstructions, based on the recent distribution ranges of species, were proposed. Consequently, two strongly contrasting evolutionary patterns were identified: (1)

rapid speciation on a continental and worldwide scale accompanied by multiple intercontinental dispersals and (2) largely diverged lineages of limited taxonomic diversity and vicariant geographic distribution.

In the third chapter, the process of polyploidisation has been analysed on an example of the *Potentilla argentea* complex. Two morphologically indistinguishable ploidy levels were found out to be persisting in sympatry: a hexaploid one, known as obligate apomict and a diploid one described either as facultative apomict or as a self-pollinator. On the basis of the Europe-wide sampling, flow cytometric ploidy screen and molecular markers (AFLP, cpDNA), conclusions about the polyploid origin, reproductive mode, phylogegraphic patterns and taxonomy have been derived.

The fourth chapter deals with the phenomenon of the hybrid speciation. Locally (western and central Alps) distributed taxon, *P. alpicola*, is presumed to be of hybrid origin between the taxa from morphologically distinct *P. argentea* and *P. verna* groups. Several populations have been studied in order to assess their hybridity, possible parental taxa involved in hybridisation and modes of reproduction. Finally, polytopic (at several localities independently) versus monotypic (at one locality and dispersed afterwards) formation of *P. alpicola* is discussed. Following the fourth chapter, an enumeration of other topic-related publications and meeting contributions, in which I was involved, is included.

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Chapter 2

A comprehensive chloroplast DNA-based phylogeny of the genus *Potentilla* (Rosaceae): implications for its geographic origin, phylogeography and generic circumscription[1]

2.1 Introduction

The genus *Potentilla* L. (cinquefoil, Rosaceae) constitutes one of the most species-rich northern hemispheric plant genera comprising according to its latest worldwide monograph (Wolf 1908) and more recent floristic surveys (cf. Soják 2005) about 300 to 430 or even up to 500 species (Airy Shaw 1973, Soják 2008). Its elements show a preference for open habitats of mountainous to alpine or arctic regions as well as for xeric communities. Most of the *Potentilla* species are hemicryptophytes or chamaephytes (Barrenscheen 1991). Other life forms such as annuals have either evolved rarely, or as shrubs and subshrubs, were considered to predate the major radiation and diversification of the genus (Wolf 1908, Panigrahi & Dikshit 1987). Accordingly, these latter life forms are exhibited by a limited number of extant species only. Agamospermy and hybridization accompanied by polyploidization of genomes was considered to have played a significant role in the evolution of the genus (e.g. Müntzing 1928; Müntzing & Müntzing 1941; Rutishauser 1943a, b, 1948; Kashin 1999). Vegetative propagation via runners or rooting shoots is known in several species (e.g. Wolf 1908). However, propagation via seeds (fruitlets), lacking specific adaptations for dispersal, is the dominant mode of reproduction. Other characters used in the description of the genus are the presence of an epicalyx, numerous free nutlets born on an exposed receptacle, lateral styles inserted in subbasal to subterminal position, androecia consisting of (4)10–30 stamina, compound – pennate or palmate – leaves, and a base chromosome number of x = 7. Nevertheless, none of these traits are exclusively found in *Potentilla* and their diagnostic applicability varied with past taxonomic concepts.

The generic circumscription of *Potentilla* was frequently altered and discussed by the taxonomists and taxa treated within *Potentilla* were transferred or described within at least 20 other genera (cf. Eriksson et al. 1998 for review). The various definitions of *Potentilla* and its discrimination from these genera were usually based on single traits or on a combination of few characters such as life form, the type of leaf partition (e.g. Adanson 1763), hairiness of nutlets (Sprengel 1818), the color of petals (Lamarck 1778), or the presence of peculiar features related to the receptacle (Smith 1810, Wolf 1908). Obvious scarcity in the number of

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available morphological characters and subjectivity in their weighting by the taxonomists resulted in different and conflicting generic concepts some of which are still used in parallel today (e.g. Soják 2004 versus Ball et al. 1968; Kalkman 2004 versus Ertter, B. in prep., *Ivesioid* sections, Flora of North America [FNA]). Recently, with the application of molecular markers, the generic limits among members of the tribe *Potentilleae*, to which *Potentilla* belongs, could be also assessed in a more objective way, using considerably enlarged sets of characters (Eriksson et al. 1998, 2003, Potter et al. 2007, Lundberg et al. 2009). These analyses, which relied on the reconstruction of phylogenetic relationships using nuclear and chloroplast DNA markers, provided strong evidence for support or rejection of several of the controversial circumscriptions of *Potentilla* and relatives. Thus, provided that the systematic concept is based on phylogenetic relationships, the phylogenetic hypotheses developed by these authors suggested that the former segregate genera *Duchesnea* Sm., *Horkelia* Rydb., and *Ivesia* Torr. & A. Gray could be included in an even narrowly defined genus *Potentilla*. Contrariwise, these studies indicated that the segregate genera *Chamaerhodos* Bunge, *Comarum* L., *Drymocallis* Fourr. ex Rydb., *Dasiphora* Raf. (*Pentaphylloides* Duhamel), *Sibbaldiopsis* Rydb. (*Potentilla tridentata* Aiton = *Sibbaldia tridentata* [Aiton] Paule & Soják) and the species *Potentilla bifurca* L. (= *Schistophyllidium bifurcum* [L.] Ikonn.) constitute quite isolated evolutionary lineages, which can only be kept within *Potentilla* if an impractically wide and taxonomically highly unconventional generic circumscription (including *Alchemilla* L., *Sibbaldia* L. sensu Soják 2008, and *Fragaria* L.) would be accepted (although such a wide definition has recently been suggested by Mabberley 2002). However, while these molecular studies provided a phylogenetic framework for the intratribal classification of the *Potentilleae*, they were still based on a limited number of species (24 out of the 306 species of *Potentilla* representing 15 out of 31 series [greges] sensu Wolf 1908), one *Horkelia* and *Ivesia* each out of approximately 50 species currently accepted (Ertter, B. in prep., *Ivesioid* sections, FNA). Therefore, inferring the phylogenetic position of the majority of *Potentilla* species and series which have not been analyzed in that respect yet, seems a next important step for a better understanding of the genus.

There has been only limited discussion and analytical study on the biogeography of *Potentilla* so far. Using and comparing distribution patterns of species, Shah et al. (1992) argued in favor of a primary center of diversity and an origin of the genus in the high mountains of Asia, in particular South-West China, and a secondary center of diversity in the European Mediterranean. However, the authors had a preponderant focus on Asian taxa, equalling the center of diversity with the center of origin, and building their inferences also upon genera, which have to be excluded from *Potentilla* according to – as later published – molecular data (Eriksson et al. 1998, 2003). They further omitted diversified specific North American lineages like those of *Ivesia* and *Horkelia*. Panigrahi & Dikshit (1987) followed a similar approach and reasoning as Shah et al. (1992) and speculated about a "principal" center of origin of *Potentilla* in India. In contrast, Wolf (1908) suggested an origin of the genus in the circumarctic belt in the early Tertiary followed by continental-specific radiations not earlier than the end of this geologic period, a view which has been partly supported by Steffen (1925). However, these hypotheses relied on assumed evolutionary ages as well as intuitive inferences of relatedness of the considered species and had to be developed based on limited evidence on the timing of major events in the evolutionary history of the genus. Nevertheless, a number of macrofossils have been recovered of *Potentilla* and relatives, which could potentially be used for dating (e.g. Axelrod 1987, Matthews & Ovenden 1990,

Mai 1995, Meyer 2003). Thus, for instance, the earliest occurrences of *Rosa* L. are from the Early to Late Eocene (Hollick 1936, Edelman 1975, Franzen 2004) or Late Eocene (Meyer 2003). *Comarum palustre* L., *Potentilla erecta* (L.) Räuschel and *Potentilla supina* L. (Mai 2001) and *Fragaria* spp. (Szafer 1961) have been reported from the Middle Miocene. This paleobotanical record as well as molecular phylogenetic analyses have not yet been exploited to date the divergences of major evolutionary lineages.

In the following we will present a comprehensive chloroplast DNA-based phylogeny of the genus *Potentilla* s.str. (the term is applied henceforward informally, i.e. inclusive of *Duchesnea*, *Fragariastrum* Heist. ex Fabr., *Horkelia*, *Horkeliella* [Rydb.] Rydb., *Ivesia*, *Tormentilla* and exclusive of *Argentina*; cf. Eriksson et al. 1998) using a worldwide sample of species and reflecting most of its diversity by choosing members from all 31 series distinguished by Theodor Wolf (1908), the author of the last worldwide monograph of *Potentilla*. The monograph is used in the following as the only comprehensive taxonomic reference, despite the existence of more-or-less differing alternative classifications for particular geographic regions (e.g. Rydberg 1898; Panigrahi & Dikshit 1987; Shah & Wilcock 1993; Li et al. 2003; Soják 2004, 2005) or taxonomic groups (e.g. Soják 1987, 1989, 1994). Wolf (1908) perceived *Potentilla* in a rather wide sense including *Argentina* Hill. (series *Anserinae*), *Comarum* and *Farinopsis* Chrtek & Soják (series *Palustres*), *Drymocallis* (series *Rupestres*) and *Potentillopsis* Opiz (series *Rivales*), *Duchesnea* and *Tormentilla* L. (series *Tormentillae*), *Dasiphora* (series *Fruticosae*), and *Sibbaldiopsis* (series *Tridentatae*), but excluded *Chamaerhodos* and the specific North American genera *Comarella* Rydb., *Horkelia*, *Horkeliella*, *Ivesia*, *Purpusia* Brandegee, and *Stellariopsis* Rydb. Hence, save the North American taxa, the monograph covers almost all of the species hitherto included in *Potentilla* (which are not members of clearly distinct and traditionally separate genera of the *Potentilleae* as *Alchemilla*, *Aphanes* L., *Fragaria* and *Sibbaldia*; but see Soják 2008 for the last mentioned genus). The present study is based on a total of 98 *Potentilla* species, 16 species of *Horkelia*, *Horkeliella*, and *Ivesia*, (treated nomenclaturally within their genera), and in addition on 21 species of *Potentilleae* (Eriksson et al. 1998, 2003; Potter et al. 2007; Soják 2008): *Alchemilla*, *Argentina*, *Chamaerhodos, Farinopsis*, *Dasiphora*, *Drymocallis*, *Fragaria*, *Piletophyllum* Soják, *Sibbaldia* including *Sibbaldiopsis*, *Schistophyllidium*, and *Tylosperma* Botsch. Three plastid markers were sequenced and used for phylogenetic reconstruction. In the following we will (i) infer the phylogenetic relationships and positions of *Potentilla* species on the background of a phylogeny of the *Potentilleae* inclusive of those series (according to Wolf`s 1908 system) which have not been assessed in previous molecular-based reconstructions, (ii) define *Potentilla* s.str. based on molecular phylogenetic evidence and the enlarged set of taxa, and (iii) make inferences on the geographic origin and biogeographic history of this taxon on a worldwide scale.

2.2 Material and Methods

2.2.1 Plant material

Plant material was obtained from the following sources: the herbaria of Missouri Botanical Garden, St. Louis (acronym MO), Heidelberg University Herbarium (HEID), the Natural History Museum Vienna (W), and the National Museum of Prague (PR), field collections carried out by Barbara Ertter, University of California, Berkeley, and the authors, and the

Index Seminum seed exchange (Appendix 1; see http://tinyurl.com/SupplMat or enclosed CD for full documentation of the collection history). Vouchers from plants collected during field trips and from cultivated plants grown from seeds were deposited in HEID (Heidelberg University Herbarium) and UC (University of California Herbarium). The studied material consisted of 162 accessions representing 141 species and 16 genera from the *Potentilleae* sensu Eriksson et al. (2003): *Alchemilla* 2 accessions/2 species, *Argentina* 1/1, *Chamaerhodos* 2/2, *Farinopsis* 1/1, *Dasiphora* 1/1, *Drymocallis* 4/4, *Fragaria* 1/1, *Horkelia* 7/6, *Horkeliella* 1/1, *Ivesia* 8/8, *Potentilla* 125/98, *Sibbaldia* incl. *Sibbaldiopsis* 5/5, and *Schistophyllidium* 2/1 as well as one representative each of *Piletophyllum* 1/1 and *Tylosperma* 1/1. A single accession each of *Agrimonia eupatoria* L., *Dryas octopetala* L., and *Geum rossii* Ser. as well as two accessions of *Rosa* 2/2 served as outgroups. Samples from *Potentilla* and from the genera *Horkelia* and *Ivesia* were selected to cover most of the morphological variation and geographic range realized by these taxa. Hence, representatives from all of Wolf's 31 series were included in the study (Appendix 1). Vouchers were labelled with a material notation (material numbers used in this study and the citation of the title of the corresponding DFG-project) to allow a consistent cross-reference between this and future studies and to ease the recovery of the material from these collections. For the purpose of documentation and map-based presentation of data, localities noted on the voucher labels were translated into geographic coordinates (WGS84). The entire plant material was critically revised by Barbara Ertter, Jiří Soják, and the authors according to the latest taxonomic treatment of the particular groups.

2.2.2 DNA extraction

Total DNA was obtained from freshly-collected and silicagel-dried leaf tissue or taken from preserved herbarium specimens from single individuals. At the date of isolation the age of the herbarium material ranged from 0 to 44 years. Extraction of total genomic DNA followed the procedure of Doyle & Doyle (1987) (CTAB method) with some modifications such as grinding of only 5–15 mg of dry leaf tissue in 2-ml tubes using a Precellys 24 homogeniser (Bertin Technologies), addition of 2 units (U) of ribonuclease per extraction to the isolation buffer, and washing of the DNA pellet twice with 70% ethanol. DNA was finally dissolved in 50µl TE-buffer for long-term storage at –20 °C.

2.2.3 PCR and DNA sequencing

The following three chloroplast DNA markers were chosen for analysis with amplification primers taken from Shaw et al. (2005) (except ycf9-M). Amplification of the trnS^{uga}-ycf9 IGS involved the primers trnS^{uga} 5′-GAG AGA GAG GGA TTC GAA CC-3′ and ycf9-M 5′-CAA AMA CAG CCA ATT GGA AAG C-3′ (Heinze 2007). Amplified sequences included the last 71 bp of the trnS gene, the complete IGS, and the first 10 bp of the ycf9 gene (= psbZ gene). The trnL^{uaa}-trnF^{gaa} IGS was amplified using the forward primer trnL^{uaa}R 5′-GGT TCA AGT CCC TCT ATC CC-3′ and the reverse primer trnF^{gaa} 5′-ATT TGA ACT GGT GAC ACG AG-3′. Sequences comprised the complete IGS, the last single base pair of the second exon of the trnL gene, and the first 40 bp of the trnF gene. The trnC^{gca}-ycf6 IGS was amplified using the primer pair trnC^{gca}F 5′-CCA GTT CRA ATC YGG GTG-3′/ycf6R 5′-GCC CAA GCR AGA CTT ACT ATA TCC AT-3′. The sequenced fragment comprised part of the IGS. All forward and

reverse primers carried a 5′-end extension complementary in sequence to the M13 forward (5′-GCA TGT TTT CCC AGT CAC GAC-3′) and reverse (5′-ACT TCA GGA AAC AGC TAT GAC-3′) primers, respectively.

Twenty-five microliters PCR reactions were performed in a master mix containing 1x PCR buffer (10 mM TRIS/50 mM KCl buffer, pH 8.0), 1.5 mM MgCl, 0.2 μM of each primer, 0.4 mM of each dNTP, 0.5 U Taq DNA polymerase (Promega GoTaq), and 10–100 ng of template DNA using an PTC-200 (MJ Research) thermal cycler. Thermal cycling started with a denaturation step at 95 °C lasting 5 min, followed by 30 cycles each of 60 s denaturation at 95 °C, 30 s annealing at 48 °C for all markers, and 60 s elongation at 72 °C. Amplification ended with an elongation phase at 72 °C lasting 10 min and a final hold at 4 °C.

PCR products were checked for length and concentrations on 1.5% agarose gels. PCR products were purified using the NucleoFast Kit (Macherey-Nagel, Germany) and sent to GATC (Germany) for commercial sequencing or IPK Gatersleben using M13 primers. Cycle sequencing was performed on both strands. Runs resulting in sequences of low quality were repeated. In the majority of cases each forward and reverse reaction spanned the complete sequence. All sequences were edited and a consensus was made of forward and reverse reactions using Seqman 4.00 (DNASTAR, USA).

2.2.4 Phylogenetic reconstruction

The analyzed chloroplast markers were joined for each accession into a single sequence and aligned manually by aid of GeneDoc version 2.7 (Nicholas et al. 1997). Several regions (listed in the Results part) within the completed alignment had to be excluded from further analysis because of ambiguous homology among sequences due to numerous indels and repeated sequence motifs. Collapse version 1.2 (http://darwin.uvigo.es/software/collapse.html) was used to identify haplotypes and their duplicates which were excluded prior to the phylogenetic analyses in order to spare computer resources. Gaps were treated as fifth state and missing data were not considered as differences in the definition of haplotypes when using Collapse. The GapCoder program (Young & Healy 2003) was applied to identify and code indels present in the alignment. GapCoder considers homologous gaps to be those with the same start and end positions and codes their presence or absence with 1 or 0, respectively. When one or more indels are contained completely within a larger indel, all sequences carrying the larger indel are coded with inapplicable characters ('-') for the smaller indels. These codes were included as an additional block of binary characters in the file containing the sequence alignment. Two cpDNA-based analyses differing in the degree of phylogenetic divergence of the species were performed: analysis 1 was based on *trn*S-*ycf*9, *trn*L-*trn*F, and *trn*C-*ycf*6 regions including coded indels and 69 selected accessions covering the whole taxonomic and molecular variability within the data set. The obtained phylogeny and the same set of accession were further used for the reconstruction of ancestral geographic areas and the molecular clock estimate (see below). For the reconstruction of the phylogenetic relationships among species of *Potentilla* s.str. and in particular of a species-rich *Potentilla* core group observed in analysis 1, a separate taxonomically much more comprehensive analysis 2 was performed: accessions were limited to those taxa grouped by analysis 1 in the major clade containing *Potentilla* s.str. and complemented by additional 99 accessions

and 71 species. Analysis 2 was based on single nucleotide polymorphisms (SNPs) and markers *trn*S-*ycf*9, *trn*L-*trn*F, and *trn*C-*ycf*6. MrBayes 3.1 was used for a Bayesian inference of these phylogenies (http://mrbayes.csit.fsu.edu/index.php; Ronquist 2004). The parallel version of MrBayes was compiled and run on the "Schrödinger" UNIX computer cluster located at the University of Vienna. The most likely DNA substitution model was selected using ModelTest version 3.7 (Posada & Crandall 2002) and the Akaike Information Criterion. Therefore, the cpDNA alignment exclusive of the regions of ambiguous homology was saved in nexus format, the PAUP command block as included in the modelblockPAUPb10.txt-file of the Modeltest package was accordingly added and the completed file executed in PAUP version 4.0b10 (Swofford 2002). The obtained output file (model.scores), containing a matrix of the log likelihood scores corresponding to the tested models, was finally executed in Modeltest under the default mode. The most likely model and the values of the estimated model parameters were entered into the input file of MrBayes as specification of the evolutionary model: *prset*: *revmatpr* = *dirichlet* (1.28, 1.6, 0.38, 0.77, 1.61, 1), *statefreqpr* = *dirichlet* (0.32, 0.16, 0.16, 0.36), *shapepr* = *uniform* (0.5, 1). The parameter *nst* was set to 6 and *rates* changed to *invgamma*. These settings applied to the first partition of the data containing the sequences only. The second partition providing the block of coded indels was analyzed under the default settings of the program. The analyses were run for 2000000 generations each with every 400th generation sampled and a temperature of 0.2. The obtained 5000 samples of substitution model parameters, trees and branch lengths were summarized with a *burnin* of 4000 and graphically represented on the corresponding 50% majority rule consensus tree. The statistical support of tree clades was estimated using the posterior node probabilities calculated by MrBayes and in addition by a heuristic bootstrap analysis carried out under the parsimony optimality criterion using the same alignments and PAUP. PAUP was run under its standard settings, except for the maximum number of retained trees and the number of bootstrap replicates which both were increased to 1000. Bootstrap values for clades only which held identical sets of accessions in the maximum parsimony as well as in the Bayesian analysis were finally plotted onto the Bayesian 50% majority rule consensus tree.

2.2.5 Molecular clock estimates

In order to infer the age of the major evolutionary lineages, a molecular clock analysis was performed on the same set of 69 accessions and the same cpDNA markers analyzed in Bayesian analysis 1 but exclusive of indel information. A variable rate approach as implemented in the BEAST program (Drummond et al. 2005) was chosen for that purpose. BEAST uses Bayesian inference and the Markov chain Monte Carlo procedure to derive the posterior distribution of substitution rates modelled along and among tree branches and of associated divergence times (i.e. age of nodes). The program reconstructs tree topologies and rates simultaneously based on the chosen input sequence data. BEAST was run using the following key settings: substitution model = GTR, molecular clock model = relaxed model: uncorrelated lognormal, priors: tree prior = speciation: Yule process, and the following initial values of priors of uniform distribution: gtr.ac = 0.8, gtr.ag = 1.0, gtr.at = 0.23, gtr.cg = 0.48, gtr.gt = 0.62, site.model (alpha) = 0.8, ucld.mean = 6.0×10^{-10}, ucld.stdv = 5.0×10^{-10}, Yule.birthRate = 1, treeModel.rootHeight = Using Tree Prior, and tmrca(ingroup) = lower/upper bound = $4.9 \times 10^{7}/5.6 \times 10^{7}$. The tuning of parameters of the operators was per-

formed automatically by the program. Length of chain was 1.0×10^7 with every 1000^{th} parameter logged resulting in 10000 samples. The set mean of the ucld (= mean of the branch rates under the uncorrelated lognormal relaxed molecular clock) was calculated manually from an approximate average number of substitutions per site of 0.06 separating *Rosa* (cf. Fig. 1) from all terminals included in its sister clade. The age of the node joining *Rosa* and its sister was assumed to be 50 million years (mya). This approximate date was derived from the oldest fossil record of the genus *Rosa* (cf. Palaeobiology database http://paleodb.org; 55.8–48.6 mya: USA, Idaho: *Rosa germerensis*) and was also used to set the prior age of this node in the BEAST analysis (leaf fragments of equal age supposed to belong to *Rosa* were also reported by Hollick 1936). *Rosa* was recently shown to constitute an evolutionary lineage of its own in sister relationship to the *Potentilleae* (Potter et al. 2007). The TreeAnnotater module was run to produce a single target tree onto which the posterior probabilities of its nodes, the posterior estimates and confidence limits of the node ages and the substitution rates calculated from 2000 trees (burnin = 8000) produced by BEAST were summarized. Finally, the program FigTree was used to visualize the target tree and to design the tree graphics.

An alternative age of the origin of the *Rosa* lineage was inferred using a *rbc*L gene-based molecular phylogeny of nine representatives of the Rosaceae, and an estimated age of 76 million years ago (mya) for this family as published by Wikström et al. (2001). The BEAST Program settings were identical with the analysis of the *Potentilleae* save the following parameters estimated using ModelTest: gtr.ac = 0.37, gtr.at = 0.20, gtr.cg = 0.53, gtr.gt = 0.37, site.model (alpha) = 1.1, tmrca(ingroup) = lower/upper bound = $7.5 \times 10^7/7.7 \times 10^7$. Sequences of these rosaceous genera including two outgroup taxa were taken from the NCBI Genbank (*Alchemilla* accession number U06792, *Cercocarpus* U06796, *Dasiphora* U06818, *Dryas* U59818, *Fragaria* U06805, *Geum* L01921, *Prunus* AF206813, *Rosa* U06824, *Spiraea* L11206; *Rhamnus* L13189 and *Ceanothus* U06795 served as outgroup) and aligned using GeneDoc version 2.7. The obtained age of the node bearing *Rosa* of 29.3–46.6 mya finally was used to run an alternative BEAST analysis for the *Potentilleae* under the same parameters (save the alternative age of the node: tmrca[ingroup] = lower/upper bound = $2.93 \times 10^7/4.46 \times 10^7$) applied in the fossil calibrated analysis and using the same data.

2.2.6 Biogeography

The 50% majority rule consensus tree obtained from Bayesian analysis 1 (Fig. 1) was taken to reconstruct the ancestral geographic areas of the clade bearing *Potentilla* s.str. and its sister clade using Mesquite version 2.6 (Maddison & Maddison 2009). In order to limit effects of species selection on the reconstruction of ancestral areas, Clade A of the phylogenetic tree was excluded from the analyses as most of the genera contained in this lineage were of geographic wide distribution but sampled with one or few species and accessions only. Two strategies of geographic coding were applied: 1) The geographic distribution ranges of the studied species were taken as the basis for coding. This analysis considers all geographic regions covered by a species and relies on the assumption that the observed haplotype is representative for the whole analyzed species, which may not be the case in lineages of reticulate evolution. 2) The actual geographic location of the studied accessions, i.e. collection sites, was used for coding. This approach infers the place of origin of clades and the migration of populations (which is concomitantly bound to fruitlets) from the geographic

distribution of haplotypic variation carried by the studied accessions irrespective of its taxonomic identity. The results of the alternative analyses can be compared with hypotheses obtained from the first approach and discussed together with additional evidence for hybridization. The recent distribution ranges of species were extracted from published data (Wolf 1908; Meusel et al. 1965a, b; Kurtto et al. 2004). Species distribution ranges were finally transformed into a categorical variable with the following continental regions used as character states: Europe, East Asia, South Asia, Central Asia, North Asia, South-West Asia, Eastern North America, Western North America, and rest of the World. Character states were treated as unordered. The geographic units South Asia and South-West Asia included the primary center of species diversity and the secondary plus the adjacent part of the tertiary center of species diversity of *Potentilla*, respectively, as suggested by Shah et al. (1992). Multiple states were assigned if taxa occurred in more than one of the defined areas. A Mesquite input file in nexus format was created including a taxa block defining the taxon labels, a character block containing the coded geographic variables, and a tree block providing the Bayesian 50% majority rule consensus tree. The file was executed in Mesquite under the parsimony method of ancestral character reconstruction using the TRACE CHARACTER HISTORY command.

2.2.7 Diversity

In order to make inferences about the phylogeographic history of a phylogenetically poorly resolved derived group of *Potentilla* s.str. accessions, we followed a method developed by Avise (2000) which is based on the comparison of nucleotide diversity with gene diversity. Gene diversity is calculated on the basis of numbers of genetic variants within a population only while nucleotide diversity also incorporates mutational differences between the observed alleles. We apply the basic idea of this concept to the derived evolutionary lineage of *Potentilla* s.str. and argue that low nucleotide diversity compared to gene diversity is indicative for colonization (expectedly by a limited number of genetic variants) followed by population growth, while an increased nucleotide diversity is indicative of stable populations over prolonged time periods or multiple colonization events adding alleles to the respective area. Nucleotide diversity (π; Nei 1987) using uncorrected *p*-distances and gene diversity *H* was calculated using Arlequin (Excoffier et al. 2005) as implemented in this software package. Both SNPs and coded indels were considered when calculating *p*-distances among sequences. The data were divided based on their geographic origin into three groups: Asia, Europe, and North America comprising 19, 35, and 52 accessions from this group of *Potentilla* s.str., respectively.

2.3 Results

2.3.1 Sequence variation

At least two cpDNA markers were successfully sequenced for 162 ingroup accessions as well as for additional five outgroup samples (Appendix 1). The length of the entire alignment comprising *trn*S-*ycf*9, *trn*L-*trn*F, and *trn*C-*ycf*6 was 2604 bp (for the alignments see enclosed CD with the Supplementary Data). The length of the individual single markers varied

between 440 bp and 500 bp (*N* = 165 sequences), 389–524 bp (*N* = 166), and 760–1103 bp (*N* = 149). After exclusion of regions of ambiguous alignment (alignment positions 272–317, 372–388, 491–505, 1699–1796, 2004–2108, 2406–2460), the effective length of the alignment was reduced to 2268 bp with *trn*S-*ycf*9, *trn*L-*trn*F, and *trn*C-*ycf*6 each contributing 517 bp, 702 bp, and 1049 bp. The following measures were deduced from this reduced alignment for the ingroup and – in parentheses – the entire dataset including the outgroup. 623 (713) sites were variable. Of these 392 (415) sites were parsimony informative. In addition 178 (209) indels as identified by GAPCODER were included in the phylogenetic analyses, with 94 (103) of these being parsimony informative. 132 haplotypes were identified by Collapse among the 167 accessions sequenced for the combined *trn*S-*ycf*9, *trn*L-*trn*F, and *trn*C-*ycf*6 regions. Haplotype designations and duplicates are given together with the taxonomic identity of the carrier of a haplotype in Appendix 1. Sequences were submitted to GenBank (GQ384460-GQ384945).

2.3.2 Phylogenetic relationships

The most basal split in the Bayesian phylogenetic reconstructions (Figs. 1 and 3) separated the ingroup taxa into two major clades, one comprising the genera *Alchemilla*, *Chamaerhodos*, *Farinopsis*, *Dasiphora*, *Drymocallis*, *Fragaria*, *Schistophyllidium* as well as *Sibbaldia parviflora* Willd. and *Sibbaldia procumbens* together with the two accessions from the former genus *Sibbaldiopsis* (*Sibbaldiopsis tridentata* [Aiton] Rydb. = *Sibbaldia tridentata*; *Sibbaldiopsis cuneifolia* [Bertol.] Soják = *Sibbaldia cuneifolia* [Bertol.] Paule & Soják). The other clade joined the genera *Argentina*, *Horkelia*, *Horkeliella*, *Ivesia*, *Piletophyllum*, *Tylosperma*, *Sibbaldia tetrandra* Bunge, and all remaining *Potentilla* accessions including species which formerly have been treated as representatives of the genera *Duchesnea*, *Fragariastrum*, and *Tormentilla*. This most basal split was supported by the Bayesian reconstruction with posterior node probabilities/bootstrap values of 1/85, 1/100, and 1/100 for the whole ingroup as well as its two most basal clades (Fig. 1).

Potentilla s.str., as informally used here, was resolved as a monophyletic group as it comprised all *Potentilla* and *Horkelia*/*Horkeliella*/*Ivesia* accessions save *Potentilla leuconota* D. Don., which belongs morphologically to the genus *Argentina* (but have not been transferred to this genus yet), along with the single sample of *Sibbaldia tetrandra*. Genera other than *Potentilla* accepted in current taxonomic concepts and represented with at least two accessions within this study were clustered into monophyletic groups or placed together with the members of other genera: The genera *Alchemilla* (supported by a posterior probability/bootstrap value of 1/100), *Chamaerhodos* (1/100), *Drymocallis* (1/100), *Schisto-phyllidium* (1/100), *Horkelia*, *Horkeliella* plus *Ivesia* (1/100), and *Sibbaldia* (1/100) were mono-phyletic, while the genera *Argentina* and *Piletophyllum* (1/96) were grouped together in a single clade. However, their phylogenetic positions were inconsistent with the monophyly of *Argentina*.

The cpDNA-based phylogenies of the *Potentilleae* (Figs. 1 and 3) were fully congruent with the *trn*L-*trn*F IGS-based reconstruction published by Eriksson et al. (2003) (cf. Fig. 3). Nevertheless, the combined cpDNA-nDNA reconstruction of Eriksson et al. (2003) (cf. Fig. 4) differed from the present results in a derived placement of *Agrimonia* compared to *Rosa* (Fig. 3) and in the sister group relationship between *Chamaerhodos* and *Potentilla arguta*

(*Drymocallis*). While the phylogenetic relationship between *Rosa* and *Agrimonia* was only weakly supported in our analyses, the positions of *Chamaerhodos* and *Drymocallis* were statistically well founded. However, the closer phylogenetic relationship of *Rosa* with the *Potentilleae* compared to *Agrimonia*, as supported by our data, was also suggested by a recently published analysis using eight nuclear and chloroplast markers (Potter et al. 2007). In the following we refer to the phylogenetic relationship and position of those taxa which have not been phylogenetically studied in the analysis of the *Potentilleae* by Eriksson et al. (2003) or have been studied for the first time. *Piletophyllum* and *Argentina* together were sister to *Tylosperma lignosa* (Willd. ex Schlechtend.) Botsch. (Clade B). *Sibbaldia tetrandra* Bunge, the studied representatives of series *Biflorae*, and *Potentilla curviseta* J.D. Hook. plus *Potentilla eriocarpa* Wall. ex Lehm. (Clade H) were paraphyletic to a derived clade of taxa geographically centered in Europe and adjacent South-West Asia (Clade G). The series *Tormentillae* (Clade F), *Potentilla biennis* Greene together with *Ivesia*, *Horkeliella* and *Horkelia* (Clade E), *Potentilla fragarioides* L. and *Potentilla freyniana* Bornm. (Clade D), and *Potentilla newberryi* A. Gray were successively sister to a large, but genetically little diverged group of 106 *Potentilla* accessions representing 80 species (large polytomy in Clade C) (except *P. biennis*) exclusively from Wolf's series *Argenteae*, *Aureae*, *Chrysanthae*, *Graciles*, *Grandiflorae*, *Haematochroae*, *Multifidae*, *Multijugae*, *Niveae*, *Persicae*, *Ranunculoides*, *Rectae*, *Rivales*, and *Tanacetifoliae*.

The phylogenetic relationships inferred among the representatives of *Potentilla* s.str. were congruent among the reconstructions (Figs. 1 and 2) and supported by maximal posterior probabilities and bootstrap values for nodes of Clade C, D, E, F, G, H, and I of 1/100, 1/100, 0.76/50 (1/100 exclusive of *P. biennis*), 1/100, 1/73, 1/99, and 1/99, respectively.

The relationships among *Potentilla* accessions within the large Clade C – which we henceforth call the *Potentilla* core group – are shown in Fig. 2. The core group was poorly resolved as this lineage contained several multiple-taxon polytomies aside from a variety of more derived but mostly small clades. Forty-nine accessions were clustered in comparably small clades according to geography, i.e. continents (Fig. 2), Europe: clades 3, 7, 8, 10, 11, and 13 (N = 17 accessions), North America: clades 4, 5, 15, 17, 19, 20, and 21 (N = 30), and Asia: clade 14 (N = 2). A smaller but still considerable fraction of accessions (N = 36) was placed in clades of mixed geography, Asia and North America: clades 1, 2b, 6, 16, 18 (N = 20 = 10 + 10), Asia and Europe: clades 2a, 12 (N = 10 = 3 + 7), and Europe and North America: clade 9 (N = 6 = 2 + 4). This distribution indicated a considerable degree of sharing of genetic variants among continents in terms of unique haplotypes and evolutionary lineages. In contrast, the resolved clades showed in general little association with taxonomy. Covered series of Wolf's worldwide system were either polyphyletic or remained unresolved and none of these taxonomic groups present with two accessions at least were monophyletic. Using the generic classification proposed by B. Ertter (in prep., *Potentilla*, FNA: valid the native North American species only) five out of the nine sections present in the data with two or more accessions were either paraphyletic or polyphyletic (*Pensylvanicae*, *Aureae*, *Multijugae*, *Graciles*, *Leucophylleae*). Contrariwise phylogenetic placement of sections *Concinnae*, *Haematochri*, and *Subviscosae* was compatible with monophyly of these groups. The statistic support of the clades was 0.5 (posterior node probability) or 50% (bootstrap value) at least, save for clade 5. Support values for clades made up of duplicate haplotypes only were not calculated.

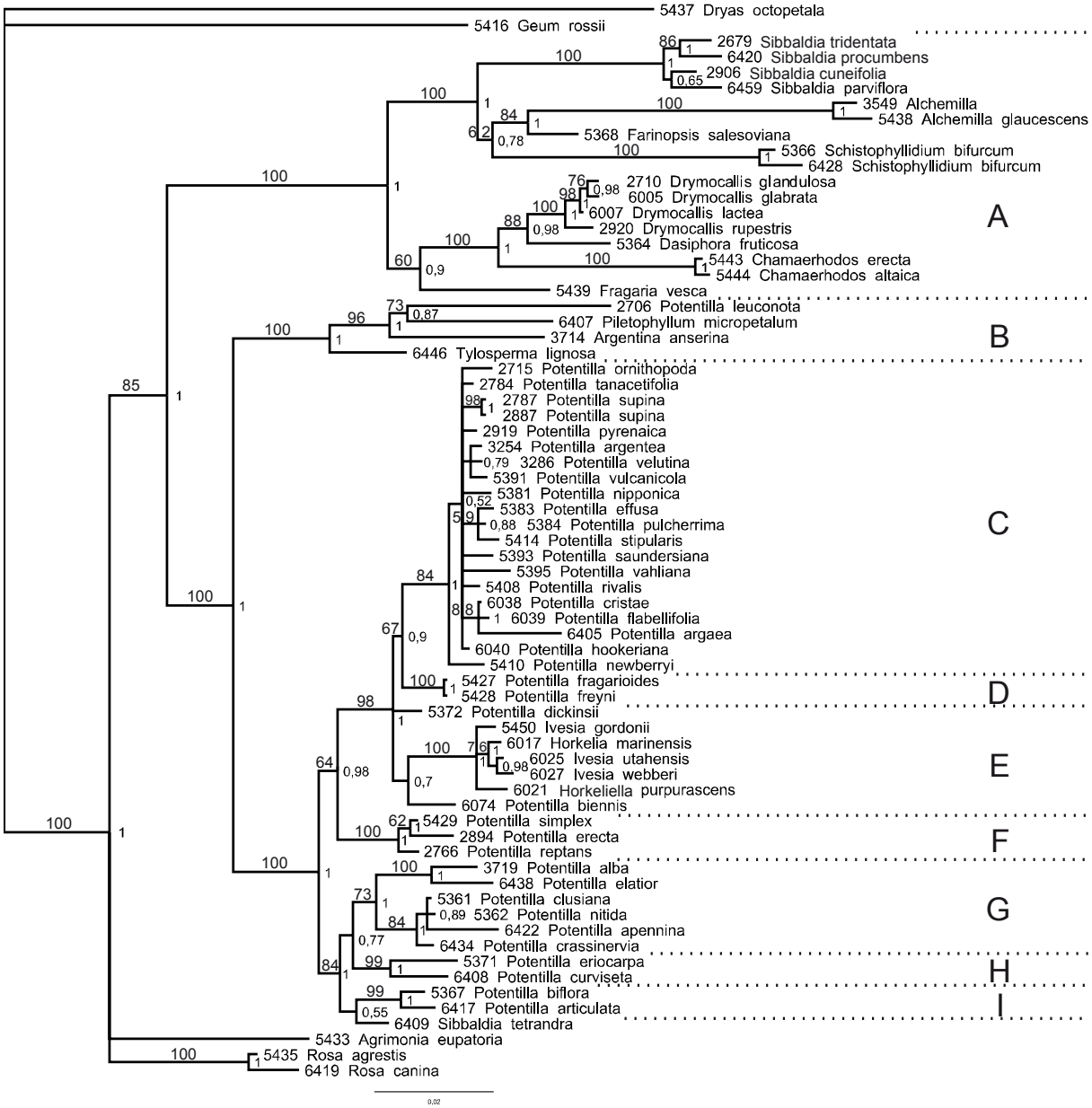

Figure 1. 50% majority rule consensus tree of 69 selected accessions from the Potentilleae based on the combined trnS-ycf9, trnL-trnF, and trnC-ycf6 IGSs data set and SNPs and coded indel present in these regions. The tree was reconstructed from 1000 retained trees (out of 5000; burnin = 4000) inferred using MrBayes. Major clades are denoted A to I as referred to in the text. Posterior probabilities are given to the right of nodes. Numbers above branches are bootstrap values obtained for taxonomically equivalent clades running an independent maximum parsimony analysis on the same character set and accessions using PAUP (number of bootstrap replicates was 1000). The scale bar denotes the number of substitutions per site.

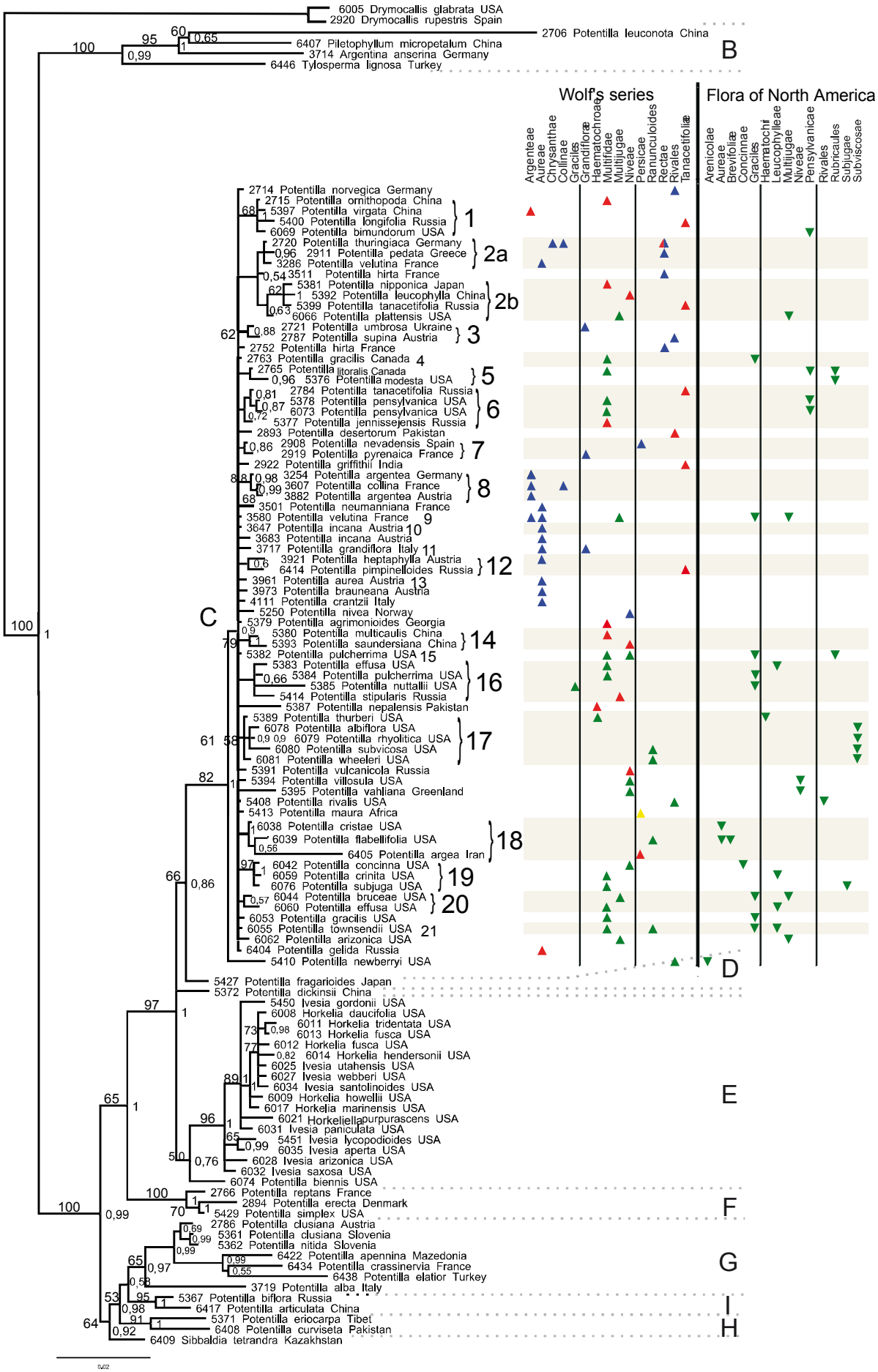
Wolf's series
Flora of North America
Argenteae
Aureae
Chrysanthae
Collinae
Gracilis
Grandiflorae
Haematochroae
Multifidae
Multijugae
Niveae
Persicae
Ranunculoides
Rectae
Rivales
Tanacetifoliae
Arenicolae
Aureae
Brevifoliae
Concinnae
Gracilis
Haematochi
Leucophylleae
Multijugae
Niveae
Pensylvanicae
Rivales
Rubricaules
Subjugae
Subviscosae
6005 Drymocallis glabrata USA
2920 Drymocallis rupestris Spain
2706 Potentilla leuconota China
6407 Piletophyllum micropetalum China
3714 Argentina anserina Germany
6446 Tylosperma lignosa Turkey
B
2714 Potentilla norvegica Germany
2715 Potentilla ornithopoda China
5397 Potentilla virgata China
5400 Potentilla longifolia Russia
6069 Potentilla bimundorum USA
1
2720 Potentilla thuringiaca Germany
2911 Potentilla pedata Greece
3286 Potentilla velutina France
2a
3511 Potentilla hirta France
5381 Potentilla nipponica Japan
5392 Potentilla leucophylla China
5399 Potentilla tanacetifolia Russia
6066 Potentilla plattensis USA
2b
2721 Potentilla umbrosa Ukraine
2787 Potentilla supina Austria
3
2752 Potentilla hirta France
2763 Potentilla gracilis Canada
4
2765 Potentilla litoralis Canada
5376 Potentilla modesta USA
5
2784 Potentilla tanacetifolia Russia
5378 Potentilla pensylvanica USA
6073 Potentilla pensylvanica USA
5377 Potentilla jennissejensis Russia
6
2893 Potentilla desertorum Pakistan
2908 Potentilla nevadensis Spain
2919 Potentilla pyrenaica France
7
2922 Potentilla griffithii India
3254 Potentilla argentea Germany
3607 Potentilla collina France
3882 Potentilla argentea Austria
8
3501 Potentilla neumanniana France
3580 Potentilla velutina France
9
3647 Potentilla incana Austria
10
3683 Potentilla incana Austria
3717 Potentilla grandiflora Italy
11
3921 Potentilla heptaphylla Austria
6414 Potentilla pimpinelloides Russia
12
3961 Potentilla aurea Austria
13
3973 Potentilla brauneana Austria
4111 Potentilla crantzii Italy
5250 Potentilla nivea Norway
C
5379 Potentilla agrimonioides Georgia
5380 Potentilla multicaulis China
5393 Potentilla saundersiana China
14
5382 Potentilla pulcherrima USA
15
5383 Potentilla effusa USA
5384 Potentilla pulcherrima USA
5385 Potentilla nuttallii USA
5414 Potentilla stipularis Russia
16
5387 Potentilla nepalensis Pakistan
5389 Potentilla thurberi USA
6078 Potentilla albiflora USA
6079 Potentilla rhyolitica USA
6080 Potentilla subvicosa USA
6081 Potentilla wheeleri USA
17
5391 Potentilla vulcanicola Russia
5394 Potentilla villosula USA
5395 Potentilla vahliana Greenland
5408 Potentilla rivalis USA
5413 Potentilla maura Africa
6038 Potentilla cristae USA
6039 Potentilla flabellifolia USA
6405 Potentilla argea Iran
18
6042 Potentilla concinna USA
6059 Potentilla crinita USA
6076 Potentilla subjuga USA
19
6044 Potentilla bruceae USA
6060 Potentilla effusa USA
20
6053 Potentilla gracilis USA
6055 Potentilla townsendii USA
21
6062 Potentilla arizonica USA
6404 Potentilla gelida Russia
5410 Potentilla newberryi USA
D
5427 Potentilla fragarioides Japan
5372 Potentilla dickinsii China
5450 Ivesia gordonii USA
6008 Horkelia daucifolia USA
6011 Horkelia tridentata USA
6013 Horkelia fusca USA
6012 Horkelia fusca USA
6014 Horkelia hendersonii USA
6025 Ivesia utahensis USA
6027 Ivesia webberi USA
6034 Ivesia santolinoides USA
6009 Horkelia howellii USA
6017 Horkelia marinensis USA
6021 Horkeliella purpurascens USA
6031 Ivesia paniculata USA
5451 Ivesia lycopodioides USA
6035 Ivesia aperta USA
6028 Ivesia arizonica USA
6032 Ivesia saxosa USA
6074 Potentilla biennis USA
E
2766 Potentilla reptans France
2894 Potentilla erecta Denmark
5429 Potentilla simplex USA
F
2786 Potentilla clusiana Austria
5361 Potentilla clusiana Slovenia
5362 Potentilla nitida Slovenia
6422 Potentilla apennina Mazedonia
6434 Potentilla crassinervia France
6438 Potentilla elatior Turkey
3719 Potentilla alba Italy
G
5367 Potentilla biflora Russia
I
6417 Potentilla articulata China
5371 Potentilla eriocarpa Tibet
6408 Potentilla curviseta Pakistan
H
6409 Sibbaldia tetrandra Kazakhstan

Figure 2. 50% majority rule consensus tree of 110 haplotypes carried by 145 accessions from Clades B to I as reconstructed by analysis 1 (Fig. 1) and using the same markers and SNP characters. Clades are accordingly designated. Subclades as well as haplotypes present more than once in the data within Clade C are enumerated 1 to 21. The tree is a consensus of 1000 single trees (out of 5000 retained; burnin = 4000) inferred by MrBayes. *Drymocallis glabrata* and *D. rupestris* served as outgroup. Values above branches (or left to nodes) are bootstrap values inferred by maximum parsimony analysis based on the same data using PAUP (number of replicates was 1000). Posterior probabilities of nodes are given to their right. The geographic origin of samples and their taxonomic affiliation according to Wolf's (1908) series and to the most recent sectional classification available for the North American *Potentilla* species (Ertter unpubl., *Potentilla* in Flora of North America) grouped in Clade C are provided to its right in a conjoined table. Colors of the triangles mark the different continents: blue indicates a European origin of accessions carrying a given haplotype, red is Asia, green is North America and yellow means Africa. Accessions carrying duplicate haplotypes are not shown in the tree and only their affiliation to series and geographic origin is shown in the matrix (see Table 1 for duplicates and their carriers).

2.3.4 Diversification in time

The phylogenetic tree of 69 accessions based on single nucleotide polymorphisms and markers *trn*S-*ycf*9, *trn*L-*trn*F, and *trn*C-*ycf*6 reconstructed by the BEAST program (Fig. 3) was largely consistent in topology with the equivalent reconstruction based on SNPs and indel polymorphisms using MrBayes (Fig. 1). Topological incongruence between these two phylogenetic reconstructions as represented by the respective 50% majority rule consensus trees referred to an interchanged position of *P. apennina* and *P. crassinervia* in Clade G only. The estimated ages of prominent nodes in million years ago (mya) given as their 95% confidence intervals are summarized in Table 1. These divergence times of clades were calculated using the node bearing *Rosa* as calibration point for which a prior of 4.9×10^6 to 5.6×10^6 years according to the oldest fossil record of the genus was set. Alternatively, ages were inferred by applying a molecularly inferred age of the *Rosa* node (29.3–46.6 mya; Fig. 4). As the root of the clade bearing *Rosa* was used to calibrate the phylogeny, inferred ages should be considered minimum time estimates: an age of 45.1–53.4 mya (Eocene) and 26.9–42.5 mya (Eocene to Early Oligocene) was estimated for the node joining the *Potentilleae* in the fossil and molecularly inferred calibration, respectively. Estimates of the earliest origin of other selected main lineages were 25.6–33.9/15.6–26.3 mya (fossil calibration/molecular calibration: henceforward the two estimates are displayed this way) *Sibbaldia*, 24.1–32.3/14.2–24.6 mya *Schistophyllidium* and *Alchemilla* plus *Farinopsis*, 21.2–32.0/13.2–24.0 mya *Argentina*, 15.1–25.8/9.8–19.4 mya *Chamaerhodos*, 13.9–21.4/8.4–16.0 mya series *Tormentillae* (Clade F), 12.3–19.4/7.6–14.6 mya Clade G, 7.1–14.7/4.5–10.5 mya *Drymocallis*, 7.2–12.7/4.3–9.2 mya *Horkelia*/*Horkeliella*/*Ivesia*, and 4.6–8.1/2.7–5.9 mya the *Potentilla* core group (excluding *P. newberryi*).

2.3.5 Biogeography

The parsimony reconstructions of ancestral geographic areas of *Potentilla* s.str. joined in Clade B to I based on continental regions and the 50% majority rule consensus tree of Bayesian analysis 1 using Mesquite suggested East Asia in the collection site-based analysis as most ancient geographic area of this evolutionary lineage (Fig. 5b). When distribution

Table 1. 95% confidence intervals of ages of major phylogenetic splits and ages of basal nodes of clades, respectively, within the *Potentilleae* as discussed in the text. Topology and age estimates refer to analysis 1 and the taxonomically equivalent BEAST analysis (Figs. 1 and 3). Ages listed in the column were calculated based on the oldest fossil record known for *Rosa* (Palaebiology database http://paleodb.org; 55.8-48.6 mya: USA, Idaho: *Rosa germerensis*) used for calibration of the phylogeny. Values provided in the second column used a time of origin of 29.3-46.6 mya for the *Rosa* lineage as inferred using a molecular phylogeny of nine representatives of Rosaceae, an estimated age of 76 mya for this family published by Wikström et al. (2001) and BEAST (Fig. 4). Ages are given in million years ago (mya). Major evolutionary events are provided in the third column.

Phylogenetic split or age of clade base	**Age of node, fossil calibration**	**Age of node, molecular estimate**	**Major biological events**
Chamaerhodos	0.0013–1.4	0.0011–1.0	intercontinental dispersal
P. articulata/*P. biflora*	1.4–7.3	1.0–5.2	dispersal from Asia to North America
P. erecta/*P. simplex*	1.5–5.2	1.6–5.5	dispersal from Eurasia to North America
Horkelia + *Ivesia*	2.1–5.4	1.3–3.8	rapid radiation in Western North America followed by multiple intercontinental dispersals
Drymocallis	2.3–6.7	1.4–4.6	intercontinental dispersal
Clade F	2.6–7.2	1.6–5.5	
Potentilla core group excl. *P. newberryi*	3.6–6.7	2.1–4.8	rapid worldwide radiation
Potentilla core group = Clade C	4.6–8.1	2.7–5.9	
Sibbaldia (including *Sibbaldiopsis*)	4.0–8.6	2.5–6.4	
Drymocallis/*Dasiphora*	7.1–14.7	4.5–10.5	
Clade E	7.2–12.7	4.3–9.2	
Clade G	9.3–15.7	7.6–14.6	continuous radiation in Europe and West Asia
Clades C–E	9.8–15.2	5.7–11.0	earliest most possible arrival of extant *Potentilla* s.str. in North America: uncertain hypothesis
Clade G–I	12.3–19.4	7.6–14.6	continuous radiation of the *Potentillae trichocarpae*
Clades C–F	13.9–21.4	8.4–16.0	
Drymocallis etc./*Chamaerhodos*	15.1–25.8	9.8–19.4	
Alchemilla/*Farinopsis*	20.4–29.1	12.1–22.1	
Argentina/*Tylosperma* = CladeB	21.2–32.0	13.2–24.0	
Alchemilla etc./*Schistophyllidium*.	24.1–32.3	14.2–24.6	
Sibbaldiopsis etc./*Schistophyllidium* etc.	25.6–33.9	15.6–26.3	
Fragariinae = Clade A	29.7–38.4	18.0–30.0	
Potentillinae	34.3–45.2	20.5–34.5	origin of *Potentilla* s.str.
Fragariinae/*Potentillinae* = *Potentilleae*	45.1–53.4	26.9–42.5	major split in the *Potentilleae*

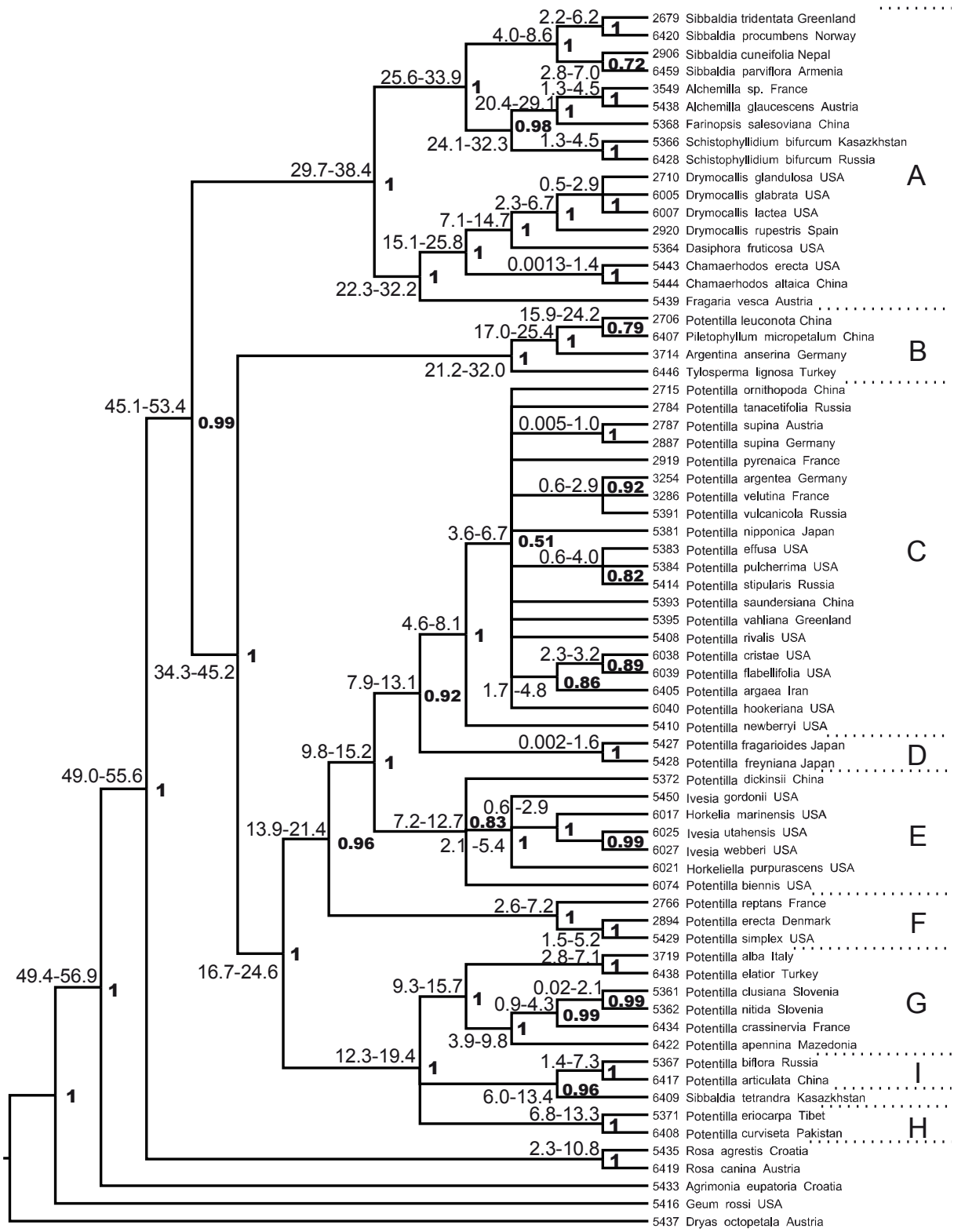

Figure 3. 50% majority rule consensus tree of 69 selected accessions from the *Potentilleae* reconstructed from 2000 retained single trees – after discarding 8000 of 10000 obtained trees during the burnin – using the combined *trn*S-*ycf*9, *trn*L-*trn*F, and *trn*C-*ycf*6 IGSs and BEAST. The tree topology was simultaneously reconstructed together with the divergence times of nodes using Bayesian inference. The 95%-confidence interval of node ages in million years is given to their left. The node bearing *Rosa* was used to calibrate the tree based on the oldest known fossil of the genus (48.6–55.8 mya). Bold numbers are the posterior probabilities of clades as estimated by the BEAST algorithm. The analysis was based on SNPs only. The topology of the tree is largely congruent with the Bayesian reconstruction including indel information shown in Fig. 1 and major clades are accordingly designated.

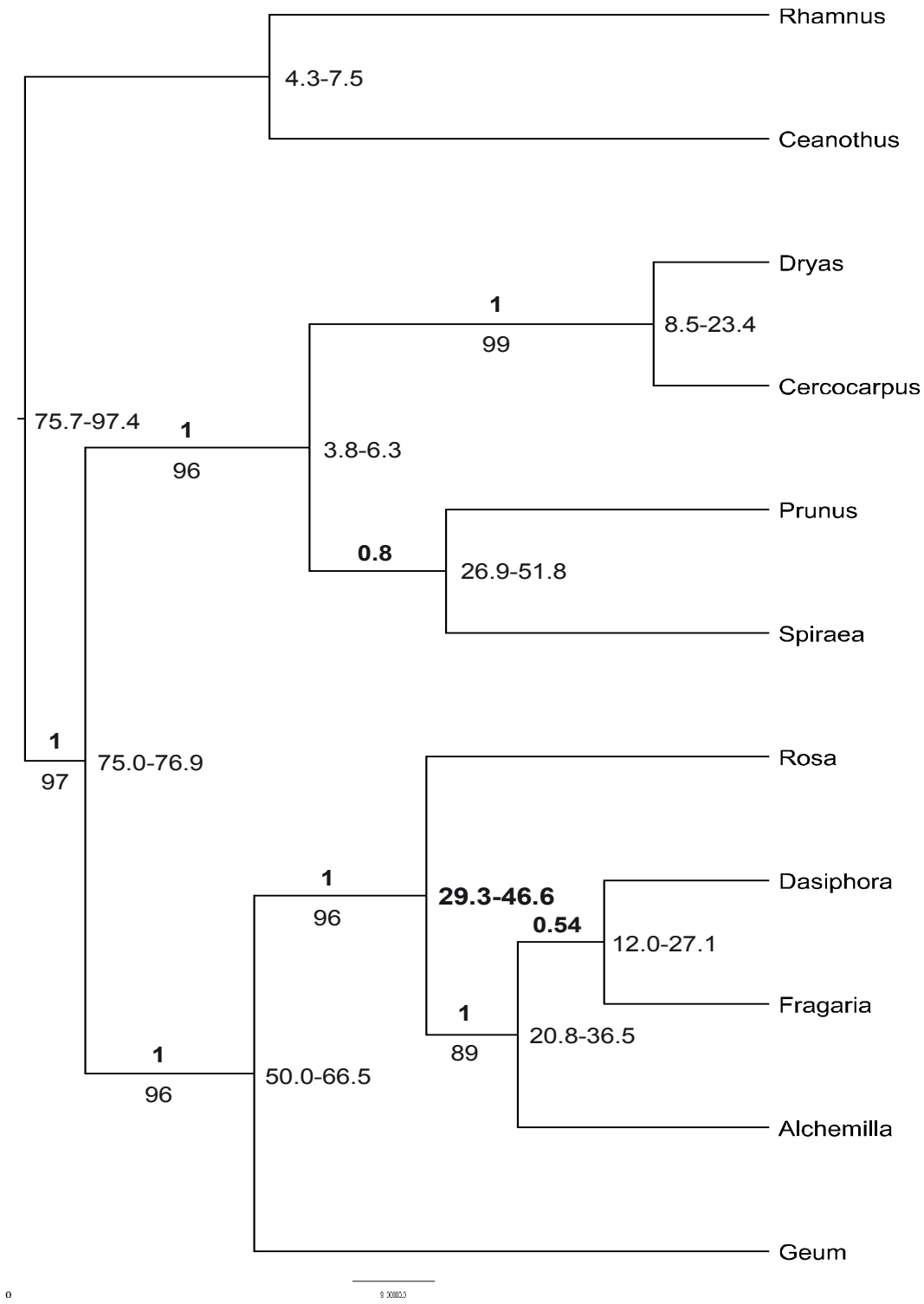

Figure 4. 50% majority rule consensus tree of nine rosaceous genera representing the two major evolutionary lineages recently recognized in Rosaceae (Potter et al. 2007) and two outgroup taxa from Rhamnaceae and reconstructed from published rbcL gene sequences using BEAST (2000 retained trees, burnin=8000). The age of the ingroup was set to 75–77 mya according to a suggested age of Rosaceae of 76 mya (Wikström et al. 2001). The 95%-confidence interval of node ages in million years is given to their right. The estimate for the node bearing Rosa used as an alternative time calibration in the phylogeny of the Potentilleae (Fig. 1) is printed in bold letters. Bold numbers above the branches are posterior probabilities of clades. Numbers below branches are bootstrap values obtained from 1000 replicates running a maximum parsimony analysis using PAUP.

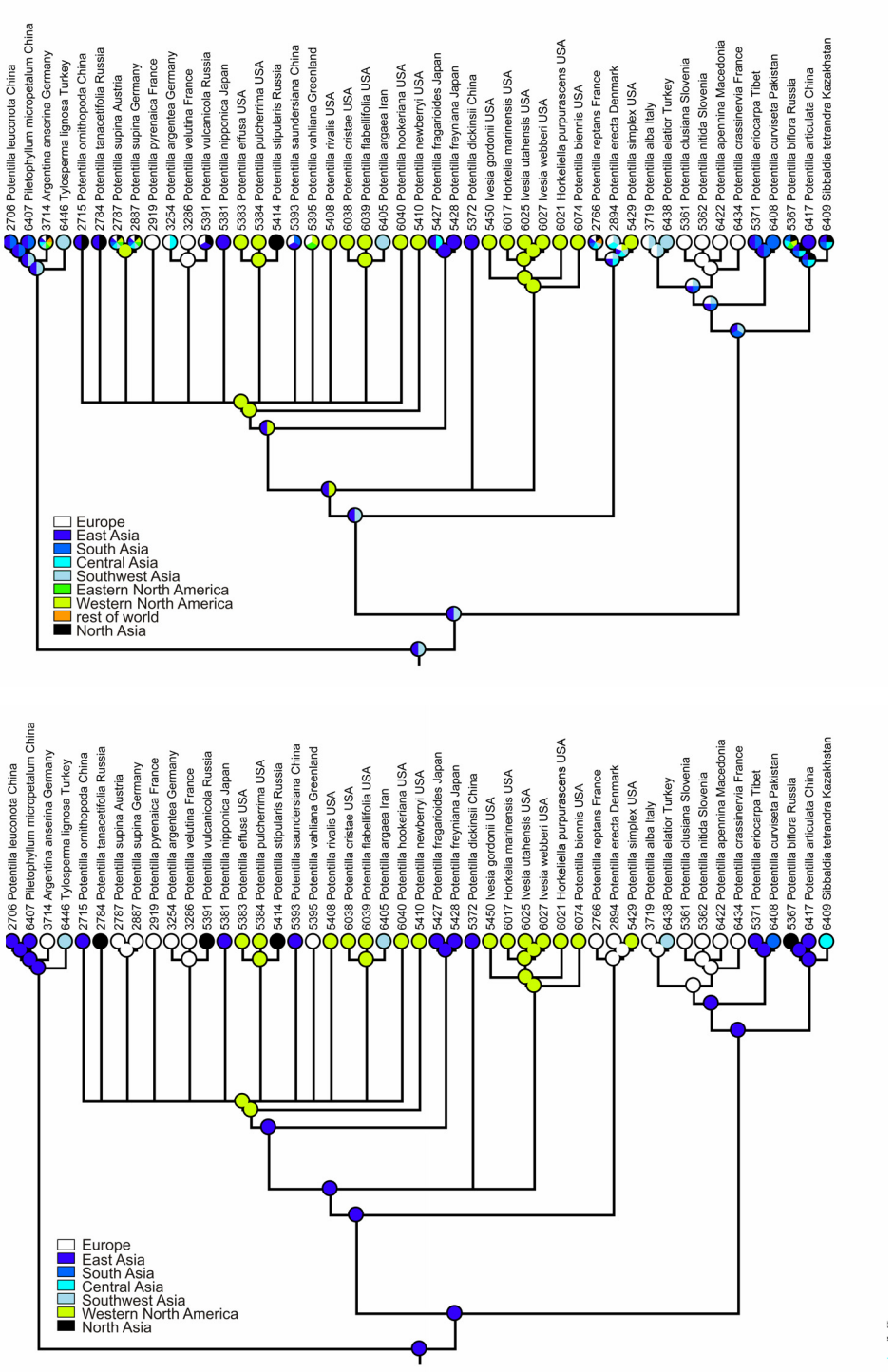

Figure 5. Ancestral area reconstructions based on the 50% majority rule consensus tree obtained in analysis 1 (Fig. 1). The geographic ranges of ancestral nodes were inferred using Mesquite under the parsimony criterion. (a) the contemporary occurrence of species was coded by transforming the recent geographic distribution range of species into continental regions as listed in the legend. (b) collection sites of accessions were used for coding using the same geographic regions.

ranges of species were used for coding, South-West Asia was additionally inferred (Fig. 5a). South-West Asia and East Asia were also alternatively suggested as ancestral areas of Clade B, *Argentina*, the combined Clades C to E, and Clade F. In addition to these two regions South Asia was inferred as origin of Clades G to I.

North America was colonized according to the reconstruction by the studied taxa from Asia at least four times independently: by the genus *Argentina* (i.e. *Argentina anserina* Rydb.), clades C to E, the series *Tormentillae* (Clade F), and *Potentilla biennis*. If East Asia instead of North America, as alternatively suggested by the distribution range-based analysis as well as by the collection site-based reconstruction is accepted as ancestral area of clades C to D and clades C to E, one additional dispersal event from Asia to North America involving Clade E (joining *Horkelia*, *Horkeliella*, *Ivesia*, and *Potentilla biennis*) and the *Potentilla* species grouped in Clade C has to be assumed. The occurrence of the resolved evolutionary lineages in Europe was explained by both analyses by four colonization events starting from Asia and involving again *Argentina,* series *Tormentillae*, *Potentilla* Clade C, and in addition Clade G. The two alternative reconstructions are not contradictory, but congruent insofar as the distribution range-based analysis suggested additional geographic areas compared to the collection site-based one.

Gene diversity H and nucleotide diversity π calculated for the core group of *Potentilla* for Asia, Europe, and North America are provided in Table 2. Gene diversity was similar among the three areas (H = 0.96 to 0.97) – a result presumably reflective of the low number of duplicates of haplotypes observed – while nucleotide diversity was increased in Asia (π = 0.0219) relative to North America (π = 0.0142) and Europe (π = 0.0116).

Table 2. Nucleotide diversity π and gene diversity H calculated based on the combined *trn*S-*ycf*9, *trn*L-*trn*F, and *trn*C-*ycf*6 regions for the *Potentilla* core group (Clade C; Fig. 2) on a continental basis.

Continent	π	H
Asia	0.0219 ± 0.011	0.968 ± 0.028
North America	0.0142 ± 0.007	0.971 ± 0.010
Europe	0.0116 ± 0.006	0.968 ± 0.015

2.4 Discussion

2.4.1 Phylogenetic relationships and taxonomic implications

Our molecular-based reconstructions using three cpDNA markers (Fig. 1) distinguished two major evolutionary lineages within the *Potentilleae*: Clade A comprising the genera *Alchemilla*, *Chamaerhodos*, *Farinopsis*, *Dasiphora*, *Drymocallis*, *Fragaria*, *Schistophyllidium*, *Sibbaldia* p.p. including former genus *Sibbaldiopsis* on one hand and the combined Clades B to I, joining the genera *Argentina*, *Horkelia*, *Horkeliella*, *Ivesia*, *Piletophyllum*, *Tylosperma*, and *Potentilla* inclusive of *Duchesnea* and *Tormentilla* as well as *Sibbaldia tetrandra* on the other hand. This phylogenetic split is in agreement with the combined nuclear and chloroplast DNA-based phylogenetic reconstruction of the *Potentilleae* published by Eriksson et al. (2003) and Potter

et al. (2007) and corresponds to their clades *Fragariinae* and "*Potentilla*" a division originally proposed by Soják (1989) (subtribes *Fragariinae* and *Potentillinae*). The genera included in Clade A and represented by two accessions at least: *Alchemilla*, *Schistophyllidium*, *Drymocallis*, and *Chamaerhodos* were each monophyletic supporting and validating the taxonomic concept to treat these lineages as separate genera as also favored by Eriksson et al. (1998) and accepted as working hypothesis in the present study. The two former members of the genus *Sibbaldiopsis* (*S. cuneifolia* and *S. tridentata*) are nested within *Sibbaldia*, similar as in Lundberg et al. (2009). However, the *Sibbaldiopsis* species have been currently transferred to the genus *Sibbaldia* (Paule & Soják 2009), making this genus a monophyletic taxon too. The presence of *Sibbaldia tetrandra* in Clade I agrees with the anther morphology of this clade (Soják 2004) and supports the use of the previous name *Potentilla tetrandra* (Bunge) J. D. Hooker (Soják 2008). At this point, it is also necessary to mention the monotypic genus *Potaninia* Maxim., which was not included in our study. However, based on the anther type (Soják 2008) and previously published molecular data it clearly belongs to the Clade A, nested within *Dasiphora* (Lundberg et al. 2009). Using anther morphology and the insertion height of styles, Soják (2008) distinguished four subtribes within the *Potentilleae*. This classification was highly consistent with the molecular divergence of the tribe with the subtribe *Potentillinae* (subterminal styles, anthers with two thecae) represented by Clades B to I and the *Fragariinae*, *Chamaerhodotinae*, and *Alchemillinae* (lateral to subbasal styles, anthers with one theca) forming the sister Clade A. However, separation of *Chamaerhodotinae* and *Alchemillinae* based on the orientation and adhesion of the theca on the connective and their phylogenetic placement within the *Fragariinae* made the subtribe *Fragariinae* a paraphyletic taxonomic group. It seems reasonable to assume, that the different types of anthers with one theca are morphological variants derived from one ancestral type common to all genera combined in Clade B. Therefore, it appears more appropriate to join these lineages within one subtribe only. This classification is also more appropiate with respect to the evolutionary age of the subtribes and their divergence in terms of comprised major evolutionary lineages. Thus the age estimates for divergence of the extant representatives of both lineages are of similar magnitude (*Fragariinae* 29.7–38.4/18.0–30.0 mya, *Potentillinae* 34.3–45.2/20.5–34.5 mya). The analysis suggested in comparison a divergence of the investigated *Chamaerhodos* species from Asia and North America not earlier than 0.0013–1.4/0.0011–1.0 mya. The *Fragariinae* and *Potentillinae* would also be comparable in terms of number of main evolutionary lineages present. While most of these lineages in the *Fragariinae* were recently recognized and accepted as separate genera, the taxonomic delimitation of lineages of similar molecular divergence is not satisfactorily solved within the subtribe *Potentillinae* yet.

Soják (2008) included *Horkelia, Ivesia*, *Piletophyllum*, *Stellariopsis*, and *Tylosperma* in the *Potentillinae* beside *Potentilla* (inclusive of *Argentina*, *Duchesnea*, and *Tormentilla*). Considering the molecular data, the phylogenetic relationships among these genera appear largely solved now. This allows to develop a generic concept on the basis of monophyletic groups. Due to the observed molecular distances, the *Potentillinae* may be split into separate genera each represented by a major evolutionary lineage as in the *Fragariinae*. Such a phylogeny-based generic concept supports also the recognition of currently accepted genera such as *Argentina*, *Horkelia* and *Ivesia*: However, under this concept it should also be accepted, that Clade C and D (*Potentilla* core group inclusive of *P. fragarioides* and *P. freyniana*), Clade F (series *Tormentillae*, which holds with *Potentilla reptans* L. the nomenclatural type of the genus *Potentilla*), and combined Clades G to I each have to be treated as a separate genus. This

would require, as discussed also by Eriksson et al. (1998), to transfer an inappropriate high number of taxa from *Potentilla* to these genera.

The placement of *Argentina* in a sister group related to *Potentilla* s.str. in the present reconstructions supports its current distinction as a separate genus. Nevertheless, it also included the genus *Piletophyllum*. *Tylosperma* was sister to these two genera, which further supports its recognition as a genus of its own right.

Clade G to I corresponds to a large phylogenetically related group of taxa comprising 12 out of 19 series of the section *Potentillae trichocarpae* Th. Wolf distinguished by Soják (1987). This molecularly defined lineage consists almost exclusively (except *Sibbaldia tetrandra*) of taxa from this section, particularly of all studied species of the series *Herbaceae* (Wolf 1908) together with the series *Biflorae*, *P. eriocarpa*, and *P. elatior* from the sister series *Suffruticolosae*. Consequently, it may be argued that the resolved evolutionary lineage of those originally morphologically recognised groups represents a natural taxon. This view is substantiated by the geographic distribution of the clade which is predominantly European to South Asian. Our molecular-based results are furthermore in agreement with Soják's (1987) proposal to exclude *P. elatior* from Wolf's series *Eriocarpae* and *P. dickinsii* from the *Potentillae trichocarpae* as both of these taxa were phylogenetically separated from *P. eriocarpa* (Clade H) outside (*P. dickinsii*) or within (Clade G: *P. elatior*) Clade G to I.

The sister group of *P. biennis* (series *Rivales*) in Clade E comprises the genera *Ivesia*, *Horkelia*, *Horkelliela*, and the former genus *Stellariopsis* (*Stellariopsis santalinoides* [Gray] Rydb. = *Ivesia santalinoides* Gray) which is here treated within *Ivesia*. These genera are morphologically distinguished by several conspicuous characters (Rydberg 1898; Ertter 1989) justifying their taxonomic separation from *Potentilla*. However, a clear separation of the genera of Clade E from each other is not seen in the molecular data. Finally, Rydberg's genus *Comarella*, has not been included in any phylogenetic study yet, but based on morphology it is clearly a member of this group (Ertter, B. in prep., *Ivesioid* sections, FNA).

Clade F, the *Tormentillae*, and Clade D, *P. fragarioides and P. freyniana*, are both two morphologically largely isolated lineages comprising about eight and two species only (Wolf 1908). In contrast, Clade C holds the majority of species currently treated within *Potentilla*. This is reflected by 106 accessions representing 80 out of 98 studied *Potentilla* species grouped within this lineage and represented by Wolf's series *Argenteae*, *Aureae*, *Chrysanthae*, *Graciles*, *Grandiflorae*, *Haematochroae*, *Multifidae*, *Multijugae*, *Niveae*, *Persicae*, *Ranunculoides*, *Rectae*, *Rivales*, and *Tanacetifoliae*. Phylogenetic lineages within this clade showed only poor association with hitherto suggested taxonomic concepts (Wolf 1908; Ertter 2009b, Fig. 2). This lack of congruence could potentially be explained by several factors including extensive lineage sorting, hybridization among sections and series, or inappropriate taxonomic classification but may also simply be a consequence of the observed limited molecular divergence of the clade. Obviously, speciation rates in the core group outcompeted molecular divergence rates and consequently was not followed by genetic differentiation of taxa for the markers used. Only the sections defined in the North American taxonomic treatment of *Potentilla* (sections *Concinnae*, *Haematochri*, and *Subviscosae*) were recovered as monophyletic lineages while others were either polyphyletic or paraphyletic at least (Fig. 2). Even though these associations may have been observed due to the reduced number of

included accessions and species only, sequence-based phylogenetic reconstructions might be successfully used for delimitation of some evolutionary lineages within the core group on regional geographic scales at least.

In summary, the phylogenetic reconstruction of species relationships was largely congruent for most major lineages with the taxonomy of *Potentilla* s.str. as the morphologically defined infrageneric groups were monophyletic according to the molecular data even in case of extensively sampled lineages. This pattern suggested that hybridization among distant lineages did not play a significant role in the history of the *Potentillinae*, an assumption supported by a largely congruent nrDNA ITS-based phylogeny performed for a subset of the studied species (Paule & Dobeš unpubl.). Exceptions involved the series grouped within the poorly resolved core group and in addition the series *Rivales*, the representatives of which were classified either as members of Clade C (*P. desertorum* Bunge, *P. newberryi*, *P. norvegica*, *P. rivalis*, *P. supina*) or placed at the basis of Clade E (Figs. 1 and 2). The polyphyly observed for the particular case of the *Rivales* could be explained by an incongruence of the cpDNA-based phylogenetic analysis with the phylogeny of this species as the representatives of this series (*P. newberryi*, *P. norvegica*, *P. rivalis*, and *P. supina*) clustered in the ITS phylogeny together with *P. biennis* (Paule & Dobeš unpubl.). The phylogenetic conflict between the cpDNA and nDNA analysis thereby may be explained by hybridization of the *Rivales* with the core *Potentilla*, which was assumed by several authors (cf. Asker 1970). Phylogeographic interpretation can not be made for these lineages in a taxonomic context, as taxa may carry haplotypes of a phylogeography history different from the history of species.

A timely concept for the classification of the *Potentillinae* is not provided yet. However, in a next step, the wealth of available data on the morphology (Wolf 1908; Panigrahi & Dikshit 1987), anatomy (Stepanova et al. 2007), reproductive modes (e.g. Rutishauser 1943b, 1960; Håkansson 1946; Asker 1986), karyological differentiation (e.g. Skalinska & Czapik 1958; Elkington 1969; Markova 1972; Asker 1985, 1986; Dobeš 1999), and geographic distribution (Wolf 1908) can be re-evaluated in order to verify if and which of these traits discriminate the main evolutionary lineages resolved by the molecular analysis. In addition, analogous analyses of nuclear DNA-markers are needed to complement the cpDNA-based dataset.

2.4.2 Biogeographic and evolutionary history

As an important consequence of the resolved molecular-based phylogenetic relationships among species of the tribe *Potentilleae* (Figs. 1 and 3), biogeographic studies can be confounded to natural evolutionary lineages. Thus the phylogeography of *Potentilla* s.str. can now be addressed without confusion with its segregate genera. The early concept of Wolf (1908) could not clearly separate these lineages based on morphological characters on which he put strong emphasis. Although the author primarily did not intend to reflect evolutionary relationships, he made a clear distinction between phylogenetically basal forms on one hand and modern derived groups on the other hand. About 40 species belonging to the *Anserinae*, *Aureae* p.p, *Biflorae*, *Bifurcae*, *Caulescentes*, *Crassinerviae*, *Curvisetae*, *Eriocarpae*, *Fruticosae*, *Nitidae*, *Niveae*, *Palustres*, *Rupestres*, *Speciosae*, *Tridentatae*, *Xylorrhizae*, were assigned by Wolf (1908) to the "paleotypic *Potentillas*", opposed to a highly diversified species-rich "neogenic" group comprising the remaining species. According to the molecular-based results several of

these groups are indeed of an early origin (Oligocene and Early Miocene) but belong to the *Fragariinae*, and therefore are only of limited interest for the phylogeography of *Potentilla* s.str.: i.e. the *Bifurcae* (*Schistophyllidium*): ≤ 24.1–32.3/14.2–24.6 mya, *Fruticosae* (*Dasiphora*) and *Rupestres* (*Drymocallis*): ≤ 15.1–25.8/9.8–19.4 mya, *Palustres* (*Farinopsis*): ≤ 20.4–29.1/12.1–22.1 mya, and *Tridentatae* (former genus *Sibbaldiopsis*): ≤ 25.6–33.9/15.6–26.3 mya. As we did not analyse the phylogeography of *Fragariinae* in detail, we only refer here to Wolf's (1908) circumarctic Tertiary theory developed to explain the intercontinental geographic distribution shown by each of these groups. Given the early origin of the lineages, the molecular data would principally be in accordance with this theory to explain their distribution. Although they may have diversified and expanded their distribution areas to the whole Northern Hemisphere in those time periods, the observed continental disjunctions of extant species should have been completed much later as can be exemplarily deduced from the molecular distances among accessions from Europe, Asia and North America: *Drymocallis* (2.3–6.7/1.4–4.6 mya), former genus *Sibbaldiopsis* (4.0–8.6/2.5–6.4 mya), and in addition *Chamaerhodos* (0.0013–1.4/0.0011–1.0 mya).

Wolf's other "paleotypic *Potentilla*s", the *Anserinae*, *Aureae* p.p., *Biflorae*, *Caulescentes*, *Crassinerviae*, *Curvisetae*, *Eriocarpae*, *Nitidae*, *Niveae*, *Speciosae*, and *Xylorrhizae* are, however, either sister or members of *Potentilla* s.str. and are therefore relevant for its phylogeography (although they may not be of an early evolutionary origin as in the case of the *Aureae* p.p. and the *Niveae*. These series were instead resolved as constituents of the derived phylogenetically young *Potentilla* core group).

2.4.2.1 Biogeographic history of the Potentillinae and Potentilla s.str.

The early split at the basis of the sister group of *Potentilla* s.str., Clade B, about 21.2–32.0/13.2–24.0 mya in the Oligocene approximately marks the evolutionary origin of the genus *Argentina* (*Anserinae*) supporting the proposed old age of this taxon (Wolf 1908). *Argentina* is centered with at least 24 species mainly in Eastern Asia and South-East Asia, i.e. the Himalaya and adjacent regions (from Afghanistan to China and the Malesian archipelago), and only two species (*A. anserina*, *A. anserinoides* [Raoul] J. Holub) occur outside of its center of diversity (Ikeda & Ohba 1999). Similarily all other taxa joined with *Argentina* in this clade are of Asian distribution: *Tylosperma lignosa*, basal in Clade B, belongs to the taxonomically isolated series *Xylorrhizae*, which is distributed in the Far East and adjacent South Asia (Wolf 1908). *Piletophyllum* as treated by Soják (2008) is of South Asian to East Asian distribution with the studied representative *Piletophyllum micropetalum* (D. Don.) Soják being centered in the Himalaya (Li et al. 2009). Hence, the combined taxonomic and geographic data indicate that Clade B is predominantly Asian which contributed to the inferred Asian origin of this lineage as well as of its sister *Potentilla* s.str. (Figs. 5a, b). The origin of the *Potentillinae* and the diversification of its major lineages Clades G to I and Clades C to F in Asia is a central and well-supported finding which rejects Wolf's circum-arctic Tertiary theory as an alternative explanation.

Clades G to I represents the *Biflorae*, *Caulescentes*, *Crassinerviae*, *Curvisetae*, *Eriocarpae*, *Fragariastra*, *Nitidae*, and *Speciosae* (together with *Sibbaldia tetrandra*) which diversified 12.3–19.4/7.6–14.6 mya in the Early to Middle Miocene. The majority of species classified within these series are of limited geographic distribution and are elements of the high mountains of

Europe and Asia. The representatives of the *Biflorae*, *Potentilla articulata* Franch. and *P. biflora*, were recovered by the phylogenetic reconstruction together with *P. curviseta*, *P. eriocarpa* and *S. tetrandra* either as sisters to the predominantly European Clade G (Fig. 3) or in paraphyletic position to this lineage (Fig. 1). These taxa were reported to be restricted to or centered in South and East Asia with the *Curvisetae* (represented by 2–3 species) occurring in the Himalayas, Kashmir, and Afghanistan, and *P. eriocarpa* and *S. tetrandra* extending also to Central and East Asia and the *Biflorae* in addition to North America (Wolf 1908). Occurrence of these taxa basal or as a sister to clade G and their highest species diversity in South Asia provide evidence for their main diversification within this area. Asia was inferred by the ancestral area analysis as geographic origin of these taxa, with South Asia, East Asia and South-West Asia suggested as alternatives (Figs. 5a, b).

The studied representatives of Wolf's series *Caulescentes*, *Crassinerviae*, *Eriocarpae*, *Fragariastra*, *Nitidae*, and *Speciosae* clustered in Clade G show a conspicuous concentration of species in the European high mountains. As already mentioned, these taxa were also recognised as phylogenetic group by Soják (1987). In particular the *Caulescentes*, *Crassinerviae*, *Fragariastra*, and *Nitidae* are centered with 2, 5, 4, and 3 species on the European continent (Wolf 1908). Except the series *Fragariastra* and *Caulescentes* (cf. Kurrto et al. 2004), species are mostly geographically isolated from each other as vicariants in different mountain chains. Based on our molecular data and the taxonomic relationships of the covered species, Clade G appears to be geographically restricted to a quite limited area comprising approximately Europe and the adjacent parts of western Asia till the Caucasus. The inferred age of Clade G of about 9.3–15.7 mya together with its geographic restriction and the obvious disjunctions among species likely suggests that this lineage evolved within this area involving fragmentation of a formerly more continuous distribution as already assumed by Wolf (1908). The ancestral area reconstructions inferred East Asia, South-West Asia, South Asia, and Europe (Figs. 5a, b) as alternative areas of origin of this lineage. Therefore, no precise conclusion about its Eurasian origin can be drawn using this approach. However, a diversity center of this lineage in Europe and South-Western Asia can be proposed based on molecular and taxonomic evidence.

Clade F, representing the series *Tormentillae*, was sampled with three species and accessions from Europe and North America only. The ancestral area reconstruction suggested an Asian origin for this lineage and a Eurasian distribution of the most common ancestor of the three species studied. Despite the inferred most early origin of this lineage about 13.9–21.4/8.4–16.0 mya in the Miocene, at least the studied extant species did not diversify earlier than an estimated 2.6–7.2/1.6–5.5 mya (Late Miocene to Pliocene).

2.4.2.2 The colonization of North America

The late diversification between Eurasian and North American *Tormentillae* is particularly interesting with respect to the date of occurrence of the only North American member of this series, *P. simplex* (Wolf 1908). As deduced from the BEAST analysis, this event did not occur earlier than about 1.5–5.2/0.9–3.7 mya during the Early Pleistocene and Pliocene. A similar time estimate was found for the colonization of North America by the combined clades G to I. The only species of North American distribution which was assigned to this lineage, *P. biflora* (Wolf 1908), thus split from its Asian sister *P. articulata* about 1.4–7.3/1.0–5.2 mya.

According to its Asian origin, it must have arrived later than that date in North America, an idea already introduced by Steffen (1925).

The earliest arrival of *Potentilla* s.str. on the North American continent, as suggested by the molecular data, already may have taken place about 9.8–15.2/5.7–11.0 mya during the Miocene. This suggestion applies in case, that the scenario of a North American distribution of the most common ancestor of Clades C to E, as inferred by the ancestral area reconstruction based on species distribution (Fig. 5a), is accepted. Alternatively, if an Asian ancestral distribution is accepted (Figs. 5a, b), the earliest occurrence of *Potentilla* s.str. in North America would be represented by the basal node of Clade E, 7.2–12.7/4.3–9.2 mya (*Horkelia*, *Horkeliella*, *Ivesia*, *P. biennis*). Under this scenario *Ivesia*, *Horkeliella* and *Horkelia* together with *P. biennis* would constitute the oldest North American "*Potentilla*"-lineage. *Potentilla biennis* was grouped in a nrDNA ITS phylogeny together with other members of the series *Rivales* of North American (*P. newberryi*, *P. rivalis*) and northern hemispheric distribution (*P. supina*, *P. norvegica*) (Paule & Dobeš unpubl.). This phylogenetic conflict can be explained by hybridization between Clade C and Clade E. The sister group relationship of a monophyletic series *Rivales* to *Ivesia*, *Horkeliella*, and *Horkelia* would agree with the North American origin of Clade E. However, this would also request an additional intercontinental dispersal event to explain the Eurasian occurrence of the *Rivales*. Clade E was statistically weakly supported (Figs. 1 and 3) involving an alternative placement of *P. dickinsii* as its sister (Fig. 3). That further opens the possibility, that the proposed members of this clade may not have arrived earlier than 2.1–5.4/1.3–3.8 mya (age of the base of the *Horkelia*/*Horkeliella Ivesia*/*P. biennis*-clade) at North America if the weakly supported branches of Clade E are collapsed and the inferred age of divergence of *Horkelia* and *Ivesia* is equalled with the arrival of these genera.

The geographic origin of the *Potentilla* core group remained unsolved as both an Asian and North American origin was suggested by the ancestral area reconstructions (Figs. 5a, b). However, the East Asian distribution, including Japan, of the phylogenetic sister of the core group Clade D (*P. fragarioides* and *P. freyniana*) and the coincidental availability of the geographically close Beringian land bridge (Gladenkov et al. 2002) at the time of the split of these lineages, at least suggest, that this continental link was of particular importance for the phylogeographic history of the core group.

Migration from Asia to North America across the Bering link was recognised as a major pattern in the movement of temperate plants lineages (Donoghue 2008) as has been demonstrated for the emfimbriate lineage of *Gentianella* (Gentianaceae) (von Hagen & Kadereit 2001). The Bering bridge was the only land connection between these continents since the Oligocene or possibly Middle Miocene at least, the period when the North Atlantic land bridge finally sundered (Tiffney 1985, Milne & Abbott 2002). It existed till the end of the Miocene (5.4–5.5 mya; Gladenkov et al. 2002) and has been re-established in course of falling water levels in the Pleistocene (Cox & Moore 2000). Although our data do not provide hard evidence about the route of Asian-North American dispersals, the geological history of Eurasian-North American land connections on the background of the timing of respective splits in our phylogeny and contemporary distributions patterns are at least in accordance with the Beringian colonization of North America or possible back migrations. These arguments apply to the *Potentilla* core group as well as to the other studied taxa of *Potentilla* s.str. such as the *Biflorae*, *Tormentillae*, and possibly the genus *Argentina*.

2.4.2.3 Biogeography of the Potentilla core group

The biogeographic history of the *Potentilla* core group can be tentatively inferred from our results only. The molecular data unravelled on one hand a considerable amount of plastid sharing in terms of evolutionary lineages and haplotype duplicates among the continents of the Northern Hemisphere. These results are strongly suggestive for repeated migrations of *Potentilla* populations across the continental borders following the origin of the core group (about 4.6–8.1/2.7–5.9 mya; 3.6–6.7/2.1–4.8 mya exclusive of *P. newberryi*) or its sublineages. The sharing of identical or closely related haplotypes was much lower between Europe and North America (N = 6 accessions) than between Asia and North America (N = 20) and Europe and Asia (N = 10). This distribution strongly suggested repeated intercontinental dispersals of these haplotypes by its carriers and assumable preferentially via Beringa. On the other hand, the majority of resolved evolutionary lineages within the core group were continent-specific indicating diversification in place. The highest number of continent-specific clades was found for North America (7 clades) and Europe (6 clades) while only one lineage was purely Asian (Fig. 2). These counts are in good accordance with the proportion of species endemic to North America (86 species out of 98 occurring) and Europe (54 species out of 90) as listed by Wolf (1908), but do not reflect the high degree of taxonomic differentiation in Asia (126 species out of 165). However, the number of haplotypes shared among Asian taxa only may have been downwardly biased by undersampling, particularly within the proposed Asian centers of species diversity of *Potentilla* (Shah et al. 1992). The poor congruence of cpDNA molecular variation and morphological differentiation of species in the core group furthermore prevented to interpret the phylogeny of its lineages in a taxonomic context.

The ratio of nucleotide diversity π to gene diversity H was highest in Asia (π/H = 0.0219 /0.968 = 0.023) compared to North America (0.0142/0.971 = 0.015) and Europe (0.0116/0.968 = 0.012). The increased level of nucleotide diversity in Asia is in accordance with the proposed centers of diversity and a center of origin of *Potentilla* on this continent (Shah et al. 1992) reflecting continuous evolution of populations over prolonged time periods. However, multiple colonizations of this area involving diverged genetic variants would theoretically also explain the obtained value. In contrast, the diversity measures calculated for North America and Europe are indicative for diversification of the *Potentilla* core group on these continents from genetically less diverse gene pools which might include a relatively recent establishment. The analyses of geographic distribution of genetic diversity were therefore in accordance with an Asian origin of this lineage, but not with an origin in North America or Europe. In order to test this scenario, we suggest that more accession from Asia and additional molecular markers need to be added. Further on, population-based approaches (cf. Lessa et al. 2003, Waltari et al. 2007, Li et al. 2010) to phylogeography of selected species should be performed to obtain more conclusive results for the phylogenetically little diverged core group.

2.4.2.4 The fossil record

In order to compare the molecular phylogeny with the fossil record of *Potentilla*, published references were exploited including the Palaeobiology database (http://paleodb.org), a review of Rosaceae fossil record (Kirchheimer 1973) and citations given therein as well as

selected specific palaeobotanical treatments of particular geographic localities and geologic periods (Mai & Walther 1988, Wolfe & Schorn 1990, Matthews & Ovenden 1990, Mai 2001). Macrofossils from *Potentilla* sensu lato were reported from all Northern Hemispheric continents in particular from Europe and North America (DeVore & Pigg 2007). In order to interpret the palaeobotanic record, we relied on the published age estimates as well as provided taxonomic identifications. Specimens reported to be of doubtful taxonomic identification or age estimate were not further considered. A summary of these records is provided in Table 3. Among the representatives of extant species records from members of the series *Rivales* (*P. norvegica*, *P. supina*) and *Tormentillae* (*P. erecta*, *P. reptans*) as well as the genera *Argentina* and *Comarum* were found. These species were reported from Europe (*P. erecta*, *P. reptans*), Europe and Asia (*P. supina*), North America (*P. norvegica*, *Argentina*) or from all of these continents (*Comarum*) (see Mai & Walther 1988 for additional records not provided in Table 3). In addition, Reid (1920) reported *P. argentea* fruits from the European Upper and Middle Pliocene. This identification was, however, reduced to the level of the genus by Kirchheimer (1973). *Argentina*, *Comarum*, and the *Tormentillae* constitute early lineages as inferred by our molecular data, i.e. from the Late Oligocene or Early Miocene (Fig. 3). The fossil and molecular data are in accordance for these taxa, as the origin of respective evolutionary lineages predate the age of the assigned fossils. However, the fossil record suggested that *P. erecta* constitutes an early element of the *Tormentillae*, a result not evident from the molecular data. Nevertheless, inconsistence with the molecular history was observed and involved the occurrence of *P. supina* – placed by the molecular analysis as a member of the *Potentilla* core group (Figs. 1 and 2) – in the Serravallian (Miocene; Mai 2001), and possibly the record of *Potentilla creedensis* (Axelrod) Schorne & Wolfe in the Oligocene of North America (Wolfe & Schorn 1990). The conflict between the early fossil record of *P. supina* (13.65–11.61 mya) and the inferred age of the core group (4.6–8.1/2.7–5.9 mya), however, may be explained by the discussed incongruence of the cpDNA-based phylogeny with the phylogeny of the *Rivales* species. The sister relationship of the *Rivales* with *Horkelia*/*Horkeliella*/*Ivesia* as suggested by the nuclear DNA data would be in agreement with the occurrence of a *P. supina* fossil in the Miocene. *Potentilla creedensis*, finally, described from the Oligocene Creede Flora of Southern Colorado was formerly treated as member of the genus *Ranunculus* (Ranunculaceae; Axelrod 1987) but later transferred to the genus *Potentilla* (Wolfe & Schorn 1990). Wolfe & Schorn (1990) assumed a relationship of *P. creedensis* with the pentapalmatisect (i.e. most of the members of *Potentilla* s.str.) and some tripalmatisect *Potentillas* (e.g. *P. norvegica*), an interpretation which implies an occurrence of *Potentilla* s.str. earlier than inferred from the molecular data (earliest origin 9.8–15.2/5.7–11.0 mya). Two opposing hypotheses can be formulated to solve this conflict: (i) replacement of early North American members of *Potentilla* s.str. by lineages of Asian distribution in later times, or (ii) incorrect assumptions on the taxonomic relationship of *P. creedensis*. Undersampling resulting in exclusion of old extant North American lineages may be another possibility but appears unlikely as most of the diversity of *Potentilla* s.str. was included in the present study. The additional published records listed in Table 3 from the Oligocene and Early Miocene from North America (Becker 1966, 1969, 1973; Matthews & Ovenden 1990), i.e. *P. horkelioides* Becker (Becker 1969), *P. passamariensis* Becker (Becker 1961), *P. salmonensis* Brown and *Potentilla* sp., could not be assigned with certainty to a particular lineage of *Potentilla* s.l. and therefore did not provide additional information concerning the origin of *Potentilla* s.str. in North America. Fossils of uncertain or weakly supported phylogenetic affinity of Miocene or Oligocene age from Eurasia, finally, were reported by Dorofeev (1963), Nikitin (1965), and

Mai (2001): *Potentilla pliocenica* E.M. Reid, *Potentilla proanserina* Nikitin, *Potentilla tomskiana* Nikitin, and *Potentilla* spp. Nevertheless, the occurrence of these early fossils from North America and Eurasia was in agreement with the time of origin of the various lineages of *Potentilla* in its former circumscription.

Table 3. Macrofossils from *Potentilla* sensu lato. Records were selected to include the oldest fossils known from representatives of *Potentilla* s.l., i.e., including nowadays segregate genera, and to cover the three Northern hemispheric continents. Species known from the fossil record only, are marked with an asterisk: "*". The table provides the taxonomic identification, the age of the fossil in million years ago (mya) together with the corresponding geologic period, and the geographic origin of the sample according to the published reference, as well as the assumed phylogenetic relationship of the reported accession within the Potentilleae (cf. Figs. 1 and 3).

	Taxon	**Phylogenetic relationship**	**Geologic period**	**Dating**	**Geographic origin**	**Reference**
1	*P. creedensis**	*Potentilla* s.str.	Oligocene, Chattian	28.4–23.03	North America, Colorado	Wolfe & Schorn 1990
2	*P. salmonensis**	uncertain	Oligocene		North America, Montana	Becker 1966
3	*P. passamariensis**	uncertain				
4	*Potentilla* sp.	uncertain	Late Oligocene to Early Miocene	28.4–15.97	North America, Montana	Becker 1973[+]
5	*P. proanserina**	possibly *Argentina*	Oligocene	–	Asia,	Nikitin 1965
	*P. tomskiana**	possibly core *Potentilla*			Western Siberia	
	Potentilla sp.	possibly core *Potentilla* or clade E				
6	*P. salmonensis**	uncertain	Miocene/Oligocene	23.03–23.03	North America, Montana	Becker 1969
	*P. horkelioides**	uncertain				
7	*P. pliocenica**	uncertain	Early Miocene	23.03–15.97	Asia, Russia	Dorofeev 1963[+]
8	*Potentilla* sp.	uncertain	Early Miocene	ca. 18	North America, Canada, N.W.T.	Matthews & Ovenden 1990
9	*P. pliocenica**	uncertain	Miocene	14.6	Europe, Germany	Mai 1995
10	*P. erecta*	*Tormentillae*	Miocene, Serravallian	13.65–11.61	Europe, Germany	Mai 2001[+]
	*P. pliocenica**	uncertain				
	P. supina	core *Potentilla* or clade E				
	Comarum palustre	"*Farinopsis*-lineage"				
11	*P. erecta*	*Tormentillae*	Pliocene, Piacenzian	3.6–2.59	Europe, Germany	Mai & Walther 1988
	*P. pliocenica**	*Potentilla* s.str.				
	P. reptans	*Tormentillae*				
	P. supina	core *Potentilla* or clade E				
12	*Argentina anserina*	*Argentina*	Early Pliocene	ca. 4.5	North America, USA, Alaska	Matthews & Ovenden 1990
	Comarum palustre	*Farinopsis*-lineage				
13	*P.* aff. *argentea*	*Potentilla* s.str.	Upper to Middle Pliocene		Europe, England	Kirchheimer 1973, Reid 1920
14	*P. norvegica*	core *Potentilla* or clade E	Late Pliocene	ca. 2.6	North America, USA, Alaska	Matthews & Ovenden 1990

[+] cited from the Palaeobiology database, http://paleodb.org

2.4.2.5 Pliocenic radiations

The molecular-based analyses unravelled two rapid radiation events, an almost dramatic proliferation of species in the *Potentilla* core group and the diversification of the *Horkelia-Ivesia*-lineage (Fig. 2). These processes were inferred to have taken place 4.6–8.1/2.7–5.9 mya

and 2.1–5.4/1.3–3.8 mya at the limit of the Miocene and Pliocene or in the Pliocene, respectively. Additional taxonomic groups or evolutionary lineages of the *Potentilleae* present in the data set and reported to be species-rich like *Alchemilla* (Fröhner 1990) and *Drymocallis* may have experienced similar radiation events, but the limited sampling does not allow to draw such a conclusion. Apomixis, polyploidization and hybridization were repeatedly reported from some of these genera (e.g. Rutishauser 1943b, 1960; Håkansson 1946; Asker 1986) and may be considered important factors to explain the observed increase in speciation rates. This interpretation would also be in accordance with the poor congruence of morpho-logical and molecular variation within the *Potentilla* core group. An additional factor pro-moting speciation, common to all those lineages, may be the steady cooling of the climate of the Northern Hemisphere starting in the Middle Miocene and culminating in the Quaternary (Milne & Abbott 2002), which led to a drastic modernization of the European flora, i.e. a replacement of the early flora by novel elements (Cox & Moore 2000).

2.5 Conclusions

The presented molecular cpDNA-based phylogenetic analyses are offering a framework for a taxonomic re-evaluation of the subtribe *Potentillinae* which showed an early diversification in its evolutionary history similar to the *Fragariinae*. While a valid generic concept reflecting the phylogenetic relationships has already been established for the *Fragariinae* (Eriksson et al. 2003, Lundberg et al. 2009), a comparable classification is still missing for the *Potentillinae*. The analyses included a taxonomically comprehensive worldwide sample and provide a basis on which the generic concept can be developed. This opportunity applies in particular to Clade D, currently joining representatives of the genera *Argentina*, *Piletophyllum*, and *Tylosperma*, the Clades G to I, which held largely diverged taxa of restricted European and Asian distribution, Clade F representing the well-defined section *Tormentillae* and includes the generic type of *Potentilla*, the North American Clade E comprising the genera *Ivesia* and *Horkelia*, as well as Clade C, represented by the majority of *Potentilla* species. The presented interspecific phylogeographic study provides the first molecular-based hypothesis on the origin and biogeographic history of *Potentilla*. Combined consideration of the herein presented molecular divergence dates, phylogenetic relationships, and geographic distribution – in opposition to the earlier proposed "circumarctic theory" (Wolf 1908) and partly the biogeographic interpretations by Steffen (1925) – supports the idea of an origin of *Potentilla* s.str. in non-arctic Asia (Panigrahi & Dikshit 1987, Shah et al. 1992). The results further suggested multiple colonizations of Europe and the North American continent by separate phylogenetic lineages. However, the performed ancestral area analysis was not fully explicit about the geographic origin of all recovered evolutionary lineages and consequently the timing of concomitant dispersal events. Interestingly, the phylogeographic hypothesis proposed for the *Potentillinae* identified also major lineages of strikingly different evolutionary history with respect to the rapidness of their diversification, in terms of evolved numbers of species, their absolute times of origin, as well as their geographic distribution limits. Thus two major rapid radiations in the Pliocene to Pleistocene in the *Potentilla* core group and *Horkelia* plus *Ivesia* contrasted with the conservative evolution of a European and Asian clade of Miocene origin. Finally, the fossil record largely supported the inferred phylogeography, with the exception of a fossil interpreted as a member of *Potentilla* s.str. (Wolfe &

Schorn 1990) . This may suggest an occurrence of this still informal taxon in North America much earlier than suggested by the molecular record. However, uncertainties in the taxonomic identification and phylogenetic assignment of the fossils calls for a critical revision of the paleobotanical record of the *Potentilleae*, in particular of the genus *Potentilla*.

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Chapter 3

Polyploidy in *Potentilla argentea* L. group

3.1 Introduction

Polyploidy (a condition in which individuals carry more than two homologous sets of chromosomes) is currently considered an important mechanism of sympatric speciation and a significant source of plant diversity (Soltis & Soltis 2000; Hegarty & Hiscock 2008), at least with regard to its increased evolutionary diversification potential (Otto & Whitton 2000). It not only promotes variability due to change in the number of chromosomes by itself (so-called nucleotypic effect, Levin 1983), rise of the allelic diversity in allopolyploids (Lewis 1980) or structural reorganisation of polyploid genomes (Wendel 2000, Osborn et al. 2003), but the possible evolutionary effects of the polyploidy are also related to an almost instant and effective barrier to the gene flow among the emerging cytotypes (Coyne & Orr 2004).

Co-occurrence of the different cytotypes within a single plant species (without recognition of infraspecific taxa) is relatively frequent in plant kingdom, both in sexual (e.g. Lihová et al. 2003; Trávníček et al. 2004; Schönswetter et al. 2007) as well as in asexual systems, in particular apomicts (Dobeš 1999, Rotreklová et al. 2005). However, the significance of the cytological differentiation for evolutionary divergence of populations and speciation is much better studied in sexuals (e.g. van Dijk et al. 1992, Felber-Girard et al. 1996, Soltis et al. 2007) than in apomicts. The difference in ploidy as a crossing barrier seems to be particularly relevant in sexual systems and should apply only to a lesser extent to asexual populations as in the case of apomicts.

Extensive variation in ploidy as well as several observations of the apomictic mode of reproduction are, among other taxa, characteristic for the genus *Potentilla* (Rosaceae). In a series of studies, starting in the early twentieth century (Fohrenbacher 1913), it was demonstrated (e.g. Håkansson 1946, Hunzinker 1954, Rutishauser 1943b, Mandryk 1994) that apomixis is accomplished either as somatic apospory or generative apospory (sensu Chiarugi 1926). These modes of reproduction have been documented for at least 15 species of this genus (Gentscheff 1938, Gustafsson 1947, Löve 1954, Asker 1970a, Nyléhn et al. 2003) and were in most cases accompanied by polyploidy (basic chromosome number x = 7).

One of the most intensively studied taxa within the genus *Potentilla*, able of the apomictic mode of reproduction, is the *Potentilla argentea* group (grex/series *Argenteae* according to Wolf 1908). Since the very early studies, two dominant ploidy levels have been recognised in this taxon (Müntzing 1931, Marklund 1933): the diploid (2n = 2x = 14) and the most common hexaploid one (2n = 6x = 42) (Müntzing 1931, Müntzing & Müntzing 1941, Müntzing 1958b, Holm 1995, Holm & Ghatnekar 1996a). Rarely reported were also tetraploid (2n = 28),

pentaploid (2n = 35) and possibly octaploid (2n = 56) individuals (Müntzing & Müntzing 1941, Müntzing 1958b). The reproductive modes have been determined in *P. argentea* by a series of crossing and castration experiments testing for maternal versus recombined origin of the offspring assessed by means of morphology and sometimes the chromosome numbers of the offspring (e.g. Müntzing 1928, Popoff 1935, Rutishauser & Hunziker 1954, Asker 1967, Asker 1970c). In the majority of these experiments, apomixis was inferred for all studied ploidy levels, i.e. diploid, tetraploid, hexaploid and octoploids races. The only exceptions involved a sexual synthetic autotetraploid (Asker 1970b, Asker 1970c, Asker 1971) and Müntzing's diploid *P. argentea* type "A-C", actually *Potentilla calabra* Ten. from Southern Europe, which were purely sexual or exhibited a considerable degree of sexuality (Müntzing 1957, Müntzing & Müntzing 1941, Müntzing & Müntzing 1945).

Embryological studies were performed particularly on hexaploid individuals and they confirmed apomixis in this cytotype (Rutishauser 1943a, Hunzinker 1954) as suggested by the previous crossing experiments. Further, the embryology confirmed sexuality in type A-C and apomixis in diploid *P. argentea* based on an accession from Sweden (Håkansson 1946). In the mid-90s, the reproduction of Swedish *P. argentea* populations was studied in a series of publications by Holm (1995), Holm & Ghatnekar (1996a, b), and Holm et al. (1997) based on the protein (isozymes) and DNA polymorphisms (RAPD) in combination with the flow cytometric ploidy determination. Conclusions from these studies were in strong contrast to the previous findings as the population genetic patterns and the segregation of parental genetic markers strongly suggested the sexuality and autogamy for diploid *P. argentea* and found no evidence for the apomictic reproduction for individuals of this cytoytype (Holm 1995, Holm et al. 1997, Holm & Ghatnekar 1996b). The controversy in these results deos not seem to be completely solved yet, but it may be explained by the dependence of the reproductive mode on the chosen pollen donor (Müntzing & Müntzing 1945), by methodological pitfalls of the applied crossing technique including the effects of the changed microenvironmental conditions present in the experimental gardens, and by obviously geographically limited sampling in the previous studies. However, for the hexaploids, the results of the molecular marker-based approach were in accordance with that of the crossing experiments and embryological studies and approved the apomictic mode of reproduction (with some residual sexuality) of that cytotype (Holm & Ghatnekar 1996a). In addition, the molecular data provided insights into the evolutionary relationship of the different cytotypes. Thus, the presence of extra alleles observed in the hexaploids, apart from those known from the diploids, suggested an allopolyploid origin of the hexaploids involving the diploids as one putative parent.

Apomeiotic as well as parthenogenetic formation of seeds was repeatedly reported as obligate or close to obligate in apomictic individuals of *P. argentea* (Müntzing 1928, Müntzing 1958b, Rutishauser 1943a, Rutishauser 1949, Asker 1970c, Asker 1983; but see also Mandryk 1994). Consequently, the majority of the offspring of this species carried the maternal genotype. However, the processes causing deviation from the apomictic pathway of the reproduction involved fertilisation of reduced (B_{II}-hybrids) and unreduced egg cells (B_{III}-hybrids) (Rutishauser 1949, Asker 1970b, Asker 1970c), instantaneous doubling of the somatic genome (Asker 1970b), as well as parthenogenetic development of the reduced egg cells (i.e. formation of polyhaploids: Asker 1983). These mechanisms, if effective, have a potential to change the cytological constitution of the offspring compared to its mother, thus

giving rise to the novel cytotypes. In contrast to female gametogenesis, pollen is almost exclusively produced via meiosis (Müntzing 1928, Rutishauser 1943a, Asker 1970b, Asker 1985). These processes are particularly relevant for understanding of the evolutionary relationships among the established cytotypes, in particular those of different ploidy. As a consequence, a genetically intricate relationships among cytotypes can be expected because the cytotypic differentiation in apomictic populations should not be necessarily accompanied by population genetic differentiation as expected in sexuals (as a result of genetic isolation and minority cytotype exclusion in sexuals: Levin 1975).

The *P. argentea* group is distributed in most of Europe and adjacent Asia (Meusel et al. 1965a, Meusel et al. 1965b). It was assumed that the taxon has its diversity centre in South-eastern Europe to South-western Asia and several, although poorly known, species were reported from this area (Wolf 1908). In the remaining European part of the distribution, two taxa have been distinguished: an aggregate *P. argentea* (*P. argentea* sensu lato) and a morphologically quite distinct south European *P. calabra* (but intermediates between *P. calabra* and *P. argentea* are known from the Balkan Peninsula; Ball et al. 1968, Kurtto et al. 2004). *Potentilla argentea* sensu lato proved to have extreme morphological variability. Accordingly, a variety of narrowly defined species have been described. However, these were mostly not accepted as species of their own (cf. Wolf 1908). The taxonomic value of the two dominant cytotypes has remained unclear until now. On one hand, *P. argentea* has been treated as one single species (a concept followed by Gerstberger 2002), and on the other hand, the diploids and hexaploids were considered separate species as done in Flora Europaea (Ball et al. 1968) and the Atlas Florae Europaeae (Kurtto et al. 2004). In this concept, the diploids refer to *Potentilla argentea* L. s.str. and the hexaploids to *Potentilla neglecta* Baumg. They both correspond to Marklund's (1933) *P. argentea* and *P. impolita* Wahlenb., respectively, who originally proposed this concept. Interestingly, both contrasting taxonomic treatments were underpinned by statistical analysis of the morphology of the cytotypes with Müntzing & Müntzing's (1941) study supporting and Leht & Paal's (1998) analysis disapproving the distinctness of the cytotypes. The analyses of the genetic differentiation of populations or subpopulations of differing ploidy, which may help solve the problem, have not been performed yet. The question should further be considered together with the frequency and distribution of intermediate cytotypes which potentially serve as bridges of gene flow among the diploid and hexaploid individuals. Despite these facts, we have decided to stick here to Gerstberger's (2002) taxonomy as the simplest concept and use the term *P. argentea* sensu lato for all populations except *P. calabra*.

In order to study polyploid complexes, a detailed knowledge of the ploidy level as well as their distribution at various spatial scales is desired (e.g. Mráz et al. 2008, Kolář et al. 2009). The fast and precise data acquisition based on plant flow cytometry is an ideal tool to collect this kind of information (Kron et al. 2007). Combining the ploidy level information with the molecular markers, such as amplified fragment length polymorphisms (AFLPs) and chloroplast DNA (cpDNA) sequences, has made it possible to deepen the insights into the evolution of polyploid genomes (e.g. Martelotto et al. 2007), to differentiate colonisation events among taxa of different ploidy (Schönswetter et al. 2007, Tremetsberger et al. 2009), to revise taxonomic concepts (Hörandl & Greilhuber 2002) and to assess the asexual behaviour (e.g. van der Hulst et al. 2003, Paun et al. 2006).

In the present study we will analyse populations of the *P. argentea* group from a broader European sampling than considered in the previous studies, which were focused on local variability. By combining nuclear (AFLPs) and plastidic markers with a flow cytometric ploidy screen, we intend to (i) elucidate the genetic and evolutionary relationships among populations and particularly among cytotypes, (ii) conclude on prevailing reproductive modes by comparing the observed with the expected genotypic population structure, (iii) infer phylogeographic and karyogeographic patterns, and (iv) discuss taxonomic consequences emerging from these results.

3.2 Material and Methods

3.2.1 Plant material

Plant material was collected from a total of 56 localities or obtained through the *Index Seminum* seed exchange (additional 7 localities: Pop119, 121, 122, 131, 133, 138, 144). The sampling strategy aimed at covering most of the European distribution of the studied taxa. In total, 382 accessions representing 59 populations of *Potentilla argentea* sensu lato (for simplicity referred to as *Potentilla argentea* in the following) and 4 populations of *Potentilla calabra* Ten. were investigated (1–23 samples per population). Individuals were collected from a distance of at least 5 m from each other. Geographic origin and collection history of the material is shown in the Appendix 2.

Vouchers from plants collected during field trips as well as from transplanted plants and those grown from seeds are deposited in HEID (Herbarium of the University of Heidelberg). The vouchers are labelled with material numbers so as to assure a consistent cross-reference between this and future studies. For the purposes of documentation and map-based presentation of the data, collection localities given for the *Index Seminum* material were switched into geographic coordinates (WGS84). The coordinates for the field-collected material were obtained using a handheld GPS and for all kinds of geographical presentation ArcView-ArcGIS 9.1 (ESRI, USA) software with a Hillshade WMS-Layer (Auer et al. 2009) was used.

3.2.2 Chromosome counts and DNA ploidy level estimation

The DNA ploidy levels were determined by flow cytometry from fresh leaf petioles using the Partec Ploidy Analyser PA (Partec, Germany) at the IPK, Gatersleben and at the Department of Pharmacognosy, University of Vienna. The samples were prepared in terms of the two-step protocol involving Otto buffers, as summarised by Doležel et al. (2007), with an internal standard [*Lycopersicon esculentum* cv. Stupické polní tyčkové rané (Doležel & Bartoš 2005); 2C = 1,96 pg]. After chopping of the material using a razor blade in Otto I-buffer and staining in Otto II-buffer containing 4 $\mu g \cdot ml^{-1}$ 4'-6-diamidino-2-phenylindol (DAPI) for 10 min at room temperature, the fluorescence intensity of 5,000–20,000 nuclei was recorded. The sample/standard ratios were calculated from the means of the sample and standard fluorescence histogram. Only histograms with coefficients of variation (CVs) for the G_0/G_1

peak of the analysed sample below 5.0% were considered. In order to obtain a reliable reference for the DNA ploidy estimation, the chromosomes of six individuals of *P. argentea* and two of *P. calabra* individuals were counted either using the methodology of Murín (1960) or that followed by Dobeš (1999). The sample/standard ratios of these individuals were regressed against their determined chromosome numbers. The DNA ploidy level has been attributed to the individuals, measured only by flow cytometry, based on the obtained regression equation. Regressions for the measurements performed in Gatersleben and in Vienna have been computed separately. Further, a literature review of previously published chromosome numbers has been carried out. The full list of references is provided in Appendix 3.

3.2.3 DNA extraction

The total DNA was isolated from freshly-collected and silica gel dried leaf tissue from single individuals. Extraction of the total genomic DNA followed the procedure of Doyle & Doyle (1987; CTAB method), with some modifications applied: grinding of 5–15 mg dry leaf tissue in 2 ml tubes using a Precellys 24 homogeniser (Bertin Technologies, France), addition of 2 U of ribonuclease per extraction to the isolation buffer, and washing of the DNA pellet twice with 70% ethanol. The DNA was finally dissolved in 50 µl TE-buffer and stored at –20 °C.

3.2.4 CpDNA amplification and sequencing

The plastomic *trn*H(gug)-*psb*A intergenic spacer (IGS) was amplified using the primers: *trn*H(gug) 5′-CGC GCA TGG TGG ATT CAC AAT CC-3′ and *psb*A 5′-GTT ATG CAT GAA CGT AAT GCT C-3′ (Shaw et al. 2005). Sequences covered the last 29 bp of the *trn*H gene, the complete IGS, and the first 53 bp of the *psb*A gene. Forward and reverse primers carried a 5′-end M13 extension modified from Messing (1983): 5′-GCA TGT TTT CCC AGT CAC GAC-3′ for forward and 5′-ACT TCA GGA AAC AGC TAT GAC-3′ for reverse primer.

The PCR reactions were performed in a total volume of 25µl containing 1×GoTaq PCR buffer (Promega, USA), 1.5 mM MgCl, 0.2 µM of each primer, 0.4 mM of each dNTP, 0.5 U Taq DNA polymerase (Promega GoTaq), and 10–100 ng of template DNA using an PTC-200 (MJ Research, USA) thermal cycler. The thermal cycling started with a denaturation step at 95 °C lasting 5 min; followed by 30 cycles each of 60 s denaturation at 95 °C, 30 s annealing at 48 °C *trn*H-*psb*A IGS and 60 s elongation at 72 °C. Consequent elongation phase lasted 10 min at 72 °C with a subsequent final hold at 4 °C. The PCR products were checked for length and intensity on 1.5% agarose gels and consequently sent to GATC (Germany) for commercial sequencing using modified M13 primers. The cycle sequencing was performed on both strands. Runs resulting in sequences of low quality were repeated. In the majority of cases, each forward and reverse reaction spanned the complete sequence. All sequences were edited and a consensus was made of forward and reverse reactions using the software Seqman 4.0 (DNASTAR, USA).

3.2.5 AFLP analysis

Prior to AFLP analysis, the DNA-concentration of each sample was measured using the Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies, USA) and each sample was diluted down to 100 ng·μl^{-1}. The AFLP analysis was performed using the protocol established by Vos et al. (1995) with the following modifications: approximately 550 ng of DNA was digested and ligated in a 15 μl reaction mix containing 1× T4 ligase buffer and 1× ATP Solution (Bioline, USA), 50 mM NaCl, 0.75 μg BSA, 1.5 U T4 ligase (Bioline), 1 U MseI and 5 U EcoRI (New England Biolabs, USA), and 0.37 μM of EcoRI-adapter and 3.67 μM of MseI adapter. The reaction mix was incubated in a tube for 3 h at 37 °C followed by an inactivation step for 10 minutes at 65 °C and a final hold at 4 °C. The restriction-ligation product was afterwards diluted ten-fold.

In the pre-selective PCR, 2.5 μl of the diluted restriction-ligation product was used in a total reaction volume of 12.5 μl containing 1× PCR buffer II (Applied Biosystems, USA), 2 mM $MgCl_2$, 0.8 mM dNTP mix, 0.2 μM EcoRI-A primer (5'-GACTGCGTACCAATTCA-A-3'), 0.2 μM MseI-C primer (5'-GATGAGTCCTGAG TAAC-C-3'), and 0.25 U AmpliTaq polymerase (Applied Biosystems). The reactions were held at 72 °C for 2 min followed by 20 cycles of: 94 °C for 20 s, 56 °C for 30 s, and 72 °C for 2 min, with a final 30 s extension at 60 °C. The pre-selective PCR product was visualised on a 1.5% agarose gel and diluted ten-fold.

For selective PCR, we used 2.5 μl of the diluted pre-selective PCR as a template in total reaction volume of 12.5 μl. The PCR-mix contained 1× GoldTaq buffer (Applied Biosystems), 2.5 mM $MgCl_2$, 0.8 mM dNTP mix, 0.08 μM EcoRI-fluorescence-labelled primer, 0.2 μM Mse primer and 0.5 U AmpliTaq Gold (Applied Biosystems). The reactions were held at 95 °C for 5 min followed by 13 cycles of: 94 °C for 30 s, 65 °C → 56 °C (–0.7 °C per cycle) for 1 min and 72 °C for 1 min, followed by 23 cycles of: 94 °C for 30 s, 56 °C for 1 min and 72 °C for 1.5 min, with a final 8 min extension at 72 °C. The selective PCR products were checked on a 1.5% agarose gel.

First of all, 64 selective primer combinations were tested with four geographically distinct individuals for variability and reproducibility. Subsequently, the following three differentially fluorescence-labelled primer combinations were chosen for further AFLP analysis: EcoRI-AGG (TET)/MseI-CTC, EcoRI-AAC (6-FAM)/MseI-CTT, EcoRI-AGC (HEX) /MseI-CTG.

Three differentially fluorescence labelled PCR products of the same sample were multiplexed and diluted (2 μl TET, 2 μl 6-FAM, 5 μl HEX and 50 μl ultra-pure H_2O). One microlitre of the multiplexed selective PCR product was further mixed with 6 μl H_2O and 0.2 μl ET-ROX 550 size standard (Amersham Biosciences, USA). Fragments were electrophoretically separated on a MegaBase 500 DNA capillary-sequencer (Amersham Biosciences). In each run, a total of 48 samples were analysed, including one standard sample applied to each run, one negative control, one repeat within the runs and several other repeats (altogether 5%) as recommended by Bonin et al. (2004). Raw data were visualised and the fragments manually scored using GeneMarker v1.8 (SoftGenetics, USA). Processed data were exported as presence/absence matrix.

3.2.6 Data Analyses

3.2.6.1 Chloroplast sequence data analysis and phylogenetic inference

The DNA-sequences were multiply aligned by means of ClustalX v1.83 (Thompson et al. 1997) and the alignments were manually refined using the GeneDoc software version 2.7 (Nicholas et al. 1997). Two regions were excluded from the alignment due to repeated sequence motifs (poly-A stretches). Three indels were manually coded for presence and absence. Phylogenetic relationships among cpDNA haplotypes were evaluated by means of the network analysis using TCS 1.2 (Clement et al. 2000) with a default connection limit of 95%.

3.2.6.2 AFLP data analyses

Diversity estimates: Several statistical parameters were computed using the R-script AFLPdat [Ehrich 2006; R 2.9.2 environment (R Development Core Team 2009)] for taxa, ploidy levels and single cytologically uniform populations or (in case of cytotype mixture) subpopulations divided according to cytotype ($N \geq 3$): total number of the fragments, proportion of polymorphic fragments, number of private fragments and Nei's gene diversity $\{D = n/(n-1) \times [1 - freq(1)^2 + freq(0)^2]\}$ (Nei 1987) for the whole dataset or for subrgroups revealed in later analyses.

Network reconstruction: In order to visualise the phylogenetic relationships among the AFLP genotypes (actually phenotype[2] in a genetic sense), a neighbor-net analysis (as implemented in SplitsTree 4.5, Huson & Bryant 2006) based on uncorrected *p*-distances has been carried out. The neighbor-net diagram represents all inferred splits in a phylogenetic network and is composed of parallel edges, rather than a bifurcating phylogenetic tree, which suggests one phylogenetic hypothesis represented by a single optimal tree or a consensus trees.

Genotypic similarity patterns: In order to analyse and display the similarity among the AFLP genotypes, a principal coordinate analysis (PCoA) was performed using MVSP 3.1 (Kovach Computing Services, UK). Pairwise Euclidean distance was applied as a distance measure and, alternatively, a simple match coefficient, as suggested by Kosman & Leonard (2005) for the dominant data.

Genetic structure of populations: Partition of the molecular variation within and among populations was assessed by analyses of molecular variance (AMOVA) using the software Arlequin 3.1 (Excoffier et al. 2005). The analyses were carried out based on pairwise squared Euclidean distances among the genotypes. Hierarchical AMOVA was conducted in order to assign the proportion of the variation to ploidy levels, population and to individuals within the whole sampling. Non-hierarchical AMOVAs were carried out to compare the partitioning of the variation between the taxa, ploidy levels, single populations or subpopulations divided according to cytotype and single individuals.

[2] Individual AFLP profiles are often referred to as AFLP phentotypes. However, the use of the term "phenotype" to address the lowest genetic level is impractical in the present context.

For the purpose of detecting the population structure on a finer scale, a genetic mixture analysis using the program Structure 2.2 (Pritchard et al. 2000) was applied. The structure implements a model-based Bayesian clustering algorithm in order to estimate the likelihood for pre-defined number of clusters (K). The admixture model with independent allele frequencies and the first row containing 0 for the recessive allele in the input data matrix, as implemented by Falush et al. (2007) was used. Firstly, the data were tested with K ranging from 2–10, with 10 replicate runs for each K, and a burn-in period of 2×10^4 and 1×10^5 iterations. In order to find the most probable value of K, the Structure output files were analysed with the R script Structure.sum (Ehrich 2006; available from http://tinyurl.com/StructureSUM) by the means of Evanno's delta K (ΔK) (Evanno et al. 2005). ΔK statistics is based on the rate of change in the log probability of the data between the successive K values. In order to obtain more accurate estimation of the clustering, three replicate runs of the analysis with the same parameters and the most probable value of the K, but with a burn-in period of 2×10^5 and 1×10^6 MCMC repetitions were run. For comparison, we also ran admixture model with correlated frequencies. Subsequently, groups determined by first round of Structure analyses were analysed separately in order to decide whether they are more subdivided (Pritchard et al. 2007, Rosenberg et al. 2002, Ehrich et al. 2007).

Geographic structure of genetic variation: Isolation by distance was evaluated by Mantel test (Mantel, 1967) with 9999 random permutations. The analysis was performed both on two ploidy levels observed for *P. argentea* separately.

Clonal assignment and genotypic variability of populations: The number of different AFLP genotypes in each population was estimated using the programs Genotype 1.1 and Genodive 1.2 (Meirmans & van Tienderen 2004). The functions allow entering a threshold/error rate, estimated from the observed differences among the replicates (maximum number) or alternatively from histogram of the observed pairwise differences between the genotypes. Consequently, the Genodive 1.2 computes several indices of clonal diversity such as Nei´s genotype diversity $\{D_g = n/(n-1) \times [1 - \Sigma (\text{genotype frequencies}^2)]\}$ (Nei 1979) and effective number of genotypes $[N_e = 1/\Sigma (\text{genotype frequencies}^2)]$ (Parker 1979).

Furthermore, character incompatibility analyses (CI, Mes 1998) were applied in order to compare the extent to which sexual recombination might be responsible for phenotypic diversity. Variation between multilocus AFLP genotypes is generated by both mutation and sexual recombination. This method calculates "matrix incompatibility count" (MIC) for pairs of individuals, the total sum of "incompatibilities" with clonal evolution found between any pair of markers present in the binary data. Two polymorphic AFLP loci can exhibit four possible character combinations (11, 01, 10 and 00). The chance of all four combinations occurring through mutation alone in a clonal lineage is very small. In contrast, genetic exchange among individuals may yield all possible character combinations. The total MIC has been counted for each population and the contribution of each genotype to MIC was calculated by jack-knifing using jactax.exe from the PICA 4.0 software package (Wilkinson 2001). Because of small populations sizes, the step of removing the genotype with the highest contribution has not been carried out.

3.3 Results

3.3.1 DNA ploidy level and cytotype distribution

Two hundred sixty-nine individuals from 47 populations of *P. argentea* have been investigated by the means of flow cytometry (Appendix 2). One hundred ninety-five samples were measured at the IPK, Gatersleben and 74 samples at the University of Vienna. Coefficients of variation (CVs) for the G_0/G_1 peak of the analysed sample ranged from 1.43 to 5.30 ($\bar{x}$ = 3.02). Two distinct classes of sample/standard ratios were identified in *P. argentea*. The individual values were normally distributed (Kolmogorov-Smirnov-test) within each of the classes with a mean of 0.218/0.179 (samples analysed at the IPK, Gatersleben /University of Vienna) and 0.667/0.651 with a coefficient of variation 0.017/0.067 and 0.019/0.019. The classes corresponded to two ploidy levels, the diploid and hexaploid, as chromosome-counted individuals (2n = 14: individual Ptl6138; 2n = 42: Ptl3878, Ptl3665, Ptl6275, Ptl6301 and Ptl6307) felt within each of these classes (Scherbatin 2009, Scherbatin et al. 2009). Chromosome numbers were euploid for all six counted individuals. DNA ploidy levels were calculated using the following two regression equations: DNA ploidy level = 0.1689 + 62.8814 × sample/standard ratio (IPK Gatersleben) and DNA ploidy level = 3.4284 + 59.4269 × sample/standard ratio (University of Vienna). Values obtained for the measured individuals ranged between 13.26–14.04–15.23 (P_5 – $\bar{x}$ – P_{95}) and 40.96–42.04–43.12 for the two classes. Sixty-five accessions from 16 populations corresponded to the diploid DNA ploidy level and 204 accessions from 37 populations to the hexaploid one. The three studied populations (4 individuals) of *P. calabra* revealed the same class of the samples/standard ratio (0.238–0.244), which corresponded with the counted diploid individuals (2n = 14; Ptl4701, Ptl4731).

The geographic distribution of the *P. argentea* and *P. calabra* cytotypes analysed in this study and complemented by literature data is shown in (Figs. 6a, 6b). Diploid populations were found mainly on a SW-NE axis, stretching from the Iberian Peninsula to Scandinavia. Hexaploids were distributed throughout the Alps, in Central Europe as well as in Scandinavia and the Baltic region (i.e. Baltic countries). Corresponding to the previously published observations (Marklund 1933; Müntzing 1931, 1958b; Holm 1995; Holm et al. 1997), several populations (Pop54, 57, 88, 91, 100, 202 and 323) were found to be mixed – with both diploid and hexaploid cytotypes present. These populations were from the Swedish islands Gotland and Öland and the central Alps (South Tyrol). Our data coincide well with the previously published ones as the hexaploids and diploids represent the most frequent cytotypes: 173 published diploids, 18 tetraploids, 8 pentaploids, 142 hexaploids and 2 octoploids (Appendix 3, Fig. 6a). The majority (77.55%) of the chromosome counts were from Fenno-Scandinavia. The tetraploid, pentaploid and octaploid individuals were not detected within our Europe-wide sampling. The *P. calabra* accessions were confirmed to be diploid as also previously shown by Müntzing (1928, 1931) and Larsen & Laegaard (1971).

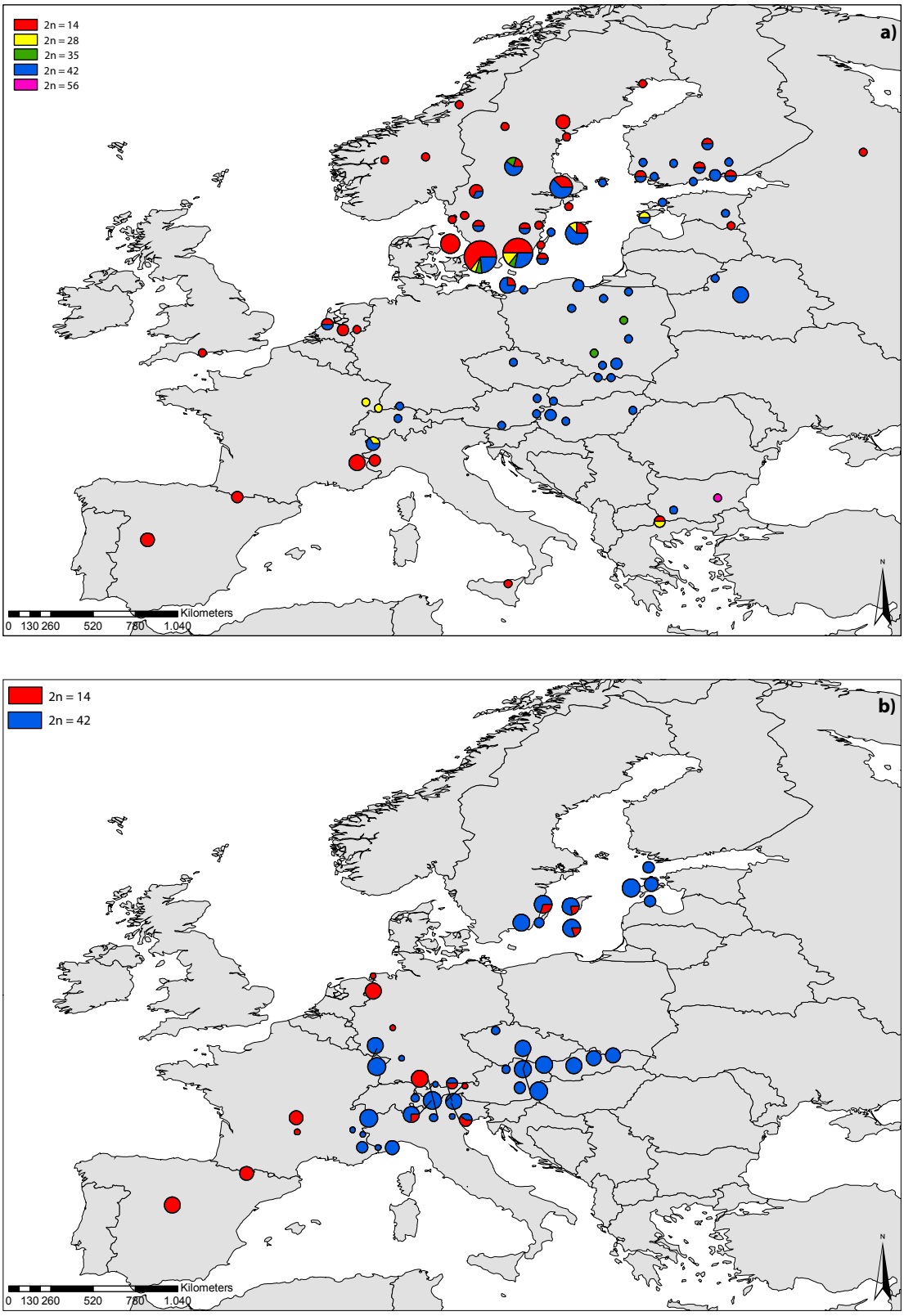


Figure 6. Europe-wide distribution of the cytotypes from *P. argentea* group: (a) literature-based data (the numerous counts from southern Sweden are downscaled because of the lucidity of the visualisation) (b) data newly acquired within this study.

3.3.2 CpDNA sequence data and haplotype distribution

Chloroplast DNA sequences were obtained for 57 individuals, for at least one sample of the majority of the studied populations (Appendix 2). The length of the *trn*H-*psb*A IGS ranged from 439 bp to 487 bp. Seventeen nucleotide substitutions, three indels and two poly-A stretches were detected. The length of the alignment was 488 bp. After manual coding of the indels for presence and absence and removal of the poly-A stretches, the total length of the alignment was reduced to 442 bp and 17 parsimony informative sites were considered. The alignments are provided on an enclosed CD with the Supplementary Data.

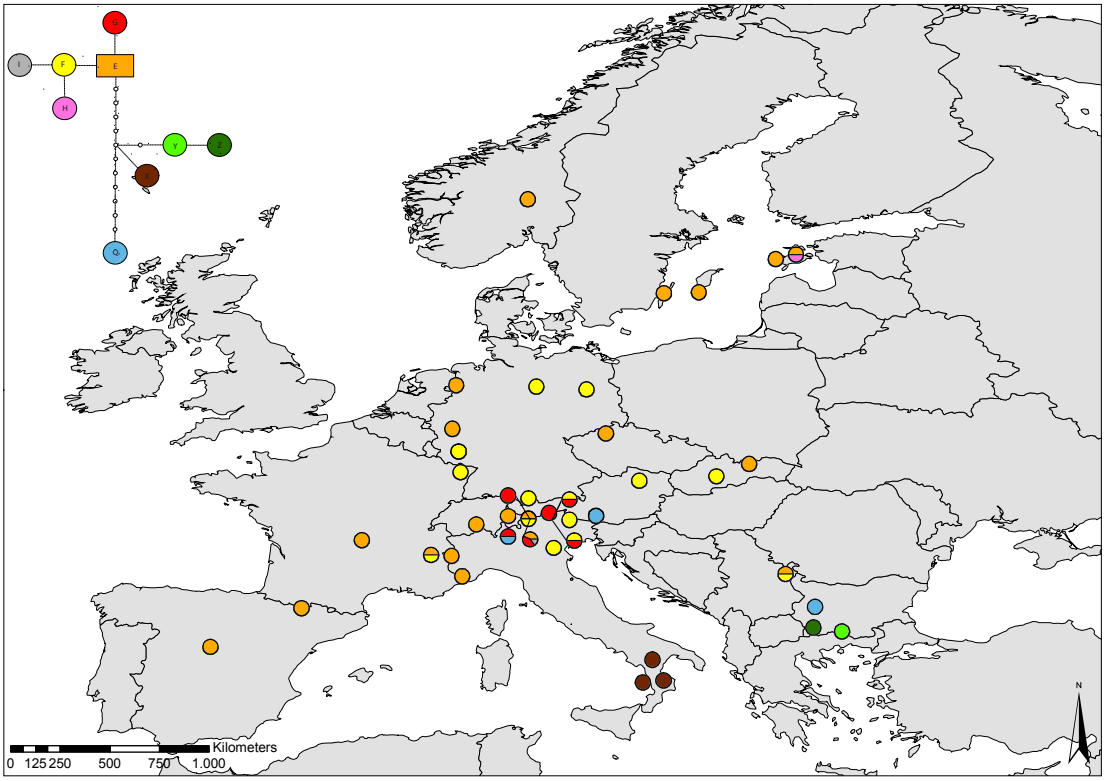


Figure 7. TCS based statistical parsimony network based on the *trn*H-*psb*A cpDNA sequences of the individuals from the *P. argentea* group and the Europe-wide distribution of the haplotypes. Small empty circles represent haplotypes that are not present, but necessary to link all observed haplotypes to the network. All haplotypes are separated from the nearest haplotype by one nucleotide difference.

Nine different *trn*H-*psb*A IGS cpDNA haplotypes were identified within the 57 sequenced individuals. The TCS network analysis revealed three groups of haplotypes (Fig. 7) separated from each other by 6–12 mutations. The first group consisted of the haplotypes E, F, G, H and I carried by *P. argentea* (referred to as the "*P. argentea* haplotype group" in the following). Haplotype G was exclusive to diploid individuals. Haplotype E comprised both, diploids and hexaploids and haplotypes the F, H, and I were observed in hexaploids only. The most common haplotype within the studied material was the haplotype E, the distribution of which follows the same SW-NE geographic axis (Iberian Peninsula to Scandinavia) as the diploid cytotype (Fig. 7). However, it is also present in the hexaploid individuals from the Alps, Central Europe, and the Balkan Peninsula. The second most common haplotype is the haplotype F and it is spread throughout the distribution range of

the hexaploid cytotype (the Balkan, the Alps, Central Europe) except Scandinavia. The derived haplotypes H and I were each found only once in the Alps and the Baltic region, respectively. A solely diploid haplotype G was restricted to the central Alps. It constituted either pure diploid populations (Pop98, possibly Pop77) or it was found in cytologically mixed populations together with haplotypes F (Pop88, 91); or E and I (Pop100). Concerning the analysed mixed population from Scandinavia (Pop57) both diploid and hexaploid individuals possessed the same haplotype E.

Haplotypes X, Y, and Z constituted a second group. Haplotypes Y and Z were specific to what is sometimes referred to as *P. argentea* L. var *pseudocalabra* Th.Wolf, a taxon restricted to the Balkan Peninsula (Wolf 1908). These haplotypes were separated by 3–4 mutation from the haplotype X, which was exclusive to *P. calabra*. Hence this group was assigned as "*P. calabra* haplotype group." Single haplotype Q constituted the third group. It was observed in hexaploid *P. argentea* only and it is distributed in the Balkans and the Alps. Separation by twelve mutation steps from the closest haplotype of the "*P. argentea* haplotype group" and its presence in distantly related taxa (Paule & Dobeš, unpubl.) suggest that this haplotype may have resulted from chloroplast capture or incomplete lineage sorting.

3.3.3 AFLP analyses

In total, 219 accessions representing 34 populations of *Potentilla argentea* and 3 populations *Potentilla calabra* Ten. were investigated. Sample size per population was 2–23 individuals, but mostly 5 individuals (Appendix 2). Three AFLP primer combinations resulted in 243 clearly scorable bands sized from 65–537 bp. 93.83% of them were polymorphic across the dataset. Mean Nei's gene diversity (D) of all accessions was 0.221.

The data quality test also confirmed a high reliability and repeatability of the data within a range of 98.35–100% and with a mean repeat accuracy of 98.93%.

3.3.3.1 Strong genetic division of cytotypes

The neighbor-net analysis recovered three well separated groups of AFLP genotypes (Fig. 8). Splits of the highest weight separated *P. calabra* plus *P. argentea* var. *pseudocalabra*, diploid and hexaploid *P. argentea* from each other. The hexaploid *P. argentea* group comprised 27 populations represented by 144 individuals, with 77 of determined ploidy. The diploid cluster of *P. argentea* combined 56 individuals (35 of known ploidy). The perfect separation of accessions according to the ploidy suggested that all individuals within each group belong to identical cytotype. The third group was made of three *P. calabra* populations (14 individuals) and one analysed population (5 individuals) of *P. argentea* var. *pseudocalabra*. This group corresponded with the "*P. calabra* haplotype group."

Two dimensional PCoA based on Euclidean distances (Fig. 9) showed a separation of accessions congruent with the neighbor-net analysis. The first axis explained 26.98%, of the variation and the second one 9.87%. An identical pattern was recovered after using the simple match coefficient. This division was further supported by nested AMOVA (Table 4).

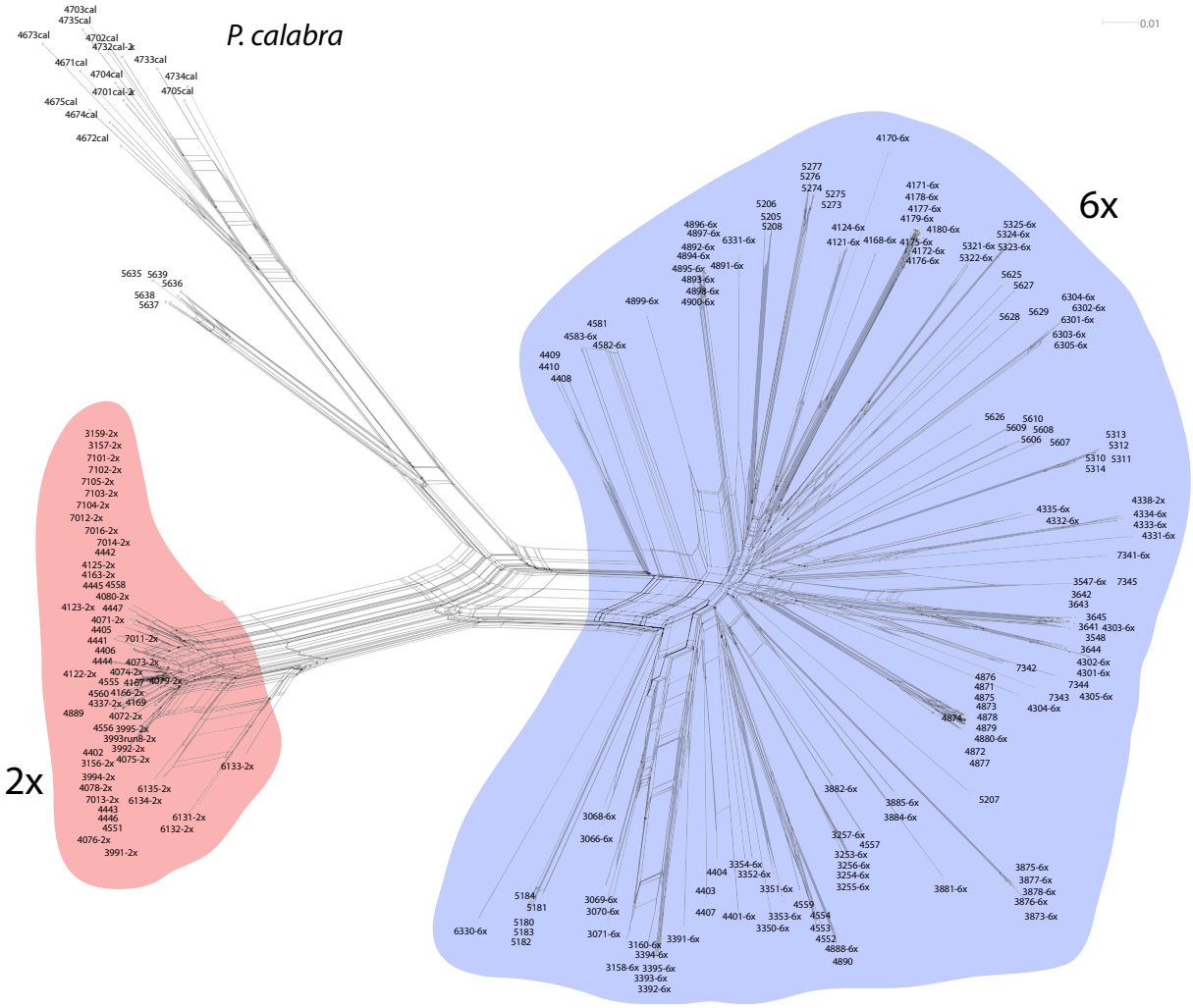


Figure 8. Phylogenetic relationships inferred on the basis of AFLP data using the distance-based neighbor-net method as implemented in SplitsTree 4. Blue colour codes the hexaploid *P. argentea* cluster, red colour codes the diploid *P. argentea* and the uncoloured clusters represents *P. calabra* and *P. argentea* var. *pseudocalabra*. Accessions are labelled according to their material number, "2x" or "6x" behind the material number indicates determined ploidy level.

Table 4. Analysis of molecular variance (AMOVA) for AFLP data in *P. argentea* group.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among ploidy levels (2x/6x)	1	1484.52	18.19	50.89
Within ploidy levels	198	3475.34	17.55	49.11
Among 2x and 6x	1	1467.52	17.39	48.76
Among populations	35	2470.30	12.11	33.96
Within populations	162	998.40	6.16	17.28
Among 6x populations	26	2297.75	15.28	66.62
Within 6x popualtions	117	895.43	7.65	33.38
Among 2x populations	9	172.55	3.12	57.68
Within 2x populations	45	102.98	2.29	42.32

The AMOVA analysis [with "*P. calabra* haplotype group" (Pop166, 218, 221, 223) excluded] attributed 48.76% of the overall diversity to the variation between ploidy levels, 33.96% to variation between populations and 17.28% to the variation within populations.

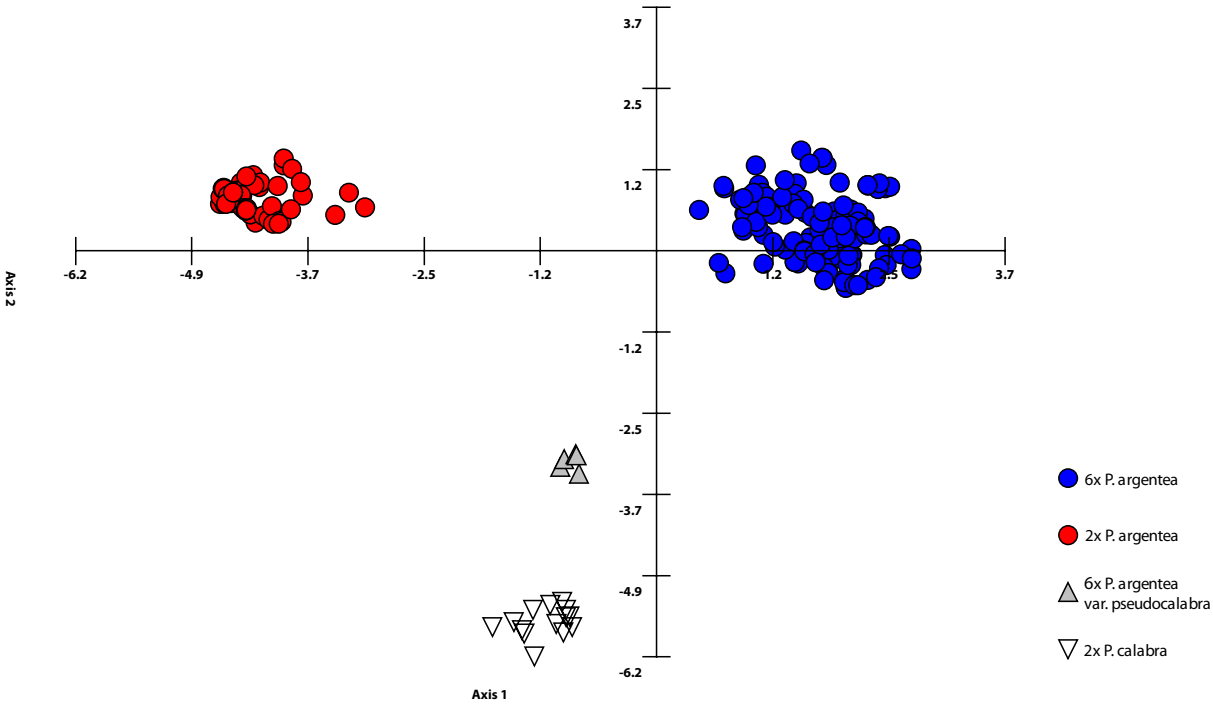
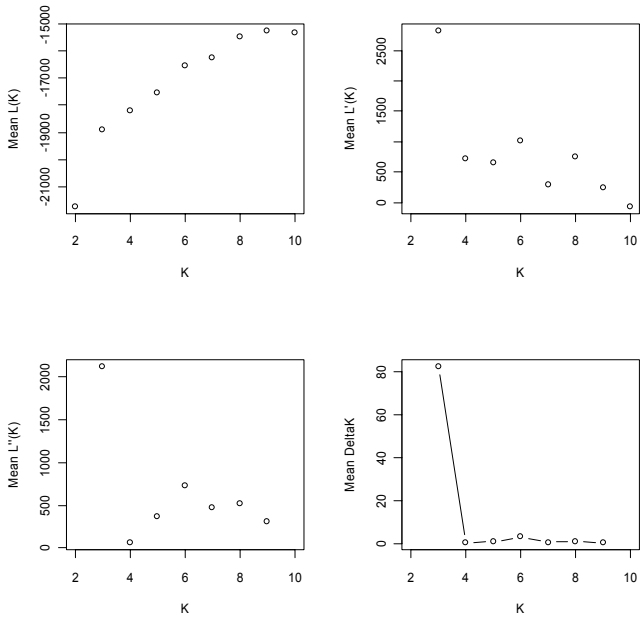


Figure 9. Principal coordinate analysis (PCoA) of the AFLP genotypes from the *P. argentea* group. The first two axes explained 26.98% and 9.87% of the total variation.

The grouping of individuals in the Structure analysis also confirmed the inferred cytotype division. The Bayesian mixture modelling yielded similar results using both correlated and independent allele frequencies. The highest ΔK value was assigned to the K = 3 (Fig. 10a). The three identified genetic groups fully corresponded with the two ploidy levels of *P. argentea* and the "*P. calabra* haplotype group" (Fig. 10b). Additionally, hexaploid individuals from mixed populations from Scandinavia (Pop48, 57), the Alps (Pop100, 202) as well as populations Pop121 and Pop292 carried small portions (8.4–19.8%; 0.3–11.4% and 0.6–2.5%, respectively) of the diploid genetic cluster in their genomes. Similarly *P. argentea* var. *pseudocalabra* population (Pop166) was admixed with the *P. calabra* genetic cluster and the hexaploid *P. argentea* cluster (18.7–22.0%). These findings suggest a limited geneflow between the two ploidy levels of *P. argentea*, however being probably more significant in Scandinavia. On the other hand, the genetic exchange between the hexaploid cytotype of *P. argentea* and *P. calabra* seems to play a more active role because of relatively higher portions of both genetic clusters in individuals of Pop166. Other K values, such as K = 4 or K = 5 were also tested, but the clustering patterns of the hexaploid individuals were inconsistent within 10 performed repeats of shorter runs (2×10^4 burn-in; 10×10^4 MCMC iterations) as well as within 3 repeats of the long runs. Nevertheless, clustering of the diploid *P. argentea* and *P. calabra* was stable within all repeats.

a)



b)

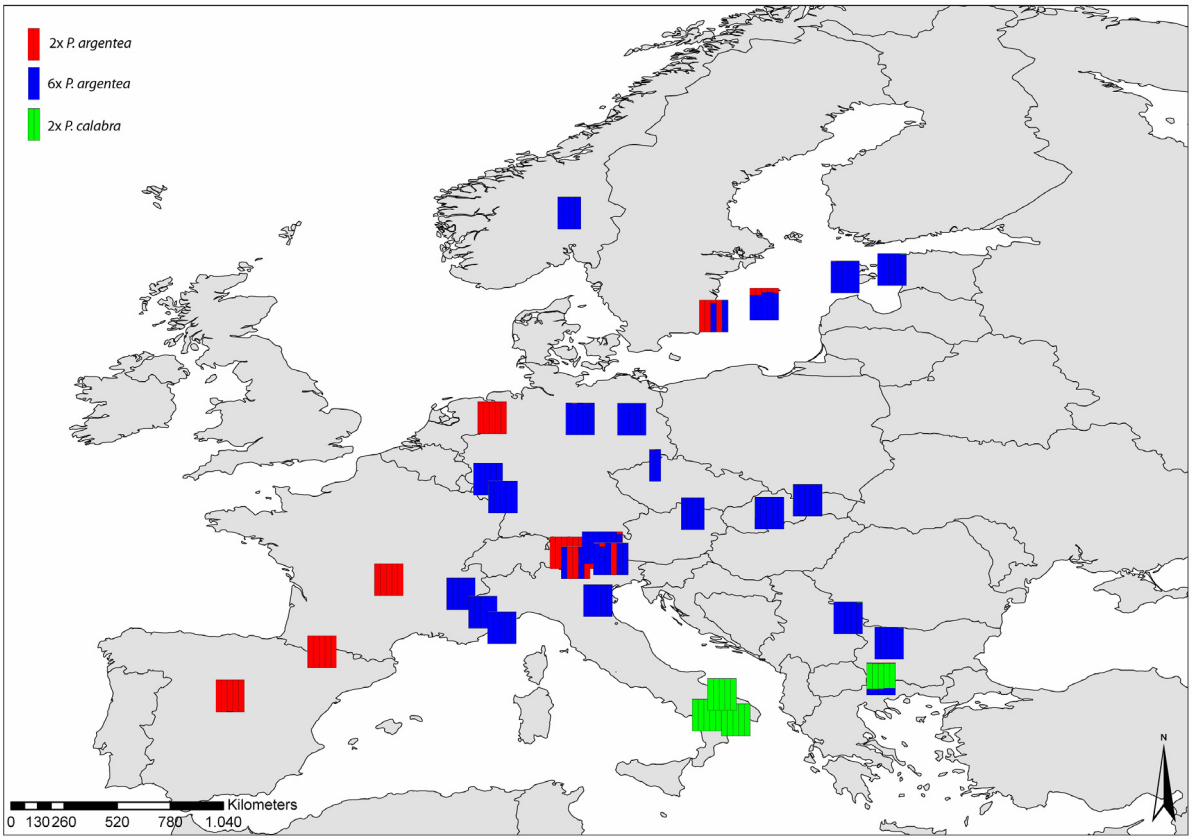


Figure 10. Population structure examined by genetic admixture analysis using the program Structure with K = 3. (a) The Structure output analysis of the Ks ranging from 2 to 10 by means of ΔK is shown, demonstrating K = 3 with the highest probability of the data. (b) Barcharts are plotted on the map in order to visualize the geographic organisations of the genetic clusters within populations.

3.3.3.2 Population genetic structure and diversity

Separate analyses were conducted to detect the organisation of genetic variation within diploid and hexaploid *P. argentea*. The highest proportion of AFLP fragments present in the whole dataset was found in hexaploid *P. argentea* (228 fragments = 93.83%), (Table 5). Diploid *P. argentea* possessed 43.62% (106 fragments), *P. calabra* 44.86% (109) and the one *P. argentea* var. *pseudocalabra* population 30.04% (73) of the observed fragments. Among all four groups 15.64% of the markers were shared. 30.86% (75) of the fragments were specific to the hexaploids, 0.41% (1) to the diploids, and 3.29% (8) to *P. calabra*. No fragments were exclusively found in *P. argentea* var. *pseudocalabra*. The highest proportion of shared fragments among the different subgroups was between diploid and hexaploid *P. argentea* (102 fragments) as well as between hexaploid *P. argentea* and *P. calabra* (98 fragments).

Hexaploid populations showed a relatively high variability (mean D = 0.184). Individuals were grouped according to their population of origin in the PCoA and the neighbor-net analysis (Fig. 11, Fig. 12). This grouping was supported also by non-hierarchical AMOVA, which assigned 66.62% of the variation distributed among and 33.38% within populations (Table 4). The clusters revealed by the Structure analyses using K = 4 and K = 5 showed only limited geographic pattern which was also confirmed by the Mantel test (r_M = 0.189, P = 0.904). On the contrary, within the diploid cytotype, a strong geographic pattern was inferred by Mantel test (r_M = 0.585, P = 0.005). A geographic structure could also be seen from the Structure analyses (K = 3), even though the clustering of popualtion Pop72 was uncertain (Fig. 13). Population Pop269 from the central Iberian Peninsula comprised all three clusters with individuals beeing composed of one major genetic cluster or of two major and one marginal one. Other studied populations were built either from one or two clusters (found in Pop269 to be marginal and major cluster) and several individuals have shown to be admixed. Diploids comprised a relatively low variability (D = 0.046) (Table 5). The variability is divided by AMOVA in a similar way as in the hexaploid cytotype: 57.68% among populations and 42.32% within populations (Table 4). Due to insufficient sampling and limited geographic coverage, the population genetic structure of *P. calabra* and *P. argentea* var. *pseudocalabra* have not been analysed separately.

3.3.3.3 Clonal assignment analysis and genotypic variability of populations

We assume that the same AFLP genotype represents a "clone". If taken strictly, clones with no difference in banding patterns have been recognised within both hexaploid and diploid populations. Interestingly, one clone has also been identified among several diploid populations in Central Alps (Pop77, 98 and Pop100). However, based on the data repeatability, a threshold of 4 band differences (the highest error rate) has been taken for assignment of AFLP clonal assignment analyses. The analyses have been carried out for each cytologically uniform population/subpopulation as well as for diploid and hexaploid *P. argentea*, *P. calabra* and *P. argentea* var. *pseudocalabra* separately and the indices of clonal diversity consider the given threshold. Alternatively, based on the pairwise distances between genotypes (Figs. 14a, b), a threshold of 8 band differences has also been applied in the case hexaploid *P. argentea*.

Table 5. Descriptive statistics of taxa, ploidy levels, single populations or subpopulations divided according to cytotype from *P. argentea* group based on AFLP data. *Nb*, number of samples; *FT*, total number of fragments; *FP*, proportion of poylmorphic bands (%); *FPP*, number of private fragments; *D*, Nei's gene diversity

Groups	Nb	FT	FP	FPP	D
All	219	243	93.83	-	0.221
6x	144	228	79.84	75	0.184
2x	56	106	24.28	1	0.042
2x-calabra	14	109	31.28	8	0.106
pseudocal	5	73	5.35	0	0.024
2x					
*Pop057	3	72	2.47	0	0.016
Pop072	5	73	3.29	0	0.019
Pop077	7	72	2.47	0	0.010
Pop088	3	74	2.88	0	0.019
Pop091	4	75	3.29	0	0.018
Pop098	9	72	2.47	1	0.013
Pop100	9	79	8.23	0	0.024
Pop237	5	71	2.06	0	0.010
Pop269	5	80	9.05	0	0.046
Pop299	5	72	2.88	0	0.016
6x					
Pop019	7	104	3.29	0	0.013
Pop034	5	96	2.47	1	0.012
Pop042	4	117	18.11	0	0.100
Pop048	5	121	16.46	0	0.082
Pop057	2	116	2.06	0	0.021
Pop069	5	100	4.12	1	0.019
Pop088	2	109	0.82	0	0.008
Pop091	2	123	16.05	1	0.160
Pop093	8	111	1.65	0	0.007
Pop100	14	150	39.92	0	0.143
Pop117	5	125	14.81	1	0.084
Pop121	5	106	2.47	2	0.012
Pop127	4	122	18.52	1	0.095
Pop138	5	103	2.06	1	0.011
Pop144	5	112	2.06	0	0.012
Pop163	5	129	21.40	0	0.113
Pop165	5	147	31.69	1	0.153
Pop196	3	99	2.47	0	0.016
Pop198	10	120	13.58	1	0.030
*Pop202	6	139	24.69	0	0.113
Pop206	10	96	2.88	0	0.010
Pop230	5	126	23.46	1	0.119
Pop234	5	118	12.76	0	0.053
Pop248	5	116	14.81	0	0.063
Pop258	5	133	25.51	0	0.127
Pop289	5	117	1.65	1	0.008
Pop292	2	121	16.46	2	0.165
pseudocal					
Pop166	5	73	5.35	0	0.024
Calabra					
Pop218	5	88	18.93	0	0.093
Pop221	5	87	16.05	0	0.078
Pop223	4	87	15.23	1	0.084

* without Pop202 because of the only one (Ptl4337) diploid plant present

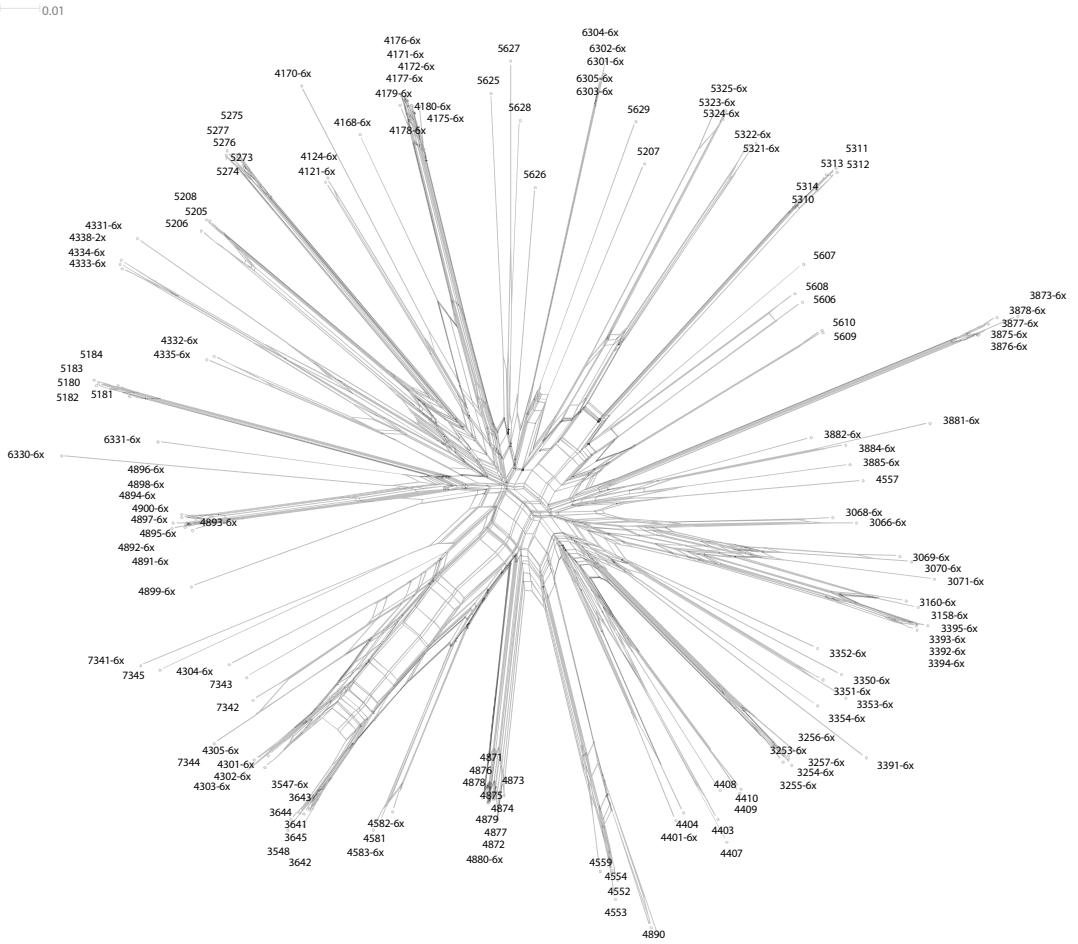


Figure 12. A neighbor-net of AFLP genotypes belonging to the hexaploid populations from the *P. argentea* group. Accessions are labeled according to their material number, "6x" behind the material number indicates a determined ploidy level.

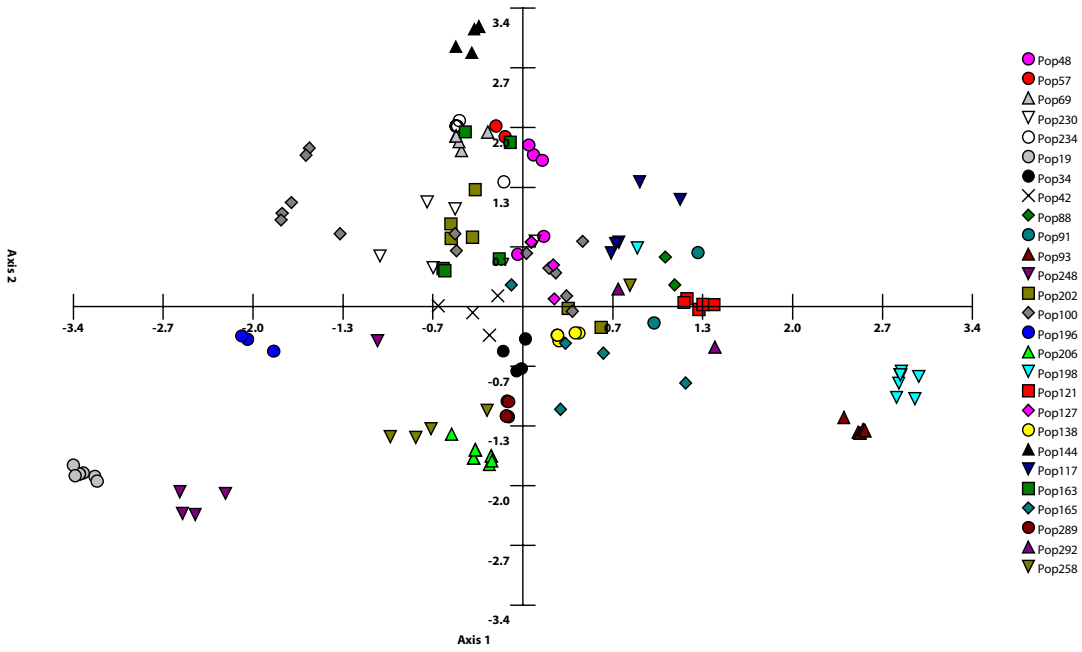


Figure 11. Principal coordinate analysis (PCoA) of the AFLP genotypes of the hexaploid populations from the *P. argentea* group. The first two axes explained 8.85% and 7.76% of the total variation.

Table 6. Indices of clonal diversity for taxa, ploidy levels, single populations or subpopulations divided according to cytotype as computed by software Genotype and Genodive based on the AFLP data. *Nb*, number of samples; Nb_{gen}, number of genotypes considering a threshold of 4 fragments differences; N_e, effective number of genotypes; D_g, genotypic diversity. In hexaploids two different thresholds are considered: 4 and 8. Values for both thresholds are separated by "/".

Groups	*Nb*	Nb_{gen}	N_e	D_g
All	219	102		
6x	144	73/61	36.64/34.33	0.979/0.977
2x	56	12	2.43	0.599
2x-calabra	14	14	14.00	1.000
pseudocal	5	3/1	2.27/1.00	0.700/0.000
2x				
*Pop57	3	1	1.00	0.000
Pop72	5	1	1.00	0.000
Pop77	7	1	1.00	0.000
Pop88	3	1	1.00	0.000
Pop91	4	1	1.00	0.000
Pop98	9	1	1.00	0.000
Pop100	9	3	1.59	0.417
Pop237	5	1	1.00	0.000
Pop269	5	5	5.00	1.000
Pop299	5	1	1.00	0.000
6x				
Pop19	7	1/1	1.00/1.00	0.000/0.000
Pop34	5	1/1	1.00/1.00	0.000/0.000
Pop42	4	4/4	4.00/4.00	1.000/1.000
Pop48	5	4/3	3.57/2.78	0.900/0.800
Pop57	2	2/1	2.00/1.00	1.000/0.000
Pop69	5	2/1	1.47/1.00	0.400/0.000
Pop88	2	1/1	1.00/1.00	0.000/0.000
Pop91	2	2/2	2.00/2.00	1.000/1.000
Pop93	8	1/1	1.00/1.00	0.000/0.000
Pop100	14	11/9	8.91/7.54	0.956/0.934
Pop117	5	3/2	2.27/1.92	0.700/0.600
Pop121	5	1/1	1.00/1.00	0.000/0.000
Pop127	4	3/2	2.67/1.60	0.833/0.500
Pop138	5	1/1	1.00/1.00	0.000/0.000
Pop144	5	1/1	1.00/1.00	0.000/0.000
Pop163	5	3/3	2.78/2.78	0.800/0.800
Pop165	5	5/5	5.00/5.00	1.000/1.000
Pop196	3	1/1	1.00/1.00	0.000/0.000
Pop198	10	2/2	1.22/1.22	0.200/0.200
Pop202	6	5/3	4.50/2.57	0.933/0.733
Pop206	10	1/1	1.00/1.00	0.000/0.000
Pop230	5	5/4	5.00/3.57	1.000/0.900
Pop234	5	2/2	1.47/1.47	0.400/0.400
Pop248	5	3/2	2.27/1.47	0.700/0.400
Pop258	5	5/4	5.00/3.57	1.000/0.900
Pop289	5	1/1	1.00/1.00	0.000/0.000
Pop292	2	2/2	2.00/2.00	1.000/1.000
pseudocal				
Pop166	5	3/1	2.27/1.00	0.700/0.000
calabra				
Pop218	5	5	5.00	1.000
Pop221	5	5	5.00	1.000
Pop223	4	4	4.00	1.000

* without Pop202 because of the only one (Ptl4337) diploid plant present

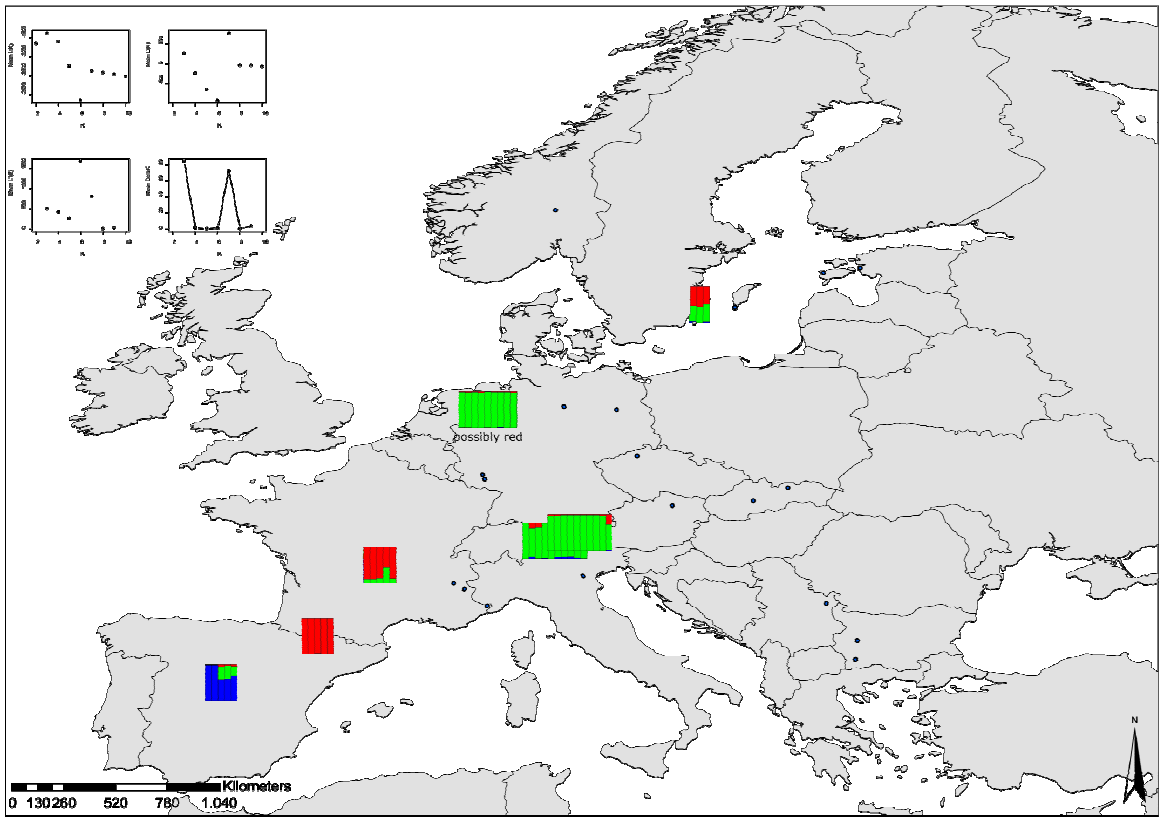


Figure 13. Population structure of the diploid *P. argentea* examined by genetic admixture analysis using the program Structure with K = 3. The Structure output analysis of the Ks ranging from 2 to 10 by mean of ΔK is shown, in the upper left corner, demonstrating K = 3 with the highest probability of the data. Barcharts are plotted on the map in order to visualize the geographic continuity of the populations.

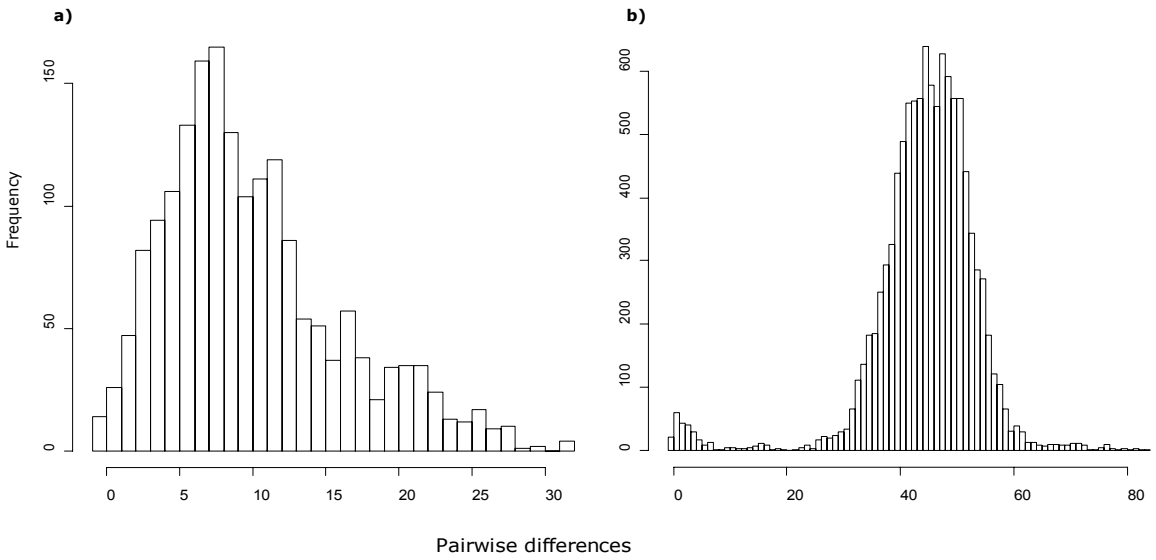


Figure 14. Frequency distribution of pairwise distances between individuals in (a) diploid and (b) hexaploid *P. argentea*, calculated with AFLPdat.

On one hand, 73/61 genotypes (threshold 4/8) have been identified among the 144 hexaploid individuals of *P. argentea* (D_g = 0.98; regarding both thresholds), 12 among the 56 diploid individuals (D_g = 0.60) and 3 within 5 samples of *P. argentea* var. *pseudocalabra* (D_g = 0.70/0.00). On the other hand, 14 different genotypes among the 14 samples of *P. calabra* (D_g = 1.00) (Tab. 6) have been observed.

The diploid *P. argentea* population with the highest number of genotypes was the population Pop269 from the central Spain (D_g = 1.00). Population Pop100 (2x subpopulation) from the central Alps was built out of 3 genotypes (D_g = 0.42), one dominant genotype (7 individuals) and two minor ones each (1 individual, N_e = 1.59). The remaining diploid populations were genotypically uniform ("clonal"). Populations Pop299 (Pyrenees), Pop237 (Massif Central) and the diploid subpopulation Pop57 (Öland) consisted each of one genotype, differing from each other by more than 4 band differences. Population Pop72 (Emsland) and all populations from the Alps (apart Pop100, see above) are built of possibly the same genotype, even though they possess different haplotypes (E and G, respectively).

Hexaploid populations proved to be genotypically more variable than the diploid ones. Out of 27 analysed hexaploid populations just nine (Pop19, 34, 93, 121, 138, 144, 196, 206, and 289) were phenotypically uniform (D_g = 0.00). They were distributed from the Maritime Alps throughout the Alps to Central Europe. The diversity of Pop121, 138, and 144 may be down-biased, as plants have been raised from seeds obtained through the *Index Seminum* exchange, and were possibly gathered from a limited number of mothers. Increased variability was observed in 3 populations (D_g = 0.20–0.40: Pop69, 198 and 234) and high variability for 11 populations (D_g = 0.60–1.00: Pop42, 48, 100, 117, 127, 163, 165, 202, 230, 248, and 258). However, the variability sunk to zero with a higher threshold in Pop69 and to slightly lower values in Pop127 and 248. The most diverse populations were Pop42, 100, 165, 230, and Pop258 (D_g = 0.90–1.00) found in the Maritime Alps, the central Alps, the Baltic region and the Wachau valley in Lower Austria and Mt. Lozen in Bulgaria. Populations and subpopulations with only two analysed individuals (Pop57, 88, 91, and Pop292) were not considered. Populations with increased variability have one dominant and one additional genotype (4 + 1 and 9 + 1 respectively, N_e = 1.22–1.47). None of the AFLP genotypes are shared among different populations using both threshold values.

When allowing more mutations as an error threshold (up to 12), the number of AFLP genotypes remains the same in *P. calabra* populations. In other populations, the number of genotypes gradually declines, e.g. diploid *P. argentea* population Pop269 starts to lose genotypic diversity already with 5 mutation thresholds.

The character incompatibility analyses have been applied to the original AFLP genotypes and to populations/subpopulations with more than four genotypes. Hence, populations Pop57, 88, 91, 127, 196, 237, 292 have been excluded. The pairwise MIC values in diploids ranged from 1–35 ($\bar{x}$ = 11.57). The highest value (MIC = 35) was found in Pop269 from central Spain. For the remaining diploid populations, lower MIC values were revealed (MIC = 1–13).

In hexaploids, the MIC values were in general higher than in diploids (MIC = 0–978 [$\bar{x}$ = 86.55]), which is also due to the higher number of fragments. The highest value (MIC = 978) was found in Pop100. Phenotypically uniform populations and those with moderate

variability (D_g = 0.00–0.40) showed also very low matrix incompatibility counts (MIC = 0–3), which implies that the genotypic diversity is caused almost solely by mutations. One population with a high genotypic diversity (Pop248) also revealed low MI counts (MIC = 0–4) suggesting a non-recombinant population structure. In the remaining populations (Pop42, 48, 100, 165, 202, 230, 258; MIC = 35–303), at least some recombination or other source of variation could be assumed. The matrix incompatibility counts in *P. calabra* ranged from 32 to 124, in *P. argentea* var. *pseudocalabra* was zero, despite relatively high phenotypic variability (D_g = 0.7).

3.4 Discussion

3.4.1 Ploidy differentiation

The AFLP data suggested a strong genetic division between *P. calabra* plus morphologically associated *P. argentea* subsp. *pseudocalabra* as well as both of the two ploidy levels observed in *P. argentea*. The inferred differentiation agreed well with the morphological distinctness of *P. calabra* compared to *P. argentea*, but it was less expected for the separation of the often sympatric diploid and hexaploid *P. argentea*. The strong division of cytotypes corresponds with the observation of the ploidy-specific RAPD banding patterns by Holm (1995). However, the Structure analyses revealed a certain proportion of the diploid genetic cluster admixed in the hexaploid *P. argentea*. Several hexaploid individuals from the Central Europe and the Alps contained 0.3–11.3% and the hexaploids from the island Gotland revealed 11.2–19.8% ($\bar{x}$ = 17.1%) of the diploid genetic cluster. Higher proportions of the diploid admixture with hexaploids are in agreement with the rare findings of tetraploids and pentaploids in the southern Sweden (4.51% for 4x and 2.26% for 5x out of all counts from Fennoscandia; Müntzing & Müntzing 1941, Müntzing 1958b, Leht & Paal 1998, Lövkist & Hultgard 1999). Both of these findings suggest certain genetic exchange among cytotypes. However, the rarity of tetraploids and pentaploids in the rest of Europe (e.g. Skalińska & Czapik 1950, Leht & Paal 1998, Ilnicki & Kołodziejek 2008) together with a strong and clear genetic separation of diploids and hexaploids indicate a very restricted genetic contact between these two cytotypes (Asker 1986). The main reasons for the genetic isolation are most probably the different mating systems which strongly limit the outcrossing. Autogamy was suggested for the dipoloids (Holm et al. 1997) and apomixis for hexaploids (Rutishauser 1943a, Hunziker 1954). However, genetic isolation may be, apart from the reproductive barriers and reduced fitness of inter-cytotype hybrids, caused also by several other factors. One possibility may be a balanced selection by means of the ecological sorting on a microscale or flowering time divergence (van Dijk & Bijlsma 1994, Felber-Girard et al. 1996, Suda et al. 2007). A certain degree of ecological sorting was observed by Holm (1995) for hexaploid plants, which were growing primarily in ruderal microenvironments with relatively tall vegetation. Flowering time divergence has been reported for cultivated plants by Müntzing & Müntzing (1941), but in nature, flowering times are overlapping (Holm & Ghatnekar 1996a). However, in order to determine types and effectiveness of the reproductive isolation mechanisms between the cytotypes, experimental ecological and more detailed genetic and cytogenetic studies of mixed populations should be carried out. In particular, reproductive modes and the origin as well as success of inter-cytotype offspring should be studied.

3.4.2 Origin of polyploid cytotypes

Two dominant ploidy levels within the *P. argentea* indicate, that at least one evolutionary significant polyploidisation event must have happened in this taxon. Assumptions on the origin of the hexaploid cytotype have already been made by Holm (1995) and Holm & Ghatnekar (1996a) using RAPDs and isozymes. The presence of the hexaploid-specific RAPD fragments indicated that hexaploids contain also genetic material from a different genome than the diploid one. Moreover, the allopolyploidisation was confirmed by these authors also on the basis of the isozyme analysis. For dimeric isozymes, heterodimers are expected in diploids and autopolyploids carrying different electrophoretic alleles at the same locus. However, the hybrid bands were present only in 2 out of 11 studied hexaploids and several unique isozyme alleles were found in the hexaploid cytotype. These patterns suggested a considerable evolutionary divergence among the individual genomes of the hexpaloids.

When studying the hybridisation or allopolyploidy, "parental additivity" of AFLP bands can be assumed with regard to the number and distribution of the bands, in the first or early generation progeny (Wendel 2000). However, in synthetic F1 allohexaploid between the tetraploid *Triticum turgidum* ssp. *dicocoides* and diploid *Aegilops tauschii* (Dong et al. 2005), 84% of the bands were additive, 17% of both parental origin were absent and, 2.4% appeared de novo. Thus, about 20% of the bands showed a deviation from parental additivity, which was explained by changes in functional genes and transposable elements. On the contrary, in F1 autotetraploid *Paspalum* sp. resulting either from fusion of unreduced gametes or from colchicine treatment (Martelotto et al. 2007), band loss (8.2% out of all bands) was significantly higher than the gain of novel bands (1.6%) in the autotetraploid. Genomic southern blots could not fully confirm a sequence deletion, but alternative de novo insertion of repetitive elements that, are mobilised by "genomic shock" events, has been declared as a possible cause of this phenomenon.

Our data revealed that the hexaploids possessed almost all fragments found in diploids. Additionally, hexaploids also revealed a considerable amount of hexaploid-specific fragments. Out of 228 bands observed in the hexaploids, 126 (55.3%) were missing in the diploid cytotype. On the contrary, there is just one diploid band missing in the hexaploid cytotype. In addition, 30.86% (75 fragments) of all fragments are hexaploid-specific and just 0.41% (1 fragment) was diploid-specific. Recovery of almost all bands from the diploids in the hexaploid contradicts genomic reorganisations as an explanation for the high proportion of extra bands observed in the polyploids. One may alternatively assume that diploids and hexaploids represent long-term diverged lineages with associated accumulation of mutations in the hexaploid cytotype. However, this hypothesis fails to explain the observed negligible diploid specific number of fragments. A straightforward comparison between monocots (*Paspalum*, *Triticum*) and dicots (*Potentilla*), or of different taxa in general, is probably questionable, but we consider the results a strong indication for an allopolyploid origin of the hexaploid *P. argentea* involving strong additivity of parental molecular polymorphisms.

The distribution of maternally inherited cpDNA haplotypes was in agreement with the AFLP based hypothesis of the allopolyploid origin of the hexaploid cytotype. The

widespread haplotype E is shared by both cytotypes. Hence, the maternal contribution of the diploid cytotype in the hexaploid can be assumed. The occurrence of the hexaploid-specific haplotypes F, H, and I derived from the haplotype E may be explained by further diversification of the hexaploid lineage. Alternatively, the hexaploid-specific haplotypes may have been contributed by a third taxon, not involved in our sampling. This taxon could have been potentially also involved in the origin of the allopolyploid. The absence of a suitable second "parent" among the investigated accessions suggests the origin of the allohexaploid outside or in the marginal position of the studied European area. Furthermore, the occasional presence of phylogenetically isolated haplotype Q in the Alps and its co-occurence in several taxa of the *P. verna* agg. (Paule & Dobeš, unpubl., see also next chapter) also indicate some genetic contact with morphologically largely divergent groups.

Allopolyploidy can also be assumed based on zero occurence of tetravalents during the male gametogenesis (Müntzing 1931, Asker 1985). Furthermore, allopolyploid speciation has also been shown for other *Potentilla* species and relatives, e.g. for *P. anglica* Laich. (Matfield & Ellis 1972) and assumed for other species of the genus (e.g. Asker 1970a, Ehrendorfer 1970). Similarly, allopolyploidy was inferred based on incongruence of the cpDNA and nuclear DNA phylogenies in the subtribe *Fragariinae* (Lundberg et al. 2009) – phylogenetic sister to *Potentilla*.

3.4.3 Reproductive modes and population genetic structure

As already mentioned in the Introduction, there is a certain controversy concerning the reproduction modes in *P. argentea*. Apospory and pseudogamy have been attributed to hexaploids on the basis of the crossing experiments, chromosome counting (Müntzing 1928, 1931), detailed cytological studies (Rutishauser 1943a, Håkansson 1946, Hunziker 1954) and validated using isozyme and RAPD analyses (Holm & Ghatnekar 1996a). Hexaploid *P. argentea* is thus considered an apomict, with the ability to produce functional pollen as well as B_{III} hybrids. Similarly to Holm & Ghatnekar (1996a), most of the genetic variation in hexaploid *P. argentea* was observed among the populations (66.62%) compared to the within-population diversity (33.38%). Analogous patterns of population genetic structure were observed in other apomictic plants (Gornall 1999). Uniparental apomictic reproduction lacks the contribution of a pollen donor and the geneflow is reduced. Hence, the populations tend to become genetically differentiated from one another due to genetic drift (Hartl & Clark 1997). Twelve out of 23 analysed hexaploid populations were built of one AFLP genotype or of one dominant and a second rare one (D_g = 0.0–0.4). Additionally, all of these populations showed low MIC values indicating that the residual variability was not created by recombination. Within the other 11 studied populations, relatively high levels of genetic diversity (D_g = 0.7–1.0) have been observed. One of these populations (Pop248) also reveals low MIC suggesting a clonal population structure and its diversity decreased significantly when a higher threshold was used. However, for the other populations (MIC = 27–303; 978), at least some sexual recombination or process deviating from mutation accumulation can be assumed. These populations were found in the Balkans, across the Alps, in the Wachau valley, in the Slovak Carpathians and in the Baltic region and Scandinavia. The frequency of the distribution of pairwise distances between the hexaploid individuals revealed two categories (Figs. 13a, b): the first peak close to null distance represents distances between

similar genotypes that are probably different only due to mutations; the subsequent large peak comprises the individuals which then have arisen through recombination. However, none of the clones are shared among populations and the clonality is found only locally.

AFLPs, as a dominant marker system dealing with a certain error rate, do not allow to make explicit assumptions about followed reproductive modes. In apomictic plants one would usually expect a low variability of populations. However, as demonstrated by several studies, genotypic variability observed in our dataset has also been shown in other apomictic taxa. (Ellstrand & Roose 1987, Gornall 1999, Mes at al. 2002). The variability within populations of apomictic taxa has two main sources: mutational accumulation within clones, multiple origin of the clones and occasional or past sexual recombination. On one hand, the mutations have been attributed to several populations based on the CI analyses, on the other hand, the distribution of the variable populations with higher MIC values strongly suggest that several localities have been colonised by different clones independently (discussed later). Furthermore, the rare sexuality within this taxon (Holm & Ghatnekar 1996a) may also occur more often than assumed. Mandryk (1994) observed sexual types within the embryologically studied Ukrainian material, however, without mentioning the ploidy. Based on the literature chromosome count review, it may concern the hexaploids.

The diploid *P. argentea* has been classified as one of the rare diploid facultative apomicts (Asker 1967, 1971), however contrasting results were obtained by detailed isozyme analyses of two southern Swedish populations (Holm et al. 1997). Both populations showed a high proportion of selfing (f = 0.78–0.94), partial outcrossing, but no apomixis, which was further confirmed by analysis of the progeny coming from castration and hybridisation experiments. Self-compatibility in a diploid sexual as well as in polyploid apomict has been recored very rarely (reviewd by Hörandl 2010). *P. argentea* is thus the other example besides the genus *Boechera*, where both sexuals and apomicts are mostly self-compatible (Roy 1995). The variation in diploids was distributed in a similar way as in hexaploids, 57.68% among and 42.32% within populations. Considering the error rate of 4 fragments, all individuals within each population belong to one genotype, apart from population Pop269, which seems to be sexual and predominantly outcrossing. Outcrossing was also supported by the highest MIC value (MIC = 35). Considering the given threshold, one genotype was also suggested for all individuals from Alps and Emsland (Pop72). This is however in conflict with the possession of different haplotypes between populations in Alps and Emsland (G and E respectively). The software Genotype/Genodive assigns the individuals to particular genotypes. In this way it recognises more "clonality" because individuals may be, due to a given threshold, interconnected. For example: if the distance A-B = 4 and B-C = 4, all three individuals: A, B and C, will be assigned to the same clone, even though the distance A-C could be more than 4. Moreover, the error rate, counted for the whole dataset, is in diploids probably overestimated, because of a lower number of AFLP fragments in the diploid cytotype. The frequency distribution of pairwise distances among the diploid individuals, showing only one peak and significantly lower distances than in the hexaploids, suggested to use a threshold of 0. This is however, inapplicable because of the error rate comprised in the dataset. When taking a closer look at the frequency distribution of pairwise distances within the populations, we may observe that the population Pop299 is possibly more diverse (pairwise distances between individuals 2–6) than population Pop237 (0–5). The reduction of diversity in this spatial pattern and in the area, where only cytotypically uniform diploid

populations occur, could be explained with the fast postglacial colonisation of a self-compatible taxon accompanied by one or several founder effects. The reduction is also reflected in the decrease of the MIC values, i.e. decrease of recombination. Therefore, we consider it an indirect evidence for the self-pollination as a reproductive mode in diploid *P.argentea*.

Another indirect evidence supporting a sexual rather than asexual mode of reproduction in diploids is the relatively higher abundance of the tetraploid cytotypes (5.25%) compared to pentaploids (2.3%). Considering all the possible gamete combinations with regard to diploids and hexaploids, tetraploids are most likely to arise from combination of x and 3x gametes. That implies either a fertilisation of a haploid egg cell with triploid pollen, or vice versa. It has been shown that the hexaploids produce triploid functional pollen (Müntzing 1931, Rutishauser 1949, Asker 1985), but no cases of reductional division in the hexaploid female gametophyte have been recorded. As a result, we assume that the first scenario is the most probable.

One population of *P. argentea* var. *pseudocalabra* (Pop166) revealed a relatively high genetic variability (D_g = 0.7) but, the zero MIC value and is thus considered apomictic, which is also reflected in the decrease of the variability after application of the higher threshold. All studied populations of *P. calabra* had different genotypes (D_g = 1.00), as expected for outcrossing plants, similarly as the diploid *P. argentea* from the Iberian Peninsula (Pop269), also revealed comparable values of MIC (MIC = 32–124). Thus, our data are further confirmation of the sexual and outcrossing mode of reproduction of this lineage.

3.4.4 Phylogeographic implications

Phylogeographic implications have been derived from the cpDNA and AFLP data. As the three studied lineages have shown strong genetic division, they were analysed separately. Because of the small number of analysed populations within the "*P. calabra* halpotype group", no phylogeographic conclusions have been made for this group.

Concerning the *P. argentea* diploid cytotype, we have discovered a highly significant geographic structure within the AFLP dataset using the Mantel test (r_M = 0.585, P = 0.005). Furthermore, the highest genetic diversity within the sampling has been revealed for the population Pop269 (D_g = 1.0), which suggests that the Iberian Peninsula is the glacial refuge area for this lineage. This finding is also confirmed by the Structure analysis. The population Pop269 reveled all three genetic clusters whereas the other populations contain just two. Combining these data with the distribution of the cpDNA, haplotype E enables us to reconstruct a Late Quaternary migration route from Iberian Peninsula throughout the Western Europe to Scandinavia and probably also farther to the Baltic region, with a diverged diploid lineage represented by haplotype G in the Alps. This scenario fits into the broadly accepted Iberian refuge area followed by a colonisation of Western Europe up to Scandinavia (reviewed by Comes & Kadereit 1998; Taberlet et al. 1998). Fast postglacial colonisation in a self-compatible species often results in reduced genetic diversity. Self-compatible species are able to establish new populations from a very small number of individuals from a larger population (so-called founder effect). Founder effect was possibly

observed in populations from Pyrenees (Pop299) and further in Massif Central (Pop237), based on the differences within populations (discussed above) and it is also reflected in the Structure analysis. Minor genetic cluster from the Pop269 is the dominating one in Pop299 and Pop237. Potential increased diversity in the population Pop72 from Emsland as well as in diploid subpopulation Pop57 from Öland may consequently be explained by the influence of the hexaploid cytotype or by larger, better established populations. An alternative mechanism could also be a switch from outcrossing to selfing as shown in *Hornungia alpina* (Winkler et al. 2010), when colonising harsh habitats. However, no self-pollination tests with the dipoid *P. argentea* population Pop269 have been carried out, hence we cannot comment about its possible self-incompatibility.

On the contrary, for the hexaploid cytotype, there have been no clear geographical patterns detected within the AFLP dataset. It is most probably due to independent immigration of genetically divergent lineages, which resulted in an overlap of several immigration routes. Concerning the cpDNA, the majority of the samples belong to the haplotype E and F. The hexaploid individuals with the haplotype E are distributed throughout the Central Europe, in the Alps and Balkans, Scandinavia and the Baltic region. The haplotype F is exclusively hexaploid, sympatric with the haplotype E, but missing in Scandinavia and the Baltic region. Haplotype H, derived from haplotype F is restricted to the Baltic region and haplotype I is found only in one population in the Alps. Additionally, haplotype Q, unrelated to the "*P. argentea* haplotype group" is found in the Alps and the Balkans. It is obvious that the highest haplotype diversity is in the Alps, but this could be strongly biased due to our sampling. The Alps have been proved to be an important glacial refuge area for several plant taxa (e.g. Schönswetter et al. 2005) but the presence of four different haplotypes may also be explained by the recolonisation of the Alps several times (Csaikl et al. 2002). Nevertheless, the hypothesis of Alps serving as a refuge area is supported by the fact, that *P. argentea* is almost completely missing in the Apennine peninsula (Kurtto et al. 2004, C. Dobeš pers. comm.). The scattered distribution could be then considered as a postglacial recolonisation from the Alps. Balkans have probably also played an important role as a refuge area for hexaploids, suggesting from the presence of the haplotype Q in both the Balkans and Alps. However, the undersampling does not allow us to make any accurate conclusions. Furthermore, the presence of the derived haplotype H in the Baltic region and the absence of the haplotype F in Scandinavia suggest that these regions have been colonised via 2 different ways: through Scandinavia and central Europe.

On the basis of the chromosome counts from the literature (Fig. 6a) it is obviuos that diploids are reaching more northwards that the polyploids. This is in strong contrast with the generally accepted scenario of the fast colonisation of the derived polyploids in the Holocene (e.g. Franzke & Hurka 2000) as well as with the the concept of "geographical parthenogenesis" (Bierzychudek 1985, Hörnadl et al. 2008). The diploid *P. argentea* seems to be at least as effective coloniser as the hexaploid one. As selfers, they possess the same advantage of no necessity of the sex, thus they can disperse fast. However, the occasional outcrossing keeps sufficient genetic diversity which is favoured when colonising new harsh habitats.

3.4.5 Taxonomic assumptions

The molecular results presented in this study are in agreement with the taxonomic treatment of *P. argentea* used in Flora Europaea (Ball et al. 1968, Kurtto et al 2004). The two observed cytotypes were genetically clearly distinct and appeard to be reproductively isolated from each other. Although, we have not analysed the morphology of the studied material in detail yet, the molecular separation perfectly correlated with karyological differentiation and fully correspond with two different taxa originally proposed by Marklund (1933): diploid cytotype, which corresponds with *P. argentea* s.str. and the hexaploid one matching *P. neglecta* (≡ *P. impolita*). Based on Marklund (1933), the main morphological feature distinguishing between the taxa was the hairiness of the upper leaf surface. Differences in habitus, leaflet shape, stipule morphology, hairiness of calyx and style as well as flowering time provided other discriminating characters. Müntzing & Müntzing (1941) and Müntzing (1958b) confirmed this taxonomic concept by studying the plant material cultivated in common garden under comparable conditions. According to their observations, hexaploid types were strictly perennial and diploids scarcely more than biennal. Furthermore, Holm (1995) confirmed that the transplanted plants of different ploidy level could be separated on the basis of plant size and hairiness. However, Müntzing (1958b) and Holm & Ghatnekar (1996b, based also on pers. comm. with Sven Asker), denoted, that none of the above mentioned characters are sufficiently discriminating in the field and are strongly influenced by local environment.

For most individuals included in this study, one herbarium specimen collected in the field and the other one obtained from material transplanted and raised in the garden is available. No assumptions regarding the life cycle could have been made, mainly due to the fact that the plants were kept in the culture two vegetation periods only. When studying the herbarium specimens, we made similar observations as Holm & Ghatnekar (1996). The hairiness of adaxial leaf side strongly varies within the cytotypes and is probably affected by local conditions. When studying plants cultivated under controlled conditions, we could observe a trend in that the leaf upper-surface is generally less hairy in diploid plants. However, there have also been diploid individuals with dense hairs observed (mixed population Pop54, Pop57 as well as Pop269). Nevertheless, the strong genetic division is in favour of the two separate taxa. Hence, we strongly suggest a morphometric study of the material from the whole distribution area.

The recognition of *P. calabra* as a separate taxon is supported by both the AFLP and cpDNA data. Molecular data support the morphological distinctiveness of *P. calabra* (Ball et al. 1968). Nevertheless, the characters mentioned in Flora Europaea have to be reconsidered as the hairiness of adaxial leaf side varies and in some cases it is almost identical to *P. argentea*. The length of petals and the branching of the caudex are probably better characters for the delimitation of *P. calabra* (Zangheri 1976, Jiří Soják pers. comm.).

Some individuals from Balkan display an intermediate genetic structure between hexaploid *P. argentea* and *P. calabra* based on the neighbor-net and Structure analyses as well as the presence of no specific bands and sharing of 68.5–94.5% of its bands with other studied taxa. It has been identified as *P. argentea* var. *pseudocalabra* Th.Wolf, firstly described by Theodor

Wolf in Asch. & Gr. Syn. VI. 720 (1904). According to Wolf (1908) it is morphologically intermediate among *P. calabra* and *P. argentea* var. *typica*, var. *incanescens* and mainly var. *dissecta*. This taxon is supposed to be restricted to SE Europe and the Balkans. Hence, the genetic intermediacy of the population Pop166 would correspond to this description and would strongly suggest *P. argentea* var. *pseudocalabra* Th.Wolf as a valid taxon.

Nevertheless, to draw up more solid taxonomic conclusions, a detailed sampling and a subsequent genetic and morphometric survey in the whole Balkan Peninsula would be desirable, covering also Asia Minor, where *P. calabra* is reported (Peşmen 1972).

3.5 Conclusions

Combined analysis of AFLPs, cpDNA sequences and ploidy levels identified four main lineages within the *Potentilla argentea* group: diploid *P. argentea*, hexaploid *P. argentea*, *P. argentea* var. *pseudocalabra* and *P. calabra*. AFLP and cpDNA data seem to agree upon an allopolyploid origin of the hexaploid *P. argentea*. Furthermore, hexaploid *P. argentea* apears to be apomictic, whereas the diploid *P. argentea* is a self-pollinator with a highly reduced genetic variability and *P. calabra* reproduces sexually. We also reconstructed a Late Quaternary migration route from Iberian Peninsula throughout the Western Europe to Scandinavia and probably also farther to the Baltic region, with a diverged diploid lineage represented by haplotype G in the Alps for the the diploid *P. argentea*. On the contrary, for the hexaploid cytotype there have been no clear geographical patterns detected, most probably due to independent immigration of genetically divergent lineages, which resulted in an overlap of several immigration routes. Finally, genetics favours the taxonomy of Flora Europaea although realiable morphological characters are missing.

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Chapter 4

Hybridisation in the genus *Potentilla* – a case study of *P. alpicola*

4.1 Introduction

Interspecific hybridisation has long been considered a potentially innovative evolutionary force playing an important role in speciation and phenotypic diversification (Kerner 1894–1895, Lotsy 1916, Anderson 1949, Stebbins 1959, Barton & Hewit 1985, Arnold 1997, Soltis & Soltis 2009). If successful, hybridisation facilitates the rise of new genotypes by means of combining previously isolated gene pools and at the population level, it may result in significant shifts of allele frequencies. In general, character intermediacy in the progeny is usually considered, but hybrids often exhibit extreme phenotypes or novel characters, referred to as "transgressive segregation" (Rieseberg et al. 1999). Nevertheless, hybridisation often results in sterile offspring, mainly because the precise pairing of chromosomes during meiosis is not fulfilled.

The occurrence of allopolyploidy is very closely related to hybridisation, following mainly heteroploid crosses (Soltis & Soltis 2000). After hybridisation between two (or more) distantly related species, doubling of the genome may occur thus overcoming the common sterility in hybrids by providing each chromosome with a pairing partner. It usually results in instantaneous speciation, because the backcrossing with the parents produces usually unviable or sterile offspring (Seehausen 2004).

The hybridisation is also believed to be fundamental to the occurrence of apomixis (asexual reproduction through seeds). Apomixis is found almost exclusively in polyploids and highly heterozygous species (Savidan 2000). In sterile amphiploids, it may serve as another mechanism for "escaping the sterility" (De Wet et al. 1974), but beyond that, it provides an additional selective advantage for overcoming any minority-related disadvantage in sympatry with parents (Levin 1975). Moreover, apomixis also preserves favourable characters and features related to the fixed heterozygosity or hybrid vigour (Richards 2003).

Hybridisation, often followed by polyploidisation or introgression, is suggested to be an important mechanism in the speciation and evolution of the highly polymorphic genus *Potentilla*. **Possible hybrid origin** of several taxa, **morphological variability,** intermediacy and consequent taxonomic complexity, mainly among the taxa from grex/series *Aureae* T. Wolf and *Argenteae* T. Wolf, were a major issue already in the 19th and at the beginning of the 20th century (e.g. Krašan 1867, Čelakovský 1889, Sauter 1888, Błocki 1889, Heinricher 1907, Domin 1908). Later on, the presence of **apomixis** (Gentscheff 1938, Gustafsson 1947, Hunziker 1954, Löve 1954) and extensive **intraspecific ploidy variation** (Müntzing & Müntzing 1941, Ehrendorfer 1970, Asker 1985, Dobeš 1999) supported this view.

Potentilla collina group, from the series *Argenteae* T. Wolf., seems to be a suitable model system for studying the influence and contribution of the above mentioned phenomena to the evolution of the genus. Fifteen to twenty or more species (Kurtto et al. 2004, Gregor 2008) belonging to this group are considered either locally to regionally distributed microspecies or are representing a taxonomically complicated Eurasian hybrid complex. The observed morphological variability and exclusive polyploidy (x = 7; 2n = 5–12x), with occasional observation of chromosome aberrations (Müntzing 1958a), are explained by the hybrid origin of the group (Wolf 1908, Asker & Fröst 1970, Soják 1995, Gerstberger 2002, Gregor et al. 2002). Within the group, the development of both female and male gametophytes was reported to be absent or disturbed. Obligate or close to obligate apomixis by means of apospory and pseudogamy was identified on the basis of crossing and castration experiments (Müntzing 1928, 1931) and later confirmed by detailed cytological studies (Gentscheff & Gustafsson 1940, Håkansson 1946). Full or partial male sterility (9–44%) has also been found in several studied individuals (Müntzing 1928, Gentscheff & Gustafsson 1940). Furthermore, in a hexaploid *P. collina* biotype only uni- and bivalents, but no tri- or tetravalents were observed (Müntzing & Müntzing 1943). This suggests a contribution of at least two different genomes.

The presumed parents are members of *P. argentea* and *P. verna* groups. Besides the apomictic polyploids, also sexual diploid taxa are found within these groups (e.g. *P. calabra* Ten., *P. subacaulis* L., *P. velutina* Lehm.). The experimental hybridisations confirmed interfertility between different taxa and ploidy levels from these groups (e.g. Rutishauser 1948, Asker & Fröst 1970). Additionally, fertilisation of reduced (B_{II}-hybrids) and unreduced egg cells (B_{III}-hybrids) (Rutishauser 1948, Asker 1966), as well as spontaneous doubling of the somatic genome (Asker 1970), representing mechanisms potentially resulting in cytotypic variability, have also been reported. The individual taxa of *P. collina* group are regarded as hybrid combinations among sexual as well as among sexual and apomictic taxa, subsequently stabilised via apomixis and whose intermediate morphology reflects the contributions of different genomes. However, the exact evolutionary scenarios are still rather speculative. Some authors considered also the series *Rectae* sensū Wolf (Wolf 1908, Asker & Fröst 1970) as their possible parental influence and the artificial crosses between several presumed parental taxa were successful, but the vitality of the progeny was restricted (summarised in Asker & Fröst 1970).

One example from the *P. collina* group is the *Potentilla alpicola* La Soie, a microspecies restricted to the western and central Alps (Käsermann & Moser 1999). It occupies montane – subalpine and is often found in close sympatry with *P. argentea* (s.l.) and *P. pusilla* Host from the *P. verna* group. Chromosomes, counted so far, revealed polyploidy in this taxon (2n = 5x, 6x, 12x; Käsermann & Moser 1999). Concerning the morphology, P. *alpicola* is usually intermediate in most morphological characters, with some individuals tending to be more similar to *P. argentea*. However, the leaf indumentum is similar to that of *P. verna* group taxa.

In order to unravel the evolutionary history of the *P. alpicola*, a ploidy level estimation and parentage analyses using molecular markers were applied in sympatric populations with presumed parents, where the geneflow is still possibly present. We employed rapid and precise ploidy level estimation by means of the flow cytometry (Kron et al. 2007), amplified fragment lengths polymorphisms (AFLPs) in order to generate a representative sample of

reproducible nuclear markers and cpDNA sequences in order to assess the haplotype diversity and to follow the maternal line. The applicability of the AFLPs for studying different, distantly related, plant groups, even within a genus, is controversial because of the uncertainty concerning the fragments of the same length representing the same alleles. However, based on the most recent phylogenetic analyses (Dobeš & Paule 2010), all studied taxa belong to the same clade C. Hence, we assume that the fragments of the same size are allelic. Furthermore, AFLPs have already been successfully employed in studies dealing with hybrid origin of several taxa of higher plants and together with the cpDNA sequences, they provide a sufficient resolution in hybridisation studies (e.g. Perný et al 2005, Bleeker 2007, Lakušić et al 2009).

In the following, four main questions will be answered: (i) Is *P. alpicola* of hybrid origin? (ii) If yes, which taxa are involved in its formation? (iii) Did *P. alpicola* arise at several localities independently (polytopicaly), or did it arise on one locality and spread afterwards throughout the area of Central Alps? (iv) What are the reproductive modes in *P. alpicola* and possible parental taxa? Finally, we comment also (v) the taxonomy.

4.2 Material and Methods

4.2.1 Plant material

Plant material was collected from a total of 13 localities within the central Alps (South Tyrol, Switzerland and North Tyrol). *P. alpicola* was sampled together with the sympatrically co-occurring possible parental taxa (*P. argentea* L. (s.l.), *P. verna* group – *P. pusilla* Host, *P. incana* G.Gaertn., B.Mey. & Scherb. Within the distribution range, populations determined as *P. collina* were also sampled.

On the basis of the geographical proximity, thirteen localities were reduced to seven broader localities (Fig. 15). Additional 8 localities with species, which do not occur in these localities, but are present in the same area of Central Alps [additional *P. argentea* L. population, *P. aurea* L., *P. brauneana* Hoppe, *P. crantzii* (Crantz) Beck ex Fritsch, *P. frigida* Vill., *P. thuringiaca* Bernh. ex Link, *P. thuringiaca* × *pusilla*] and could have been potentially involved in the genesis of *P. alpicola* were also sampled. Each individual was collected from a distance of at least 5 m from each other. The origin of the material is shown in the Appendix 4. In total, 321 accessions representing 34 populations of 11 taxa were investigated, 5–27, but mostly 10 samples per population.

Vouchers from plants collected during field trips as well as from transplanted plants are deposited in HEID (Herbarium of the University of Heidelberg) and WUP (Herbarium of the Department of Pharmacognosy, University of Vienna). The vouchers are labelled with the material numbers so as to assure a consistent cross-reference with other studies. The coordinates for the collected material were obtained using a handheld GPS. In order to present the geographical data ArcView-ArcGIS 9.1 (ESRI, USA) software was used. In Figure 15 a Hillshade WMS-layer (Auer et al. 2009) was used.

4.2.2 Chromosome counts and DNA ploidy level estimation

The DNA ploidy levels were determined by flow cytometry from fresh leaf petioles using the Partec Ploidy Analyser PA (Partec, Germany) at the IPK, Gatersleben and at the Department of Pharmacognosy, University of Vienna. The samples were prepared according to the two-step protocol involving Otto buffers, as summarised by Doležel et al. (2007), with an internal standard [*Lycopersicon esculentum* cv. Stupické polní tyčkové rané (Doležel & Bartoš 2005), 2C = 1,96 pg; *Potentilla incana* Ptl4311]. After chopping of the material using a razor blade in Otto I-buffer and staining in Otto II-buffer containing 4 $\mu g \cdot ml^{-1}$ 4'-6-diamidino-2-phenylindol (DAPI) for 10 min at room temperature, the fluorescence intensity of 5,000–20,000 nuclei was recorded. The sample/standard ratios were calculated from the means of the sample and standard fluorescence histograms. Only histograms with coefficients of variation (CVs) for the G_0/G_1 peak of the analysed sample below 5.0% were considered. In order to obtain a reliable reference for the DNA ploidy estimation, chromosome numbers of individuals of all studied taxa were counted using either the methodology of Murín (1960) or that followed by Dobeš (1999) (Appendix 4). In a case that counts unravelled more than one ploidy level for a species, the sample/standard ratios of individuals were regressed against their determined chromosome numbers. The DNA ploidy level has been attributed to the individuals measured only by flow cytometry based on the regression equation. Regressions for the measurements performed in Gatersleben and in Vienna have been computed separately. DNA-ploidy levels were directly assigned to individuals, if species showed a total variation of sample/standard ratios explainable by the error of measurement.

4.2.3 DNA extraction

The total DNA was isolated from freshly-collected and silicagel dried leaf tissue from single individuals. Extraction of the total genomic DNA followed the protocol of Doyle & Doyle (1987; CTAB method), with some modifications applied: grinding of 5–15 mg dry leaf tissue in 2 ml tubes using a Precellys 24 homogeniser (Bertin Technologies, France), addition of 2 units (U) of ribonuclease A per extraction to the isolation buffer and washing of the DNA pellet twice with 70% ethanol. The DNA was finally dissolved in 50µl TE-buffer and stored at –20 °C.

4.2.4 CpDNA amplification and sequencing

The plastomic *trn*H(gug)-*psb*A intergenic spacer (IGS) was amplified using the primers: *trn*H(gug) 5'-CGC GCA TGG TGG ATT CAC AAT CC-3' and *psb*A 5'-GTT ATG CAT GAA CGT AAT GCT C-3' (Shaw et al. 2005). Sequences covered the last 29 bp of the *trn*H gene, the complete IGS, and the first 53 bp of the *psb*A gene. Forward and reverse primers carried a 5'-end M13 extension modified from Messing (1983): 5'-GCA TGT TTT CCC AGT CAC GAC -3' for forward and 5'-ACT TCA GGA AAC AGC TAT GAC-3' for reverse primer.

The PCR reactions were performed in a total volume of 25µl containing 1×GoTaq PCR buffer (Promega, USA), 1.5 mM MgCl, 0.2 µM of each primer, 0.4 mM of each dNTP, 0.5 U Taq DNA polymerase (Promega GoTaq), and 10–100 ng of template DNA using an PTC-200 (MJ Research) thermal cycler. The thermal cycling started with a denaturation step at 95 °C lasting 5 min; followed by 30 cycles each of 60 s denaturation at 95 °C, 30 s annealing at 48 °C *trn*H-*psb*A IGS and 60 s elongation at 72 °C. Consequent elongation phase lasted 10 min at 72 °C with a subsequent final hold at 4 °C. The amplicons were checked for length and intensity on 1.5% agarose gels and consequently sent to GATC (Germany) for commercial sequencing using modified M13 primers. The cycle sequencing was performed on both strands. Runs resulting in sequences of low quality were repeated. In the majority of cases each forward and reverse reaction spanned the complete sequence. All sequences were edited and a consensus was made of forward and reverse reactions using the software Seqman 4.0 (DNASTAR, USA).

4.2.5 AFLP analysis

Prior to the AFLP analysis, the DNA-concentration of each sample was measured using the Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies, USA) and each sample was diluted down to 100 ng·µl^{-1}. The AFLP analysis was performed using the protocol established by Vos et al. (1995) with the following modifications: approximately 550 ng of DNA was digested and ligated in a 15 µl reaction mix containing 1× T4 ligase buffer and 1× ATP Solution (Bioline, USA), 50 mM NaCl, 0.75 µg BSA, 1.5 U T4 ligase (Bioline), 1 U MseI and 5 U EcoRI (New England Biolabs, USA), and 0.37 µM of EcoRI-adapter and 3.67 µM of MseI adapter. The reaction mix was incubated in a tube for 3 h at 37 °C followed by an inactivation step for 10 minutes at 65 °C and a final hold at 4 °C. Afterwards the restriction-ligation product was diluted ten-fold.

In the pre-selective PCR, 2.5 µl of the diluted restriction-ligation product was used in a total reaction volume of 12.5 µl containing 1× PCR buffer II (Applied Biosystems, USA), 2 mM $MgCl_2$, 0.8 mM dNTP mix, 0.2 µM EcoRI-A primer (5′-GACTGCGTACCAATTCA-A-3′), 0.2 µM MseI-C primer (5′-GATGAGTCCTGAG TAAC-C-3′), and 0.25 U AmpliTaq polymerase (Applied Biosystems). The reactions were held at 72 °C for 2 min followed by 20 cycles of: 94 °C for 20 s, 56 °C for 30 s, and 72 °C for 2 min, with a final 30 s extension at 60 °C. The pre-selective PCR product was then visualised on a 1.5% agarose gel and diluted ten-fold.

For selective PCR we used 2.5 µl of the diluted pre-selective PCR as a template in total reaction volume of 12.5 µl. The PCR-mix contained 1× GoldTaq buffer (Applied Biosystems), 2.5 mM $MgCl_2$, 0.8 mM dNTP mix, 0.08 µM EcoRI-fluorescence-labelled primer, 0.2 µM Mse primer and 0.5 U AmpliTaq Gold (Applied Biosystems). The reactions were held at 95 °C for 5 min followed by 13 cycles of: 94 °C for 30 s, 65 °C → 56 °C (–0.7 °C per cycle) for 1 min and 72 °C for 1 min, followed by 23 cycles of: 94 °C for 30 s, 56 °C for 1 min and 72 °C for 1.5 min, with a final 8 min extension at 72 °C. The selective PCR products were checked on a 1.5% agarose gel.

First of all, 64 selective primer combinations were tested with four geographically distinct individuals for variability and reproducibility. Subsequently, the following three

differentially fluorescence-labelled primer combinations were chosen for further AFLP analysis: EcoRI-AGG (TET)/MseI-CTC, EcoRI-AAC (6-FAM)/MseI-CTT, EcoRI-AGC (HEX)/MseI-CTG.

Three fluorescence labelled PCR products of the same sample were multiplexed and diluted (2 µl TET, 2 µl 6-FAM, 5 µl HEX and 50 µl ultra-pure H_2O). One microliter of the multiplexed selective PCR product was further mixed with 6 µl H_2O and 0.2 µl ET-ROX 550 size standard (Amersham Biosciences, USA). Fragments were electrophoretically separated on a MegaBase 500 DNA capillary-sequencer (Amersham Biosciences). In each run, a total of 48 samples were analysed including one standard sample applied to each run, one negative control and one repeat within the run. Raw data were visualised and the fragments manually scored using GeneMarker v1.8 (SoftGenetics, USA). Processed data were exported as presence /absence matrix.

4.2.6 Data Analyses

4.2.6.1 Chloroplast sequence data analysis and phylogenetic inference

The DNA-sequences were multiply aligned by means of ClustalX v1.83 (Thompson et al. 1997) and the alignments were manually refined using the GeneDoc software version 2.7 (Nicholas et al. 1997). Two regions were excluded from the alignment due to repeated sequence motifs (poly-A stretches) and three indels were manually coded for presence and absence. Phylogenetic relationships among cpDNA haplotypes were evaluated by means of the statistical parsimony network analysis using TCS 1.2 (Clement et al. 2000) with a default connection limit of 95%.

4.2.6.2 AFLP data analyses

Diversity estimates: Several statistical parameters were computed using the R-script AFLPdat [Ehrich 2006; R 2.9.2 environment (R Development Core Team 2009)] for the whole dataset, taxa or clusters revealed by later analyses: total number of the fragments, proportion of polymorphic fragments, number of private fragments. Furthermore a percentage of the shared and "contained" fragments among groups revealed by later analyses were also computed.

Network reconstruction: In order to visualise the phylogenetic relationships among the genotypes (in sense of AFLP phenotype, see Chapter 3), a neighbor-net analysis (as implemented in SplitsTree 4.5, Huson & Bryant 2006) based on uncorrected *p*-distances has been carried out. The neighbor-net diagram represents all inferred splits in a phylogenetic network and is composed of parallel edges, rather than a bifurcating phylogenetic tree, which suggests one phylogenetic hypothesis represented by a single optimal tree or a consensus trees. Hence, it is suitable to depict the reticulate relationships.

Genotypic similarity patterns: A principal coordinate analysis (PCoA) using MVSP 3.1 (Kovach Computing Services, UK) was used with the aim to analyse the similarity among the AFLP genotypes. Pairwise Euclidean distance was applied as a distance measure and,

alternatively, a simple match coefficient, as suggested by Kosman & Leonard (2005) for the dominant data.

Genetic structure of populations/taxa: For the purpose of detecting the population structure, a genetic mixture analysis using the program Structure 2.2 (Pritchard et al. 2000) was applied. The structure implements a model-based Bayesian clustering algorithm in order to estimate the likelihood for different number of clusters (K) defined by user. The admixture model with independent allele frequencies and a first row containing 0 for the recessive allele, as implemented in Falush et al. (2007), was used. Firstly, the data were tested with K ranging from 2–10, with 10 replicate runs for each K, and a burn-in period of 2×10^4 and 10×10^4 iterations. In order to find the most probable value of K, the Structure output files were analysed with the R script Structure.sum (Ehrich 2006; available from http://tinyurl.com/StructureSUM) by the means of Evanno's delta K (Evanno et al. 2005). Delta K (ΔK) statistics is based on the rate of change in the log probability of the data between the successive K values. In order to obtain more accurate estimation of the clustering, three replicate runs of the analysis with the same parameters and the most probable value of the K, but with a burn-in period of 2×10^5 and 1×10^6 MCMC repetitions were run. For comparison, we also ran admixture model with correlated frequencies with the same MCMC parameters. Moreover, groups determined by first round of the Structure analyses were analysed separately in order to decide whether they are more subdivided (Pritchard et al. 2007, Rosenberg et al. 2002, Ehrich et al. 2007).

Clonal assignment and genotypic variability of populations: The number of different AFLP genotypes in the *P. alpicola* populations was estimated using the programs Genotype 1.1 and Genodive 1.2 (Meirmans & van Tienderen 2004). The functions allow entering a threshold/error rate, estimated from the observed differences among the replicates (maximum number) or alternatively from histogram of the observed pairwise differences between the genotypes. Consequently, the Genodive 1.2 computes several indices of clonal diversity such as Nei's genotype diversity {$D_g = n/(n-1) \times [1 - \Sigma (\text{genotype frequencies}^2)]$} (Nei 1979) and effective number of genotypes [$N_e = 1/\Sigma (\text{genotype frequencies}^2)$] (Parker 1979).

4.3 Results

4.3.1 DNA ploidy levels

In total, 221 individuals from 28 populations of nine studied taxa have been investigated by means of the flow cytometry (Appendix 4). 141 samples were measured at the IPK, Gatersleben and 80 samples at the University of Vienna. The obtained data have been analysed in an analogical way as shown in the previous chapter. A detailed data analysis is also listed in Scherbatin (2009) and Scherbatin et al. (2009). Coefficients of variation (CVs) for the G_0/G_1 peak of the analysed sample ranged from 1.50 to 5.13 ($\bar{x}$ = 2.70). The ploidy level has not been determined for the two populations of *P. frigida* and one population of *P. crantzii*. However, based on the previously published data (Dobeš & Vitek 2000), both of the taxa may be tetraploid or *P. crantzii* possibly even of higher ploidy. The only size class of the sample/standard ratio found within *P. aurea* referred to one chromosome-counted individual

(2n = 14: Ptl3961). The studied *P. incana* population also revealed one class of sample /standard ratio, to which a tetraploid level (2n = 4x) has been attributed after comparison with chromosome-counted *P. incana* individuals beyond this study (data not shown). Two ploidy levels (2n = 2x and 2n = 6x) have been revealed in *P. argentea* s.l. (see previous chapter). One DNA ploidy level has been determined for the *P. collina*. As no reference chromosomes were counted, the ratios were compared with *P. argentea* measurements (because of the genetic affinity; see later) and a hexaploid level has been assigned. Furthermore, two distinct classes of DNA ploidy levels were identified in *P. thuringiaca*: the nonaploid (2n = 9x) for the majority of the individuals (18) and the hexaploid for the individual Ptl4608 only. The highest cytotypic variability was found in *P. pusilla.* The studied individuals have shown five distinct sample/standard ratio classes referring to the following DNA ploidy levels: tetraploid (26 individuals), pentaploid (11; 2n = 5x), hexpaloid (1), heptaploid (6) and one individual was aneuheptaploid (2n = 48). Each DNA ploidy level, apart from the hexaploid individual (DNA ploidy inferred by interpolation), were inferred from a regression obtained from chromosome-counted individuals (2n = 28: Ptl4048; 2n = 35: Ptl4184; 2n = 48: Ptl4132; 2n = 49: Ptl4133, Ptl4187, Ptl4188). Three ploidy levels have been detected within *P. thuringiaca* × *pusilla*: a tetraploid (11), pentaploid (1) and hexaploid (10). Tetraploid and pentaploid level was assigned on the basis of linear extrapolation of the ratios of the three chromosome-counted individuals (2n= 42: Ptl 4491, Ptl4497, Ptl4500). Finally, *P. alpicola* individuals were pentaploid (4), hexaploid (36) and heptaploid (10) and for each DNA ploidy, a chromosome count was obtained (2n = 35: Ptl4081, Ptl4149; 2n = 42: Ptl4026, Ptl4141, Ptl4881, Ptl4887, Ptl4911, Ptl4913; 2n = 49: Ptl4325, Ptl4328). On the basis of the flow cytometry measurements, two individuals (Ptl4146, Ptl4148; Pop86) could be possibly aneuhexaploid. However, chromosomes have not been counted for these individuals, hence, the measurement mistake cannot be excluded.

The distribution of the cytotypes within populations has shown that there are cytotypically uniform and cytotypically diverse populations. Populations of *P. aurea*, *P. incana* and *P. collina* were homogeneous, exhibiting just one ploidy level. Within the populations of *P. argentea*, *P. pusilla*, *P. thuringiaca* × *pusilla*, *P. thuringiaca*, and *P. alpicola*, typically two ploidy levels were detected. However, the populations Pop189 (*P. thuringiaca*), Pop201 (*P. pusilla*) and Pop203 (*P. alpicola*) were also cytotypically uniform.

4.3.2 CpDNA sequence data and haplotype distribution

The chloroplast DNA sequences were obtained for a total of 91 individuals. The sequences were obtained for at least one sample per population (Appendix 4). For *P. alpicola* populations, 5–9 sequences have been generated. The length of the *trn*H-*psb*A IGS ranged from 439 bp to 487 bp. Fifteen nucleotide substitutions, six indels and two poly-A stretches were detected. The length of the alignment was 510 bp. After manual coding of the indels for the presence and absence and removal of the poly-A stretches, the total length of the alignment was reduced to 441 bp and 19 parsimony informative sites were considered. The alignments are provided on an enclosed CD with the Supplementary Data.

Altogether fourteen *trn*H-*psb*A cpDNA haplotypes were identified within the 88 sequenced individuals. The TCS network analysis revealed three groups of haplotypes (Fig. 16) sepa-

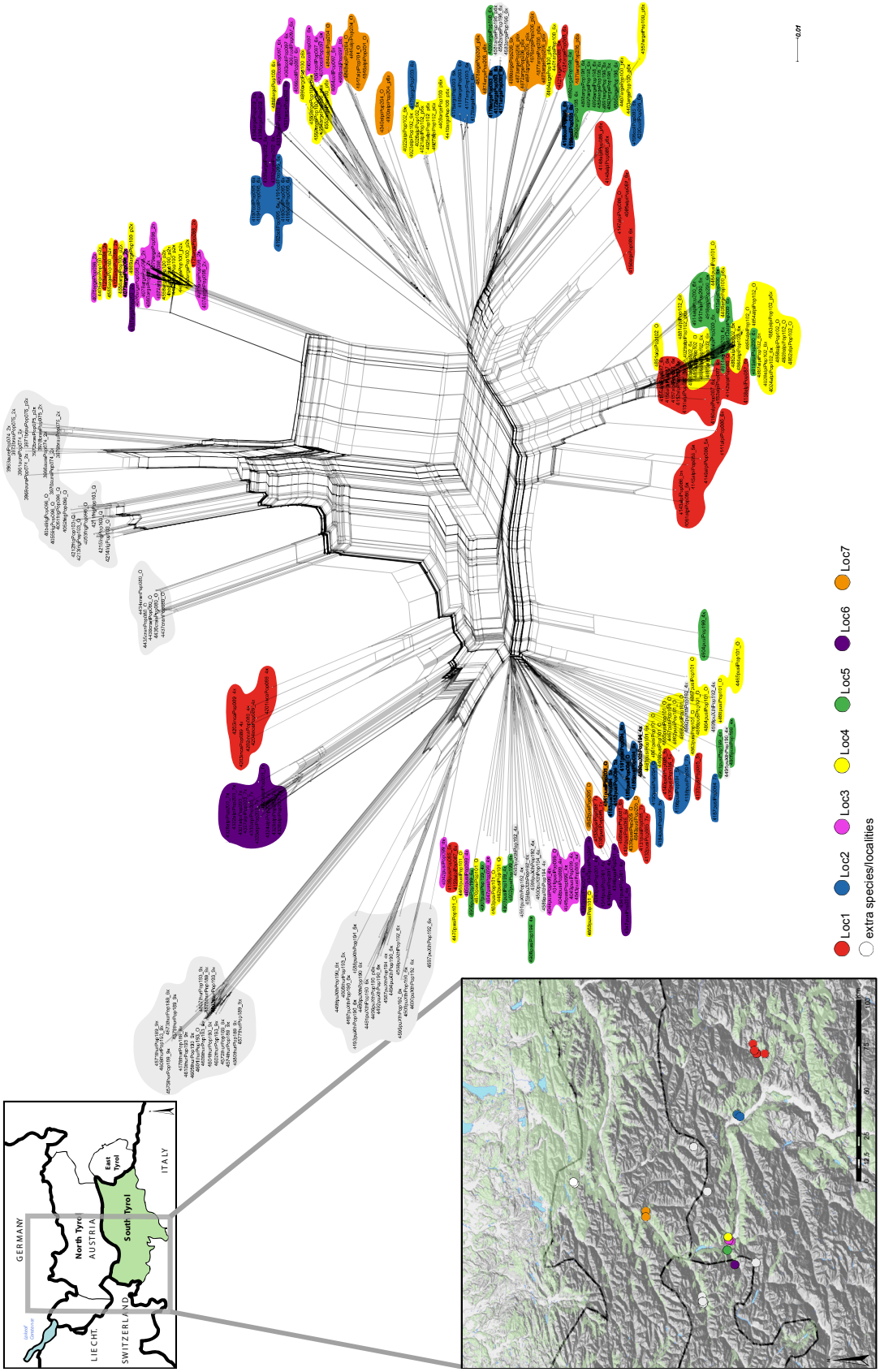

Figure 15. Phylogenetic relationships inferred on the basis of AFLP data using the distance-based neighbor-net method as implemented in SplitsTree 4. Accessions are labeled according to their material number, population number and ploidy. Colour-coding refers to the collection locality of the population as shown on the accompanied map.

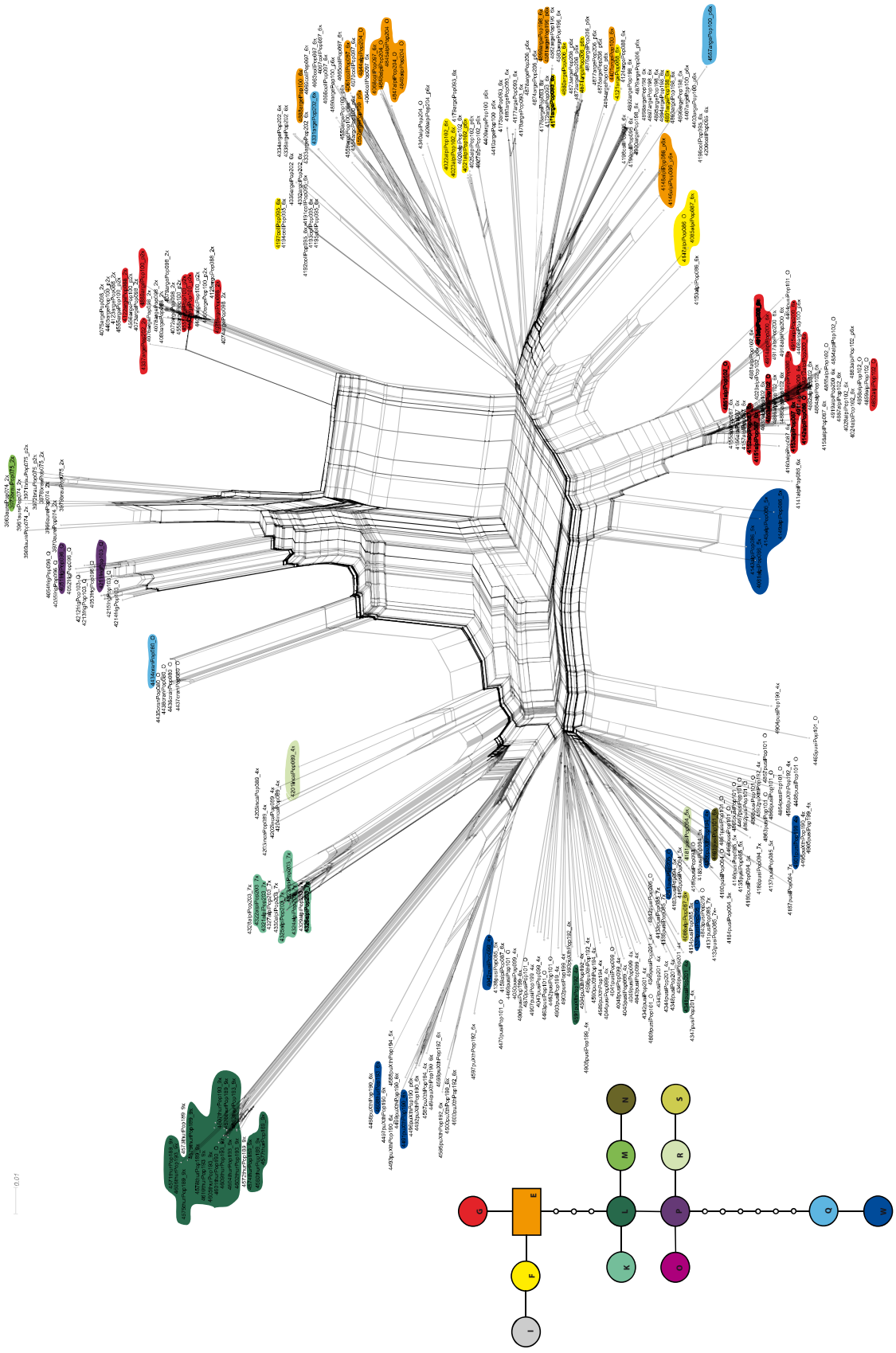

Figure 16. Phylogenetic relationships inferred on the basis of AFLP data using the distance-based neighbor-net method as implemented in SplitsTree 4. Accessions are labeled according to their material number, population number and ploidy. Colour-coding refers to the statistical parsimony network based on the *trn*H-*psb*A cpDNA sequences of the studied individuals, situated next to the neighbor-net. Small empty circles represent haplotypes that are not present, but necessary to link all observed haplotypes to the network. All haplotypes are separated from the nearest haplotype by one nucleotide difference.

-rated from each other by 4–12 mutations. Similarly, as in previous chapter, the first group consisted of the haplotypes E, F, G and I carried just by *P. argentea* s.l. ("*P. argentea* haplotype group"). The haplotype G was exclusive to diploid individuals, the haplotype E comprised both, diploids and hexaploids and the haplotypes F and I were observed in hexaploids only. The most of the individuals determined as *P. alpicola* and *P. collina* share the haplotypes from the *P. argentea* haplotype group (Fig. 16). The second group was composed of haplotypes K, L, M, N, O, P, R, and S. They included *P. thuringiaca*, the taxa from the *P. verna* group (*P. pusilla*, *P. incana*, *P. thuringiaca* × *pusilla*) as well as the additional species *P. aurea*, *P. brauneana* and *P. frigida*. Two of these haplotypes (K and S) are also shared by *P. alpicola*. Haplotypes Q and W constituted the third group. Haplotype Q was observed in hexaploid *P. argentea* s.l. and in *P. crantzii* and haplotype W was found in *P. pusilla*, *P. thuringiaca*, *P. thuringiaca* × *pusilla* and *P. alpicola*. The division of the *P. argentea* haplotype group and the haplotypes of *P. verna* group enables us to follow the maternal line in the studied hybrids. The presence of the haplotype Q in *P. argentea* was unexpected as the *P. argentea* haplotype group seems to be lineage specific (Paule & Dobeš, unpubl.). However, the presence of the haplotype Q in the *P. argentea* could be explained either by hybridisation or by incomplete lineage sorting.

4.3.3 AFLP analyses

In total, 311 accessions representing 34 populations of the above mentioned taxa were investigated, 4–27 individuals, but mostly 10 individuals per population (Appendix 4). Three AFLP primer combinations resulted in 241 clearly scorable fragments sized from 63–537 bp and 97.10% of them were polymorphic across the dataset. The number of fragments in the different taxa ranged from 58 in *P. brauneana* to 204 in *P. pusilla*. The data quality test confirmed a high reliability and repeatability of the data within a range of 98.33–100%. The mean repeat accuracy was 98.76%.

4.3.3.1 Division among different taxonomic groups

The neighbor-net analysis revealed four well separated groups of AFLP genotypes (Fig. 18). Splits of the highest weight separated *P. verna* group, hexaploid *P. argentea* plus the majority of *P. alpicola*, the diploid *P. argentea* and additional taxa *P. aurea*, *P. brauneana*, *P. frigida* and *P. crantzii*. Within the four recovered main groups, several subgroups were detected. As for the cluster of *P. verna* group, *P.incana*, *P. thuringiaca* and *P. pusilla* were well delimited from each other. *Potentilla pusilla* × *thuringiaca* individuals formed either a separate group or were clustered within *P. pusilla*, most probably due to misidentification. *P. alpicola* individuals were clustered either together with hexaploid *P. argentea* or built three more or less well separated clusters. Furthermore, *P.aurea*, *P. frigida* and *P. crantzii* constituted also separate fractions.

Two dimensional PCoA based on the Euclidean distances (Fig. 19) showed a congruent pattern with the neighbor-net analysis. The following seven main groups were recognised: 1. diploid *P. argentea*; 2. hexaploid *P. argentea*; 3. *P. aurea* and *P. frigida*; 4. *P. crantzii*, 5. *P. verna* group (*P. incana* and *P. pusilla* together with *P. pusilla* × *thuringiaca*); 6. *P. thuringiaca* and 7. *P. alpicola*. The first axis explained 23.06% of the total variation and the second one 11.41%.

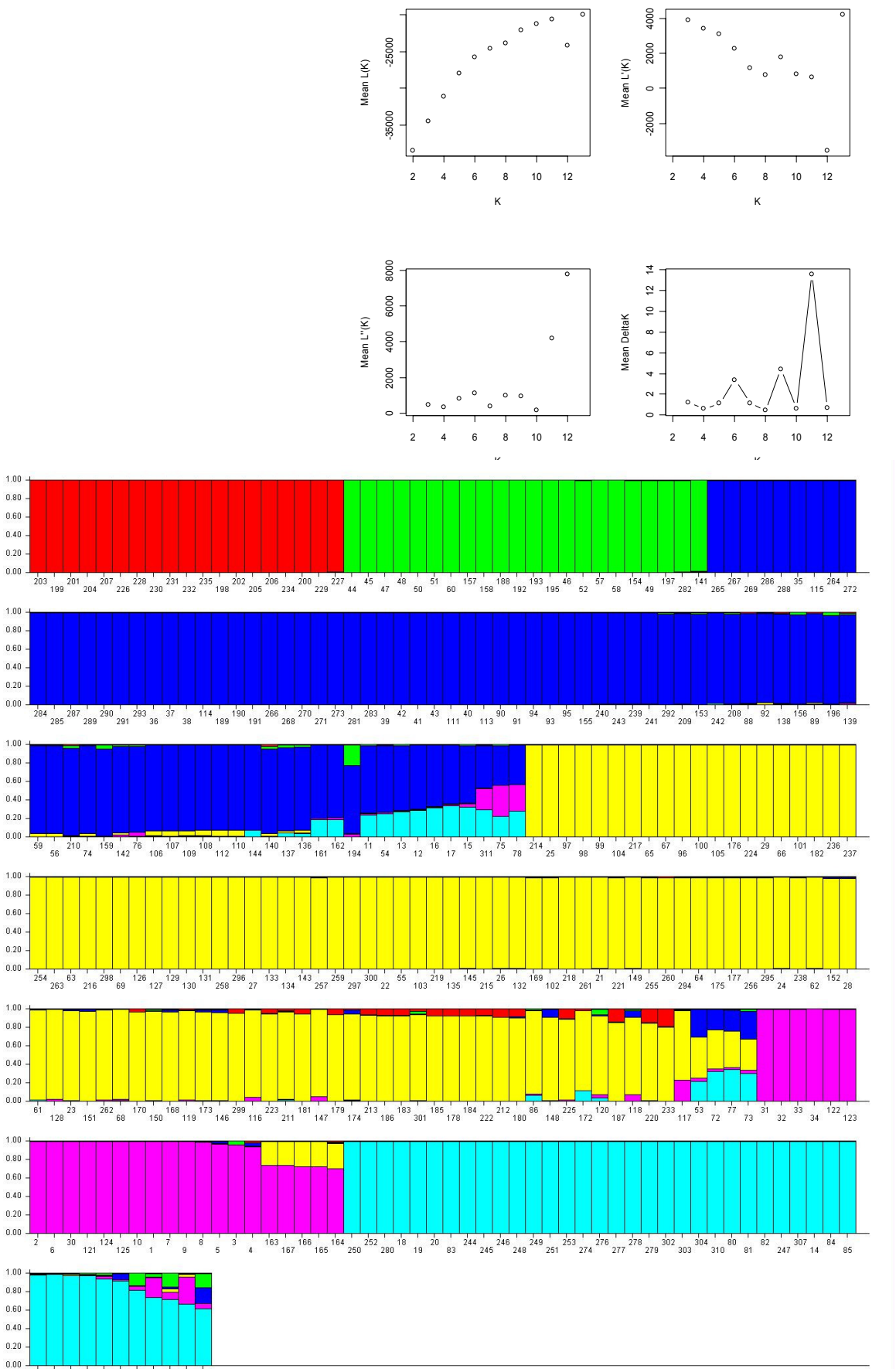

Figure 17. Population structure of the studied taxa examined by genetic admixture analysis using the program Structure with K = 6. The Structure output analysis of the Ks ranging from 2 to 10 by mean of ΔK is shown demonstrating K = 6 with the highest probability of the data. Barcharts represent individuals, showing either pure or mixed genetic identity.

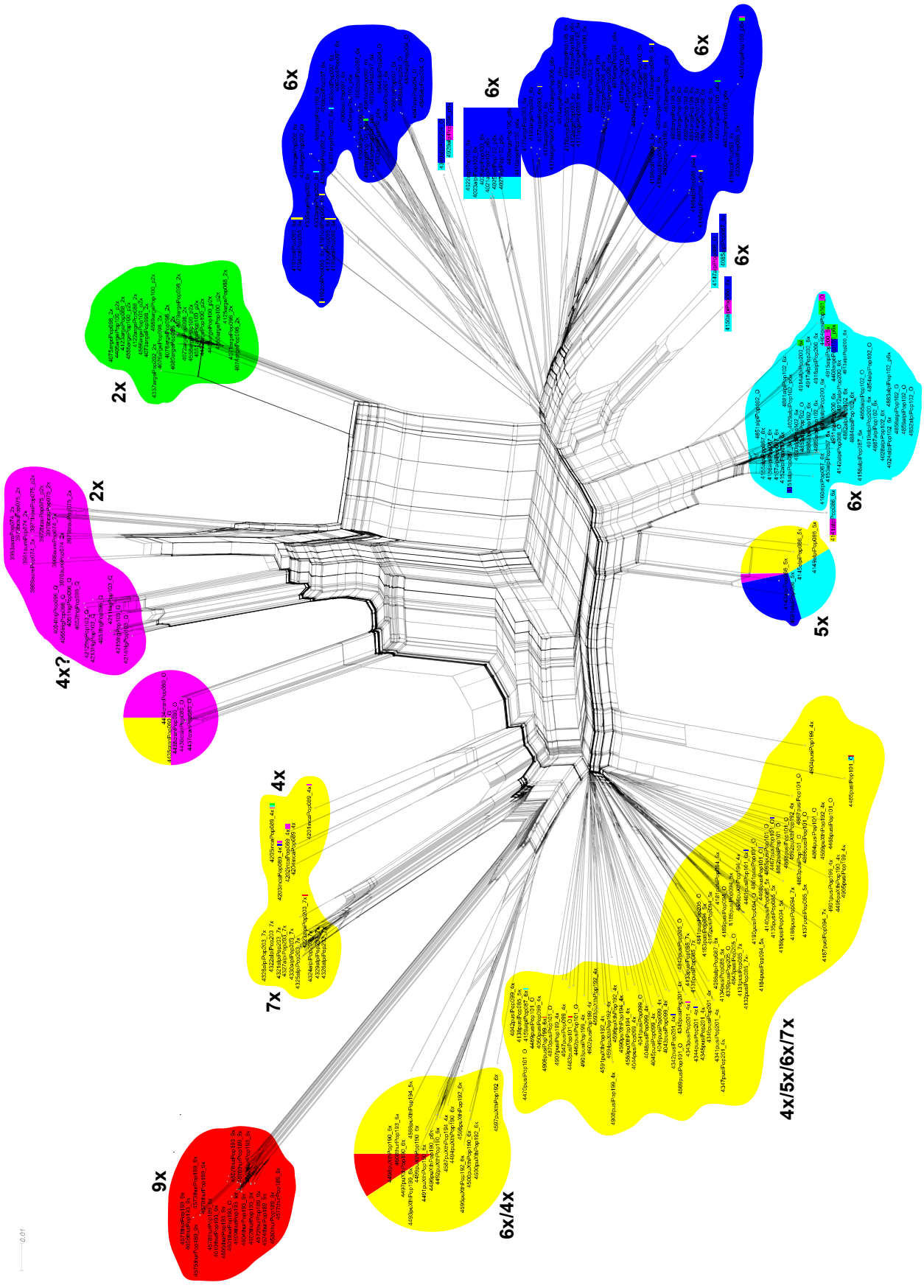

Figure 18. Phylogenetic relationships inferred on the basis of AFLP data using the distance-based neighbor-net method as implemented in SplitsTree 4. Accessions are labeled according to their material number, population number and ploidy. Colour-coding refers to the Structure analysis, showed in Fig. 17.

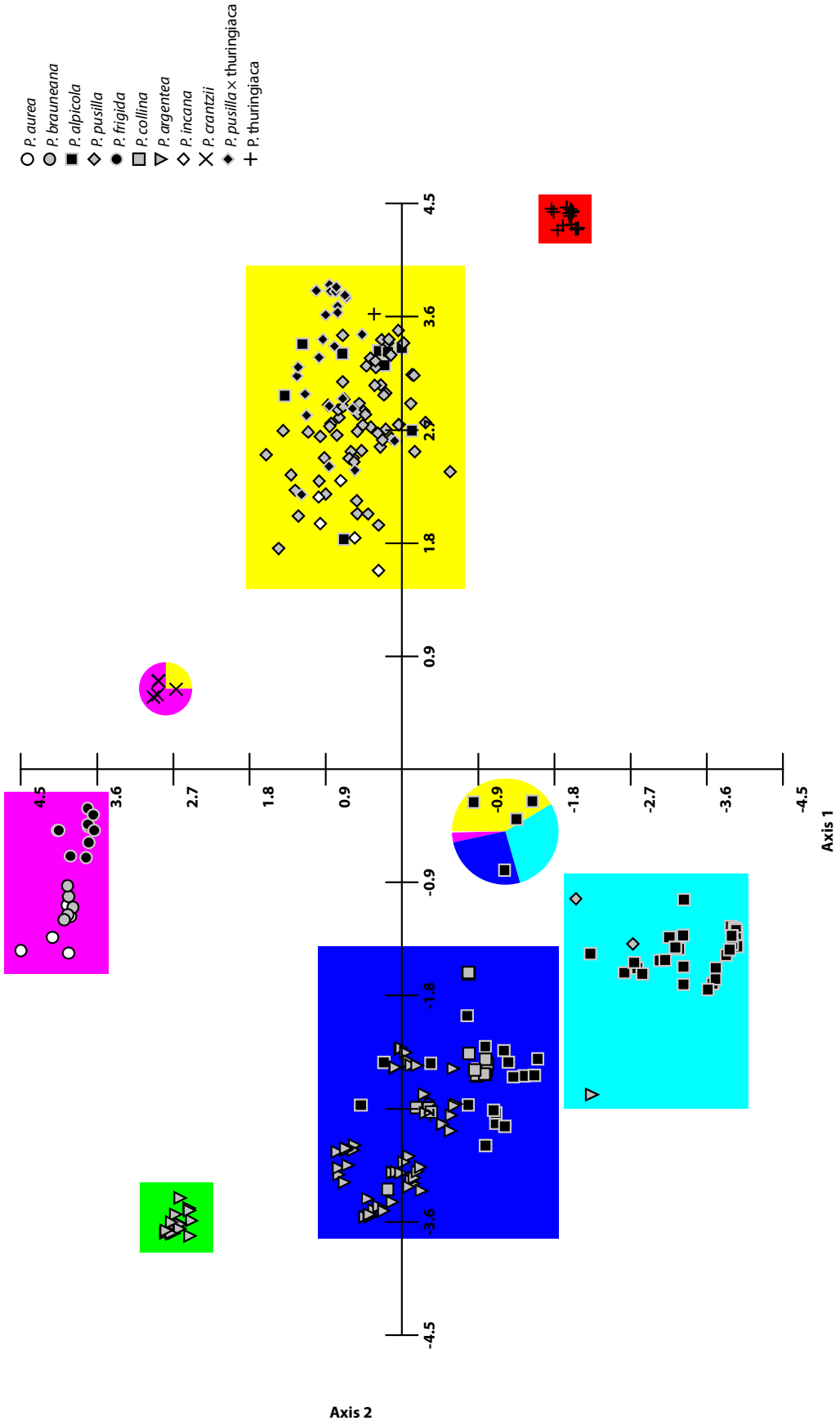

Figure 19. Principal coordinate analysis (PCoA) of the AFLP genotypes. The first two axes explained 23.06% and 11.41% of the total variation. Colour-coding refers to the Structure analysis, showed in Fig. 17.

The grouping of individuals in the Structure analysis also confirmed the inferred taxonomic division (Fig. 17). The Bayesian mixture modelling yielded similar results using both "independent" and "correlated" allele frequencies. The lowest appropriate ΔK value was assigned to the K = 6, which also proved to be biologically the most meaningful one. Six identified genetic units highly corresponded with the following taxonomic groups: 1. diploid *P. argentea*; 2. hexaploid *P.argentea*; 3. *P. thuringiaca*; 4. *P. verna* group (*P. pusilla* and *P. incana*); 5. *P. aurea* and *P. frigida*; and 6. *P. alpicola*. *P. crantzii* and *P. pusilla* × *thuringiaca* revealed an intermediate structure between *P. aurea/frigid*a and *P. verna* (25.4–27.5/69.8–74.1%) or *P. thuringiaca* and *P. verna* (0.0–10.5%/83.1–99.7%), respectively. Several *P. pusilla* and *P. incana* as well as *P. argentea* individuals also revealed a minor admixture from other groups.

Concerning the number of AFLP fragments, a cytotypic division was observed within previously defined clusters. The number of the fragments correlated with the ploidy and two main groups could be recognised: diploids (48-63 fragments) and polyploids (112 -190 fragments). However, the expected polyploid *P. frigida* and *P. crantzii* reveal 66 and 76 fragments only. Furthermore, 1–4 unique fragments have been revealed for *P. thuringiaca*, *P. pusilla*, *P. pusilla* × *thuringiaca* and for both diploid and hexaploid *P. argentea*

4.3.3.2 Identity of Potentilla alpicola *and* P. collina *individuals*

The neighbor-net analysis revealed different positions of *P. alpicola* in the phylogenetic network, suggesting different evolutionary origin for particular populations. The majority of the individuals (44 out of 74), representing three localities (Localities 1, 4 and 5; Pop86, Pop87, Pop101, Pop102 and Pop200), formed a separate cluster in the proximity to the hexaploid *P. argentea* with three possible subclusters. Within this separate group, one *P. pusilla* (Ptl4464) and one *P. argentea* (Ptl4408) were found as well. Furthermore, ten individuals from the population Pop203 (Locality 6) formed another separate cluster between *P. thuringiaca* and *P. incana*. Two individuals (Ptl4159, Ptl4086; Pop87) grouped within *P. pusilla* and 15 individuals (Pop102 and Pop204) were clustered within hexaploid *P. argentea*. Similarly, two studied populations of *P. collina* (Pop97 and Pop95) were also clustered together with the hexaploid *P. argentea*.

PCoA confirmed the partition of the *P. alpicola* individuals. The majority, comprising the same individuals as revealed by the neighbor-net analysis, built an almost exclusive *P. alpicola* cluster. Similarly, the same individuals, including *P. collina*, were grouped together with hexaploid *P. argentea*. The only difference was that the population Pop203 (Locality 6) was clustered within the *P. verna* group.

The Structure analysis further validated the *P. alpicola* specific group (Fig. 18, Fig. 19). However, several individuals, apart from the *P. alpicola* specific fraction, showed also a significant admixture from other groups: hexaploid *P. argentea* (72.4%, Ptl4085), hexaploid *P. argentea*, *P. verna* and *P. aurea/frigida* (21.8–30.0%/33.8–44.5%/2.4-3.4%; Ptl4081, Ptl4143, Ptl4145, Ptl4149); diploid *P. argentea* and *P.aurea/frigida* (1.1–13.2%/1.2–21.6%; Ptl4914, Ptl4915, Ptl4917, Ptl4918); and hexaploid *P. argentea* and *P.aurea/frigida* (42.6–42.8%/28.6–33.6%; Ptl4147, Ptl4150). Population Pop203 showed an affiliation to *P. verna* group, with the individual Ptl4323 containing 2.4% of *P. thuringiaca* fraction. Furthermore, individuals clustered in previous analyses with the hexaploid *P. argentea* showed either a complete or

almost complete hexaploid identity (Ptl4146, Ptl4148, Ptl4844–48; Pop86, Pop204), admixture with *P. alpicola* fraction (7.1–34.1%; Ptl4021–23, Ptl4025–27, Ptl4340) or admixture with both *P. alpicola* and a fraction of *P.aurea/frigida* (29.8%/22.9; Ptl4920). *P. collina* revealed either purely hexaploid *P. argentea* identity (Ptl4196, Ptl4198–200, Pop97) or a minimal admixture with *P. verna* (1.1–1.3%; Ptl4191–95, Ptl4197).

Potentilla alpicola specific cluster, as defined by neighbor-net and Structure analyses (Fig. 18, see also later), revealed altogether 157 fragments. The highest proportion was shared with *P. pusilla* (144 fragments shared, 91.72%) and hexaploid *P. argentea* (141, 89.81%) (Table 7). With other polyploid taxa, it shared from 68.15% (*P. incana*) to 75.80% (*P. thuringiaca*) fragments and with possible polyploid *P. frigida* and *P. crantzii* only 37.58% and 40.13%, respectively. *P. alpicola* also revealed a significant number of the bands present in the diploid taxa. It contained 92.31% (69/78) of the bands present in diploid *P. argentea*, 82.43% (63/74) of *P. aurea* and 82.76% (48/58) of *P. brauneana*.

P. alpicola population Pop203 revealed 118 bands and has been compared separately due to affinity within the *P. verna* group. Consequently, it shared the highest proportion of the bands (116, 98.31%) with *P. pusilla*. The proportion ranged from 77.97% to 88.98% in other polyploids (*P. incana, P. thuringiaca, P. pusilla* × *thuringiaca*) and in *P. frigida* and *P. crantzii* it was 42.37% and 50.85%, respectively. Concerning the diploid taxa, Pop203 covered 61.54–70.69%.

P. collina displayed 149 fragments in total and shared 95.97% with the hexaploid *P. argentea*. As for other polyploids, the proportion ranged from 34.23% (*P. frigida*) to 87.92% (*P. pusilla*). Furthermore, 31.54% (*P. brauneana*) to 45.64% (diploid *P. argentea*) of the fragments contained *P. collina* with diploids in common.

Furthermore, when comparing only potential parental taxa (discussed below) several of them revealed a different number of specific fragments: diploid *P. argentea* – 2 specific fragments, hexaploid *P. argentea* – 9, *P. incana* – 1, *P. pusilla* – 6, *P. thuringiaca* – 5. *P. aurea*, *P. brauneana* and *P. frigida* showed no specific fragments. Out of these specific fragments, *P. alpicola* contained two of *P. pusilla* and two of *P. argentea*. *P. alpicola* Pop203 contained two specific fragments, one from *P. incana* and one from *P. thuringiaca* and *P. collina* contained six, belongig all to hexaploid *P. argnetea*.

Prior to the comparisons of shared bands, individuals found sporadically intermixed in the AFLP-based neighbor-net and PCoA analyses in clusters of differing taxonomy have been excluded from the analysis (*P. argentea*: Ptl4408–4410; *P. pusilla*: Ptl4464; *P. pusilla* × *thuringiaca*: Ptl4495, Ptl4586, Ptl4589–4594, Ptl4596, Ptl4599; *P. alpicola* Ptl4086, Ptl4146, Ptl4148, Ptl4159, Ptl4340, Ptl4844–4848). These individuals were either taxonomically misidentified when collected in the field or showed morphological affinities not reflected by the molecular data (a detailed biometric study is currently under development: Gafaar, Paule & Dobeš, in prep.). Even though, *P. alpicola* individuals grouped by neigbor-net with the hexaploid *P. argentea* (Ptl4021–23, Ptl4025–27) were analysed as *P. alpicola* because the Structure analysis revealed a considerable *P. alpicola* specific identity.

Table 7. Comparisons of the number of the shared AFLP fragments among the studied taxa. Number (Nb) of fragments for each taxon are in brackets behind the taxon name, Nb of shared fragments for the taxon combinations, % of shared fragments (Nb of shared fragments/Nb of fragments in *P. alpicola*, Pop203, *P. collina*, respectively) and the% of the „contained" fragments (Nb of shared fragments/Nb of fragments in presumed parental taxa) are in the field of conjunction.

Nb shared fragments/ % shared fragments/ % „contained" fragments	*P. alpicola* (**157**)	*P. alpicola* Pop203 (**118**)	*P. collina* (**149**)
P. argentea 2x (**78**)	**69**/43.95/88.46	**48**/40.68/61.54	**68**/45.64/87.18
P. aurea (**74**)	**61**/38.85/82.43	**50**/42.37/67.57	**60**/40.27/81.08
P. brauneana (**58**)	**48**/30.57/82.76	**41**/34.75/70.69	**47**/31.54/81.03
P. frigida (**66**)	**59**/37.58/89.39	**50**/42.37/75.76	**51**/34.23/77.27
P. crantzii (**76**)	**63**/40.13/82.89	**60**/50.85/78.95	**59**/39.60/77.63
P. incana (**130**)	**107**/68.15/82.31	**92**/77.97/70.77	**97**/65.10/74.62
P. argentea 6x (**184**)	**141**/89.81/76.63	**101**/85.59/54.89	**143**/95.97/77.72
P. pusilla (**204**)	**144**/91.72/70.59	**116**/98.31/56.86	**131**/87.92/64.22
P. thuringiaca (**167**)	**119**/75.80/71.26	**105**/88.98/62.87	**111**/74.50/66.47
P. pusilla × *thuringiaca* (**153**)	**110**/70.06/71.90	**103**/87.29/67.32	**106**/71.14/69.28

4.3.3.3 Genotypic/Clonal assignment analysis

Similarly as in the previous chapter, we assumed that the same AFLP genotype represents a "clone". If taken strictly, clones with no difference in banding patterns have been recognised in several populations of *P. alpicola* (Pop87, Pop102, Pop200 and Pop203) and *P. collina* (Pop95). However, based on the data repeatability and the pairwise distances between genotypes, a threshold of 4 and 5 (Fig. 20), respectively, have been suggested. Hence, the 5 was chosen as a threshold in the clonal assignment analysis. The analyses have been carried out for each *P. alpicola* and *P. collina* population or alternatively for the groups identified by previous analyses (neighbor-net, Structure): pentaploids (Pop086), hexaploids (specific *P. alpicola* cluster as recognised by Structure), *P. alpicola* placed between the specific cluster and hexaploid *P. argnetea* (as recognised by neighbor-net, Ptl4085, Ptl4147, Ptl4150), Pop102 clustered within the hexaploid *P. argentea* and Pop203 clustered with the *P. verna* group.

The majority of the *P. alpicola* populations were built out of 1 or 2 abundant clones plus other individuals (N_e = 1.38–3.52; Table 8), with an exception of a diverse population Pop86 (D_g = 1.00). Most of the identified clones were population specific, but one clone was shared between populations Pop102 and Pop200. Concerning the groups, clones have been identified within each one (D_g = 0.333–0.587; Table 8), apart from the three pentaploid individuals and three individuals placed by neighbor-net between the specific cluster and hexaploid *P. argnetea*. Both studied *P. collina* populations consisted of 2 and 3 clones (Pop97 and Pop95, respectively) which were more or less equally distributed.

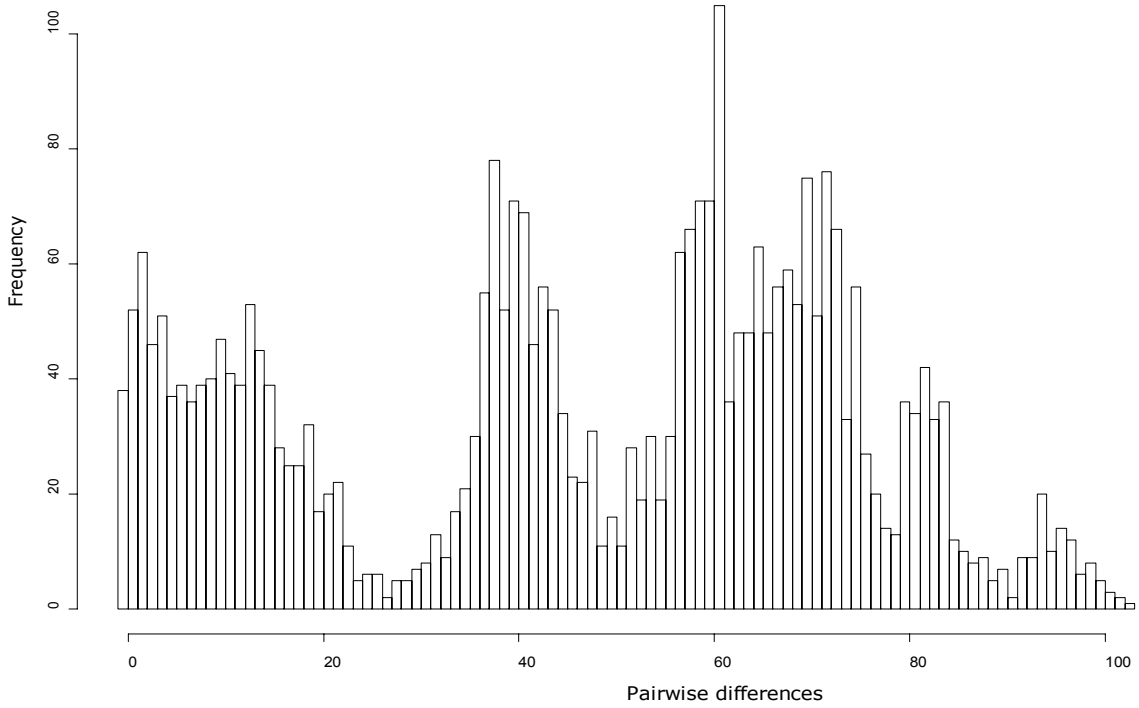

Figure 20. Frequency distribution of pairwise distances between individuals of *P. alpicola* and *P.collina*.

Table 8. Indices of clonal diversity for groups defined by previous analyses as computed by software Genotype and Genodive based on the AFLP data. *Nb*, number of samples; Nb_{gen}, number of genotypes considering a threshold of 5 fragments differences; N_e, effective number of genotypes; D_g, genotypic diversity.

	Nb	Nb_{gen}	N_e	D_g
***P. alpicola* - populations**				
Pop86	8	8	8.00	1.000
Pop87	9	3	1.59	0.417
Pop102	27	3	1.56	0.373
Pop200	9	5	3.52	0.806
Pop203	10	4	1.92	0.533
***P. alpicola* - groups**				
Pop86-5x	4	4	4.00	1.000
Alpi-specific	40	9	2.34	0.587
Alpi/6xArge	3	3	3.00	1.000
Pop102Arge	6	2	1.38	0.333
P. collina				
Pop95	10	3	2.17	0.600
Pop97	9	2	1.80	0.500

4.4 Discussion

4.4.1 Origin of Potentilla collina group in central Alps

Considering our data, we can generally assume the presence of two units: the taxa which evolved most probably gradually (we will refer to them as possible parental taxa) and the hybrid taxa. The first indication for the possible parental taxa may be the ploidy. No recent hybrid origin can be assumed for the diplods: *P. aurea*, *P. argentea* and *P. brauneana*. Secondly, the distinct morphology could be a further indication for the identification of the parents. Hence, the hexaploid *P. argentea*, *P. pusilla*, *P. incana* and *P. thuringiaca* are regarded as possible parents. This is also supported by the genetic data (Structure analysis) as all the presumed possible parental taxa constitute separate, sharply defined groups. On the contrary, several other taxa (Table 9) are polyploid and reveal either intermediate morphology and/or intermediate genetic structure and they are considered hybrids. The taxa of our interest are *P. alpicola* and *P. collina*. Both are polyploid, show intermediate morphology and intermediate or specific genetic structure.

Table 9. Features revelaed by each of the studied taxonomic group. Morphology describes either the presence of distinct morphological characters (distinct), or those present also in other taxa (intermediate). Genetic strucutre describes the assigmnement to a specific genetic cluster by the Sturcture analysis (specific) or to the combination of several specific clusters (intermediate).

	Ploidy	Morphology	Genetic structure
P. aurea	diploid	distinct	specific
P. brauneana	diploid	distinct	specific
P. argentea 2x	diploid	distinct	specific
P. frigida	polyploid	distinct	specific
P. thuringiaca	polyploid	distinct	specific
P. pusilla	polyploid	distinct	specific
P.incana	polyploid	distinct	specific
P. argentea 6x	polyploid	distinct	specific
P. crantzii	**polyploid**	**distinct**	**intermediate**
P. pusilla × *thuringiaca*	**polyploid**	**intermediate**	**intermediate**
P. alpicola	**polyploid**	**intermediate**	**intermediate/specific**
P. collina	**polyploid**	**intermediate**	**specific**

On the basis of the AFLP dataset (neighbor-net, PCoA), the majority of *P. alpicola* samples (44 out of 67 accessions) build a separate cluster between hexaploid *P. argentea* and *P. pusilla*. It shared the majority of the fragments with these taxa (91.72 and 89.81%, respectively) and possesed one unique fragment (0.4%) concerning the whole dataset. Hence, hexaploid/diploid *P. argentea* and *P. pusilla* are considered as parental taxa involved in the hybrid origin of *P. alpicola*. Furthermore, out of the specific fragments identified in the possible parental taxa, *P. alpicola* revealed 2 fragments from both *P. pusilla* and hexaploid *P. argentea* and no fragments from other taxa. Hence, *P. alpicola* combines alleles of its putative parents with an exception of one fragment. However, in the synthetic F1 allohexaploid

between the tetraploid *Triticum turgidum* ssp. *dicocoides* and diploid *Aegilops tauschii* (Dong et al. 2005), 84% of the bands were additive, 17% of both parental origin were absent and, 2.4% appeared de novo. Structure analysis assigned a specific genetic group to the majority of the *P. alpicola* individuals. Even though, as there is only one specific AFLP fragment, we presume that the specific group has been recognised because of the distinct combination of the alleles resulting from the hybridisation. The lack of unique AFLP fragments further suggests that *P. alpicola* has not evolved as a lineage of its own for times sufficient to accumulate specific molecular polymorphisms. This corresponds to its limited geographic distribution. In contrast, both *P. argentea* and *P. pusilla* group are geographically widespread and both possess several unique fragments (9 and 6, respectively), which suggests their ancient origin.

The presence of three different haplotypes coming from three distinct haplotype groups (4–13 mutation steps from each other) indicates that the taxon did not arise through differentiation but rather via other evolutionary processes. This pattern principally agrees with the putative hybrid origin of *P. alpicola*. Due to the fact that chloroplast genome is maternally inherited in the majority of flowering plants (Reboud & Zeyl 1994, Mogensen 1996), the cpDNA bear on directionality of hybridisation. *P. alpicola* specific cluster exhibits three cpDNA haplotypes coming from two haplotype groups. Hence, both *P. pusilla* and *P. argentea* were potentially mother plants in interspecific hybridisations.

Furthermore, clonality, observed within each population of *P. alpicola* (apart from the Pop86) can be attributed to the apomictic mode of reproduction, which was already observed in other taxa of *P. collina* group (Gentscheff & Gustafsson 1940, Håkansson 1946). As apomixis is considered a mechanism through which fertility can be restored in otherwise sterile amphiploids (Dobeš et al. 2007), we consider it another indirect evidence for the hybrid origin.

Three different ploidy levels have been detected in the studied populations of *P. alpicola* – a rare pentalploid, an abundant hexaploid one and a heptaploid one. Four pentaploid individuals from population Pop86 were grouped separately (PCoA, neigbor-net) to the *P. alpicola* specific cluster. The presence of the cpDNA haplotype from the *P. verna* group (W) indicate that a taxon from this group was involved in hybridisation as a mother. This is also supported by the ability of producing gametes by means of reductional division by the tetraploid *P. pusilla* (based on the flow cytometric seed screen, data not shown here; Håkansson 1946). The most probable crossing scenario might have been the fertilisation of the reduced *P. pusilla* egg cell by reduced triploid pollen coming from the hexaploid *P. argentea*, which is also reflected in the Structure analysis. A minor fraction of *P.aurea/frigida* was also revealed, however, we consider it an artefact resulting from fragments generally shared in the whole dataset. It may possibly be a fraction of diploid *P. argentea* missing in our sampling, but due to the tendency of the diploid taxa clustering because of the small amount of AFLP fragments, *P.aurea/frigida* fraction has been recovered. The uncertainity is also reflected in the reticulate structure between *P.aurea/brauneana/frigida* and diploid *P. argentea* in the neighbour-net analysis.

Hexaploid *P. alpicola*, on the other hand, comes from several populations (Pop86, Pop87, Pop102, Pop200 and Pop204) from three different localities (Locality 1, 4, 5 and 7). The majority of them (36) constitute a separate cluster (PCoA, neigbor-net, Structure), three

individuals (Ptl4085, Ptl4147, Ptl4150; Pop86, Pop87) are intermediate between hexaploid P. argentea and the specific cluster (neighbor-net) and 13 individuals (Pop102 and Pop204) are nested with hexaploid *P. argentea*. Haplotype G, found exclusively in diploid *P. argentea* (previous chapter, unpublished data), was found in all individuals from the separate specific cluster. It implies that the diploid *P. argentea*, as proposed also by Gregor et al. (2002), served as a mother in the hybrid genesis. Individuals clustered with the hexaploid *P. argentea* displayed the haplotype F, an implication for the hexaploid *P. argentea* matherhood. However, the Structure analysis attributed them 7.1–34.1% of the specific *P. alpicola* fraction. Hence, we consider these individuals as products of rare events of introgression from *P. alpicola* into otherwise apomictic hexaploid *P. argentea*. Such rare sexual events have been documented by Holm & Ghatnekar (1996).

Heptaploid chromosome number (2n = 7x = 63) strongly suggests a hybrid origin of the population Pop203 from the Locality 6 (Müstair). Permanent odd polyploidy is, according to Grant (1981), one of the mechanisms for stabilising of the breeding behaviour of hybrids. Pop203 possesses the chloroplast DNA haplotype from the *P. verna* haplotype group (K) as well as AFLP-based *P. verna* group identity (Structure). Hence, we conclude that the population Pop203 is most probably a result of introgression of the taxa within the *P. verna* group.

P. collina in the central Alps has several features in common with the hexaploid *P. argentea*. Both studied populations are hexpaloid, shareing the haplotypes with the hexaploid *P. argentea* (F – Pop95, E – Pop97) and based on AFLP, they are nested within the hexaploid *P. argentea*. Out of the fragments found in *P. collina*, 95.97% (143/149) were shared with the hexaploid *P. argentea*. The six remaining fragments were shared with the taxa from *P. verna* group which has also been reflected in the Structure analysis (5.3–6.0%). However, this is also the case in several hexaploid *P. argentea* individuals (e.g. Ptl4121, Ptl4124, Ptl4177). Hence, we do not consider it an indication for a recent hybrid origin, but rather a reflection of historical introgression. These data also suggest that *P. collina* populations in central Alps may actually be a recent derivative of the hexaploid *P. argentea* rather than stabilised hybrids as proposed in the introduction. On the other hand, there are always some difficulties concerning the taxonomic determination of *P. collina* group due to the environmental variability of several diagnostic characters. The determination of our material may also be uncertain, as it is based just on the field determinations. Hence, as in all previous cases, a detailed morphometric study involving also transplanted material cultivated under standardised conditions is currently going on.

4.4.2 Multiple Hybrid Formation

Within our data there is no evidence that *P. alpicola* populations have a common ancestor and have arisen monotopically and spread afterwards. The majority of the studied populations possess different haplotypes and AFLP genotypes and clones were mostly population specific. The only group, which could be considered as hacing the common origin, is the Structure- and neighbour-net-defined *P. alpicola* specific group, composed of individuals from three different localities (Locality 1, 4 and 5; Fig. 15). These populations share the same haplotype G (Fig. 16). However, just individuals from the populations Pop102 and Pop200 also share one AFLP genotype. The distance between the populations is approximately 8 km,

which would involve a certain seed dispersal. Seeds are considered wind-dispersed and travel an average 0.27 m from the parent plant (Dwire et al. 2003; studied in *P. recta*, whose seeds can be regarded as morphologically similar with those of *P. alpicola*). Long-distance dispersal is also known and could be accomplished via animals (in fur, hooves etc.) and seeds may also be carried in melting snow and surface flows (Endress & Parks 2004). Hence, in this case we may assume that the *P. alpicola* arose at, or near the Locality 4, because of the presence of the diploid *P. argentea*, which possesses the same haplotype G, and was consequently distributed further.

Three studied pentaploid individuals could also be regarded as those originated locally, even though none of the sympatrically occurring *P. pusilla* (Locality 1; Pop85) shared the same haplotype (W). Tetraploid *P. pusilla* sharing the haplotype W was found in Locality 3 and Locality 5 (at a distance of approximately 100 km). However, it is possible that we missed it in our sampling from the Locality 1 or the surrounding area. The presence of two haplotypes and no sharing of the clones between the populations also suggest that they arose independently.

4.4.3 Taxonomic comments

Potentilla alpicola De la Soie was first described by Gaspard Abdon de la Soie (1876; Bull. Trav. Soc. Murith. 5: 18). However, the type specimen could not be traced back. Therefore, a neotype from one of the original localities (Mont Clou, Wallis, Switzelrand) has been introduced by Gregor (2008; Kochia 3: 63), originally collected by F.O. Wolf in 1890 and 1896 (Herbarium von Sion). The occurrence of *P. alpicola* in the locality is nowadays very restricted (observations of T. Gregor & B. Wolf 2003 and T. Gregor & F. Dunkel 2006) and in keeping with the IUCN criteria, it is critically endangered in Switzerland (Käsermann & Moser 1999). Hence, we did not include it in our sampling.

We studied morphologically similar forms from the South Tyrol and within this material, several units have been identified. In order to clarify and validate the taxon identity, a genetic comparison is desirable. If the individuals from the type locality fit genetically with one of our groups or even show identity (e.g. due to the apomictic mode of reproduction), we may consider this taxon as broadly distributed, which evolved at least once and was afterwards distributed throughout the Southern Alps. If not, populations from South Tyrol have to be considered as different members of *P. collina* group and its origin should be regarded more like as a matter of parallel morphological formation. Consequently, this should also be reflected in the taxonomic treatment of these populations. Even though, at least some of the individuals from populations Pop87, Pop102 and Pop200 may, according to genetic analysis, carry one name.

4.5 Conclusions

Combined analysis of AFLPs, cpDNA sequences and ploidy levels revealed a hybrid origin of *P. alpicola* and *P. collina* populations in the South Tyrol. On one hand, *P. argentea* and *P. pusilla* have been identified as parental taxa for *P. alpicola*. On the other hand, *P. collina*

populations have been regarded rather as a recent derivatives of the hexaploid *P. argentea*. Several clones have been identified within both *P. alpicola* and *P. collina* suggesting an apomictic mode of reproduction and studied populations seem to evolve multiply, at each locality separately. Finally, taxa from three populations could possibly carry one name. However, they have to be compared with the individuals from the type locality.

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Enumeration of other related publications and meeting contributions

Publications

1. Paule J & Soják J 2009. Taxonomic comments on the genus *Sibbaldiopsis* Rydb. (Rosaceae). Journal of the National Museum (Prague), Natural History Series 178: 15–16.

One of the results of the phylogenetic study (Chapter 1) is that several taxa have to be taxonomically revised. The molecular data supported previous morphological observations concerning the genus *Sibbaldiopsis*. Hence, three species originally published as members of the genus *Potentilla*, later classified as *Sibbaldiopsis*, were transferred to the genus *Sibbladia*. Due to the copyright reasons the article can not be shown in presented thesis. However, it can be viewed here: **http://tinyurl.com/Sibbaldiopsis**

Meeting contributions

1. Systematics 2008, 7–11 April 2008, Göttingen, Germany – poster presentation

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Origin and evolution of agamospermic *Potentilla* species (Rosaceae) in Central Europe

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The aggregates of *Potentilla verna* and *Potentilla argentea* are both agamospermic species complexes of overlapping geographic distribution, which integrate into each other by extensive hybridisation. Hybrid populations are found in a multitude of isolated places throughout Europe and are currently treated as species within the *Potentilla collina* agg. (Collinae sensu Th. Wolf). Chloroplast and nuclear DNA sequence polymorphisms within the putative paternal aggregates but also within other related taxa were analysed in order to identify those parental evolutionary lineages which gave rise to *P. collina* forms. In a first phase of the project, we have focused on the origin of *Potentilla alpicola* De la Soie, a member of the Collinae, in southern Tyrol and adjacent areas. In this study on hybrid speciation we assessed the question whether *P. alpicola* populations are of single or multiple origin and whether they have been established by a uniform evolutionary process or if a more complex scenario of evolution applies. For that purpose AFLP, morphological and cytological data were combined. Finally, we address the question which criteria should be fulfilled in order to give *P. collina* hybrid forms the rank of a species.

2. Xth Symposium of the International Organization of Plant Biosystematists, 2–4 July 2008 Vysoké Tatry, Slovakia – poster presentation.

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Origin and evolution of agamospermic *Potentilla* species (*Rosaceae*) in Central Europe

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The aggregates of *Potentilla verna* and *P. argentea* are both agamospermic species complexes of overlapping geographic distribution, which integrate into each other by extensive hybridisation. Hybrid populations are found in a multitude of isolated places throughout Europe and are currently treated as species within the *Potentilla collina* agg (*Collinae* sensu Th. Wolf). Chloroplast DNA sequence polymorphisms within the putative paternal aggregates but also within other related taxa were analysed in order to identify those parental evolutionary lineages which gave rise to *P. collina* forms. In a first phase of the project, we have focused on the origin of *Potentilla alpicola* De la Soie, a member of the *Collinae*, in Southern Tyrol and adjacent areas. In this study on hybrid speciation we assessed the question whether *P. alpicola* populations are of single or multiple origin and whether they have been established by a uniform evolutionary process or if a more complex scenario of evolution applies. For that purpose cpDNA, morphological and cytological data were combined.

3. 21st Scientific Congress of the Austrian Pharmaceutical Society, 16–18 April 2009, Vienna, Austria – poster presentation.

Scherbantin A, Paule J & Dobeš C 2009. Karyogeography and hybrid origin of European *Potentilla* species (Rosaceae). Scientia Pharmaceutica 77: 264. doi:10.3797/scipharm.oephg.21.PO-65

Appendices

Appendix 1: List of 167 investigated accessions, subgeneric classification according to Wolf (1908), and sequenced chloroplast DNA markers – Samples are ordered alphabetically by taxon names and within taxa by material numbers assigned by the authors. Haplotypes are defined by the combined plastid regions sequenced. Genebank accession numbers are provided for all DNA sequences. Full documentation of collection history and the depository of vouchers are provided in supplemetary material - http://tinyurl.com/SupplMat.

Taxon	material number	grex sensu Wolf 1908	haplo-type	*trn*L^{uaa}-*trn*F^{gaa} IGS	*trn*S^{uga}-*ycf*9 IGS	*trn*C^{gca}-*ycf*6 IGS
Agrimonia eupatoria L.	Ptl5433		AA	GQ384718	GQ384550	-
Alchemilla glaucescens Wallr. emend. Sam.	Ptl5438		AB	GQ384721	GQ384553	GQ384878
Alchemilla sp.	Ptl3549		AC	GQ384656	GQ384489	GQ384824
Argentina anserina Rydb.	Ptl3714	Anserinae	AD	GQ384662	GQ384495	GQ384830
Chamaerhodos altaica (Laxm.) Bunge	Ptl5444		AE	GQ384724	GQ384556	-
Ch. erecta var. parviflora C.L. Hitchc.	Ptl5443		AF	GQ384723	GQ384555	-
Dasiphora fruticosa (L.) Rydb.	Ptl5364	Fruticosae	AG	GQ384680	GQ384512	GQ384848
Dryas octopetala L. (outgroup)	Ptl5437		AH	GQ384720	GQ384552	-
Drymocallis glabrata Rydb.	Ptl6005	Rupestres	AI	GQ384728	GQ384560	GQ384882
D. glandulosa (Lindl.) Rydb.	Ptl2710	Rupestres	AJ	GQ384629	GQ384462	GQ384797
D. lactea var. austiniae (Jeps.) Ertter	Ptl6007		AK	GQ384729	GQ384561	GQ384883
D. rupestris (L.) Soják	Ptl2920	Rupestres	AL	GQ384650	GQ384483	GQ384818
Farinopsis salesoviana (Steph.) Chrtek & Soják	Ptl5368	Palustres	AM	GQ384683	GQ384515	GQ384851
Fragaria vesca L.	Ptl5439		AN	GQ384722	GQ384554	GQ384879
Geum rossi Ser. (outgroup)	Ptl5416		AO	GQ384713	GQ384545	-
Horkelia daucifolia var. caruifolia (Rydb.) Ertter & Reveal	Ptl6008		AP	GQ384730	GQ384562	GQ384884
H. fusca var. filicoides (Crum) M. Peck	Ptl6013		AQ	GQ384734	GQ384566	GQ384888
var. parviflora (Nutt.) D.D. Keck	Ptl6012		AR	GQ384733	GQ384565	GQ384887
H. hendersonii Howell	Ptl6014		AS	GQ384735	GQ384567	GQ384889
H. howellii Rydb.	Ptl6009		AT	GQ384731	GQ384563	GQ384885
H. marinensis (Elmer) Crum	Ptl6017		AU	GQ384736	GQ384568	GQ384890
H. tridentata Torr. var. tridentata	Ptl6011		AV	GQ384732	GQ384564	GQ384886
Horkeliella purpurascens Rydb.	Ptl6021		AW	GQ384737	GQ384569	GQ384891
Ivesia aperta (Howell) Munz var. aperta	Ptl6035		AX	GQ384744	GQ384576	GQ384898
I. arizonica var. saxosa (Brandegee) Ertter	Ptl6028		AY	GQ384740	GQ384572	GQ384894
I. gordonii (Hook) T. & G.	Ptl5450		AZ	GQ384725	GQ384557	-
I. paniculata T.W. Nelson & J.P. Nelson	Ptl6031		BA	GQ384741	GQ384573	GQ384895
I. santolinoides A. Gray	Ptl6034		BB	GQ384743	GQ384575	GQ384897
I. saxosa (Greene) Ertter	Ptl6032		BC	GQ384742	GQ384574	GQ384896
I. utahensis S. Watson	Ptl6025		BD	GQ384738	GQ384570	GQ384892
I. webberi A. Gray	Ptl6027		BE	GQ384739	GQ384571	GQ384893
Piletophyllum micropetalum (D. Don) Soják	Ptl6407		BF	GQ384783	GQ384615	GQ384935
Potentilla agrimonioides M. Bieb.	Ptl5379	Multifidae	BG	GQ384690	GQ384522	GQ384857
P. alba L.	Ptl3719	Fragariastra	BH	GQ384664	-	GQ384832
P. albiflora L.O. Williams	Ptl6078	Subviscosae	BI	GQ384777	GQ384609	GQ384930
P. alpicola De la Soie	Ptl4021	Argenteae	BJ	GQ384669	GQ384501	GQ384837
P. apennina Ten.	Ptl6422	Speciosae	BK	GQ384790	GQ384621	GQ384941
P. argaea Boiss.	Ptl6405	Persicae	BL	GQ384782	GQ384614	-
P. argentea L.	Ptl3254	Argenteae	BM	GQ384652	GQ384485	GQ384820
	Ptl3882	Argenteae	BN	GQ384665	GQ384497	GQ384833
	Ptl5167	Argenteae	BJ	GQ384675	GQ384507	GQ384843
var. pseudocalabra T. Wolf	Ptl5187	Argenteae	BO	GQ384676	GQ384508	GQ384844
× P. inclinata Vill.	Ptl2764	Rectae	BP	GQ384636	GQ384469	GQ384804
P. arizonica Greene	Ptl6062	Multijugae	BQ	GQ384764	GQ384596	GQ384917
P. articulata Franch.	Ptl6417	Biflorae	BR	GQ384787	GQ384618	-
P. aurea L.	Ptl3961	Aureae	BS	GQ384667	GQ384499	GQ384835
	Ptl4225	Aureae	BS	GQ384673	GQ384505	GQ384841
P. biennis Greene	Ptl6074	Rivales	BT	GQ384775	GQ384607	GQ384928
P. biflora Willd. ex Schlecht.	Ptl5367	Biflorae	BU	GQ384682	GQ384514	GQ384850
P. bimundorum Soják	Ptl6069	Pensylvanicae	BV	GQ384770	GQ384602	GQ384923
P. bipinnatifida Dougl.	Ptl6070	Multifidae	BW	GQ384771	GQ384603	GQ384924
P. brauneana L.	Ptl3973	Aureae	BX	GQ384668	GQ384500	GQ384836
P. brevifolia Nutt. ex Torr. & A. Gray	Ptl6041	Ranunculoides	BY	GQ384748	GQ384580	GQ384901
P. bruceae Rydb.	Ptl6044	Graciles	BZ	GQ384751	GQ384583	GQ384904
P. caulescens L.	Ptl5361	Caulescentes	CA	GQ384678	GQ384510	GQ384846
P. clusiana Jacq.	Ptl2786	Caulescentes	CB	GQ384640	GQ384473	GQ384808
P. collina agg.	Ptl5008	Collinae	BP	GQ384674	GQ384506	GQ384842
P. concinna Richards.	Ptl6042	Niveae	CC	GQ384749	GQ384581	GQ384902
P. crantzii (Crantz) Beck ex Fritsch	Ptl4111	Aureae	CD	GQ384671	GQ384503	GQ384839
P. crassinervia Viv.	Ptl6434	Crassinerviae	CE	GQ384792	GQ384623	GQ384943
P. crinita A. Gray	Ptl6059	Multifidae	CF	GQ384761	GQ384593	GQ384914
P. cristae Ferlatte & Strother	Ptl6038	Aureae	CG	GQ384745	GQ384577	GQ384899
P. curviseta var. colletiana (Aitch. & Hemsl.) Soják	Ptl6408	Curvisetae	CH	GQ384784	GQ384616	GQ384936
P. desertorum Bunge var. arnavatensis T. Wolf	Ptl2893	Rivales	CI	GQ384643	GQ384476	GQ384811
P. dickinsii var. dickinsii (Franchet & Savatier) Koidzumi	Ptl5372	Eriocarpae	CJ	GQ384685	GQ384517	-
P. drummondii Lehm.	Ptl6047	Multijugae	BZ	GQ384753	GQ384585	GQ384906
P. effusa Dougl. ex Lehm. var. effusa	Ptl6060	Multifidae	CK	GQ384762	GQ384594	GQ384915

var. effusa	Ptl5383	Multifidae	CL	GQ384694	GQ384526	-
P. elatior Willd. ex Schlecht.	Ptl6438	Eriocarpae	CM	-	GQ384624	GQ384944
P. erecta (L.) Raeusch.	Ptl2894	Tormentillae	CN	GQ384644	GQ384477	GQ384812
P. eriocarpa Wallr. ex Lehm.	Ptl5371	Eriocarpae	CO	GQ384684	GQ384516	GQ384852
P. flabellifolia Hook.	Ptl6039	Ranunculoides	BY	GQ384746	GQ384578	GQ384900
P. fragaroides var. major Maxim.	Ptl5427	Fragarioides	CQ	GQ384715	GQ384547	GQ384875
P. freyniana Bornm.	Ptl5428	Fragarioides	CQ	GQ384716	GQ384548	GQ384876
P. frigida Vill.	Ptl4051	Aureae	CS	GQ384670	GQ384502	GQ384838
P. gelida C.A. Mey.	Ptl6404	Aureae	CT	GQ384781	GQ384613	GQ384934
P. glaucophylla Lehm.	Ptl5417	Multijugae	BO	GQ384714	GQ384546	GQ384874
var. glaucophylla Lehm.	Ptl6046	Multijugae	BO	GQ384752	GQ384584	GQ384905
P. gracilis Dougl.	Ptl2763	Multifidae	CU	GQ384635	GQ384468	GQ384803
var. flabelliformis (Lehm.) Nutt. ex Torrey & A. Gray	Ptl6052	Multifidae	CU	GQ384755	GQ384587	GQ384908
var. glabrata (Lehm.) C.L. Hitchc.	Ptl6053	Multifidae	CV	GQ384756	GQ384588	GQ384909
var. owyheensis Ertter & Mansfield	Ptl6051	Multifidae	CU	GQ384754	GQ384586	GQ384907
P. grandiflora L.	Ptl3717	Grandiflorae	CS	GQ384663	GQ384496	GQ384831
P. griffithii Hook. f.	Ptl2922	Tanacetifoliae	CW	GQ384651	GQ384484	GQ384819
P. heptaphylla L.	Ptl3921	Aureae	CX	GQ384666	GQ384498	GQ384834
P. hippiana Lehm.	Ptl6061	Multifidae	CY	GQ384763	GQ384595	GQ384916
P. hirta L.	Ptl2752	Rectae	CZ	GQ384634	GQ384467	GQ384802
ssp. laete (Focke) Prodán	Ptl3511	Rectae	DA	GQ384655	GQ384488	GQ384823
P. hookeriana Lehm.	Ptl6040	Niveae	DB	GQ384747	GQ384579	-
P. incana Gaertn., Mey. & Scherb.	Ptl3647	Aureae	DC	GQ384659	GQ384492	GQ384827
	Ptl3683	Aureae	DD	GQ384660	GQ384493	GQ384828
P. inclinata Vill.	Ptl3607	Collinae	BJ	GQ384658	GQ384491	GQ384826
P. jennissejensis Polozhij et W. Smirn.	Ptl5377	Multifidae	DE	GQ384688	GQ384520	GQ384855
P. jepsonii Ertter	Ptl6071	Pensylvanicae	BW	GQ384772	GQ384604	GQ384925
P. leuconota D. Don.	Ptl2706	Anserinae	DF	GQ384628	GQ384461	GQ384796
P. leucophylla Pall.	Ptl5392	Niveae	DG	GQ384700	GQ384532	GQ384864
P. litoralis Rydb.	Ptl2765	Multifidae	BW	GQ384637	GQ384470	GQ384805
P. longifolia Willd. ex Schlecht.	Ptl5400	Tanacetifoliae	DH	GQ384706	GQ384538	GQ384868
P. maura T. Wolf	Ptl5413	Persicae	DI	GQ384711	GQ384543	GQ384873
P. millefolia Rydb.	Ptl6063	Multijugae	BZ	GQ384765	GQ384597	GQ384918
P. modesta Rydb.	Ptl5376	Rubricaules	DJ	GQ384687	GQ384519	GQ384854
	Ptl6072	Rubricaules	BW	GQ384773	GQ384605	GQ384926
P. morefieldii Ertter	Ptl6043	Concinnae	CC	GQ384750	GQ384582	GQ384903
P. multicaulis Bunge	Ptl5380	Multifidae	DK	GQ384691	GQ384523	GQ384858
P. nepalensis Hook.	Ptl5387	Haematochroae	DL	GQ384697	GQ384529	GQ384861
P. neumanniana Rchb.	Ptl3501	Aureae	DM	GQ384654	GQ384487	GQ384822
	Ptl3684	Aureae	DC	GQ384661	GQ384494	GQ384829
P. nevadensis Boiss.	Ptl2908	Persicae	DN	GQ384647	GQ384480	GQ384815
P. newberryi A. Gray	Ptl5410	Rivales	DO	GQ384710	GQ384542	GQ384872
P. nipponica T. Wolf	Ptl5381	Multifidae	DP	GQ384692	GQ384524	-
P. nitida L.	Ptl5362	Nitidae	DQ	GQ384679	GQ384511	GQ384847
P. nivea L.	Ptl5250	Niveae	DR	GQ384677	GQ384509	GQ384845
P. norvegica L.	Ptl2714	Rivales	DS	GQ384630	GQ384463	GQ384798
P. ornithopoda Tausch	Ptl2715	Multifidae	DT	GQ384631	GQ384464	GQ384799
P. ovina var. decurrens (Wats.) S.L. Welsh & B.C. Johnst.	Ptl6065	Multijugae	BO	GQ384767	GQ384599	GQ384920
var. ovina	Ptl6064	Multijugae	BO	GQ384766	GQ384598	GQ384919
P. pedata Nestl.	Ptl2911	Rectae	DU	GQ384648	GQ384481	GQ384816
P. pensylvanica L.	Ptl5378	Multifidae	DV	GQ384689	GQ384521	GQ384856
	Ptl6073	Multifidae	DW	GQ384774	GQ384606	GQ384927
P. pimpinelloides L.	Ptl6414	Tanacetifoliae	DX	GQ384786	-	GQ384938
P. plattensis Nutt.	Ptl6066	Multijugae	DY	GQ384768	GQ384600	GQ384921
P. pulcherrima Lehm.	Ptl5384	Multifidae	DZ	GQ384695	GQ384527	-
	Ptl5386	Multifidae	DB	GQ384696	GQ384528	GQ384860
	Ptl6054	Multifidae	DB	GQ384757	GQ384589	GQ384910
	Ptl5382	Multifidae	DB	GQ384693	GQ384525	GQ384859
P. pusilla Host	Ptl4135	Aureae	DC	GQ384672	GQ384504	GQ384840
P. pyrenaica Ram. ex DC.	Ptl2919	Grandiflorae	EA	GQ384649	GQ384482	GQ384817
P. recta L.	Ptl2899	Rectae	BP	GQ384645	GQ384478	GQ384813
	Ptl5405	Rectae	BP	GQ384707	GQ384539	GQ384869
	Ptl5406	Rectae	BP	GQ384708	GQ384540	GQ384870
P. reptans L.	Ptl2766	Tormentillae	EB	GQ384638	GQ384471	GQ384806
P. rhyolitica Ertter var. rhyolitica	Ptl6079	Subviscosae	EC	GQ384778	GQ384610	GQ384931
P. rivalis var. millegrana (Engelm.) S. Watson	Ptl5408	Rivales	ED	GQ384709	GQ384541	GQ384871
P. saundersiana Royle.	Ptl5393	Niveae	EE	GQ384701	GQ384533	-
P. simplex Michx.	Ptl5429	Tormentillae	EF	GQ384717	GQ384549	GQ384877
P. sp. nov. aff. bimundorum Soják	Ptl5375	Multifidae	BW	GQ384686	GQ384518	GQ384853
P. stipularis L.	Ptl5414	Multijugae	EG	GQ384712	GQ384544	-
P. subjuga Rydb. var. subjuga	Ptl6076	Multifidae	EH	GQ384776	GQ384608	GQ384929
P. subviscosa Greene var. subviscosa	Ptl6080	Ranunculoides	EI	GQ384779	GQ384611	GQ384932
P. supina L. ssp. supina	Ptl2787	Rivales	EJ	GQ384641	GQ384474	GQ384809
	Ptl2887	Rivales	EJ	GQ384642	GQ384475	GQ384810

P. tanacetifolia Willd.	Ptl2784	Tanacetifoliae	EK	GQ384639	GQ384472	GQ384807
	Ptl5399	Tanacetifoliae	EL	GQ384705	GQ384537	GQ384867
P. thurberi A. Gray	Ptl5389	Haematochroae	EM	GQ384698	GQ384530	GQ384862
sanguinea (Rydb.) Kearney & Peebles	Ptl6057	Haematochroae	EM	GQ384759	GQ384591	GQ384912
var. thurberi	Ptl6058	Haematochroae	EM	GQ384760	GQ384592	GQ384913
P. thuringiaca Bernh.	Ptl2720	Chrysanthae	BP	GQ384632	GQ384465	GQ384800
P. townsendii Rydb.	Ptl6055	Ranunculoides	CY	GQ384758	GQ384590	GQ384911
P. umbrosa Stev.	Ptl2721	Grandiflorae	EN	GQ384633	GQ384466	GQ384801
P. vahliana Lehm.	Ptl5395	Niveae	EO	GQ384703	GQ384535	-
P. velutina Lehm.	Ptl3286	Aureae	EP	GQ384653	GQ384486	GQ384821
	Ptl3580	Aureae	BO	GQ384657	GQ384490	GQ384825
P. versicolor Rydb.	Ptl6067	Multijugae	BZ	GQ384769	GQ384601	GQ384922
P. villosula Jurtz.	Ptl5394	Niveae	EQ	GQ384702	GQ384534	GQ384865
P. virgata Lehm.	Ptl5397	Argenteae	ER	GQ384704	GQ384536	GQ384866
P. vulcanicola Juz.	Ptl5391	Niveae	ES	GQ384699	GQ384531	GQ384863
P. wheeleri var. paupercula Jeps.	Ptl6081	Ranunculoides	ET	GQ384780	GQ384612	GQ384933
Rosa agrestis Gaertn. (outgroup)	Ptl5435		EU	GQ384719	GQ384551	-
R. canina L. (outgroup)	Ptl6419		EV	GQ384788	GQ384619	GQ384939
Schistophyllidium bifurcum (L.) Ikonn.	Ptl5366	Bifurcae	EN	GQ384681	GQ384513	GQ384849
	Ptl6428	Bifurcae	EX	GQ384791	GQ384622	GQ384942
Sibbaldia cuneifolia (Bertol.) Paule & Soják	Ptl2906	Tridentatae	EY	GQ384646	GQ384479	GQ384814
S. parviflora Willd.	Ptl6459		EZ	GQ384794	GQ384626	GQ384945
S. procumbens L.	Ptl6420		FA	GQ384789	GQ384620	GQ384940
S. tetrandra Bunge	Ptl6409		FB	GQ384785	GQ384617	GQ384937
S. tridentata (Aiton) Paule & Soják	Ptl2679	Tridentatae	CP	GQ384627	GQ384460	GQ384795
Tylosperma lignosa (Willd.) Botsch.	Ptl6446	Xylorrhizae	CR	GQ384793	GQ384625	-

Appendix 2. List of investigated accessions and conducted experiments from the Chapter 3. **AFLP** – amplified fragment length polymorphism (X) , **FCM** – flow cytometry (CC – chromosoems counted, p – presumed ploidy level based on the genetic data), **Haplo** – sequenced chloroplast *trn*H-*psb*A marker with attributed ha-plotype. Samples are ordered by material material numbers, country codes follows ISO 3166-1 Alpha-3; IS – *Index Seminum* material, collector abbreviations: CD – C. Dobeš, TG – T. Gregor, JP – J. Paule, F&S – B. Frajman & P. Schönswetter, RS – Roswitha Schmickl, HH – H. Hunderi, MK – M. Koch.

Mat Nr	PopID	Taxon	Locality/Collector	AFLP	FCM	Haplo
Ptl3066	Pop048	*P. argentea*	SWE; SE Sundre/TG	X	6x	E
Ptl3068	Pop048	*P. argentea*	SWE; SE Sundre/TG	X		
Ptl3069	Pop048	*P. argentea*	SWE; SE Sundre/TG	X	6x	
Ptl3070	Pop048	*P. argentea*	SWE; SE Sundre/TG	X	6x	
Ptl3071	Pop048	*P. argentea*	SWE; SE Sundre/TG	X	6x	
Ptl3072	Pop048	*P. argentea*	SWE; SE Sundre/TG		6x	
Ptl3073	Pop048	*P. argentea*	SWE; SE Sundre/TG		6x	
Ptl3074	Pop048	*P. argentea*	SWE; SE Sundre/TG		6x	
Ptl3075	Pop048	*P. argentea*	SWE; SE Sundre/TG		6x	
Ptl3126	Pop054	*P. argentea*	SWE; NE Klintehamn/TG		6x	
Ptl3127	Pop054	*P. argentea*	SWE; NE Klintehamn/TG		6x	
Ptl3128	Pop054	*P. argentea*	SWE; NE Klintehamn/TG		6x	
Ptl3129	Pop054	*P. argentea*	SWE; NE Klintehamn/TG		6x	
Ptl3130	Pop054	*P. argentea*	SWE; NE Klintehamn/TG		2x	
Ptl3131	Pop054	*P. argentea*	SWE; NE Klintehamn/TG		6x	
Ptl3132	Pop054	*P. argentea*	SWE; NE Klintehamn/TG		2x	
Ptl3133	Pop054	*P. argentea*	SWE; NE Klintehamn/TG		6x	
Ptl3134	Pop054	*P. argentea*	SWE; NE Klintehamn/TG		6x	
Ptl3156	Pop057	*P. argentea*	SWE; SE Borgholm/TG	X	2x	E
Ptl3157	Pop057	*P. argentea*	SWE; SE Borgholm/TG	X	2x	
Ptl3158	Pop057	*P. argentea*	SWE; SE Borgholm/TG	X	6x	E
Ptl3159	Pop057	*P. argentea*	SWE; SE Borgholm/TG	X	2x	
Ptl3160	Pop057	*P. argentea*	SWE; SE Borgholm/TG	X	6x	
Ptl3161	Pop057	*P. argentea*	SWE; SE Borgholm/TG		6x	
Ptl3162	Pop057	*P. argentea*	SWE; SE Borgholm/TG		6x	
Ptl3163	Pop057	*P. argentea*	SWE; SE Borgholm/TG		6x	
Ptl3164	Pop057	*P. argentea*	SWE; SE Borgholm/TG		6x	
Ptl3165	Pop057	*P. argentea*	SWE; SE Borgholm/TG		6x	
Ptl3221	Pop063	*P. argentea*	SWE; SE Mörbylanga/TG		6x	
Ptl3222	Pop063	*P. argentea*	SWE; SE Mörbylanga/TG		6x	
Ptl3223	Pop063	*P. argentea*	SWE; SE Mörbylanga/TG		6x	
Ptl3224	Pop063	*P. argentea*	SWE; SE Mörbylanga/TG		6x	
Ptl3226	Pop063	*P. argentea*	SWE; SE Mörbylanga/TG		6x	
Ptl3227	Pop063	*P. argentea*	SWE; SE Mörbylanga/TG		6x	
Ptl3228	Pop063	*P. argentea*	SWE; SE Mörbylanga/TG		6x	
Ptl3229	Pop063	*P. argentea*	SWE; SE Mörbylanga/TG		6x	
Ptl3245	Pop066	*P. argentea*	SWE; SE Mörbylanga/TG		6x	
Ptl3249	Pop066	*P. argentea*	SWE; SE Mörbylanga/TG		6x	
Ptl3251	Pop066	*P. argentea*	SWE; SE Mörbylanga/TG		6x	
Ptl3253	Pop069	*P. argentea*	DEU; Haschbach am Remigius-Berg/TG	X	6x	
Ptl3254	Pop069	*P. argentea*	DEU; Haschbach am Remigius-Berg/TG	X	6x	F

Ptl3255	Pop069	*P. argentea*	DEU; Haschbach am Remigius-Berg/TG	X	6x	
Ptl3256	Pop069	*P. argentea*	DEU; Haschbach am Remigius-Berg/TG	X	6x	
Ptl3257	Pop069	*P. argentea*	DEU; Haschbach am Remigius-Berg/TG	X	6x	
Ptl3258	Pop069	*P. argentea*	DEU; Haschbach am Remigius-Berg/TG		6x	
Ptl3259	Pop069	*P. argentea*	DEU; Haschbach am Remigius-Berg/TG		6x	
Ptl3260	Pop069	*P. argentea*	DEU; Haschbach am Remigius-Berg/TG		6x	
Ptl3261	Pop069	*P. argentea*	DEU; Haschbach am Remigius-Berg/TG		6x	
Ptl3262	Pop069	*P. argentea*	DEU; Haschbach am Remigius-Berg/TG		6x	
Ptl3300	Pop225	*P. argentea*	EST; Kaarma/TG		6x	
Ptl3305	Pop225	*P. argentea*	EST; Kaarma/TG		6x	
Ptl3307	Pop225	*P. argentea*	EST; Kaarma/TG		6x	
Ptl3308	Pop225	*P. argentea*	EST; Kaarma/TG		6x	
Ptl3323	Pop227	*P. argentea*	EST; Salme/TG		6x	
Ptl3324	Pop227	*P. argentea*	EST; Salme/TG		6x	
Ptl3325	Pop227	*P. argentea*	EST; Salme/TG		6x	
Ptl3329	Pop227	*P. argentea*	EST; Salme/TG		6x	
Ptl3350	Pop230	*P. argentea*	EST; Kihelkonna/TG	X	6x	E
Ptl3351	Pop230	*P. argentea*	EST; Kihelkonna/TG	X	6x	
Ptl3352	Pop230	*P. argentea*	EST; Kihelkonna/TG	X	6x	
Ptl3353	Pop230	*P. argentea*	EST; Kihelkonna/TG	X	6x	
Ptl3354	Pop230	*P. argentea*	EST; Kihelkonna/TG	X	6x	
Ptl3355	Pop230	*P. argentea*	EST; Kihelkonna/TG		6x	
Ptl3356	Pop230	*P. argentea*	EST; Kihelkonna/TG		6x	
Ptl3357	Pop230	*P. argentea*	EST; Kihelkonna/TG		6x	
Ptl3358	Pop230	*P. argentea*	EST; Kihelkonna/TG		6x	
Ptl3359	Pop230	*P. argentea*	EST; Kihelkonna/TG		6x	
Ptl3391	Pop234	*P. argentea*	EST; W Hanila NE Virtsu/TG	X		H
Ptl3392	Pop234	*P. argentea*	EST; W Hanila NE Virtsu/TG	X		E
Ptl3393	Pop234	*P. argentea*	EST; W Hanila NE Virtsu/TG	X	6x	
Ptl3394	Pop234	*P. argentea*	EST; W Hanila NE Virtsu/TG	X	6x	
Ptl3395	Pop234	*P. argentea*	EST; W Hanila NE Virtsu/TG	X	6x	
Ptl3396	Pop234	*P. argentea*	EST; W Hanila NE Virtsu/TG		6x	
Ptl3397	Pop234	*P. argentea*	EST; W Hanila NE Virtsu/TG		6x	
Ptl3398	Pop234	*P. argentea*	EST; W Hanila NE Virtsu/TG		6x	
Ptl3601	Pop017	*P. argentea*	FRA; Alpes Maritimes, Pont du Coq/CD		6x	
Ptl3602	Pop017	*P. argentea*	FRA; Alpes Maritimes, Pont du Coq/CD		6x	
Ptl3603	Pop017	*P. argentea*	FRA; Alpes Maritimes, Pont du Coq/CD		6x	
Ptl3604	Pop017	*P. argentea*	FRA; Alpes Maritimes, Pont du Coq/CD		6x	
Ptl3605	Pop017	*P. argentea*	FRA; Alpes Maritimes, Pont du Coq/CD		6x	
Ptl3606	Pop017	*P. argentea*	FRA; Alpes Maritimes, Pont du Coq/CD		6x	
Ptl3547	Pop019	*P. argentea*	Frau; Alpes Maritimes, Vallon de Minière/CD	X	6x	E
Ptl3548	Pop019	*P. argentea*	Frau; Alpes Maritimes, Vallon de Minière/CD	X		
Ptl3641	Pop019	*P. argentea*	Frau; Alpes Maritimes, Vallon de Minière/CD	X		
Ptl3642	Pop019	*P. argentea*	Frau; Alpes Maritimes, Vallon de Minière/CD	X		
Ptl3643	Pop019	*P. argentea*	Frau; Alpes Maritimes, Vallon de Minière/CD	X		
Ptl3644	Pop019	*P. argentea*	Frau; Alpes Maritimes, Vallon de Minière/CD	X		
Ptl3645	Pop019	*P. argentea*	Frau; Alpes Maritimes, Vallon de Minière/CD	X		
Ptl3656	Pop020	*P. argentea*	AUT; Dunkelsteiner Wald/RS		6x	
Ptl3657	Pop020	*P. argentea*	AUT; Dunkelsteiner Wald/RS		6x	
Ptl3658	Pop020	*P. argentea*	AUT; Dunkelsteiner Wald/RS		6x	

Ptl3659	Pop020	*P. argentea*	AUT; Dunkelsteiner Wald/RS		6x	
Ptl3660	Pop020	*P. argentea*	AUT; Dunkelsteiner Wald/RS		6x	
Ptl3661	Pop020	*P. argentea*	AUT; Dunkelsteiner Wald/RS		6x	
Ptl3662	Pop020	*P. argentea*	AUT; Dunkelsteiner Wald/RS		6x	
Ptl3663	Pop020	*P. argentea*	AUT; Dunkelsteiner Wald/RS		6x	
Ptl3664	Pop020	*P. argentea*	AUT; Dunkelsteiner Wald/RS		6x	
Ptl3665	Pop020	*P. argentea*	AUT; Dunkelsteiner Wald/RS		6x	
Ptl3678	Pop023	*P. argentea*	AUT; Wachau, E Dürnstein/RS		6x	
Ptl3679	Pop023	*P. argentea*	AUT; Wachau, E Dürnstein/RS		6x	
Ptl3865	Pop037	*P. argentea*	DEU; Maulbronn/CD		6x	
Ptl3869		*P. argentea*	DEU; Emsland, Papenburg/CD		2x	
Ptl3871	Pop034	*P. argentea*	DEU; Idar-Oberstein/CD		6x	
Ptl3872	Pop034	*P. argentea*	DEU; Idar-Oberstein/CD		6x	
Ptl3873	Pop034	*P. argentea*	DEU; Idar-Oberstein/CD	X	6x	F
Ptl3875	Pop034	*P. argentea*	DEU; Idar-Oberstein/CD	X	6x	
Ptl3876	Pop034	*P. argentea*	DEU; Idar-Oberstein/CD	X	6x	
Ptl3877	Pop034	*P. argentea*	DEU; Idar-Oberstein/CD	X	6x	
Ptl3878	Pop034	*P. argentea*	DEU; Idar-Oberstein/CD	X	6x	
Ptl3880	Pop034	*P. argentea*	DEU; Idar-Oberstein/CD		6x	
Ptl3881	Pop042	*P. argentea*	AUT; Wachau, Dürnstein/CD	X	6x	
Ptl3882	Pop042	*P. argentea*	AUT; Wachau, Dürnstein/CD	X		F
Ptl3883	Pop042	*P. argentea*	AUT; Wachau, Dürnstein/CD	xX	6x	
Ptl3884	Pop042	*P. argentea*	AUT; Wachau, Dürnstein/CD	X	6x	
Ptl3885	Pop042	*P. argentea*	AUT; Wachau, Dürnstein/CD	X	6x	
Ptl3886	Pop042	*P. argentea*	AUT; Wachau, Dürnstein/CD		6x	
Ptl3887	Pop042	*P. argentea*	AUT; Wachau, Dürnstein/CD		6x	
Ptl3888	Pop042	*P. argentea*	AUT; Wachau, Dürnstein/CD		6x	
Ptl3889	Pop042	*P. argentea*	AUT; Wachau, Dürnstein/CD		6x	
Ptl3901	Pop044	*P. argentea*	AUT; Melk, Wachberg/CD		6x	
Ptl3902	Pop044	*P. argentea*	AUT; Melk, Wachberg/CD		6x	
Ptl3903	Pop044	*P. argentea*	AUT; Melk, Wachberg/CD		6x	
Ptl3904	Pop044	*P. argentea*	AUT; Melk, Wachberg/CD		6x	
Ptl3991	Pop072	*P. argentea*	DEU; Emsland, 4 km NW Meppen/CD	X	2x	E
Ptl3992	Pop072	*P. argentea*	DEU; Emsland, 4 km NW Meppen/CD	X	2x	
Ptl3993	Pop072	*P. argentea*	DEU; Emsland, 4 km NW Meppen/CD	X	2x	
Ptl3994	Pop072	*P. argentea*	DEU; Emsland, 4 km NW Meppen/CD	X	2x	
Ptl3995	Pop072	*P. argentea*	DEU; Emsland, 4 km NW Meppen/CD	X	2x	
Ptl3996	Pop072	*P. argentea*	DEU; Emsland, 4 km NW Meppen/CD		2x	
Ptl3998	Pop072	*P. argentea*	DEU; Emsland, 4 km NW Meppen/CD		2x	
Ptl3999	Pop072	*P. argentea*	DEU; Emsland, 4 km NW Meppen/CD		2x	
Ptl4071	Pop098	*P. argentea*	ITA; Vinschgau, Glurns/CD	X	2x	G
Ptl4072	Pop098	*P. argentea*	ITA; Vinschgau, Glurns/CD	X	2x	
Ptl4073	Pop098	*P. argentea*	ITA; Vinschgau, Glurns/CD	X	2x	
Ptl4074	Pop098	*P. argentea*	ITA; Vinschgau, Glurns/CD	X	2x	
Ptl4075	Pop098	*P. argentea*	ITA; Vinschgau, Glurns/CD	X	2x	
Ptl4076	Pop098	*P. argentea*	ITA; Vinschgau, Glurns/CD	X	2x	
Ptl4078	Pop098	*P. argentea*	ITA; Vinschgau, Glurns/CD	X	2x	
Ptl4079	Pop098	*P. argentea*	ITA; Vinschgau, Glurns/CD	X	2x	
Ptl4080	Pop098	*P. argentea*	ITA; Vinschgau, Glurns/CD	X	2x	

Ptl4088		*P. argentea*	ITA; Mölten, Schlaneid/CD		2x	
Ptl4121	Pop088	*P. argentea*	ITA; SW Seis am Schlern/CD	X	6x	F
Ptl4122	Pop088	*P. argentea*	ITA; SW Seis am Schlern/CD	X	2x	G
Ptl4123	Pop088	*P. argentea*	ITA; SW Seis am Schlern/CD	X	2x	
Ptl4124	Pop088	*P. argentea*	ITA; SW Seis am Schlern/CD	X	6x	
Ptl4125	Pop088	*P. argentea*	ITA; SW Seis am Schlern/CD	X	2x	
Ptl4126	Pop090	*P. argentea*	ITA; Villnöss, Nafen/CD		6x	
Ptl4163	Pop091	*P. argentea*	ITA; Feldthurns/CD	X	2x	G
Ptl4166	Pop091	*P. argentea*	ITA; Feldthurns/CD	X	2x	
Ptl4167	Pop091	*P. argentea*	ITA; Feldthurns/CD	X		
Ptl4168	Pop091	*P. argentea*	ITA; Feldthurns/CD	X	6x	F
Ptl4169	Pop091	*P. argentea*	ITA; Feldthurns/CD	X		
Ptl4170	Pop091	*P. argentea*	ITA; Feldthurns/CD	X	6x	
Ptl4171	Pop093	*P. argentea*	ITA; Burgstall/CD	X	6x	F
Ptl4172	Pop093	*P. argentea*	ITA; Burgstall/CD	X	6x	
Ptl4175	Pop093	*P. argentea*	ITA; Burgstall/CD	X	6x	
Ptl4176	Pop093	*P. argentea*	ITA; Burgstall/CD	X	6x	
Ptl4177	Pop093	*P. argentea*	ITA; Burgstall/CD	X	6x	
Ptl4178	Pop093	*P. argentea*	ITA; Burgstall/CD	X	6x	
Ptl4179	Pop093	*P. argentea*	ITA; Burgstall/CD	X	6x	
Ptl4180	Pop093	*P. argentea*	ITA; Burgstall/CD	X	6x	
Ptl4301	Pop248	*P. argentea*	FRA; Durance Valley/CD	X	6x	E
Ptl4302	Pop248	*P. argentea*	FRA; Durance Valley/CD	X	6x	
Ptl4303	Pop248	*P. argentea*	FRA; Durance Valley/CD	X	6x	
Ptl4304	Pop248	*P. argentea*	FRA; Durance Valley/CD	X	6x	
Ptl4305	Pop248	*P. argentea*	FRA; Durance Valley/CD	X	6x	
Ptl4306	Pop248	*P. argentea*	FRA; Durance Valley/CD		6x	
Ptl4307	Pop248	*P. argentea*	FRA; Durance Valley/CD		6x	
Ptl4308	Pop248	*P. argentea*	FRA; Durance Valley/CD		6x	
Ptl4309	Pop248	*P. argentea*	FRA; Durance Valley/CD		6x	
Ptl4310	Pop248	*P. argentea*	FRA; Durance Valley/CD		6x	
Ptl4320	Pop239	*P. argentea*	FRA; Grandeyrolles/CD		2x	
Ptl4331	Pop202	*P. argentea*	CHE; Münstertal, NW Müstair/CD	X	6x	Q
Ptl4332	Pop202	*P. argentea*	CHE; Münstertal, NW Müstair/CD	X	6x	
Ptl4333	Pop202	*P. argentea*	CHE; Münstertal, NW Müstair/CD	X	6x	
Ptl4334	Pop202	*P. argentea*	CHE; Münstertal, NW Müstair/CD	X	6x	
Ptl4335	Pop202	*P. argentea*	CHE; Münstertal, NW Müstair/CD	X	6x	
Ptl4336	Pop202	*P. argentea*	CHE; Münstertal, NW Müstair/CD	xX	2x	
Ptl4337	Pop202	*P. argentea*	CHE; Münstertal, NW Müstair/CD	X	2x	G
Ptl4338	Pop202	*P. argentea*	CHE; Münstertal, NW Müstair/CD	X	6x	
Ptl4401	Pop100	*P. argentea*	ITA; Schluderns – Kalvarienberg/CD	X	6x	E
Ptl4402	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		G
Ptl4403	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		
Ptl4404	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		
Ptl4405	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		
Ptl4406	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		
Ptl4407	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		
Ptl4408	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		
Ptl4409	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		

Ptl4410	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		
Ptl4551	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		G
Ptl4552	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		E
Ptl4553	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		
Ptl4554	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		
Ptl4555	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		
Ptl4556	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		
Ptl4557	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		I
Ptl4558	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		
Ptl4559	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		
Ptl4560	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		
Ptl4888	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X	6x	E
Ptl4889	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		G
Ptl4890	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg/CD	X		
Ptl4441	Pop077	*P. argentea*	AUT; Ötztal, Zwieselstein/CD	X		G
Ptl4442	Pop077	*P. argentea*	AUT; Ötztal, Zwieselstein/CD	X		
Ptl4443	Pop077	*P. argentea*	AUT; Ötztal, Zwieselstein/CD	X		
Ptl4444	Pop077	*P. argentea*	AUT; Ötztal, Zwieselstein/CD	X		
Ptl4445	Pop077	*P. argentea*	AUT; Ötztal, Zwieselstein/CD	X		
Ptl4446	Pop077	*P. argentea*	AUT; Ötztal, Zwieselstein/CD	X		
Ptl4447	Pop077	*P. argentea*	AUT; Ötztal, Zwieselstein/CD	X		
Ptl4581	Pop196	*P. argentea*	CHE; Engadin, above Scoul/CD	X		E
Ptl4582	Pop196	*P. argentea*	CHE; Engadin, above Scoul/CD	X	6x	
Ptl4583	Pop196	*P. argentea*	CHE; Engadin, above Scoul/CD	X	6x	
Ptl4871	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns/CD	X		F
Ptl4872	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns/CD	X		
Ptl4873	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns/CD	X		
Ptl4874	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns/CD	X		
Ptl4875	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns/CD	X		
Ptl4876	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns/CD	X		
Ptl4877	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns/CD	X		
Ptl4878	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns/CD	X		
Ptl4879	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns/CD	X		
Ptl4880	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns/CD	X	6x	F
Ptl4891	Pop198	*P. argentea*	ITA; Laatsch/CD	X	6x	F
Ptl4892	Pop198	*P. argentea*	ITA; Laatsch/CD	X	6x	
Ptl4893	Pop198	*P. argentea*	ITA; Laatsch/CD	X	6x	
Ptl4894	Pop198	*P. argentea*	ITA; Laatsch/CD	X	6x	
Ptl4895	Pop198	*P. argentea*	ITA; Laatsch/CD	X	6x	
Ptl4896	Pop198	*P. argentea*	ITA; Laatsch/CD	X	6x	
Ptl4897	Pop198	*P. argentea*	ITA; Laatsch/CD	X	6x	
Ptl4898	Pop198	*P. argentea*	ITA; Laatsch/CD	X	6x	
Ptl4899	Pop198	*P. argentea*	ITA; Laatsch/CD	X	6x	E
Ptl4900	Pop198	*P. argentea*	ITA; Laatsch/CD	X	6x	
Ptl5167	Pop119	*P. argentea*	DEU; Bonn, Tannenbuscher Düne/IS			E
Ptl5180	Pop121	*P. argentea*	DEU; Radenbeck bei Wittingen/IS	X		F
Ptl5181	Pop121	*P. argentea*	DEU; Radenbeck bei Wittingen/IS	X		
Ptl5182	Pop121	*P. argentea*	DEU; Radenbeck bei Wittingen/IS	X		
Ptl5183	Pop121	*P. argentea*	DEU; Radenbeck bei Wittingen/IS	X		
Ptl5184	Pop121	*P. argentea*	DEU; Radenbeck bei Wittingen/IS	X		

Ptl5187	Pop122	*P. argentea* var. *pseudocalabra*	BGR; Prov. Smolyan/IS			Z
Ptl5205	Pop127	*P. argentea*	NOR; Lillehammer, Smestadmoen/HH	X		E
Ptl5206	Pop127	*P. argentea*	NOR; Lillehammer, Smestadmoen/HH	X		
Ptl5207	Pop127	*P. argentea*	NOR; Lillehammer, Smestadmoen/HH	X		
Ptl5208	Pop127	*P. argentea*	NOR; Lillehammer, Smestadmoen/HH	X		
Ptl5233	Pop131	*P. argentea*	AUT; Millstatt, Großdombra/IS			Q
Ptl5242	Pop133	*P. argentea*	CHE; Fieschertal/IS			E
Ptl5273	Pop138	*P. argentea*	ITA; Monte Venda, Teolo/IS	X		F
Ptl5274	Pop138	*P. argentea*	ITA; Monte Venda, Teolo/IS	X		
Ptl5275	Pop138	*P. argentea*	ITA; Monte Venda, Teolo/IS	X		
Ptl5276	Pop138	*P. argentea*	ITA; Monte Venda, Teolo/IS	X		
Ptl5277	Pop138	*P. argentea*	ITA; Monte Venda, Teolo/IS	X		
Ptl5310	Pop144	*P. argentea*	DEU; Berlin-Spandau, Staaken/IS	X		F
Ptl5311	Pop144	*P. argentea*	DEU; Berlin-Spandau, Staaken/IS	X		
Ptl5312	Pop144	*P. argentea*	DEU; Berlin-Spandau, Staaken/IS	X		
Ptl5313	Pop144	*P. argentea*	DEU; Berlin-Spandau, Staaken/IS	X		
Ptl5314	Pop144	*P. argentea*	DEU; Berlin-Spandau, Staaken/IS	X		
Ptl5321	Pop117	*P. argentea*	SVK; Budička/JP	X		F
Ptl5322	Pop117	*P. argentea*	SVK; Budička/JP	X	6x	
Ptl5323	Pop117	*P. argentea*	SVK; Budička/JP	X		
Ptl5324	Pop117	*P. argentea*	SVK; Budička/JP	X	6x	
Ptl5325	Pop117	*P. argentea*	SVK; Budička/JP	X	6x	
Ptl5326	Pop117	*P. argentea*	SVK; Budička/JP		6x	
Ptl5327	Pop117	*P. argentea*	SVK; Budička/JP		6x	
Ptl5328	Pop117	*P. argentea*	SVK; Budička/JP		6x	
Ptl5329	Pop117	*P. argentea*	SVK; Budička/JP		6x	
Ptl5330	Pop117	*P. argentea*	SVK; Budička/JP		6x	
Ptl5606	Pop163	*P. argentea*	SRB; Bor, from village Luka towards Stol/F&S	X		F
Ptl5607	Pop163	*P. argentea*	SRB; Bor, from village Luka towards Stol/F&S	X		
Ptl5608	Pop163	*P. argentea*	SRB; Bor, from village Luka towards Stol/F&S	X		
Ptl5609	Pop163	*P. argentea*	SRB; Bor, from village Luka towards Stol/F&S	X		E
Ptl5610	Pop163	*P. argentea*	SRB; Bor, from village Luka towards Stol/F&S	X		
Ptl5625	Pop165	*P. argentea*	BGR; Mount Lozen SE Sofia/F&S	X		Q
Ptl5626	Pop165	*P. argentea*	BGR; Mount Lozen SE Sofia/F&S	X		
Ptl5627	Pop165	*P. argentea*	BGR; Mount Lozen SE Sofia/F&S	X		
Ptl5628	Pop165	*P. argentea*	BGR; Mount Lozen SE Sofia/F&S	X		
Ptl5629	Pop165	*P. argentea*	BGR; Mount Lozen SE Sofia/F&S	X		
Ptl5635	Pop166	*P. argentea* var. *pseudocalabra*	BGR; Pirin, near the mountain hut Vihren/F&S	X		Y
Ptl5636	Pop166	*P. argentea* var. *pseudocalabra*	BGR; Pirin, near the mountain hut Vihren/F&S	X		
Ptl5637	Pop166	*P. argentea* var. *pseudocalabra*	BGR; Pirin, near the mountain hut Vihren/F&S	X		
Ptl5638	Pop166	*P. argentea* var. *pseudocalabra*	BGR; Pirin, near the mountain hut Vihren/F&S	X		
Ptl5639	Pop166	*P. argentea* var. *pseudocalabra*	BGR; Pirin, near the mountain hut Vihren/F&S	X		
Ptl6082		*P. argentea*	DEU; Hessen/MK		2x	
Ptl6131	Pop269	*P. argentea*	ESP; El Paular/JP	X	2x	E
Ptl6132	Pop269	*P. argentea*	ESP; El Paular/JP	X		
Ptl6133	Pop269	*P. argentea*	ESP; El Paular/JP	X	2x	

Ptl6134	Pop269	*P. argentea*	ESP; El Paular/JP	X	2x	
Ptl6135	Pop269	*P. argentea*	ESP; El Paular/JP	X	2x	
Ptl6136	Pop269	*P. argentea*	ESP; El Paular/JP		2x	
Ptl6137	Pop269	*P. argentea*	ESP; El Paular/JP		2x	
Ptl6138	Pop269	*P. argentea*	ESP; El Paular/JP		2x_CC	
Ptl6140	Pop269	*P. argentea*	ESP; El Paular/JP		2x	
Ptl6192	Pop275	*P. argentea*	AUT; Fehnhaube-Kogelstein/JP		6x	
Ptl6193	Pop275	*P. argentea*	AUT; Fehnhaube-Kogelstein/JP		6x	
Ptl6194	Pop275	*P. argentea*	AUT; Fehnhaube-Kogelstein/JP		6x	
Ptl6195	Pop275	*P. argentea*	AUT; Fehnhaube-Kogelstein/JP		6x	
Ptl6196	Pop275	*P. argentea*	AUT; Fehnhaube-Kogelstein/JP		6x	
Ptl6197	Pop275	*P. argentea*	AUT; Fehnhaube-Kogelstein/JP		6x	
Ptl6198	Pop275	*P. argentea*	AUT; Fehnhaube-Kogelstein/JP		6x	
Ptl6199	Pop275	*P. argentea*	AUT; Fehnhaube-Kogelstein/JP		6x	
Ptl6200	Pop275	*P. argentea*	AUT; Fehnhaube-Kogelstein/JP		6x	
Ptl6221	Pop280	*P. argentea*	AUT; Wachau, Dürnstein an der Donau/JP		6x	
Ptl6222	Pop280	*P. argentea*	AUT; Wachau, Dürnstein an der Donau/JP		6x	
Ptl6223	Pop280	*P. argentea*	AUT; Wachau, Dürnstein an der Donau/JP		6x	
Ptl6224	Pop280	*P. argentea*	AUT; Wachau, Dürnstein an der Donau/JP		6x	
Ptl6225	Pop280	*P. argentea*	AUT; Wachau, Dürnstein an der Donau/JP		6x	
Ptl6226	Pop280	*P. argentea*	AUT; Wachau, Dürnstein an der Donau/JP		6x	
Ptl6227	Pop280	*P. argentea*	AUT; Wachau, Dürnstein an der Donau/JP		6x	
Ptl6229	Pop280	*P. argentea*	AUT; Wachau, Dürnstein an der Donau/JP		6x	
Ptl6230	Pop280	*P. argentea*	AUT; Wachau, Dürnstein an der Donau/JP		6x	
Ptl6271	Pop286	*P. argentea*	SVK; Svarín/JP		6x	
Ptl6272	Pop286	*P. argentea*	SVK; Svarín/JP		6x	
Ptl6273	Pop286	*P. argentea*	SVK; Svarín/JP		6x	
Ptl6274	Pop286	*P. argentea*	SVK; Svarín/JP		6x	
Ptl6275	Pop286	*P. argentea*	SVK; Svarín/JP		6x_CC	
Ptl6276	Pop286	*P. argentea*	SVK; Svarín/JP		6x	
Ptl6277	Pop286	*P. argentea*	SVK; Svarín/JP		6x	
Ptl6279	Pop286	*P. argentea*	SVK; Svarín/JP			
Ptl6301	Pop289	*P. argentea*	SVK; Tatranská Lomnica/JP	X	6x_CC	E
Ptl6302	Pop289	*P. argentea*	SVK; Tatranská Lomnica/JP	X	6x	
Ptl6303	Pop289	*P. argentea*	SVK; Tatranská Lomnica/JP	X	6x	
Ptl6304	Pop289	*P. argentea*	SVK; Tatranská Lomnica/JP	X	6x	
Ptl6305	Pop289	*P. argentea*	SVK; Tatranská Lomnica/JP	X	6x	
Ptl6306	Pop289	*P. argentea*	SVK; Tatranská Lomnica/JP		6x	
Ptl6307	Pop289	*P. argentea*	SVK; Tatranská Lomnica/JP		6x_CC	
Ptl6330	Pop292	*P. argentea*	CZE; České stredohoří, Lovoš/JP	X	6x	E
Ptl6331	Pop292	*P. argentea*	CZE; České stredohoří, Lovoš/JP	X	6x	E
Ptl7011	Pop237	*P. argentea*	FRA; ca. 2 km SW Beaune/CD	X	2x	E
Ptl7012	Pop237	*P. argentea*	FRA; ca. 2 km SW Beaune/CD	X	2x	
Ptl7013	Pop237	*P. argentea*	FRA; ca. 2 km SW Beaune/CD	X	2x	
Ptl7014	Pop237	*P. argentea*	FRA; ca. 2 km SW Beaune/CD	X	2x	
Ptl7016	Pop237	*P. argentea*	FRA; ca. 2 km SW Beaune/CD	X	2x	
Ptl7017	Pop237	*P. argentea*	FRA; ca. 2 km SW Beaune/CD		2x	
Ptl7042	Pop245	*P. argentea*	FRA; Colmars les Alpes/CD		6x	
Ptl7044	Pop245	*P. argentea*	FRA; Colmars les Alpes/CD		6x	
Ptl7046	Pop245	*P. argentea*	FRA; Colmars les Alpes/CD		6x	

Ptl7047	Pop245	*P. argentea*	FRA; Colmars les Alpes/CD		6x	
Ptl7101	Pop299	*P. argentea*	ESP; Pyrenées, Bielsa/CD	X	2x	E
Ptl7102	Pop299	*P. argentea*	ESP; Pyrenées, Bielsa/CD	X		
Ptl7103	Pop299	*P. argentea*	ESP; Pyrenées, Bielsa/CD	X		
Ptl7104	Pop299	*P. argentea*	ESP; Pyrenées, Bielsa/CD	X	2x	
Ptl7105	Pop299	*P. argentea*	ESP; Pyrenées, Bielsa/CD	X	2x	
Ptl7108	Pop299	*P. argentea*	ESP; Pyrenées, Bielsa/CD		2x	
Ptl7109	Pop299	*P. argentea*	ESP; Pyrenées, Bielsa/CD		2x	
Ptl7110	Pop299	*P. argentea*	ESP; Pyrenées, Bielsa/CD		2x	
Ptl7181	Pop323	*P. argentea*	ITA; Vinschgau, Staben/CD		6x	
Ptl7182	Pop323	*P. argentea*	ITA; Vinschgau, Staben/CD		2x	
Ptl7183	Pop323	*P. argentea*	ITA; Vinschgau, Staben/CD		2x	
Ptl7184	Pop323	*P. argentea*	ITA; Vinschgau, Staben/CD		6x	
Ptl7185	Pop323	*P. argentea*	ITA; Vinschgau, Staben/CD		6x	
Ptl7186	Pop323	*P. argentea*	ITA; Vinschgau, Staben/CD		6x	
Ptl7187	Pop323	*P. argentea*	ITA; Vinschgau, Staben/CD		2x	
Ptl7188	Pop323	*P. argentea*	ITA; Vinschgau, Staben/CD		6x	
Ptl7281	Pop249	*P. argentea*	FRA; Durance Valley/CD		6x	
Ptl7341	Pop258	*P. argentea*	FRA; Le Clapier/CD	X	6x	E
Ptl7342	Pop258	*P. argentea*	FRA; Le Clapier/CD	X		F
Ptl7343	Pop258	*P. argentea*	FRA; Le Clapier/CD	X		
Ptl7344	Pop258	*P. argentea*	FRA; Le Clapier/CD	X		
Ptl7345	Pop258	*P. argentea*	FRA; Le Clapier/CD	X		
Ptl7421	Pop329	*P. argentea*	CHE; Unterengadin, Zernez/CD&JP		2x	
Ptl7422	Pop329	*P. argentea*	CHE; Unterengadin, Zernez/CD&JP		2x	
Ptl7424	Pop329	*P. argentea*	CHE; Unterengadin, Zernez/CD&JP		2x	
Ptl7425	Pop329	*P. argentea*	CHE; Unterengadin, Zernez/CD&JP		2x	
Ptl7426	Pop329	*P. argentea*	CHE; Unterengadin, Zernez/CD&JP		2x	
Ptl7427	Pop329	*P. argentea*	CHE; Unterengadin, Zernez/CD&JP		2x	
Ptl7428	Pop329	*P. argentea*	CHE; Unterengadin, Zernez/CD&JP		2x	
Ptl7429	Pop329	*P. argentea*	CHE; Unterengadin, Zernez/CD&JP		2x	
Ptl7430	Pop329	*P. argentea*	CHE; Unterengadin, Zernez/CD&JP		2x	
Ptl4350		*P. calabra*	ITA; Spezzano/CD		2x	
Ptl4671	Pop218	*P. calabra*	ITA; Monte Pollino-Gebiet/CD	X		X
Ptl4672	Pop218	*P. calabra*	ITA; Monte Pollino-Gebiet/CD	X		
Ptl4673	Pop218	*P. calabra*	ITA; Monte Pollino-Gebiet/CD	X		
Ptl4674	Pop218	*P. calabra*	ITA; Monte Pollino-Gebiet/CD	X		
Ptl4675	Pop218	*P. calabra*	ITA; Monte Pollino-Gebiet/CD	X		
Ptl4701	Pop221	*P. calabra*	ITA; Monte Botte Donato/CD	X	2x_CC	X
Ptl4702	Pop221	*P. calabra*	ITA; Monte Botte Donato/CD	X	2x	
Ptl4703	Pop221	*P. calabra*	ITA; Monte Botte Donato/CD	X		
Ptl4704	Pop221	*P. calabra*	ITA; Monte Botte Donato/CD	X		
Ptl4705	Pop221	*P. calabra*	ITA; Monte Botte Donato/CD	X		
Ptl4731	Pop223	*P. calabra*	ITA; Longobucco/CD	X	2x_CC	
Ptl4732	Pop223	*P. calabra*	ITA; Longobucco/CD	X		X
Ptl4733	Pop223	*P. calabra*	ITA; Longobucco/CD	X		
Ptl4734	Pop223	*P. calabra*	ITA; Longobucco/CD	X		
Ptl4735	Pop223	*P. calabra*	ITA; Longobucco	X		

Appendix 3 – *P. argentea* chromosome counts: literature reviewed

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Appendix 4. List of investigated accessions and conducted experiments from the Chapter 4. **AFLP** – amplified fragment length polymorphism (X) , **FCM** – flow cytometry (cc – chromosoems counted, p – presumed ploidy level based on the genetic data), **Haplo** – sequenced chloroplast *trn*H-*psb*A marker with attributed haplotype. Samples are ordered according to broader localities (**LOC_1 – LOC_7**), country codes follows ISO 3166-1 Alpha-3; all samples have been collected by C. Dobeš.

Mat Nr	PopID	Taxon	Locality	AFLP	FCM	Haplo
LOC_1			**Völs/Seis am Schlern (NE from Bolzano)**			
Ptl4121	Pop088	*P. argentea*	ITA; SW from Seis am Schlern	X	6x	F
Ptl4122	Pop088	*P. argentea*	ITA; SW from Seis am Schlern	X	2x	G
Ptl4123	Pop088	*P. argentea*	ITA; SW from Seis am Schlern	X	2x	
Ptl4124	Pop088	*P. argentea*	ITA; SW from Seis am Schlern	X	6x	
Ptl4125	Pop088	*P. argentea*	ITA; SW from Seis am Schlern	X	2x	
Ptl4131	Pop085	*P. pusilla*	ITA; Völs am Schlern, 0.5 km N	X	7x	
Ptl4132	Pop085	*P. pusilla*	ITA; Völs am Schlern, 0.5 km N	X	cc_7x-	
Ptl4133	Pop085	*P. pusilla*	ITA; Völs am Schlern, 0.5 km N	X	cc_7x	
Ptl4134	Pop085	*P. pusilla*	ITA; Völs am Schlern, 0.5 km N	X	5x	
Ptl4135	Pop085	*P. pusilla*	ITA; Völs am Schlern, 0.5 km N	X	5x	L
Ptl4136	Pop085	*P. pusilla*	ITA; Völs am Schlern, 0.5 km N	X	7x	
Ptl4137	Pop085	*P. pusilla*	ITA; Völs am Schlern, 0.5 km N	X	5x	
Ptl4138	Pop085	*P. pusilla*	ITA; Völs am Schlern, 0.5 km N	X	5x	
Ptl4140	Pop085	*P. pusilla*	ITA; Völs am Schlern, 0.5 km N	X	5x	
Ptl4083	Pop085	*P. pusilla*	ITA; Völs am Schlern, 0.5 km N		7x	
Ptl4201	Pop089	*P. incana*	ITA; Völs am Schlern, Mongadui	X	4x	R
Ptl4202	Pop089	*P. incana*	ITA; Völs am Schlern, Mongadui	X	4x	
Ptl4203	Pop089	*P. incana*	ITA; Völs am Schlern, Mongadui	X	4x	
Ptl4204	Pop089	*P. incana*	ITA; Völs am Schlern, Mongadui	X	4x	
Ptl4205	Pop089	*P. incana*	ITA; Völs am Schlern, Mongadui	X	4x	
Ptl4206	Pop089	*P. incana*	ITA; Völs am Schlern, Mongadui		4x	
Ptl4207	Pop089	*P. incana*	ITA; Völs am Schlern, Mongadui		4x	
Ptl4208	Pop089	*P. incana*	ITA; Völs am Schlern, Mongadui		4x	
Ptl4209	Pop089	*P. incana*	ITA; Völs am Schlern, Mongadui		4x	
Ptl4141	Pop086	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	cc_6x	
Ptl4142	Pop086	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X		G
Ptl4143	Pop086	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	5x	W
Ptl4145	Pop086	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	5x	W
Ptl4146	Pop086	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	6x-	E
Ptl4147	Pop086	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X		F
Ptl4148	Pop086	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	6x-	E
Ptl4149	Pop086	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	cc_5x	W
Ptl4150	Pop086	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	6x	
Ptl4081	Pop086	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	cc_5x	W
Ptl4082	Pop086	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin		6x	E
Ptl4151	Pop087	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	cc6x	G
Ptl4152	Pop087	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	6x	G
Ptl4153	Pop087	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	6x	G
Ptl4154	Pop087	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin		6x	
Ptl4155	Pop087	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	6x	
Ptl4156	Pop087	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	6x	
Ptl4157	Pop087	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	6x	
Ptl4158	Pop087	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	6x	

Ptl4159	Pop087	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	6x	
Ptl4160	Pop087	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	6x	
Ptl4085	Pop087	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	6x	F
Ptl4086	Pop087	*P. alpicola*	ITA; Völs am Schlern, St. Konstantin	X	6x	S
LOC_2			**Burgstall/Lana-Burgstall (SE from Merano)**			
Ptl4171	Pop093	*P. argentea*	ITA; Burgstall – western slope	X	6x	F
Ptl4172	Pop093	*P. argentea*	ITA; Burgstall – western slope	X	6x	
Ptl4175	Pop093	*P. argentea*	ITA; Burgstall – western slope	X	6x	
Ptl4176	Pop093	*P. argentea*	ITA; Burgstall – western slope	X	6x	
Ptl4177	Pop093	*P. argentea*	ITA; Burgstall – western slope	X	6x	
Ptl4178	Pop093	*P. argentea*	ITA; Burgstall – western slope	X	6x	
Ptl4179	Pop093	*P. argentea*	ITA; Burgstall – western slope	X	6x	
Ptl4180	Pop093	*P. argentea*	ITA; Burgstall – western slope	X	6x	
Ptl4181	Pop094	*P. pusilla*	ITA; Burgstall – western slope	X	5x	R
Ptl4182	Pop094	*P. pusilla*	ITA; Burgstall – western slope	X	5x	
Ptl4183	Pop094	*P. pusilla*	ITA; Burgstall – western slope	X	5x	
Ptl4184	Pop094	*P. pusilla*	ITA; Burgstall – western slope	X	5x	
Ptl4185	Pop094	*P. pusilla*	ITA; Burgstall – western slope	X	5x	
Ptl4186	Pop094	*P. pusilla*	ITA; Burgstall – western slope	X	5x	
Ptl4187	Pop094	*P. pusilla*	ITA; Burgstall – western slope	X	7x	
Ptl4188	Pop094	*P. pusilla*	ITA; Burgstall – western slope	X	7x	
Ptl4189	Pop094	*P. pusilla*	ITA; Burgstall – western slope	X		
Ptl4190	Pop094	*P. pusilla*	ITA; Burgstall – western slope	X		
Ptl4191	Pop095	*P. collina*	ITA; Burgstall/Lana – railway station	X	6x	F
Ptl4192	Pop095	*P. collina*	ITA; Burgstall/Lana – railway station	X	6x	
Ptl4193	Pop095	*P. collina*	ITA; Burgstall/Lana – railway station	X	6x	
Ptl4194	Pop095	*P. collina*	ITA; Burgstall/Lana – railway station	X	6x	
Ptl4195	Pop095	*P. collina*	ITA; Burgstall/Lana – railway station	X	6x	
Ptl4196	Pop095	*P. collina*	ITA; Burgstall/Lana – railway station	X	6x	
Ptl4197	Pop095	*P. collina*	ITA; Burgstall/Lana – railway station	X	6x	
Ptl4198	Pop095	*P. collina*	ITA; Burgstall/Lana – railway station	X	6x	
Ptl4199	Pop095	*P. collina*	ITA; Burgstall/Lana – railway station	X	6x	
Ptl4200	Pop095	*P. collina*	ITA; Burgstall/Lana – railway station	X	6x	
Ptl4089	Pop095	*P. collina*	ITA; Burgstall/Lana – railway station		6x	
LOC_3			**Glurns (Vinschgau)**			
Ptl4071	Pop098	*P. argentea*	ITA; Glurns – sedimentation tank	X	2x	G
Ptl4072	Pop098	*P. argentea*	ITA; Glurns – sedimentation tank	X	2x	
Ptl4073	Pop098	*P. argentea*	ITA; Glurns – sedimentation tank	X	2x	
Ptl4074	Pop098	*P. argentea*	ITA; Glurns – sedimentation tank	X	2x	
Ptl4075	Pop098	*P. argentea*	ITA; Glurns – sedimentation tank	X	2x	
Ptl4076	Pop098	*P. argentea*	ITA; Glurns – sedimentation tank	X	2x	
Ptl4078	Pop098	*P. argentea*	ITA; Glurns – sedimentation tank	X	2x	
Ptl4079	Pop098	*P. argentea*	ITA; Glurns – sedimentation tank	X	2x	
Ptl4080	Pop098	*P. argentea*	ITA; Glurns – sedimentation tank	X	2x	
Ptl4041	Pop099	*P. pusilla*	ITA; Glurns – settlement Sölles	X		
Ptl4042	Pop099	*P. pusilla*	ITA; Glurns – settlement Sölles	X	4x	W
Ptl4043	Pop099	*P. pusilla*	ITA; Glurns – settlement Sölles	X	4x	
Ptl4044	Pop099	*P. pusilla*	ITA; Glurns – settlement Sölles	X	4x	
Ptl4045	Pop099	*P. pusilla*	ITA; Glurns – settlement Sölles	X	4x	
Ptl4047	Pop099	*P. pusilla*	ITA; Glurns – settlement Sölles	X	4x	
Ptl4048	Pop099	*P. pusilla*	ITA; Glurns – settlement Sölles	X	4x	
Ptl4049	Pop099	*P. pusilla*	ITA; Glurns – settlement Sölles	X	4x	

Ptl4050	Pop099	*P. pusilla*	ITA; Glurns – settlement Sölles	X	4x	
Ptl4061	Pop097	*P. collina*	ITA; Glurns – sedimentation tank	X	6x	E
Ptl4063	Pop097	*P. collina*	ITA; Glurns – sedimentation tank	X	6x	
Ptl4064	Pop097	*P. collina*	ITA; Glurns – sedimentation tank	X	6x	
Ptl4065	Pop097	*P. collina*	ITA; Glurns – sedimentation tank	X	6x	
Ptl4066	Pop097	*P. collina*	ITA; Glurns – sedimentation tank	X	6x	
Ptl4067	Pop097	*P. collina*	ITA; Glurns – sedimentation tank	X	6x	
Ptl4068	Pop097	*P. collina*	ITA; Glurns – sedimentation tank	X	6x	
Ptl4069	Pop097	*P. collina*	ITA; Glurns – sedimentation tank	X	6x	
Ptl4070	Pop097	*P. collina*	ITA; Glurns – sedimentation tank	X	6x	
LOC_4			**Schluderns (Vinschgau)**			
Ptl4401	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	6x	E
Ptl4402	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p2x	G
Ptl4403	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p6x	
Ptl4404	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p6x	
Ptl4405	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p2x	
Ptl4406	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p2x	
Ptl4407	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p6x	
Ptl4408	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p6x	
Ptl4409	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p6x	
Ptl4410	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p6x	
Ptl4551	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p2x	G
Ptl4552	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p6x	E
Ptl4553	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p6x	
Ptl4554	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p6x	
Ptl4555	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p2x	
Ptl4556	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p2x	
Ptl4557	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p6x	I
Ptl4558	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p2x	
Ptl4559	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p6x	
Ptl4560	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p2x	
Ptl4888	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	6x	E
Ptl4889	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p2x	G
Ptl4890	Pop100	*P. argentea*	ITA; Schluderns - Kalvarienberg	X	p6x	
Ptl4461	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X	4x	N
Ptl4462	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4463	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4464	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4465	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4466	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4467	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4468	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4469	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4470	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4861	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4862	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4863	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4864	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4865	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4866	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4867	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4868	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4869	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		
Ptl4870	Pop101	*P. pusilla*	ITA; Schluderns - Kalvarienberg	X		

Ptl4021	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X	p6x	F
Ptl4022	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X	6x	F
Ptl4023	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X	6x	F
Ptl4024	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X	6x	
Ptl4025	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X	p6x	
Ptl4026	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X	6x	
Ptl4027	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X	p6x	
Ptl4028	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X	6x	
Ptl4029	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X	p6x	
Ptl4030	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X	6x	
Ptl4851	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X		G
Ptl4852	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X		G
Ptl4853	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X		G
Ptl4854	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X		
Ptl4855	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X		
Ptl4856	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X		
Ptl4857	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X		
Ptl4858	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X		
Ptl4859	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X		
Ptl4860	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X		
Ptl4881	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X	6x	
Ptl4882	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X	6x	
Ptl4883	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X	p6x	
Ptl4884	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X	6x	
Ptl4885	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X	6x	
Ptl4886	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X	6x	
Ptl4887	Pop102	*P. alpicola*	ITA; Schluderns - Kalvarienberg	X	6x	
LOC_5			**Laatsch/Münstertal valley**			
Ptl4891	Pop198	*P. argentea*	ITA; exit of the Münstertal valley/Laatsch	X	6x	F
Ptl4892	Pop198	*P. argentea*	ITA; exit of the Münstertal valley/Laatsch	X	6x	
Ptl4893	Pop198	*P. argentea*	ITA; exit of the Münstertal valley/Laatsch	X	6x	
Ptl4894	Pop198	*P. argentea*	ITA; exit of the Münstertal valley/Laatsch	X	6x	
Ptl4895	Pop198	*P. argentea*	ITA; exit of the Münstertal valley/Laatsch	X	6x	
Ptl4896	Pop198	*P. argentea*	ITA; exit of the Münstertal valley/Laatsch	X	6x	
Ptl4897	Pop198	*P. argentea*	ITA; exit of the Münstertal valley/Laatsch	X	6x	
Ptl4898	Pop198	*P. argentea*	ITA; exit of the Münstertal valley/Laatsch	X	6x	
Ptl4899	Pop198	*P. argentea*	ITA; exit of the Münstertal valley/Laatsch	X	6x	E
Ptl4900	Pop198	*P. argentea*	ITA; exit of the Münstertal valley/Laatsch	X	6x	
Ptl4901	Pop199	*P. pusilla*	ITA; exit of the Münstertal valley/Laatsch	X	4x	W
Ptl4902	Pop199	*P. pusilla*	ITA; exit of the Münstertal valley/Laatsch	X	4x	
Ptl4903	Pop199	*P. pusilla*	ITA; exit of the Münstertal valley/Laatsch	X	4x	
Ptl4904	Pop199	*P. pusilla*	ITA; exit of the Münstertal valley/Laatsch	X	4x	
Ptl4905	Pop199	*P. pusilla*	ITA; exit of the Münstertal valley/Laatsch	X	4x	
Ptl4906	Pop199	*P. pusilla*	ITA; exit of the Münstertal valley/Laatsch	X	6x	
Ptl4907	Pop199	*P. pusilla*	ITA; exit of the Münstertal valley/Laatsch	X	4x	
Ptl4908	Pop199	*P. pusilla*	ITA; exit of the Münstertal valley/Laatsch	X	4x	
Ptl4909	Pop199	*P. pusilla*	ITA; exit of the Münstertal valley/Laatsch		4x	
Ptl4910	Pop199	*P. pusilla*	ITA; exit of the Münstertal valley/Laatsch		4x	
Ptl4911	Pop200	*P. alpicola*	ITA; exit of the Münstertal valley/Laatsch	X	6x	G
Ptl4912	Pop200	*P. alpicola*	ITA; exit of the Münstertal valley/Laatsch	X	6x	G
Ptl4913	Pop200	*P. alpicola*	ITA; exit of the Münstertal valley/Laatsch	X	6x	G
Ptl4914	Pop200	*P. alpicola*	ITA; exit of the Münstertal valley/Laatsch	X	6x	G
Ptl4915	Pop200	*P. alpicola*	ITA; exit of the Münstertal valley/Laatsch	X	6x	G
Ptl4916	Pop200	*P. alpicola*	ITA; exit of the Münstertal valley/Laatsch	X	6x	G

Ptl4917	Pop200	*P. alpicola*	ITA; exit of the Münstertal valley/Laatsch	X	6x	
Ptl4918	Pop200	*P. alpicola*	ITA; exit of the Münstertal valley/Laatsch	X	6x	
Ptl4919	Pop200	*P. alpicola*	ITA; exit of the Münstertal valley/Laatsch	X	6x	
LOC_6			**Müstair/Münstertal valley**			
Ptl4331	Pop202	*P. argentea*	CHE; Münstertal valley, NW Müstair	X	6x	Q
Ptl4332	Pop202	*P. argentea*	CHE; Münstertal valley, NW Müstair	X	6x	
Ptl4333	Pop202	*P. argentea*	CHE; Münstertal valley, NW Müstair	X	6x	
Ptl4334	Pop202	*P. argentea*	CHE; Münstertal valley, NW Müstair	X	6x	
Ptl4335	Pop202	*P. argentea*	CHE; Münstertal valley, NW Müstair	X	6x	
Ptl4337	Pop202	*P. argentea*	CHE; Münstertal valley, NW Müstair	X	2x	G
Ptl4338	Pop202	*P. argentea*	CHE; Münstertal valley, NW Müstair	X	6x	
Ptl4341	Pop201	*P. pusilla*	CHE; Münstertal valley, NW Müstair	X	4x	L
Ptl4342	Pop201	*P. pusilla*	CHE; Münstertal valley, NW Müstair	X	4x	
Ptl4343	Pop201	*P. pusilla*	CHE; Münstertal valley, NW Müstair	X	4x	
Ptl4344	Pop201	*P. pusilla*	CHE; Münstertal valley, NW Müstair	X	4x	
Ptl4345	Pop201	*P. pusilla*	CHE; Münstertal valley, NW Müstair	X	4x	
Ptl4346	Pop201	*P. pusilla*	CHE; Münstertal valley, NW Müstair	X	4x	
Ptl4347	Pop201	*P. pusilla*	CHE; Münstertal valley, NW Müstair	X	4x	
Ptl4348	Pop201	*P. pusilla*	CHE; Münstertal valley, NW Müstair	X	4x	
Ptl4321	Pop203	*P. alpicola*	CHE; Münstertal valley, NW Müstair	X	7x	K
Ptl4322	Pop203	*P. alpicola*	CHE; Münstertal valley, NW Müstair	X	7x	K
Ptl4323	Pop203	*P. alpicola*	CHE; Münstertal valley, NW Müstair	X	7x	K
Ptl4324	Pop203	*P. alpicola*	CHE; Münstertal valley, NW Müstair	X	7x	K
Ptl4325	Pop203	*P. alpicola*	CHE; Münstertal valley, NW Müstair	X	7x	K
Ptl4326	Pop203	*P. alpicola*	CHE; Münstertal valley, NW Müstair	X	7x	K
Ptl4327	Pop203	*P. alpicola*	CHE; Münstertal valley, NW Müstair	X	7x	
Ptl4328	Pop203	*P. alpicola*	CHE; Münstertal valley, NW Müstair	X	7x	
Ptl4329	Pop203	*P. alpicola*	CHE; Münstertal valley, NW Müstair	X	7x	
Ptl4330	Pop203	*P. alpicola*	CHE; Münstertal valley, NW Müstair	X	7x	
LOC_7			**Kauns (North Tyrol)**			
Ptl4871	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns – W the church	X	p6x	F
Ptl4872	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns – W the church	X	p6x	
Ptl4873	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns – W the church	X	p6x	
Ptl4874	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns – W the church	X	p6x	
Ptl4875	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns – W the church	X	p6x	
Ptl4876	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns – W the church	X	p6x	
Ptl4877	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns – W the church	X	p6x	
Ptl4878	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns – W the church	X	p6x	
Ptl4879	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns – W the church	X	p6x	
Ptl4880	Pop206	*P. argentea*	AUT; Ötztaleralpen, Kauns – W the church	X	6x	F
Ptl4339	Pop205	*P. pusilla*	AUT; Ötztaleralpen, Kauns – ESE the church	X		
Ptl4841	Pop205	*P. pusilla*	AUT; Ötztaleralpen, Kauns – ESE the church	X		W
Ptl4842	Pop205	*P. pusilla*	AUT; Ötztaleralpen, Kauns – ESE the church	X		
Ptl4843	Pop205	*P. pusilla*	AUT; Ötztaleralpen, Kauns – ESE the church	X		
Ptl4340	Pop204	*P. alpicola*	AUT; Ötztaleralpen, Kauns – ESE the church	X		
Ptl4844	Pop204	*P. alpicola*	AUT; Ötztaleralpen, Kauns – ESE the church	X		E
Ptl4845	Pop204	*P. alpicola*	AUT; Ötztaleralpen, Kauns – ESE the church	X		E
Ptl4846	Pop204	*P. alpicola*	AUT; Ötztaleralpen, Kauns – ESE the church	X		E
Ptl4847	Pop204	*P. alpicola*	AUT; Ötztaleralpen, Kauns – ESE the church	X		E
Ptl4848	Pop204	*P. alpicola*	AUT; Ötztaleralpen, Kauns – ESE the church	X		E
Ptl4920	Pop204	*P. alpicola*	AUT; Ötztaleralpen, Kauns – ESE the church	X	p6x	

EXTRA						
Ptl3961	Pop074	*P. aurea*	AUT; Northern Kalkalpen	X	2x	O
Ptl3963	Pop074	*P. aurea*	AUT; Northern Kalkalpen	X	2x	
Ptl3966	Pop074	*P. aurea*	AUT; Northern Kalkalpen	X	2x	
Ptl3969	Pop074	*P. aurea*	AUT; Northern Kalkalpen	X	2x	
Ptl3970	Pop074	*P. aurea*	AUT; Northern Kalkalpen	X	2x	
Ptl3971	Pop075	*P. brauneana*	AUT; Northern Kalkalpen	X	p2x	
Ptl3972	Pop075	*P. brauneana*	AUT; Northern Kalkalpen	X	p2x	
Ptl3973	Pop075	*P. brauneana*	AUT; Northern Kalkalpen	X	2x	M
Ptl3978	Pop075	*P. brauneana*	AUT; Northern Kalkalpen	X	2x	
Ptl3979	Pop075	*P. brauneana*	AUT; Northern Kalkalpen	X	2x	
Ptl4051	Pop096	*P. frigida*	AUT; Ötztaleralpen, summit Fineiljoch	X		P
Ptl4052	Pop096	*P. frigida*	AUT; Ötztaleralpen, summit Fineiljoch	X		
Ptl4053	Pop096	*P. frigida*	AUT; Ötztaleralpen, summit Fineiljoch	X		
Ptl4054	Pop096	*P. frigida*	AUT; Ötztaleralpen, summit Fineiljoch	X		
Ptl4055	Pop096	*P. frigida*	AUT; Ötztaleralpen, summit Fineiljoch	X		
Ptl4211	Pop103	*P. frigida*	ITA; South Tyrol, Stilfserjoch pass	X		P
Ptl4212	Pop103	*P. frigida*	ITA; South Tyrol, Stilfserjoch pass	X		
Ptl4213	Pop103	*P. frigida*	ITA; South Tyrol, Stilfserjoch pass	X		
Ptl4214	Pop103	*P. frigida*	ITA; South Tyrol, Stilfserjoch pass	X		
Ptl4215	Pop103	*P. frigida*	ITA; South Tyrol, Stilfserjoch pass	X		
Ptl4434	Pop080	*P. crantzii*	AUT; Obergurgl, Romoostal valley	X		Q
Ptl4435	Pop080	*P. crantzii*	AUT; Obergurgl, Romoostal valley	X		
Ptl4436	Pop080	*P. crantzii*	AUT; Obergurgl, Romoostal valley	X		
Ptl4437	Pop080	*P. crantzii*	AUT; Obergurgl, Romoostal valley	X		
Ptl4438	Pop080	*P. crantzii*	AUT; Obergurgl, Romoostal valley	X		
Ptl4581	Pop196	*P. argentea*	CHE; Engadin, above Scoul	X	p6x	E
Ptl4582	Pop196	*P. argentea*	CHE; Engadin, above Scoul	X	6x	
Ptl4583	Pop196	*P. argentea*	CHE; Engadin, above Scoul	X	6x	
Ptl4571	Pop189	*P. thuringiaca*	CHE; Engadin, Ftan – railway station	X	9x	L
Ptl4572	Pop189	*P. thuringiaca*	CHE; Engadin, Ftan – railway station	X	9x	
Ptl4573	Pop189	*P. thuringiaca*	CHE; Engadin, Ftan – railway station	X	9x	
Ptl4574	Pop189	*P. thuringiaca*	CHE; Engadin, Ftan – railway station	X	9x	L
Ptl4575	Pop189	*P. thuringiaca*	CHE; Engadin, Ftan – railway station	X	9x	L
Ptl4576	Pop189	*P. thuringiaca*	CHE; Engadin, Ftan – railway station	X	9x	L
Ptl4577	Pop189	*P. thuringiaca*	CHE; Engadin, Ftan – railway station	X	9x	L
Ptl4578	Pop189	*P. thuringiaca*	CHE; Engadin, Ftan – railway station	X	9x	L
Ptl4579	Pop189	*P. thuringiaca*	CHE; Engadin, Ftan – railway station	X	9x	L
Ptl4580	Pop189	*P. thuringiaca*	CHE; Engadin, Ftan – railway station	X	9x	L
Ptl4601	Pop193	*P. thuringiaca*	CHE; Engadin, Ftan-Pitschen/Scuol	X		L
Ptl4602	Pop193	*P. thuringiaca*	CHE; Engadin, Ftan-Pitschen/Scuol	X	9x	L
Ptl4603	Pop193	*P. thuringiaca*	CHE; Engadin, Ftan-Pitschen/Scuol	X	9x	L
Ptl4604	Pop193	*P. thuringiaca*	CHE; Engadin, Ftan-Pitschen/Scuol	X	9x	L
Ptl4605	Pop193	*P. thuringiaca*	CHE; Engadin, Ftan-Pitschen/Scuol	X	9x	L
Ptl4606	Pop193	*P. thuringiaca*	CHE; Engadin, Ftan-Pitschen/Scuol	X	9x	L
Ptl4607	Pop193	*P. thuringiaca*	CHE; Engadin, Ftan-Pitschen/Scuol	X	9x	L
Ptl4608	Pop193	*P. thuringiaca*	CHE; Engadin, Ftan-Pitschen/Scuol	X	6x	W
Ptl4609	Pop193	*P. thuringiaca*	CHE; Engadin, Ftan-Pitschen/Scuol	X	9x	L
Ptl4610	Pop193	*P. thuringiaca*	CHE; Engadin, Ftan-Pitschen/Scuol	X	9x	L

Ptl4491	Pop190	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	6x	W
Ptl4492	Pop190	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	6x	
Ptl4493	Pop190	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	6x	
Ptl4494	Pop190	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	6x	
Ptl4495	Pop190	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	4x	
Ptl4496	Pop190	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	p6x	
Ptl4497	Pop190	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	6x	
Ptl4498	Pop190	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	6x	
Ptl4499	Pop190	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	6x	
Ptl4500	Pop190	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	6x	
Ptl4591	Pop192	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	4x	L
Ptl4592	Pop192	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	4x	
Ptl4593	Pop192	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	4x	
Ptl4594	Pop192	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	4x	
Ptl4595	Pop192	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	6x	
Ptl4596	Pop192	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	4x	
Ptl4597	Pop192	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	6x	
Ptl4598	Pop192	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	6x	
Ptl4599	Pop192	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	4x	
Ptl4600	Pop192	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan – railway station	X	6x	
Ptl4586	Pop194	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan-Pitschen/Scuol	X	4x	W
Ptl4587	Pop194	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan-Pitschen/Scuol	X	4x	
Ptl4588	Pop194	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan-Pitschen/Scuol	X	5x	
Ptl4589	Pop194	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan-Pitschen/Scuol	X	4x	
Ptl4590	Pop194	*P. pusilla* × *thuringiaca*	CHE; Engadin, Ftan-Pitschen/Scuol	X	4x	