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Molecular phylogenetics of *Alchemilla*, *Aphanes* and *Lachemilla* (Rosaceae) inferred from plastid and nuclear intron and spacer DNA sequences, with comments on generic classification

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ABSTRACT

Alchemilla (the lady's mantles) is a well known but inconspicuous group in the Rosaceae, notable for its ornamental leaves and pharmaceutical properties. The systematics of *Alchemilla* has remained poorly understood, most likely due to confusion resulting from apomixis, polyploidisation and hybridisation, which are frequently observed in the group, and which have led to the description of a large number of (micro-) species. A molecular phylogeny of the genus, including all sections of *Alchemilla* and *Lachemilla* as well as five representatives of *Aphanes*, based on the analysis of the chloroplast trnL–trnF and the nuclear ITS regions is presented here. Gene phylogenies reconstructed from the nuclear and chloroplast sequence data were largely congruent. Limited conflict between the data partitions was observed with respect to a small number of taxa. This is likely to be the result of hybridisation/introgression or incomplete lineage sorting. Four distinct clades were resolved, corresponding to major geographical division and life forms: Eurasian *Alchemilla*, annual *Aphanes*, South American *Lachemilla* and African *Alchemilla*. We argue for a wider circumscription of the genus *Alchemilla*, including *Lachemilla* and *Aphanes*, based on the morphology and the phylogenetic relationships between the different clades.

1. Introduction

The genus *Alchemilla* in the wide sense (Rosaceae), by some authors recognised as the subtribe *Alchemillinae* including *Alchemilla*, *Lachemilla* and *Aphanes* (e.g. Notov and Kusnetzova, 2004), is notable for its highly derived but inconspicuous flowers. *Alchemilla* was previously thought to be related to the Sanguisorbinae (e.g. Hutchinson, 1964) due to superficial similarity caused by reduction in flower parts. However, its relation to Potentilleae was noted by Schulz-Menz (1964) and the position later confirmed by DNA sequence data (Eriksson et al. 1998, 2003). The petals of *Alchemilla, Lachemilla* and *Aphanes* are lacking and the two whorls of four calyx and four epicalyx lobes form a hypanthium. One to four or more introrse or extrorse stamens are inserted at the inner or outer side of a flower disc and one to many carpels are present (Fig. 1). *Alchemilla* is a well known example of polyploidy and it is probably the best known group with autonomous apomixis (in the sense of agamospermy, in which endosperm formation is independent of the fertilization of the primary endosperm nuclei) in the Rosaceae (Czapik, 1996). In most plants apomixis is not strictly obligate but facultative to a varying extent (Asker and Jerling, 1992; Mogie, 1992; Richards, 2003; Hörandl, 2004 and many others), although this might not apply to autonomous apomicts in cases where no viable pollen is produced, as for most Eurasian *Alchemilla* species but could be the case for *Aphanes* or *Lachemilla*. In addition to (in-) complete apomixis and poly- or aneuploidy, many species of *Alchemilla* can grow clonally, they display heteroblastic plasticity (such as differing morphologies of leaves), and show variability of indumentum and instability in flower characters. The difficulties inherent in interpreting this kind of variation have led to the description of many micro-species and species complexes, a problem often associated with agamic species complexes (Asker and Jerling, 1992; Hörandl, 2004).

1.1. Alchemilla L.

The genus *Alchemilla* was described by Linnaeus (1753) and currently includes at least 250 (–1000) species (Fröhner, 1995a). Some earlier authors referred to this group as *Eualchemilla* (Table 1). It

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Table 1
Classification of *Alchemilla* in the wide sense used by different authors

Author	*Alchemilla*	*Aphanes*	*Lachemilla*	Others
Linnaeus (1753)	Genus	Genus	–	–
Scopoli (1772)	Genus	–	–	–
Persoon (1805)	Genus	Genus	In *Aphanes*	–
De Candolle (1825)	Section	Section	In *Alchemilla*	–
Focke (1888)	Section *Eualchemilla*	Section	Section	–
Lagerheim (1894)	Subg. *Alchemilla*, sect. *Eualchemilla*	Subg. *Alchemilla*, sect. *Aphanes*	Subg. *Lachemilla*, sect. *Fockella*, sect. *Eulachemilla*	–
Rydberg (1908)	Genus	Genus	Genus	*Zygalchemilla*
Murbeck (1915)	Section	Section	In *Aphanes*	Sect. *Fockella*
Perry (1929)	Section	Section	Section (6 series)	
Rothmaler (1935)	Subgenus (6 sect.)	Subgenus (3 sect.)	Subgenus (5 sect.)	–
Haumann and Balle (1936)	Subg. *Eualchemilla*	Subgenus	?	–
Rothmaler (1937b)	Genus	Genus	Genus (6 Sect.)	–
Fröhner (1995a,b)	Genus (18 sect.)	Genus (3 sect.)	Genus (6 sect.)	–
Kalkman (2004)	Subgenus	Subgenus	Subgenus	–
Notov and Kusnetzova (2004)	Genus (7 sect.)	Genus (3 sect.)	Genus (6 sect.)	–

has a mainly Holarctic distribution with a centre of species richness in western Eurasia but occurs also in South India, Sri Lanka, Java, China and Japan and on the mountains of Africa and Madagascar (Fig. 2). *Alchemilla* is characterised by introrse stamens that are inserted at the outer side of the discus (Fig. 1). However, specimens have been reported which also possess stamens at the inner side of the discus (Fröhner, 1995a). Stamens that are inserted at the outer side of the discus are common in *Potentilla* and probably represent the pleisiomorphic character state. A monograph or revision has yet to be accomplished for the whole genus, but various less than comprehensive treatments have been produced by different authors (Table 1). Linnaeus (1753) mentioned three representatives, *A. vulgaris*, *A. alpina* and *A. pentaphyllea* (Fig. 3). Many of the earliest authors have followed this division into three groups of species in the Eurasian *Alchemilla* on the basis of the level of dissection of their leaves (Focke, 1888; Buser, 1892; Rothmaler, 1934). All authors agree in placing the species from tropical and southern Africa in sections separate to those of the Eurasian species (Table 2), based on their distributions and the occurence of longer internodes in many species. However, no consistent diagnostic characters have been proposed for the African sections combined, nor it has been suggested that all African sections should be recognised together as separate taxa in their own right (Fröhner, 1986). Nevertheless, the African species display greater morphological variation than those found in Eurasia. They include many dwarf shrubs and trailing herbs that are often dominant species in the Afrotemperate regions.

1.2. Aphanes L.

Aphanes is a small group of about 20 species distributed in temperate regions across the world. The centre of species diversity is found with seven species in the western Mediterranean (Europe and North Africa). Two species occur throughout northern and central Europe, four on the pacific coast of North America, five in western South America, one in eastern South America (South Brazil to southern Argentina) and three in southern Australia (Fig. 2). Three sections are recognised according to Fröhner (1986): *Aphanes*, *Quadridentatae* and *Aequidentatae*.

The plants are mostly very small, sometimes no more than a few centimetres in height and annual or biennial (Peter Frost-Olsen pers. comm.). The flowers are similar to those of *Alchemilla*, but they have only a single extrorse episepal stamen at the inner part of the discus, and a single stigma is formed by the only carpel (Fig. 1). Occasional reports of multiple carpels in *Aphanes* have by some authors been thought to stem from the inclusion of *Lachemilla* (Fröhner, 1995a,b), even though it is not uncommon to find plants where all flowers have two carpels, especially in *Ap. arvensis* (Peter Frost-Olsen pers. comm.). In *Aphanes*, as opposed to *Alchemilla*, pseudogamous apomixis has been reported, in which pollination is necessary for endosperm formation, as the polar nuclei must be fertilised to ensure formation of viable seeds (Asker and Jerling, 1992). Unlike in *Alchemilla* diploid species with $2n = 16$ exist in *Aphanes* in addition to tetraploids and hexaploids.

1.3. Lachemilla Focke

Lachemilla is a group of ca. 80 morphologically variable perennial herbs and shrubs. They are distributed in South and Central America from Mexico and the Greater Antilles (Hispaniola) to the Andes of northern Chile and Argentina, between 2200 and 5000 m in elevation, where they can form dense stands. *Lachemilla* is considered one of the most important and most species rich groups of plants in the andean páramos (Albach and Chase, 2004; Romoleroux, 2004).

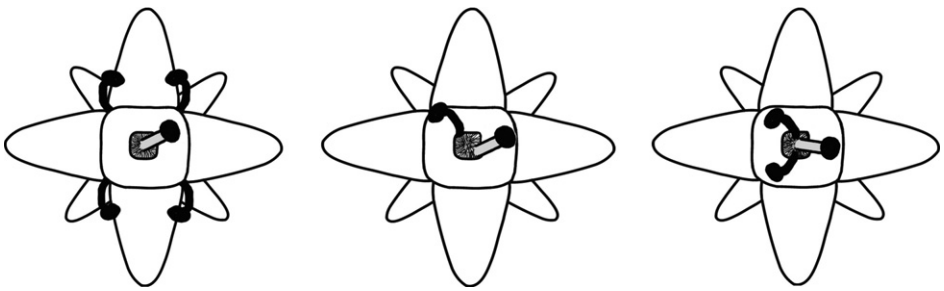

Fig. 1. Schematic drawing of *Alchemilla* flowers. From left to right Eurasian and African *Alchemilla* A4 G1 -4(-12) stamens introse; *Aphanes* A1 G1 stamens extrose; *Lachemilla* A2 (3-4) G1-2 (-8) stamens extrose.

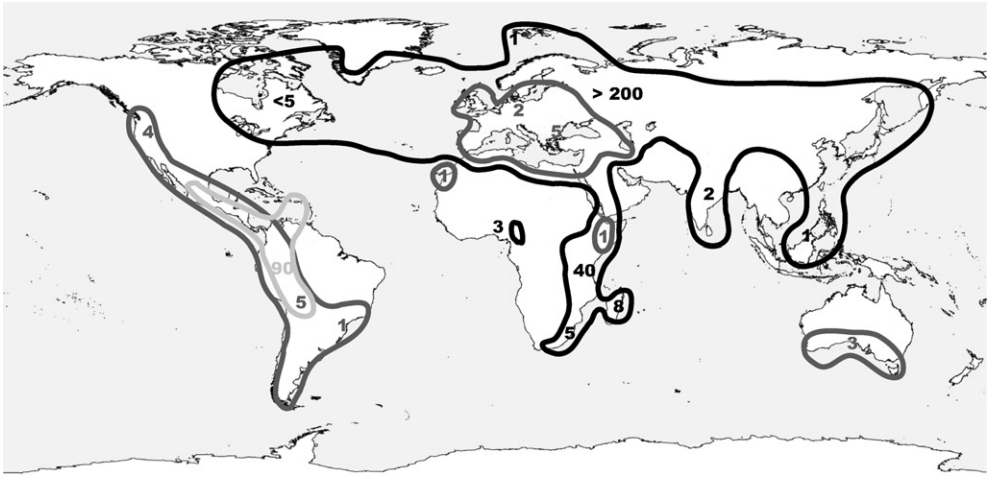

Fig. 2. Distribution of *Alchemilla*. Black lines Eurasian and African *Alchemilla*, grey lines *Aphanes* and light grey *Lachemilla*. Numbers indicate number of species in the area.

Lachemilla has sometimes been included in *Alchemilla* (Linnaeus, 1753; Focke, 1888; Fedde, 1910; Perry, 1929) or *Aphanes* (Persoon, 1805; Rothmaler, 1935; see also comments in Fröhner, 1995b) because the stamens are extrorse and inserted at the inner side of the discus. The presence of at least 2 to rarely 3 or 4 extrorse stamens (in single flowers) and 1–12 stigmas (Fig. 1), however have been considered sufficient to justify generic rank by various authors (Rydberg, 1908; Rothmaler, 1937b; Gaviria, 1996; Romoleroux, 1996).

Subdivision of *Lachemilla* has differed between the most influential treatments (Table 2) especially those of Perry (1929) and Rothmaler (1937b). Both recognised six units (series/sections) and divided one of them (*Aphanoides*) into five subunits (subseries/subsections) based on growth form, leaf characteristics or inflorescence structure. The two authors further agreed in defining two monotypic groups, *Polylepides* and *Diplophyllae*. Perry (1929) placed the remaining species in the four series. Rothmaler (1937b) however arranged these species in three different sections and created the new monotypic section *Fruticulosae,* which he described from the type material, the only known collection (Puebla, Mexico) and which is probably extinct. The holotype of *Fruticulosa* has been destroyed at B and only a small fragment at JE is preserved, therefore the status remains unclear, but affinities seem to be with to *Aphanes* rather than *Lachemilla* (Romoleroux, pers. obs.). To our knowledge no work has been done on the extent or type of apomixis in *Lachemilla* nor has the ploidy level been assessed.

Here we present the first comprehensive molecular phylogenetic analysis of *Alchemilla* sensu lato (*Alchemillinae*; including *Aphanes* and *Lachemilla*). A major goal is to provide new evidence from nuclear and chloroplast genes on higher-level relationships within the clade. With these new phylogenetic results, we evaluate relative support from genes and morphology for currently recognised genera, and establish an initial framework that can be used for future investigations of relationships, biogeography and the evolution of autonomous agamospermy.

2. Materials and methods

2.1. Phylogeny reconstruction in systems with apomixis, hybridisation and polyploidy

Apomixis influences the evolution of both genetic and morphological variability. The source of genetic variability in agamosperms is mainly derived from somatic mutations and recombination (Shi et al., 1996), or is present because of backcrossing, facultative meiotic recombination and cross-fertilization, as well as the multiple hybrid origins of apomicts from genetically divergent, usually diploid sexual ancestors (Hörandl, 2004). Nevertheless, the morphological and genetic diversity within species in a predominantly apomictic system is usually much lower than that of their sexually reproducing relatives (Asker and Jerling, 1992; Shi et al., 1996; Richards, 2003). We might therefore expect that the amount of genetic variation and number of informative characters in DNA markers used in molecular phylogenetic analysis would be lower in apomictic species. However, almost all apomictic species, arguably including all species of *Alchemilla*, are of polyploid origin and should have an enhanced rate of molecular evolution (Mogie, 1992). Notwithstanding, several studies have shown that molecular markers such as ITS, *trn*LF or *mat*K, can be used to study evolutionary relationships within genera with apomictic lineages (Alice and Campbell, 1999; Wittzell, 1999; Kirschner et al., 2003; Fehrer et al., 2007).

2.2. Taxon sampling

Sampling of the species followed the strategy to (1) include the generic and subgeneric types of *Alchemilla*, *Aphanes* and *Lachemilla;* (2) include at least two representatives of each section (sensu Rothmaler, 1935–1937a,b; Fröhner, 1995a,b; Notov and Kusnetzova, 2004), if possible these should include the type species and a second representative (3) species should represent the whole geographical and (4) the morphological range of the genus. With respect to the representation of taxonomic groups as described in point two, the material is complete except for *Aphanes* and two of Rothmaleŕs (1937b) sections of *Lachemilla, i.e. Fruticulosa* and *Polylepis,* for which fresh material was not available. Extraction from herbarium material proved difficult or impossible. Identification of *A. japonica* material that was provided by the Botanical Garden in Göttingen is uncertain as *A. japonica* in culture has been found to be a Caucasian species related to *A. speciosa* (P. Frost-Ohlsen pers. comm.). Outgroup sampling represents most genera in the Fragariinae-clade (Eriksson et al., 2003) and members of Potentilleae and other Rosoideae to test the monophyly of the genus and for rooting purposes. For some widespread and critical species, more than one accession was sequenced, but in most cases sequences proved identical and only one of them was used for phylogenetic reconstruction. Altogether 100 taxa were included in the final analysis (Table 4).

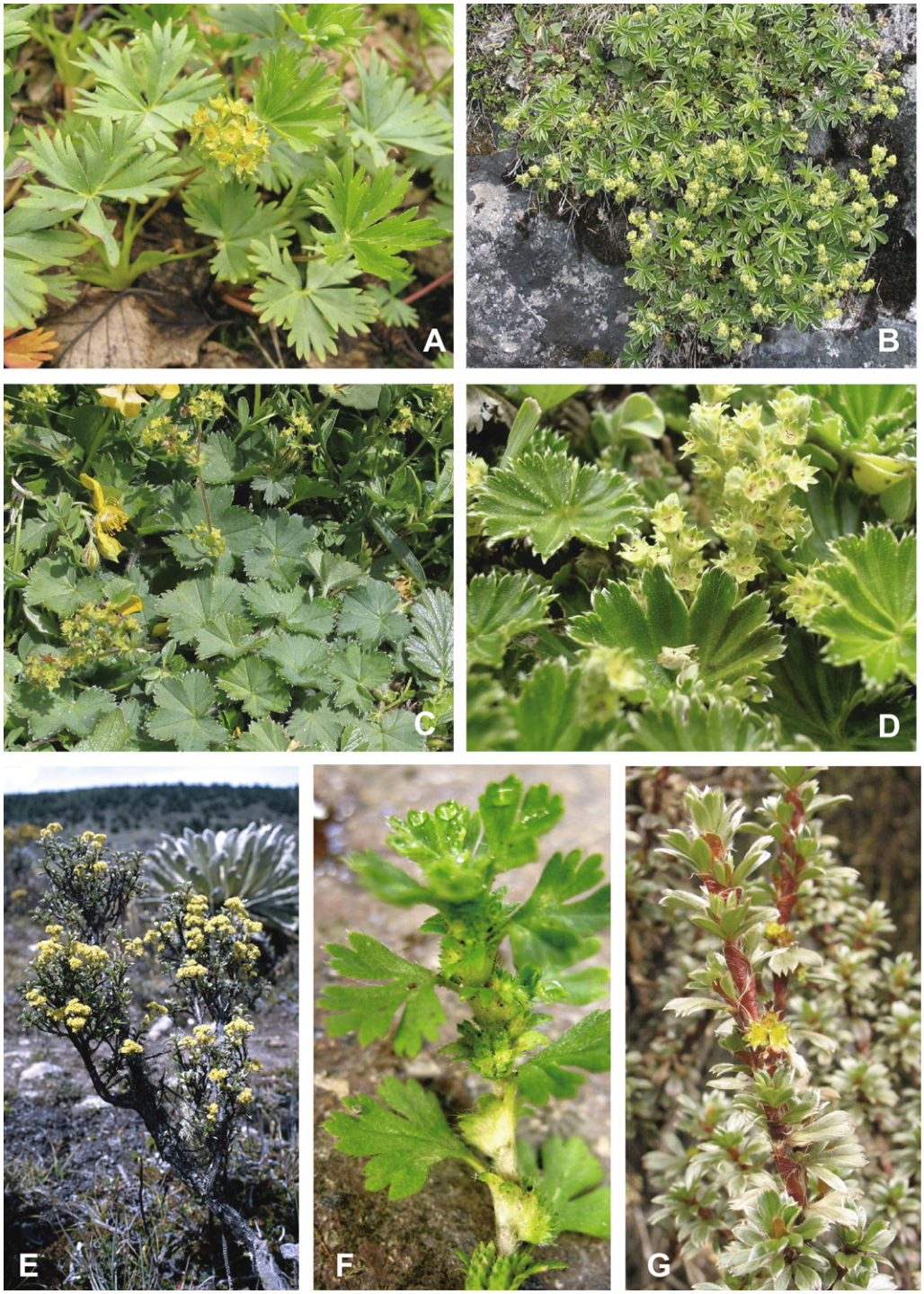

Fig. 3. Pictures of the *Alchemilla* in the wide sense: (A) *A. pentaphyllea*; (B) *A. alpina*; (C) *A. hybrida*; (D) *L. orbiculata*; (E) *L. polylepis*; (F) *Ap. arvensis*; (G) *A. argyrophylla.* Pictures (A), (B), (C), (F) and (G) from B. Gehrke; (D) from K. Romoleroux and (L) from A. Groeger.

2.3. DNA extraction, sequencing and alignment

Different protocols for DNA extraction and amplification were applied in the respective labs of the collaborating authors. At the Institute for Systematic Botany of the University of Zurich, silica dried material was homogenised using two glass beads in 2 μl reaction tubes in a Regget Machine 2 × 130 s at full speed. DNA extraction was performed using DNeasy extraction kit (Qiagen), following the manufacturer's instructions with minor modifications. Samples where diluted 1:100 in ddH_2O prior to polymerase chain reactions (PCR) that were performed in 25 μl reactions (1× PCR buffer, with 2.5 mM $MgCl_2$, 0.25 mM dNTPs, 1.6 μM primer and 1 U of Taq polymerase (Sigma–Aldrich, USA) in a Biometra Thermocycler TGradient (Biometra, Göttingen, Germany). The entire ITS1-5.8S-ITS2 region and the *trnL–F* intergenic spacer together with the *trnL* intron were amplified and sequenced as described in Eriksson et al. (2003) with the exception that in Zurich PCR products were purified using DNA band purification kit (Amersham Biosciences, Otelfingen, Switzerland). Forward and reverse strand sequences were edited using Sequencher 4.2. (Genecode Corp.).

Table 2
Important contribution to the infrageneric classification of *Alchemilla*

Infrageneric classification of Eurasian and African Alchemilla species	
Thunberg (1794)	*A. capensis* is mentioned as first *Alchemilla* species from Africa (formal desciption 1823)
De Candolle (1825)	*A. capensis* is mentioned within the section *Alchemilla,* no further division of the section
Rothmaler (1935)	*Alchmilla* is divided into seven sections; African *Alchemilla* material in five sections and Eurasian material in two sections: section *Brevicaules* (including subsect. *Alpinae* and subsect. *Vulgares*) and section *Pentaphyllea*
Haumann and Balle (1936)	All african *Alchemilla* species are placed in subg. *Eualchemilla* (not differentiated in sections)
Rothmaler (1937a)	Recircumscribtion of the five African *Alchemilla* sections (sect. *Longicaules* including material from *Lachemilla* and possibly *Aphanes*). The Eurasian material in sect. *Pentaphyllea* and sect. *Brevicaules* (including material from Australia and Africa but mentioned with question marks)
Fröhner (1995a,b)	*Alchemilla* is divided into 18 sections: African *Alchemilla* sections are recognised according to Rothmaler (1937a) and Eurasian taxa are grouped in four main and nine intermediate sections
Notov and Kusnetzova (2004)	African *Alchemilla* material in five sections and Eurasian material in two sections: section *Brevicaules* (including subsect. *Chirophyllum*, *Heliodrosium* and *Calycanthum*) and section *Pentaphyllea*
Infrageneric classification of Lachemilla	
Muits ex L. f. (1781)	*Lachemilla aphanoides* is first described as *Alchemilla aphanoides*
Focke (1888)	*Lachemilla* is mentioned as a separate section for the first time
Lagerheim (1894)	*Lachemilla* is divided in two sections, sect. *Fockella* and sect. *Eulachemilla*
Rydberg (1908)	*Lachemilla* and *Zygalchemilla* are recognised as seperate genera
Perry (1929)	The genus *Lachemilla* is divided in six series (ser. *Aphanoides*, ser. *Polylepides*, ser. *Diplophyllae*, ser. *Nivales*, ser. *Obiculatae* and ser. *Pinnatae*)
Rothmaler (1937a)	The genus *Lachemilla* divided in six sections (sect. *Polylepides*, sect. *Rupestres*, sect. *Procumbentes*, sect. *Aphanoides*, sect. *Fruticulosae* and sect. *Diplophyllae*)
Notov and Kusnetzova (2004)	The genus *Lachemilla* is divided in six sections according to Rothmaler

At Munich, total DNA was extracted from both silica gel dried and herbarium material as described earlier (Bräuchler et al., 2004) using the Macherey–Nagel Nucleo Spin Plant Kit. Standard protocols for PCR did not yield any product for either herbarium or silica gel dried material. Therefore, a different approach using Phusion™ high fidelity polymerase (Finnzymes, Finland) was used. PCR were performed following manufacturer's protocol with the following cycle profile: 1′ of initial denaturation at 98 °C, 35 cycles of 30″ at 98 °C, 30″ at 53 °C, 45″ of 72 °C and a final extention for 10′ at 72 °C. The same primers as above were used and for trnL–F additonally the primers D and E (Taberlet et al., 1991). PCR products were purified using Microcon YM 100 filter devices (Millipore, USA), sequencing was performed using the Amersham Kit (Amersham, Freiburg) and an ABI 377 automated sequencer. Sequences were edited using GeneDoc (Nicholas and Nicholas, 1997).

At the Department of Botany, Stockholm University, 0.02–0.03 g of silica gel dried or herbarium material was homogenised using a mini-beadbeater (Biospec products). Total DNA was extracted through a downscaled version of the CTAB protocol described by Doyle and Doyle (Doyle and Doyle, 1990). In PCR reactions of 25 μl we used 1× buffer, 1.5 mM MgCl2, 0.2 mM of each dNTP, 0.04% BSA, 0.3 μM of each primer, 0.5 U of Taq polymerase (Roche Applied Science, Germany), and 1 μl of DNA. PCR reactions were amplified in an Eppendorf Mastercycler gradient. Amplification products were cleaned using Montage PCR96 plates (Millipore) and a vacuum manifold. Base callings were obtained by using phred (Ewing et al., 1998; Ewing and Green, 1998) and assembled with phrap (Green, 1996) integrated in the Staden package (Staden, 1996) under GNU/Linux.

Data matrices were aligned by eye, poly A or poly A/T regions were excluded from the trnLF dataset (9 bp between 497–505 and 5 bp between 834–838). Gaps were coded by hand using the simple indel-coding of Simmons and Ochoterena (2000).

2.4. Parsimony analyses (MP)

Datasets were analysed in three different ways (1) ITS and trnL–trnF regions separately, (2) separate subdivisions of this data, partitioned into ITS1, ITS2, trnL intron and trnL–trnF intergenic spacer (IGS) (the 5.8S, trnL and trnF exons were not analysed separately due to their lack of parsimony informative characters), (3) all data combined. maximum parsimony analyses were performed using PAUP* version 4.0b10 (Swofford, 2001) using heuristic searches with 1000 replicates of random addition sequence, tree-bisection-reconnection (TBR) branch swapping, MULTREE on (keeping multiple, equally parsimonious trees), saving a maximum of 50 trees each replicate. Support was assessed using 1000 replicates of non-parametric bootstrap analysis (Felsenstein, 1985).

Potential incongruence between datasets was assessed visually (i) by comparing phylogenies from individual datasets and (ii) by comparing the robustness and resolution of phylogenies from combined data versus individual gene regions. There are two well-supported incongruences between the gene trees within the Eurasian Alchemilla-clade (Figs. 4 and 5). It has been argued that in cases where incongruence is localised to particular taxa, or to specific areas of a tree, pruning of the conflicting taxa or clades may permit the datasets to be combined for analysis (Barber et al., 2007). Therefore, taxa with incongruent gene phylogenies (*A. angustata*

Table 3
Hybrid origin of sections according to Fröhner (1995a) for species with lobed leaves which were place in the Dissected-clade

Species	Section	Parental sections	Other species of the same section
A. decumbens	Sect. *Decumbentes*	*Ultravulgares* (Lobed-clade) and *Pentaphylleae* (Dissected-clade)	*A. tenuis*
A. splendens and A. faeroensis	Sect. *Splendentes*	*Ultravulgares* (Lobed-clade), *Erectae* (Lobed-clade) and *Alpinae* (Dissected-clade)	*A. aranica, A. hispanica*
A. exigua	Sect. *Plicatae*	*Ultravulgares* (Lobed-clade), *Alpinae* (Dissected-clade) and *Pentaphylleae* (Dissected-clade)	*A. colorata, A. filicaulis, A. plicata* and *A. schmidelyana*
A. angustata	Sect. *Alchemilla*	*Ultravulgares* (Lobed-clade) and *Erectae* (Lobed-clade)	*A. crinita, A. ilerdensis, A. tenerifolia* and *A. vulgaris*

Table 4
List of all taxa used in analyses including authors, voucher information and GenBank accession numbers

Genus species author	Section	Voucher: collector/no. (herbarium)	trnLF	ITS
African Alchemilla species				
A. abyssinica Fres.	Sect. Longicaules	Gehrke/BG-Af 225 (ZH)	—	EU072507
A. andringitrensis R. Viguier & De Wild.	Sect. Schizophyllae	Gehrke/BG-Af 292 (ZH)	EU072595	EU072509
A. argyrophylla Oliv.	Sect. Parvifoliae	Gehrke/BG-Af 016 (ZH)	EU072599	EU072512
A. colura Hill.	Sect. Longicaules	Gehrke/BG-Af 464 (ZH)	EU072604	EU072517
A. cryptantha Steud. ex A. Rich.	Sect. Longicaules	Gehrke/BG-Af 248 (ZH)	EU072607	EU072520
A. dewildermanii T.C.E. Fr.	Sect. Subcuneatifoliae	Gehrke/BG-Af 066 (ZH)	EU072609	EU072522
A. ellenbeckii Engl.	Sect. Longicaules	Gehrke/BG-Af 104 (ZH)	EU072610	EU072523
A. elongata Eckl. & Zeyher	Sect. Longicaules	Gehrke/BG-Af 446 (ZH)	EU072611	EU072524
A. elongensis Mildbr.	Sect. Subcuneatifoliae	Gehrke/BG-Af 140 (ZH)	EU072612	EU072525
A. fischeri Engl.	Sect. Longicaules	Gehrke/BG-Af 205 (ZH)	—	EU072529
A. gracilipes (Engl.) Engl.	Sect. Longicaules	Gehrke/BG-Af 127 (ZH)	EU072620	EU072532
A. granvikii T.C.E. Fr.	Sect. Longicaules	Gehrke/BG-Af 023 (ZH)	EU072621	EU072533
A. hageniae T.C.E. Fr.	Sect. Grandifoliae	Gehrke/BG-Af 005 (ZH)	EU072623	EU072535
A. haumanii Engl.	Sect. Grandifoliae	Gehrke/BG-Af 204 (ZH)	EU072624	EU072536
A. hildebrandtii Engl.	Sect. Schizophyllae	Gehrke/BG-Af 258 (ZH)	EU072626	EU072538
A. johnstonii Oliv.	Sect. Geraniifoliae	Gehrke/BG-Af 364 (ZH)	EU072632	EU072544
A. kiwuensis Engl.	Sect. Longicaules	Gehrke/BG-Af 223 (ZH)	EU072633	EU072545
A. microbetula T.C.E. Fr.[b]	Sect. Parvifoliae	Gehrke/BG-Af 360 (ZH)	EU072636	EU072548
A. pedata Hochst. ex A. Rich.	Sect. Pedatae	Gehrke/BG-Af 214 (ZH)	EU072642	EU072553
A. roccatii Cort.	Sect. Geraniifoliae	Gehrke/BG-Af 365 (ZH)	—	EU072557
A. rutenbergii O. Hoffm.	Sect. Schizophyllae	Gehrke/BG-Af 253 (ZH)	—	EU072558
A. schizophylla Bak.	Sect. Schizophyllae	Gehrke/BG-Af 282 (ZH)	EU072646	EU072560
A. stuhlmanii Engl.	Sect. Subcuneatifoliae	Gehrke/BG-Af 363 (ZH)	EU072649	EU072564
A. subnivalis Bak.	Sect. Subcuneatifoliae	Gehrke/BG-Af 362 (ZH)	EU072650	EU072565
A. x subnivalis	Sect. Subcuneatifoliae	Gehrke/BG-Af 370 (ZH)	EU072659	EU072575
A. triphylla Rothm.	Sect. Subcuneatifoliae	Gehrke/BG-Af 361 (ZH)	EU072655	EU072570
A. volkensii Engl.	Sect. Longicaules	Gehrke/BG-Af 205 (ZH)	—	EU072572
A. woodii Kuntze	Sect. Longicaules	Gehrke/BG-Af 453 (ZH)	EU072658	EU072574
Eurasian Alchemilla species				
A. aff. retinervis Buser	Unknown	Frost-Olsen/5690 (ZH)	EU072594	EU072556
A. alpina L.	Sect. Alpinae ser. Saxatiles	Gehrke/BG-E 392 (ZH)	EU072595	EU072508
A. angustata S.E. Fröhner	Sect. Alchemilla	Gehrke/BG-E 403 (ZH)	EU072597	EU072510
A. aranica S.E. Fröhner	Sect. Splendentes	Frost-Olsen/7713 (ZH)	EU072598	EU072511
A. atriuscula S.E. Fröhner	Sect. Alpinae ser. Hoppeanae	Frost-Olsen/12776 (ZH)	EU072600	EU072513
A. catachnoa Rothm.	Sect. Alchemilla	Frost-Olsen/389 (ZH)	EU072601	EU072514
A. charbonelliana Buser	Sect. Alpinae ser. Hoppeanae	Frost-Olsen/12908 (ZH)	EU072602	EU072515
A. colorata Buser	Sect. Plicatae	Frost-Olsen/8986 (ZH)	EU072603	EU072516
A. coriacea Buser	Sect. Coriaceae	Frost-Olsen/10872 (ZH)	EU072605	EU072518
A. crinita Buser	Sect. Alchemilla	Gehrke/BG-E 390 (ZH)	EU072606	EU072519
A. decumbens Buser[b]	Sect. Decumbentes	Frost-Olsen/8592 (ZH)	EU072608	EU072521
A. epipsila Buser[b]	Sect. Erectae	Frost-Olsen/251 (ZH)	EU072613	EU072526
A. exigua Buser ex Paulin	Sect. Plicatae	Lippert/417 (ZH)	EU072614	—
A. faeroensis (Lange) Buser	Possibly sect. Splendentes	Frost-Olsen/2000-BI-00121 (ZH)	EU072615	EU072527
A. fallax Buser	Sect. Flabellatae	Frost-Olsen/7705A (ZH)	EU072616	EU072528
A. filicaulis Huds.	Sect. Plicatae	Gehrke/BG-E 386 (ZH)	EU072637	EU072549
A. fissa Hegetschw.[b]	Sect. Calycinae	Gehrke/BG-E 395 (ZH)	EU072617	EU072539
A. flabellata Buser[b]	Sect. Flabellatae	Frost-Olsen/11859 (ZH)	EU072618	EU072531
A. sp. sect. glacalis Buser	Sect. Glaciales	Frost-Olsen/11699 (ZH)	EU072619	EU072562
A. grenieri Guillot	Sect. Alpinae ser. Saxatiles	Frost-Olsen/12695 (ZH)	EU072622	EU072534
A. heptagona Juz.[b]	Sect. Ultravulgares	Frost-Olsen/6999 (ZH)	EU072625	EU072537
A. hispanica S.E. Fröhner	Sect. Splendentes	Frost-Olsen/9065 (ZH)	EU072627	EU072539
A. ilerdensis S.E. Fröhner	Sect. Alchemilla	Gehrke/BG-E 409 (ZH)	EU072628	EU072540
A. incisa Buser	Sect. Coriaceae	Gehrke/BG-E 399 (ZH)	EU072629	EU072541
A. indivisa (Buser) Rothm.	Sect. Erectae	Frost-Olsen/3383 (ZH)	EU072630	EU072542
A. japonica Nakai & H. Hara	Sect. Villosae	Gehrke/BG-E 419 (ZH)	EU072631	EU072543
A. lapeyrousii Buser[b]	Sect. Pubescentes	Gehrke/BG-E 494 (ZH)	EU072634	EU072546
A. longana Buser	Sect. Coriaceae	Frost-Olsen/11549 (ZH)	EU072635	EU072547
A. mollis (Buser) Rothm.	Sect. Erectae	Gehrke/BG-E 420 (ZH)	EU072638	EU072550
A. montserratii S.E. Fröhner	Sect. Ultravulgares	Lippert/402 (ZH)	EU072639	EU072551
A. nitida Buser	Sect. Glaciales	Lippert/415 (ZH)	EU072640	—
A. oscensis S.E. Fröhner	Sect. Pubescentes	Gehrke/BG-E 404 (ZH)	EU072641	EU072552
A. pentaphyllea L.[b]	Sect. Pentaphylleae	Gehrke/BG-E 400 (ZH)	EU072643	EU072554
A. plicata Buser[b]	Sect. Plicatae	Frost-Olsen/11575 (ZH)	EU072644	EU072555
A. saxatilis Buser[b]	Sect. Alpinaeser. Saxatiles	Frost-Olsen/8088 (ZH)	EU072645	EU072559
A. schmidelyana Buser	Sect. Plicatae	Gehrke/BG-E 391 (ZH)	EU072647	EU072561
A. splendens Christ[b]	Sect. Splendentes	Frost-Olsen/7587 (ZH)	EU072648	EU072563
A. subsericea Reut.	Sect. Glaciales	Frost-Olsen/11984 (ZH)	EU072651	EU072566
A. tenerifolia S.E. Fröhner	Sect. Alchemilla	Frost-Olsen/407 (ZH)	EU072652	EU072567
A. tenuis Buser	Sect. Decumbentes	Frost-Olsen/9716 (ZH)	EU072653	EU072568
A. transiens (Buser) Buser	Sect. Alpinaeser. Saxatiles	Frost-Olsen/12454 (ZH)	EU072654	EU072569
A. vetteri Buser	Sect. Flabellatae	Frost-Olsen/9097 (ZH)	EU072656	EU072571
A. vulgaris (syn. of *A. acutiloba*) Stev[a]	Sect. Alchemilla	Frost-Olsen/460 (ZH)	EU072657	EU072573

(*continued on next page*)

Table 4 (*continued*)

Genus species author	Section	Voucher: collector/no. (herbarium)	trnLF	ITS
Aphanes species				
Ap. arvensis L.[b]	Sect. Quadridentatae	Rydberg/s.n. (S)	—	U90819
Ap. arvensis L.[b]	Sect. Quadridentatae	Eriksson/s.n. (SBT)	AJ512234	—
Ap. cornucopioides Lag.	Sect. Quadridentatae	J.Lambinon/96/707 (M)	EU072660	EU072576
Ap. floribunda (Murb.) Rothm.	Sect. Quadridentatae	R.Deschartes/10292 (M)	EU072661	—
Ap. innexpectata W. Lippert	Sect. Quadridentatae	Dörr/s.n. (M)	EU072662	EU072577
Ap. minutiflora (Azn.) S. Snogerup, Bothmer & M.A. Gust.	Sect. Quadridentatae	Auguier/1723 (M)	EU072663	EU072578
Ap. sp. Bolivia -	Unknown	Beck/4635 (LPB)	EU072664	EU072579
Lachemilla species				
L. angustata Romol.	Described 1996, acc. to Romoleroux Ser. Nivalis (sensu Romoleroux)	S.Laegard & I.Grignon/19394 (QCA)	EU072666	EU072581
L. aphanoides (Mutis) Rothm.[b]	Sect. Aphanoides subsect. Glomerulatae, ser. Aphanoides	Romol./4110 (QCA)	EU072667	EU072582
L. diplophylla (Diels) Rothm.[b]	Sect. Diplophylla	E.Ternews & V.Rivera/280 (QCA)	EU072668	EU072583
L. hispidula (Perry) Rothm.	Sect. Aphanoides subsect. Nivales, ser. Nivales	Romol./4119 (QCA)	EU072669	EU072584
L. holosericea (Perry) Rothm.	Sect. Aphanoides subsect. Subnivales, ser. Aphanoides	Romol./4118 (QCA)	EU072670	EU072585
L. mandoniana (Wedd.) Rothm.	Sect. Aphanoides subsect. Pachyrrhizae, ser. Pinnatae	Romol./4111 (QCA)	—	EU072586
L. mandoniana (Wedd.) Rothm.	Sect. Aphanoides subsect. Pachyrrhizae, ser. Pinnatae	P.Skelnar & V. Kosteckova/66-2 (QCA)	EU072671	—
L. nivalis (Kunth) Rothm.	Sect. Aphanoides subsect. Nivales, ser. Nivales	Romol./4000 (MSB)	—	EU072587
L. orbiculata Rydb.	Sect. Aphanoides subsect. Radicantes; ser. Orbiculatae	Romol./4115 (QCA)	EU072672	EU072588
L. pectinata (Kunth) Rothm.	Sect. Aphanoides subsect. Radicantes, ser. Orbiculatae	K. Romoleroux/4072 (QCA)	EU072673	—
L. pinnata (Ruiz. & Pav.) Rothm.	Sect. Aphanoides subsect. Pachyrrhizae, ser. Pinnatae	J.C.Solomon/17431 (QCA)	EU072674	EU072589
L. rivulorum (Rothm.) Rothm.	Sect. Aphanoides subsect. Subnivales	P.Sklenar & V. Sklenardua/2247 (QCA)	EU072675	—
L. rupestris (Kunth) Rothm.	Sect. Rupestres, ser. Aphanoides,	P.Sklenar & V.Sklenardua/3033 (QCA)	EU072676	—
L. tanacetifolia Rothm.	Sect. Rupestres, descr. in 1935 acc. to Romoleroux ser. Pinnatae	P. Skelnar & V. Kostechova/57-2 (QCA)	EU072677	EU072590
L. vulcanica Rydb.	Sect. Procumbentes, ser. Aphanoides	S. Laegaard/17701 (QCA)	EU072678	—
L. vulcanica Rydb.	Sect. Procumbentes, ser. Aphanoides	Romol./4120 (QCA)	—	EU072591
Outgroup	tribe/subtribe			
Comarum palustre L.	Fragariinae	Gehrke/ BG-E412 (ZH)	EU072665	EU072580
Comarum salesovianum (Steph.) Aschers. & Graebn.	Fragariinae	Eriksson & Vretblad/TE751 (SBT)	AJ512228	AJ511779
Dasiphora fructicosa (L.) Rydb.	Fragariinae	Karlsson/94074 (LD)	AF348557	U90809
Fragaria viridis Weston	Fragariinae	CFRA/333 (OR)	AF163550	AF163506
Potentilla reptans L.	Potentillinae	Eriksson/650 (G)	—	U90784
Potentilla reptans L.	Potentillinae	Eriksson/822 (SBT)	AJ512241	—
Potentilla stenophylla Diels	Potentillinae	Eriksson & Vretblad/TE763 (SBT)	AJ512240	AJ511780
Rosa majalis Herrm.	Roseae	Eriksson/641 (GH)	AJ512229	U90801
Sibbaldia cuneata Hornem.	Fragariinae	Gehrke/BG-K413 (ZH)	EU072679	EU072592
Sibbaldia procumbens L.	Fragariinae	Gehrke/BG-S 397 (ZH)	EU072680	EU072593
Sibbaldianthe bifurca (L.) Kurtto & T. Erikss.	Fragariinae	Karis/412 (S)	—	PBU90786
Sibbaldianthe bifurca (L.) Kurtto & T. Erikss.	Fragariinae	Eriksson/811 (SBT)	AJ512224	—
Sibbaldiopsis tridentata (Aiton) Rydb.	Fragariinae	Hill/17146 (A)	—	PTU90791
Sibbaldiopsis tridentata (Aiton) Rydb.	Fragariinae	Eriksson & Smedmark/40 (SBT)	AJ512236	—

Authors of sections and subsections:*Alchemilla* sect. Longicaules Rothm.; *Alchemilla* sect. Schizophyllae (Rothm.) Notov; *Alchemilla* sect. Parvifoliae Rothm.; *Alchemilla* sect. Subcuneatifoliae (De Wild.) Rothm.; *Alchemilla* sect. Grandifoliae Rothm.; *Alchemilla* sect. Geraniifoliae (Haum. and Balle) Rothm.; *Alchemilla* sect. Pedatae (Rothm.) Notov; *Alchemilla* sect. Alpinae Buser ex Camus; *Alchemilla* sect. Alpinae ser. Hoppeanae Buser ex Rothm.; *Alchemilla* sect. Alpinae ser. Saxatiles Buser ex Rothm.; *Alchemilla* sect. Splendentes Buser; *Alchemilla* sect. Plicatae S.E. Fröhner; *Alchemilla* sect. Coriaceae S.E. Fröhner, *Alchemilla* sect. Decumbentes S.E. Fröhner; *Alchemilla* sect. Erectae S.E. Fröhner; *Alchemilla* sect. Flabellatae S.E. Fröhner; *Alchemilla* sect. Calycinae Buser; *Alchemilla* sect. Glaciales S.E. Fröhner; *Alchemilla* sect. Ultravulgares S.E. Fröhner; *Alchemilla* sect. Calycinae Buser; *Alchemilla* sect. Villosae Rothm; *Alchemilla* sect. Pubescentes Buser; *Alchemilla* sect. Pentaphylleae Buser ex Camus; Aphanes sect. Quadridentatae Rothm.

[a] Genus type.

[b] Section type.

and *A. decumbens*), were removed and the remaining dataset reanalysed (termed here "combined analysis").

2.5. Bayesian inference

Bayesian analysis was performed as implemented in MrBayes 3.1.2. (Huelsenbeck and Ronquist, 2001). Applying the Akaike Information Criterion using MrModeltest (Nylander et al., 2004) based on Modeltest (Posada and Crandall, 1998), the general time reversible GTR model with gamma distributed rates was identified as best fitting the sequence data of ITS1, ITS2 and trnLF. For the 5.8S, SYM + G was identified as the best fitting model and GTR + G + I as the best model in the combined dataset. Bayesian analysis was carried out for each of the partition sets including the coded gaps. GTR + G + I was then used in the combined Bayesian analysis, and the parameter values of the different partitions were allowed to vary independently. For each partition, four chains (three hot, one cold) were run in two parallel runs for 2,000,000 generations for the separate analysis and 3,000,000 generations for the combined analysis, each sampling every 1000 generations. The burnin was set to 100 tree or 100,000 generations for each run of the separate analyses and 200 trees or 200,000 generations for each run of the combined analysis (determined empirically from the log-likelihood values using Tracer; Rambaut and Drummond,

2003–2007). A combined consensus tree of the last 3802 tree or 5602 trees, respectively, was constructed and clade credibilities for the bipartitions recorded as a measure of node support.

3. Results

The length of the ITS sequences included in the final data matrix was very uniform around 624 bp. The aligned matrix including outgroups consisted of 665 bp, including 299 variable base pairs of which 208 bp were parsimony informative in addition to 14 parsimony informative characters from the indel-coding. The length of the *trn*LF sequences ranged from 643 to 853 bp. The aligned matrix consisted of 96 taxa with a total alignment of 1241 bp and 33 characters from the indel-coding, including 252 variable base pairs of which 134 bp were parsimony informative. A number of large indels were observed in the *trn*LF intergenic spacer (IGS). Unique insertions were observed in *A. pentaphyllea* (a duplication of 25 bp) and in *A. schizophylla* (28 bp); *A. kiwuensis* and *A. abyssinica* shared an identical insertion of 58 bp. There was a large deletion of 210 bp in *A. microbetula*, *A. roccatii* and *A. haumanii*. Topologies were unaffected when these indels were excluded from analyses (data not shown).

Four clades were retrieved in all analyses (Parsimony and Bayesian): *Aphanes*, Eurasian *Alchemilla*, African *Alchemilla* and *Lachemilla* with high (75–89) or very high (90–100) bootstrap support values as well as clade credibilities above 0.96 (Figs. 4–6).

3.1. The Eualchemilla-clade

The monophyly of the Eurasian species of *Alchemilla* was well supported in all analyses. We will henceforth refer to this group as the Eualchemilla-clade. It comprises two major clades, which we will refer to as the Lobed-clade and the Dissected-clade. Most taxa in the Lobed-clade clade have lobed or not entirely dissected leaves, and most taxa found in the Dissected-clade have entirely dissected leaves (or nearly so), exeptions in the Dissected-clade are *A. angustata, A. decumbens, A. exigua, A. faeroensis* and *A. splendens*.

The monophyly of the Lobed-clade received high support values in the ITS and the combined analysis but low parsimony bootstrap support in the *trn*LF analysis. Resolution within the Lobed-clade is low due to the low sequence variability (6 bp in the *trn*LF dataset and 9 bp in the ITS sequence alignment within the reduced taxon sampling). *A. japonica,* the only sample of an East Asian *Alchemilla*, was in all analyses nested well within the Lobed-clade.

The monophyly of the Dissected-clade also received high support values in the ITS and the combined analysis and low parsimony bootstrap support in the *trn*LF analysis. *A. pentaphyllea* was retrieved together with *A. decumbens* in the *trn*LF analysis as sister to the other members of the Dissected-clade and alone as sister to other members of the Dissected-clade in the combined Baysian analysis. However, this relationship collapsed in the strict consensus of the combined MP analyses. The Resolution within the Dissected-clade was low with some support for a sister species relationship between *A. saxatilis* and *A. transiens*. In the separate MP analyses of chloroplast and nuclear ITS data, *A. decumbens* and *A. splendens* were placed in the Lobed-clade (according to *trn*LF), and in the Dissected-clade (according to ITS) (marked with asterisk in Figs. 4 and 5). *A. faeroensis* and *A. angustata* were in both the *trn*LF and the ITS analyses placed well within the Dissected-clade despite their lobed leaf morphology (marked with pluses in Figs. 4–6). *A. nitida* and *A. exigua* were placed in the *trn*LF analysis in the Dissected-clade but ITS data is missing (marked with circles in Figs. 5 and 6). The combined analysis yields high support values with respect to the monophyly of the Dissected-clade but no support within the clade (Fig. 6).

3.2. The Afromilla-clade

A strongly supported clade including all the African *Alchemilla* species was received in all analyses. We will henceforth refer to this as the Afromilla-clade. The 29 taxa included in the analysis represent 50–80% of all species that occur in Sub-Saharan and southern Africa (depending on the number of species recognised). These represent all recognised sections of *Alchemilla* in Africa, their full geographical distribution, all life forms, and a broad range of ecological preferences and morphological variation. Within the Afromilla-clade the number of variable characters is low and therefore neither the topologies of the gene trees nor of the combined analysis are well resolved. Even though the resolution within the Afromilla-clade is low, there seems to be some support for patterns in distribution and life form: all taxa from southern Africa (*A. colura*, *A. woodii* and *A. elongata*) form a clade with moderate support values at least in the combined analysis. Some of the accessions from Madagascar (*A. andringitrensis*, *A. cryptantha* and *A. rutenbergii*) form a well-supported clade in the combined analyses and form a clade with low support in the combined Bayesian analyses together with the other accessions from Madagascar (*A. schizophylla* and *A. hildebrandtii*; data not shown). The third supported clade is a clade of *A. kiwuensis* and *A. volkensii*. A sister-group relationship between *A. kiwuensis* and *A. volkensii* is supported in the ITS and combined analyses, data for the *trn*LF region are missing. Two more clades with low to moderate support values in the combined analysis are present, one comprising *A. ellenbeckii* and *A. microbetula* and another comprising the two dwarf shrubs *A. roccatii* and a hybrid of *A. subnivalis*.

3.3. The Aphanes-clade

A clade including all the sampled species of *Aphanes* (the Aphanes-clade) was well supported in all analyses (100 bootstrap and 1.00 c.c., with exception of the 0.96 c.c. in the *trn*LF analysis). It is sister to the Eualchemilla-clade. The small Mediterranean *Ap. minutiflora* was well supported as sister to the widespread Eurasian *Ap. arvensis* in the ITS and the combined analyses, whereas this is contradicted in the *trn*LF analysis, however not strongly supported. The South American species form a clade together with the more robust *Ap. floribunda*, again only in the ITS and the combined analyses. *Ap. bachitii* (from Ethiopia) and *Ap. parodii* (from South America), which have been hypothesised to be the most basal members of the clade based on their morphology, were not included in the analyses due to the lack of material and/or difficulties in PCR amplification. Further conclusions on the geographical origin of this clade will have to await denser taxon sampling.

3.4. The Lachemilla-clade

The Lachemilla-clade was supported with moderate to high values in the separate analyses and support was increased considerably in the combined analysis. The clade was sister to the Afromilla-clade in the *trn*LF-analysis and sister to a combined Aphanes- and Eualchemilla-clade but with very weak support in the ITS analysis. It is notable that the support for the clade comprising the Lachemilla-clade and the Afromilla-clade decreased in the combined analysis. This indicates a conflict in the data, not just a lack of resolution in the ITS data. Combining the datasets did not result in improved resolution in this part of the topology. *Lachemilla* showed the highest amount of sequence divergence within the analysed dataset which led to a better resolution within the clade, though this was weakly supported which might also be a result of the sparse taxon sampling. Four clades however seem to be more reliable and are retrived in all analyses (where sequence information was present). One comprises *L. pectinata* and *L. orbiculata,* a

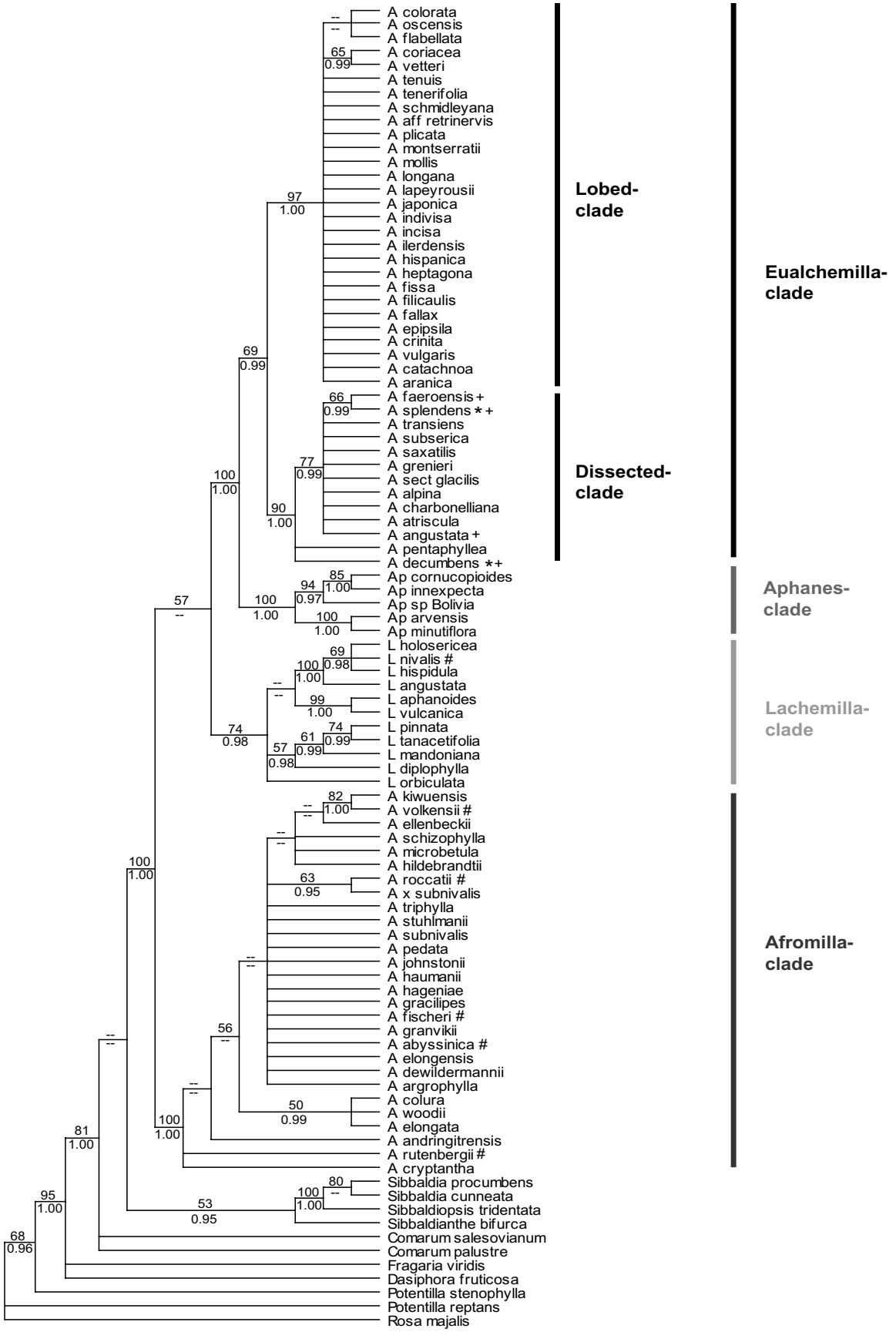

Fig. 4. Strict consensus tree based on the nuclear ITS region reconstructed using maximum parsimony; bootstrap support values above 50 are given above branches and Bayesian clade credibility values above 0.95 are given below branches. Asterisks indicate species that where removed from the dataset for the combined analysis because of incongruences between chloroplast and nuclear data. Hashes indicate species for which only nuclear sequences are present. Pluses indicate taxa placed in the Dissected-clade despite their lobed leaves.

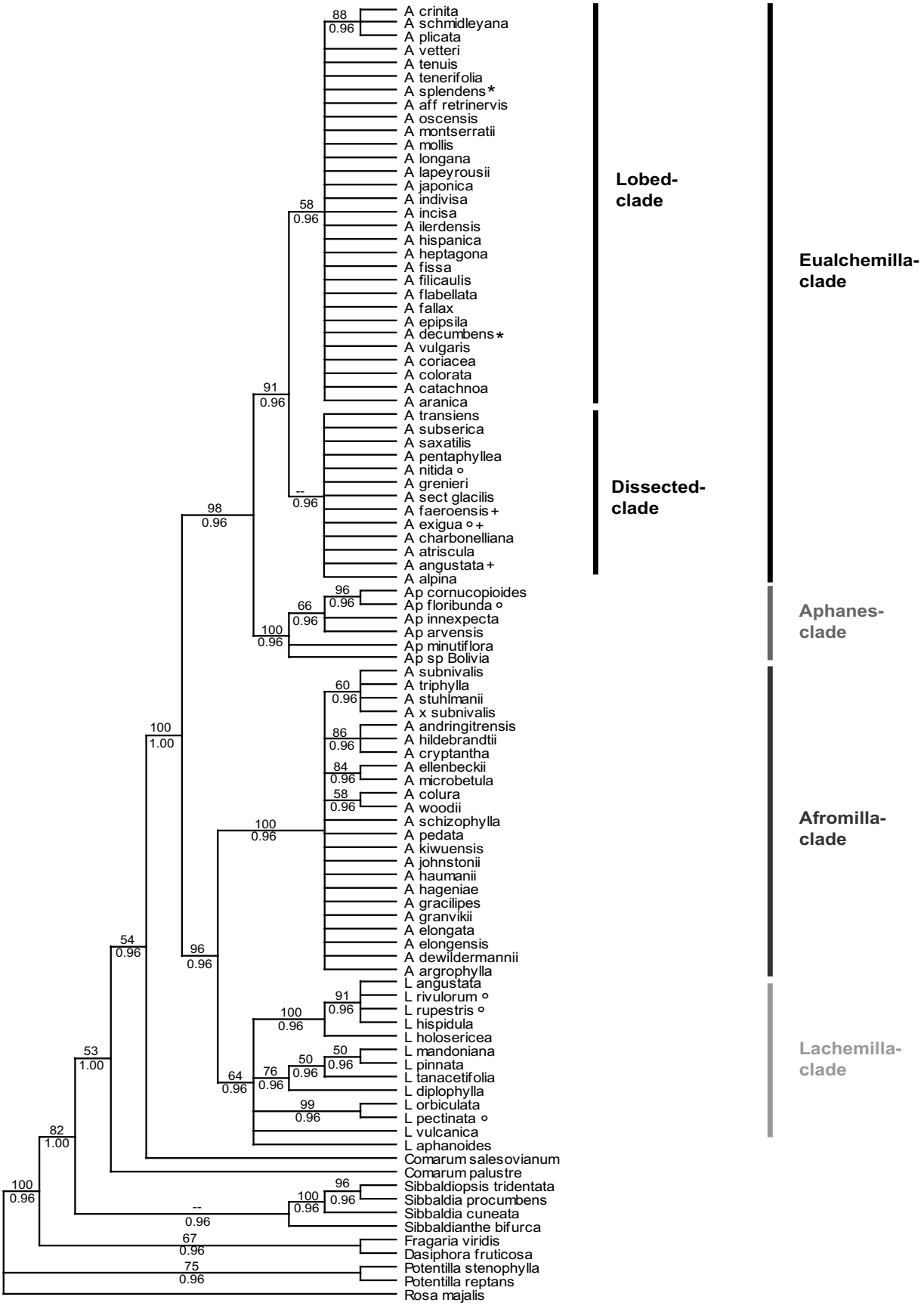

Fig. 5. Strict consensus tree based on the chloroplast *trn*L–*trn*F region reconstructed using maximum parsimony; bootstrap support values above 50 are given above branches and Bayesian clade credibility values above 0.95 are given below branches. Asterisks indicate species that where removed from the dataset for the combined analysis because of incongruences between chloroplast and nuclear data. Circles indicate species for which only chloroplast sequences are present. Pluses indicate taxa placed in the Dissected-clade despite their lobed leaves.

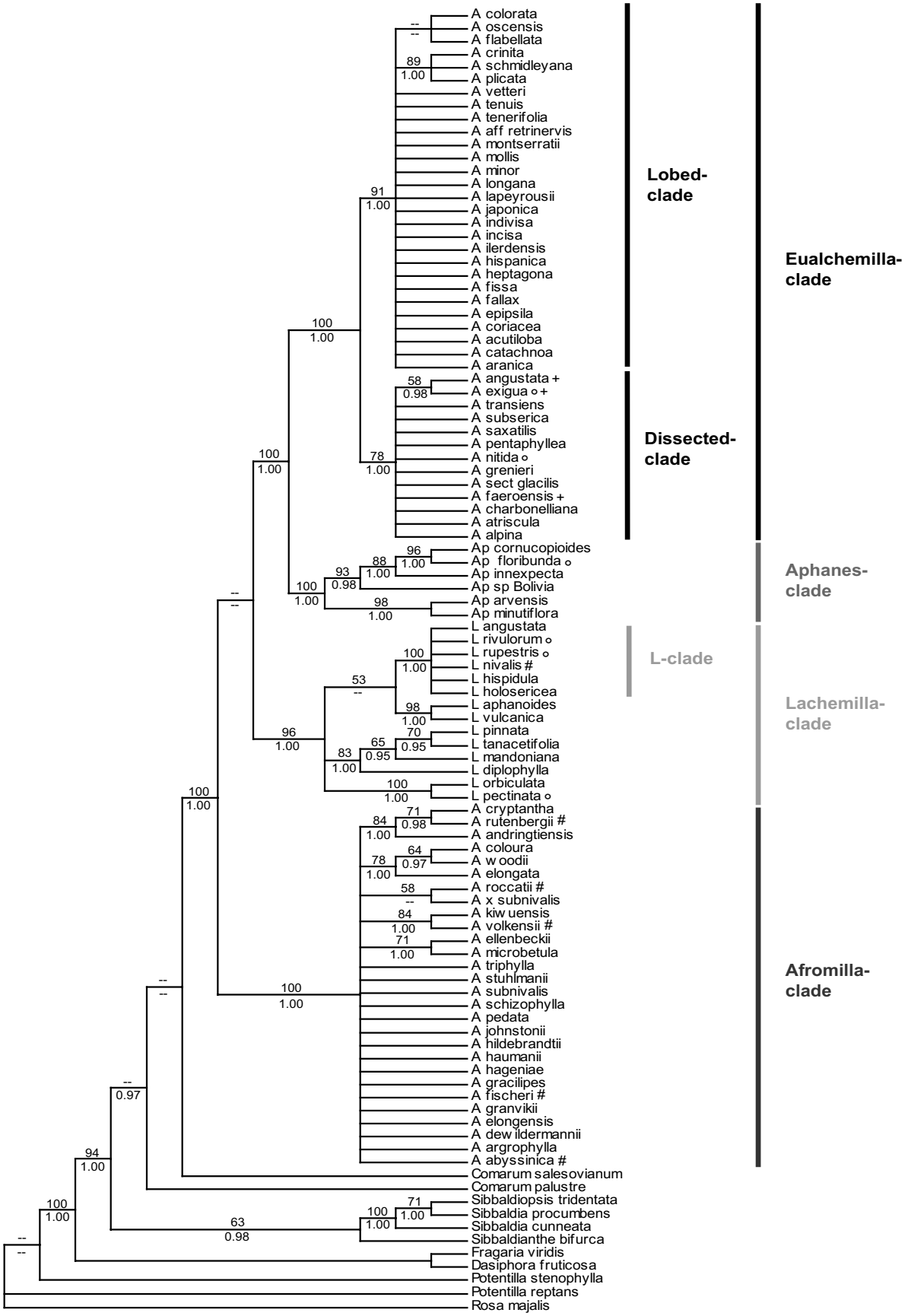

Fig. 6. Strict consensus tree based on the combined dataset reconstructed using maximum parsimony; bootstrap support values above 50 are given above branches and Bayesian clade credibility values above 0.95 are given below branches. Circles indicate species for which only chloroplast sequences are present. Hashes indicate species for which only nuclear sequences are present. Pluses indicate taxa placed in the Dissected-clade despite their lobed leaves.

second with four species, within which *L. diplophylla* is sister to a clade including *L. mandoniana*, *L. pinnata* and *L. tanacetifolia*, a third contains *L. aphanoides* and *L. vulcanica* and the fourth contains *L. holosericea, L. nivalis* and four others.

4. Discussion

The results of the molecular phylogenetic reconstruction were significant at a number of levels, despite incomplete resolution and some evidence for conflict between the data partitions.

4.1. Taxonomic implications

Pfeil and Crisp (2005) argue that "because there is no objective way to measure the degree of character similarity within a group of species, there is no phenetic criterion by which to decide whether to recognise a group of similar organisms as a genus, family or other rank". That is, genera are essentially a matter of opinion. Generic revisions tend to focus either on clarification of membership of monophyletic groups deemed a priori to be genera, or on arbitrary re-classification of accepted monophyletic groups to generic rank. Three factors are considered to be of primary importance in making taxonomic (re-)classifications, especially on assigning generic rank: (i) monophyly combined with (ii) morphological synapomorphies and (iii) nomenclatural stability. Nomenclatural stability especially applies to genera (Pfeil and Crisp, 2005; Scotland and Sanderson, 2004) since in the binominal system a change in genus name consequently changes the names of all species within.

Circumscribing *Alchemilla* in the wide sense, including the four monophyletic groups Eualchemilla-, Aphanes-, Lachemilla- and the Afromilla-clade on a subgeneric or informal level fits all three mentioned criteria.

4.1.1. Monophyly

Our analyses have shown that *Alchemilla* in the wide sense is monophyletic and nested within the subtribe Fragariinae (cf. Eriksson et al., 2003). It comprises four very well-supported clades. If *Alchemilla* in the wide sense is recognised at the rank of genus, thus including the Eualchemilla-, Aphanes-, Lachemilla- and Afromilla-clades, then the principle of monophyly will be satisfied at all taxonomic levels. The same is not true for any other of the solutions discussed. If *Alchemilla* in the wide sense is recognised at the rank of subtribe (Alchemillinae), including the three genera *Alchemilla*, *Aphanes* and *Lachemilla*, both *Alchemilla* and the subtribe *Fragariinae* are paraphyletic, the latter because Alchemillinae is nested within Fragariinae. If four genera are recognised: *Alchemilla* (the Eualchemilla-clade), *Aphanes*, *Lachemilla* and "*Afromilla*" (African *Alchemilla* as a new genus), these are monophyletic but *Fragariinae* remains paraphyletic. Therefore, if the principle of monophyly is to be applied, it will be necessary to abandon the subtribe Alchemillinae and either apply the name *Alchemilla* in a broader sense, or to group its constituent species in four genera rather than three.

4.1.2. Morphological synapomorphies

Alchemilla in the wide sense as a monophyletic group can be recognised by floral synapomorphies: lack of petals and presence of two whorls of four calyx and four epicalyx lobes that form a hypanthium. (Fig. 1). If Alchemillinae is recognised as a tribe including the four genera Eurasian *Alchemilla*, *Aphanes*, *Lachemilla* and "*Afromilla*", then new diagnostic morphological characters have to be found to separate Eurasian *Alchemilla* from African "*Afromilla*". Notov and Kusnetzova (2004), worked extensively to assess the taxonomic delimitations within Alchemillinae. They did not report any characters on which it would be possible to separate the Eurasian from the African sections of *Alchemilla*. This is also the conclusion that we have reached, despite having had the benefit of a robust phylogeny with which to focus investigation into the issue.

4.1.3. Nomenclatural stability

The classification of *Alchemilla* has undergone a number of changes (Table 1). We will here only outline the most important ones: *Alchemilla* was first described by Linnaeus (1753). The first groupings of species were presented by Buser (1892) in which he treated the Eurasian taxa only. His system was refined by Rothmaler (1934–37a, b) and Walters and Pawlowski (1968) and corrected for the Eurasian sections by Plocek (1982). Hauman and Balle (1936) based their classification mainly on life forms, shoot type and leaf dissection but did not complete their work. Hedberg (1957) concluded from his study of the Afroalpine *Alchemilla* species that these taxa cannot be split into distinct micro-species and recognised five African and East-Asian Sections. Fröhner (1995a) revised the European species for Flora Europea and for the Flora Iberica (Fröhner, 1998). Notov and Kusnetzova (2004) tried to unravel the taxonomic relationships in Alchemillinae by using architectural units. Most authors agree on recognising three different groups on a subgeneric or generic level (*Alchemilla*, *Aphanes* and *Lachemilla*), placing taxa from tropical and southern Africa in separate sections within *Alchemilla* based on biogeography, without describing diagnostic characters which would separate all African material from the Eurasian species. Thus they do not indicate that the African Alchemilla species represent a separate subgenus or genus distinct from an Eurasian Alchemilla subgenus or genus.

Aphanes was first described by Linnaeus (1753) on the basis of material of *Ap. arvensis*, but he misinterpreted the stamen that is inserted at the inner side of the discus as an additional stigma. Scopoli reunited *Aphanes* with *Alchemilla* in 1772, but Persoon resurrected *Aphanes* in 1805 after more species had been discovered in South America. De Candolle (1825) treated *Aphanes* again as a section of *Alchemilla* whereas Rothmaler initially treated *Aphanes* as a subgenus (1935) but changed his opinion in 1937 where he raised *Aphanes* and *Lachemilla* from the rank of subgenera to that of genera. Some authors have since followed his recommendation and treated them as separate genera, whilst others such as Kalkman (2004) have not.

Lachemilla was first described as a section of *Alchemilla* by Focke (1888). Lagerheim (1894) raised it to genus level, a view that was followed by Rydberg (1908) who additionally recognised the genus *Zygalchemilla*. Murbeck (1915) treated *Lachemilla* together with *Aphanes* again as a section of *Alchemilla*. The first revision of *Lachemilla* was conducted by Perry in 1929 who recognised 41 species in 6 series within the section *Lachemilla*. Rothmaler (1935) was the first to give *Lachemilla* the rank of a subgenus and then later revised this position and gave it genus rank again (Rothmaler 1937b) creating 72 new combinations. More recently, authors like Gaviria (1996) and Romoleroux (1996) have used the rank of a genus in their regional treatments of *Lachemilla*.

It is our opinion, that recognising *Alchemilla* as a single genus will lead to greater stability whilst minimising taxonomic changes. Therefore, we prefer the principle of monophyly to be given preference, and apply the name *Alchemilla* in a broader sense, rather than using *Aphanes*, *Lachemilla* and two separate genera of *Alchemilla* which cannot be distinguished on criteria other than their geographic distribution. If new characters are identified, which can be used as synapomorphies for the African clade, it might be that having four separate genera would be preferable. At this time, however, it seems that arguments for nomenclatural stability strongly support keeping all of these species in *Alchemilla*.

4.2. Robust phylogenetic hypothesis for Eualchemilla-clade

The monophyly of the Eurasian species of *Alchemilla* was well supported and this group was very well supported as sister to the Aphanes-clade. Within the Eualchemilla-clade two subsections (Dissected- and Lobed-clade) are very well supported by the molecular phylogenetic reconstruction (Fig. 6). Some of the earliest authors have proposed a differentiation between groups of species of *Alchemilla* in the strict sense on the basis of the level of dissection of their leaves. Especially noteworthy here is the classification-system proposed by Buser (1892), which was refined by Rothmaler (1934). Rothmaler recognised the section *Brevicaules* with the two subsection *Alpinae* (the Dissected-clade) and subsection *Vulgares* (the Lobed-clade) and subsequently gave *Pentaphylleae* the rank of a separate section. Later this distinction between subsections *Alpinae, Vulgares* and *Pentaphylleae* within the Eurasian *Alchemilla* was dropped and several other species groups were included at the same subsectional or sectional rank (Fröhner, 1995a). The results of the molecular phylogenetic analysis are congruent with the earlier authors that proposed three subsections (*Alpinae, Vulgares* and *Pentaphylleae*) based on their morphology. However the sister species relationship of *A. pentaphyllea* to the rest of the Dissected-clade (section *Alpinae*) is not supported in the combined analysis and there are some exceptions of species with lobed leaves in the Dissected-clade (*A. decumbens* and *A. splendens* for one marker whilst the other marker places them in the Lobed-clade; *A. exigua,* for which only one marker is available and *A. angustata* and *A. faeroensis* for both markers). However, these taxa are from sections of putative hybrid origin (Table 3). All the examples in which morphology does not appear to agree with the molecular phylogenetic results indicated an incorrect placement of taxa with lobed leaves in the Dissected-clade. No taxon with dissected leaves was placed in the Lobed-clade in any of the analyses.

4.3. Biogeography of the Afromilla-clade

The strong separation of the African and the Eurasian *Alchemilla* species is a striking pattern that has not been postulated before. It is interesting to note that there seems to have been only a single dispersal between the two areas leading to two well-supported monophyletic groups. Within the Afromilla-clade the genetic variability is higher than in the Eurasian Alchemilla-clade, which might be due to higher levels of sexual reproduction in these species as proposed by some authors on the basis of pollen viability (Hedberg, 1957; Hedberg, 1986; Fröhner, 1995a). Functional pollen is unnecessary for plants that exhibit autonomous apomixis, which is supposed to be coupled with degenerative phenomena, such as meiotic disturbance, which may interfere with pollen formation. In the European sections of *Alchemilla* the pollen is aborted and seeds develops precociously (before anthesis) in the flower (Izmailow, 1994; Fröhner, 1995a). Hedberg (1957) suggested that the Afroalpine *Alchemilla* species might not be obligate apomictic, referring to high levels of pollen production in some of these taxa. However, this could reflect pseudogamous (rather than autonomous) apomixis, and therefore does not necessarily prove that they are not apomictic at all, or that they are facultative apomicts.

The resolution within the Afromilla-clade is low, with some support for patterns in distribution (represented by the two clades in southern Africa and Madagascar). Resolution is insufficient to allow meaningful reconstruction of ancestral states, and the lack of cytological data does not allow for the reconstructions of the role of polyploidy in the occupation of the Afrotemperate regions. More variable markers or population-level molecular techniques would need to be applied to be able to make further inferences at this level of relatedness. For example, a population based analysis of the dwarf shrubs endemic to the Ruwenzori Mountain range might give more insights into the historical development of the mountain chain that has so uniquely given rise to a number of co-occurring *Alchemilla* species.

4.4. Lachemilla-clade

This analysis represents the first assessment of monophyly of *Lachemilla.* Further research, however, including analysis of more species is needed to address questions of, natural subgroupings, biogeography and the migration history of *Lachemilla.*

Relationships within *Lachemilla* as revealed by our analyses are to some extent congruent with those based on morphological data. However, the taxon sampling is low, thus a more complete sampling might reveal other relationships. We will therefore give here only a single example were molecular data point towards a possible relationship not inferred from morphological data (marked as the L-clade in Fig. 6). *L. rupestris* is nested here in section *Lachemilla* (which corresponds to series or section *Nivales* but in terms of nomenclature, the correct name should be *Lachemilla* as *L. nivalis* is the type not only of the section but also of the subgenus/genus), however its morphological characteristics do not support this. The section *Lachemilla* is highly supported in all analyses and corresponds well to the very characteristic leaf-morphology of this group, with the exception of the newly discovered relationship of *L. rupestris.*

4.5. Incongruences in the Eualchemilla-clade: hybridisation/introgression or incomplete lineage sorting?

Interspecific hybridisation, especially in case of allopolyploidy, is one of the most important factors leading to phylogenetic incongruence between loci of the plastid and nuclear genomes. The most extreme case is chloroplast capture, a process that can occur at a variety of taxonomic levels (Rieseberg and Soltis, 1991), i.e. cpDNA introgression can occur in the absence of analogous nrDNA gene introgression. As a result, clustering taxa on the basis of chloroplast DNA can fail to correspond to taxonomic units, groups supported by analysis of morphological characters, or clades indicated by nuclear markers because either the chloroplast of these taxa is derived (captured) from another taxon (e.g. in *Heuchera* group (Saxifragaceae) Soltis and Kuzoff, 1995; Veroniceae in Albach and Chase, 2004; *Achillea* (Asteraceae) Guo et al., 2004; *Hieracium* (Asteraceae) Fehrer et al., 2007) or several independent chloroplast lineages are present in a single taxon (e.g. in *Hordeum* (Poaceae) Jakob and Blattner, 2006).

In the Eualchemilla-clade, and possibly in all taxa of *Alchemilla*, recent hybridisations are thought to be extremely rare due to their reproduction via autonomous apomixis and the absence of diploids (Fröhner, 1995a). However, hybridisation events are likely to have been more frequent in the past, when there were still diploid species present. In many other well researched groups with abundant apomictic reproduction such as *Hieracium* (Fehrer et al., 2007), *Rubus* (Alice and Campbell, 1999), *Taraxacum* (Kirschner et al., 2003) or the *Ranunculus auricomus* complex (Hörandl, 2004; Hörandl et al., 2005) hybridisation, facultative apomixis and introgression, as revealed by incongruent gene phylogenies, has been hypothesised as having played an important role in the evolution of the groups. Therefore, we suggest that one possible explanation of the observed incongruence in the cpDNA and nrDNA data in the Eualchemilla-clade is due to hybridisation/introgression including cpDNA haplotype capture. All species with incongruent placement in the Lobed- or Dissected-clade have previously been placed in putative hybrid sections based on their morphology (Table 3). The observed placement of taxa (*A. decumbens* and *A. splendens)* in the Lobed-clade for the cpDNA sequences and not the Dis-

sected-clade (as according to the nrDNA) could then be interpreted as chloroplast capture as a result of hybridisation. However, it is interesting to note that not all members of putative hybrid sections show an incongruent pattern, and that multiple sequencing of different individuals of the same species resulted in identical sequences for both chloroplast and nuclear markers.

A second possible explanation of the observed data is incomplete lineage sorting, the persistence of ancestral polymorphisms through speciation events (Wendel and Doyle, 1998; Linder and Rieseberg, 2004; Jakob and Blattner, 2006). In this, different copies of ITS can homogenise to either paternal or maternal copy, and thus hide hybrid origins (Alvarez and Wendel, 2003) as possibly the case in *A. faeroensis* and *A. angustata*. This homogenisation, known as concerted evolution, arises through mechanisms such as unequal crossing over and high-frequency gene conversion (Alvarez and Wendel, 2003). Therefore, concerted evolution can only occur given meiosis. In Eurasian *Alchemilla* the central nucleus is formed without meiosis from unreduced egg cells or somatic cells and the embryo is produced pathenogenetically (without fertilisation). Thus it is asummed that in most members of Eurasian *Alchemilla* meiosis is circumvented, an assumption that is further supported by the high level of ploidy in combination with a relatively frequent occurrence of uneven number of chromosomes (Asker and Jerling, 1992). Lack of meiosis would effectively halt the processes of concerted evolution. A possible alternative explanation might be that meiosis is not always interrupted completely. As we have no reliable measurements of the level of meiosis that occurs in *Alchemilla*, it is difficult to estimate whether the observed incongruence is an artefact from the time when *Alchemilla* was reproducing sexually and that hybridisation has given rise to such a large number of lineages or micro-species, or whether there is an ongoing process of faculative sexual reproduction or meiotic processes in the formation of the egg cell.

5. Conclusions and future research

We provide here the first molecular phylogeny of the Alchemillinae. Four distinct clades are revealed: the Eurasian Alchemilla-clade, Aphanes-clade, Lachemilla-clade and the African Afromilla-clade. We suggest treating *Alchemilla*, *Aphanes* and *Lachemilla* as a single genus *Alchemilla,* based on the lack of evident characters for the identification and description of the Afromilla-clade at the rank of genus (without which *Alchemilla* would be rendered paraphyletic with respect to *Aphanes* and *Lachemilla*) and nomenclatural stability, because relatively few new combinations will have to be made.

Future research might reveal still more complex patterns in the evolution of *Alchemilla*. Sampling of many individuals and possibly cloning for multiple haplotypes may be necessary to confirm the basic patterns presented in this paper. Species relationships within the clades remain largely unresolved due to low genetic variability and possible recent speciation. Different molecular techniques or markers such as AFLPs or ISSRs, or more variable genomic regions may have to be applied to be able to resolve relationships at this level of relatedness. Taxonomic implications from this study, corroborated by partial revisions and cytological investigations of *Lachemilla* and a revision of *Aphanes* are in preparation and shall be presented elsewhere.

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References

Albach, D.C., Chase, M.W., 2004. Incongruence in Veroniceae (Plantaginaceae): evidence from two plastid and a nuclear ribosomal DNA region. Mol. Phylogenet. Evol. 32, 183–197.

Alice, L.A., Campbell, C.S., 1999. Phylogeny of Rubus (Rosaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. Am. J. Bot. 86 (1), 81–97.

Alvarez, I., Wendel, J.F., 2003. Ribosomal ITS sequences and plant phylogenetic inference. Mol. Phylogenet. Evol. 29 (3), 417–434.

Asker, S.E., Jerling, L., 1992. Apomixis in Plants. CRC Press, Boca Raton, Florida.

Barber, J.C., Finch, C.C., Francisco-Ortega, J., Santos-Guerra, A., Jansen, R.K., 2007. Hybridization in Macaronesian *Sideritis* (Lamiaceae): evidence from incongruence of multiple independent nuclear and chloroplast sequence datasets. Taxon 56 (1), 74–88.

Bräuchler, C., Meimberg, H., Heubl, G., 2004. Molecular phylogeny of the genera *Digitalis* L. and *Isoplexis* (Lindley) Loudon (Veronicaceae) based on ITS- and trnL–F sequences. Plant Syst. Evol. 248, 111–128.

Buser, R., 1892. Nouvelle classification du genre *Alchemilla*. Bull. Soc. Bot. Lyon sér 2 10, 34–35.

Czapik, R., 1996. Problems of apomictic reproduction in the families Compositae and Rosaceae. Folia Geobot. Phytotax. 31 (3), 381–387.

De Candolle, A.P., 1825. *Alchemilla*. In: Prodr. Syst. Nat. Regn. Veg., vol. 2, Treuttel et Würtz, Paris, p. 590.

Doyle, J.J., Doyle, J.L., 1990. Isolation of plant DNA from fresh tissue. Focus 12, 13–15.

Eriksson, T., Donoghue, M.J., Hibbs, M.S., 1998. Phylogenetic analysis of *Potentilla* using DNA sequences of nuclear ribosomal internal transcribed spacers (ITS), and implications for the classification of Rosoideae (Rosaceae). Plant Syst. Evol. 212, 155–179.

Eriksson, T., Hibbs, M.S., Yoder, A.D., Delwiche, C.F., Donoghue, M.J., 2003. The phylogeny of Rosoideae (Rosaceae) based on sequences of the internal transcribed spacers (ITS) of nuclear ribosomal DNA and the trnL/F region of chloroplast DNA. Int. J. Plant Sci. 164 (2), 197–211.

Ewing, B., Green, P., 1998. Basecalling of automated sequencer traces using phred. II. Error probabilities. Genom. Res. 8, 186–194.

Ewing, B., Hillier, L., Wendl, M., Green, P., 1998. Basecalling of automated sequencer traces using phred. I. Accuracy assessment. Genom. Res. 8, 175–185.

Fedde, 1910. Bot. Jarb. XXXVI (pt. 2), 496.

Fehrer, J., Gemeinholzer, B., Chrtek Jr, J., Bäutigam, S., 2007. Incongruent plastid and nuclear DNA phylogenies reveal ancient intergeneric hybridization in *Pilosella* hawkweeds (*Hieracium* Cichorieae Asteraceae). Mol. Phylogenet. Evol. 42, 347–361.

Felsenstein, J., 1985. Confidence limits on phylogenies: and approach using the bootstrap. Evolution 39 (4), 783–791.

Focke, W.O., 1888. *Alchemilla*. In: Engler, A., Prantl, K. (Eds.), Die Natürlichen Pflanzenfamilien, vol. 3, Berlin, p. 43.

Fröhner, S.E., 1986. Zur infragenerischen Gliederung der Gattung *Alchemilla* L. in Eurasien. Gleditsia 14 (1), 3–49.

Fröhner, S.E., 1995a. *Alchemilla*. In: Scholz, H., Conert, H.J., Jäger, E.J., Kadereit, J.W., Schultze-Motel, W., Wagenitz, G., Weber, H.E. (Eds.), Hegi: Illustrierte Flora von Mitteleuropa, vol. 4 Teil 2B. Verlag Paul Parey, Berlin, Germany, pp. 13–242.

Fröhner, S.E., 1995b. *Aphanes*. In: Scholz, H., Conert, H.J., Jäger, E.J., Kadereit, J.W., Schultze-Motel, W., Wagenitz, G., Weber, H.E. (Eds.), Hegi: Illustrierte Flora von Mitteleuropa, vol. 4 Part 2B. Verlag Paul Parey, Berlin, Germany, pp. 242–248.

Fröhner, S.E., 1998. Notulae taxonomicae, chorologicae, nomenclaturales, bibliographicae aut philologicae in opus "Flora Iberica" intendentes: new *Alchemilla* species (Rosaceae) of the Iberian flora: part 5. Anales Jard. Bot. Madrid 56 (2), 405–407.

Gaviria, J., 1996. Sinópsis del género *Lachemilla* (Focke) Rydberg (Rosaceae) para Venezuela. Plantula 1 (3), 189–212.
Guo, Y.P., Ehrendorfer, F., Samuel, R., 2004. Phylogeny and systematics of *Achillea* Asteraceae–Anthemideae inferred from nrITS and plastid trnL–F DNA sequences. Taxon 53, 657–672.
Hauman, L., Balle, S., 1936. Les *Alchemilla* de l'Abyssinie et de Madagascar. Bull. Jard. Bot. Bruxelles 14, 1–55.
Hedberg, O., 1957. Afroalpine vascular plants. a taxonomic revision. Symb. Bot. Upsal. XV, 411.
Hedberg, O., 1986. Taxonomic notes on Ethiopian Rosaceae. Nord. J. Bot. 6 (5), 573–579.
Hörandl, E., 2004. Comparative analysis of genetic divergence among sexual ancestors of apomictic complexes using isozyme data. Int. J. Plant Sci. 165 (4), 615–622.
Hörandl, E., Paun, O., Johansson, J.T., Lehnebach, C., Armstrong, T., Chen, L., Lockhart, P., 2005. Phylogenetic relationships and evolutionary traits in *Ranunculus* s.l. (Ranunculaceae) inferred from ITS sequence analysis. Mol. Phylogenet. Evol. 36, 305–327.
Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.
Hutchinson, J., 1964. The Genera of Flowering Plants. Oxford University Press, Oxford.
Izmailow, R., 1994. Embryo and endosperm relations at early stages of their development in *Alchemilla* subsect. *Heliodrosium* (Rosaceae). Pol. Bot. Stud. 8 (0), 61–67.
Jakob, S.S., Blattner, F.R., 2006. A chloroplast genealogy of *Hordeum* (Poaceae): long-term persisting haplotypes, incomplete lineage sorting, regional extinction, and the consequences for phylogenetic inference. Mol. Biol. Evol. 23 (8), 1602–1612.
Kalkman, C., 2004. Rosaceae: 13. *Alchemilla* group. In: Kubitzki, K. (Ed.), Flowering Plants. Dicotyledons: Celastrales, Oxalidales, Rosales, Cornales, Ericales, vol. 6. Springer, Berlin, pp. 371–372.
Kirschner, J., Stepanek, J., Mes, J.T.H.M., den Nijs, J.C.M., Oosterveld, P., Storchova, H., Kuperus, P., 2003. Principal features of the cpDNA evolution in *Taraxacum* (Asteraceae, Lactuceae): a conflict with taxonomy. Plant Syst. Evol. 239, 231–255.
Lagerheim, N.G., 1894. Ueber die andinen *Alchemilla*-Arten. Kongl. Svenska Vetensk. Acad. Handl. 51, 15–18.
Linder, C.R., Rieseberg, L.H., 2004. Reconstructing patterns of reticulate evolution in plants. Am. J. Bot. 91 (10), 1700–1708.
Linnaeus, C., 1753. *Aphanes arvensis*. Sp. Pl. 2, 123.
Mogie, M., 1992. The Evolution of Asexual Reproduction in Plants. Chapman & Hall, London, UK.
Murbeck, S.S., 1915. Zur Morphologie und Systematik der Gattung *Alchemilla*. Kgl. Fys. Sällsk. Handl. N.F. 26,8 and N.F. Afd. 2; 11,8, 17.
Muits, J.C.B., 1781. *Alchemilla*. In: Linnaeus, f. (Ed.), Suppl. Pl., Impensis Orphanotrophei, Braunschweig, p. 129.
Nicholas, K.B., Nicholas, H.B.J., 1997. GeneDoc: a tool for editing and annotating multiple sequence alignments. Distributed by the authors.
Notov, A.A., Kusnetzova, T.V., 2004. Architectural units, axiality and their taxonomic implications in *Alchemillinae*. Wulfenia 11, 85–130.
Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P., Nieves Aldrey, J.L., 2004. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53, 47–67.
Perry, L.M., 1929. A tentative revision of *Alchemilla* section *Lachemilla*. Contr. Gray Herb. 84, 1–57.
Persoon, C.H., 1805. Synopsis Plantarum. Cramer, Paris.
Pfeil, B.E., Crisp, M.D., 2005. What to do with *Hibiscus*? A proposed nomenclatural resolution for a large and well known genus of Malvaceae and comments on paraphyly. Aust. Syst. Bot. 18 (1), 49–60.
Plocek, A., 1982. Revised nomenclature of infrageneric groups of *Alchemilla* in Eurasia. Preslia 54, 45–53.
Posada, Crandall, 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.
Rambaut, A., Drummond, A.J., 2003–2007. MCMC Trace Analysis Tool v1.4. Available from: http://tree.bio.ed.ac.uk/software/tracer/.
Richards, A.J., 2003. Apomixis in flowering plants: an overview. Philos. Trans., Ser. B 358 (1434), 1085–1093.
Rieseberg, L.H., Soltis, D.E., 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. Evol. Trend. Plant. 5, 65–84.
Romoleroux, K., 1996. Rosaceae. In: Harling, G., Andersson, L. (Eds.), Flora of Ecuador, vol. 56, pp. 1–152.
Romoleroux, K., 2004. The genus *Lachemilla* (Rosaceae) in the northern Andes of South America. Lyonia 7 (1), 21–32.
Rothmaler, W., 1934. Systematische Vorarbeiten zu einer Monographie der Gattung *Alchemilla* (L) Scop. I. Fedd. Rep. 33, 342–350.
Rothmaler, W., 1935. Systematische Vorarbeiten zu einer Monographie der Gattung *Alchemilla* (L.) Scop. II. Die systematische Gliederung der Gattung. In: Fedde, F., Schwarz, O. (Eds.), Fedd. Rep., IV. Band Nr. 27–29, pp. 409–419.
Rothmaler, W., 1936. Systematische Vorarbeiten zu einer Monographie der Gattung *Alchemilla* (L.) Scop. IV. Die Gruppen der Untergattung *Eualchemilla* (Focke) Buser. Fedd. Rep. 40, 208–212.
Rothmaler, W., 1937a. Systematische Vorarbeiten zu einer Monographie der Gattung *Alchemilla* (L.) Scop. V. Die Sectionen *Subcuneatifoliae* (de Wild.) Rothm. Fedd. Rep. 42, 111–119.
Rothmaler, W., 1937b. Systematische Vorarbeiten zu einer Monographie der Gattung *Alchemilla* (L.) Scop. VII. Aufteilung der Gattung und Nomenklatur. Repert. Spec. Nov. 42, 164-173.
Rydberg, P.A., 1908. *Alchemilla*, *Aphanes*, *Lachemilla*, *Zygalchemilla*. (Rosaceae). North Amer. Flora, vol. 22, pp. 377–385.
Schulz-Menz, G.K., 1964. Rosales. In: Melchior, H. (Ed.), A. Engler's Syllabus der Pflanzenfamilien. Borntraeger, Berlin.
Scopoli, J.A., 1772. Flora Carniolica. Impensis Ioannis Pavli Krauss, Bibliopolae Vindobonensis.
Scotland, R.W., Sanderson, M.J., 2004. The significance of few versus many in the tree of life. Science 303 (5658), 643.
Shi, Y., Gornall, R.J., Draper, J., Stace, C.A., 1996. Intraspecific molecular variation in *Hieracium* sect. *Alpina* (Asteraceae), an apomictic group. Folia Geobot. Phytotax. 31, 305–313.
Simmons, M.P., Ochoterena, H., 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49 (2), 369–381.
Soltis, D.E., Kuzoff, R.K., 1995. Discordance between nuclear and chloroplast phylogenies in the *Heuchera* group (Saxifragaceae). Evol. Trend. Plant. 49, 727–742.
Staden, R., 1996. The Staden sequence analysis package. Mol. Biotechnol. 5, 233–241.
Swofford, D.L., 2001. PAUP*: Phylogenetic Analysis Using Parsimony. Sinauer Associates.
Taberlet, P., Gielly, L., Pautou, G., Bouvet, J., 1991. A set of universal primers for amplifications of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Plant. Mol. Biol. 17, 1105–1119.
Thunberg, C.P., 1794. *Alchemilla*. In: Prodromus Plantarum Capensium, 1, Edman, J., Uppsala.
Walters, S.M., Pawlowski, B., 1968. *Alchemilla*. In: Tutin, H.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), Flora Europaea 2. Rosaceae to Umbelliferae. Cambridge University Press, Cambridge, pp. 8–64.
Wendel, J.F., Doyle, J.J., 1998. Phylogenetic incongruence: window into genome history and molecular evolution. In: Soltis, D.E., Soltis, P.S., Doyle, J.J. (Eds.), Molecular systematics of plants II: DNA Sequencing. Kluwer, Dordrecht, the Netherlands, pp. 265–296.
Wittzell, H., 1999. Chloroplast DNA variation and reticulate evolution in sexual and apomictic sections of dandelions. Mol. Ecol. 8 (12), 2023–2035.