

A molecular phylogeny, morphology and classification of genera of Ranunculaceae (Ranunculaceae)

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Abstract Ranunculaceae represent a highly diverse and cosmopolitan tribe within Ranunculaceae. Because of the great diversity of morphological features and lack of molecular phylogeny for the tribe, the classification of its genera has always been controversial. We report here molecular phylogenetic analyses based on nuclear and plastid markers (nrITS, *matK*, *trnK*, *psbJ-petA*) that provide a framework for understanding relationships and character evolution within the tribe. Maximum parsimony analyses suggest a weakly supported basal dichotomy, while Neighbor Net analysis indicates strong support for five distinct lineages. Both methods revealed several well-supported, small terminal clades which correspond to previously described genera, characterised by unique morphological features and character combinations. Anatomical structures of the achenes suggested relationships with greatest concordance to those in the molecular phylogeny. Macroscopic analysis of achene morphology often indicated parallel evolution of structures related to certain dispersal mechanisms. Characters of perianth, androecium, gynoecium and pollen are highly homoplasious, but several features characteristic of small terminal clades and terminal branches can be observed. Geographic isolation and adaptations may have triggered the evolution of morphological characters. We conclude that a classification accepting several small genera (*Arcteranthis*, *Beckwithia*, *Callianthemoides*, *Ceratocephala*, *Coptidium*, *Cyrtorhyncha*, *Ficaria*, *Halerpestes*, *Hamadryas*, *Krapfia*, *Kumlienia*, *Laccopetalum*, *Myosurus*, *Oxygraphis*, *Paroxygraphis*, *Peltocalathos*, *Trautvetteria*) and a large genus *Ranunculus* s.str. (including *Batrachium*, *Aphanostemma* and *Gampsoceras*) reflects best the molecular phylogeny and morphological diversity of the tribe.

Keywords anatomy; molecular systematics; morphology; Ranunculaceae; SEM; taxonomy

■ INTRODUCTION

Ranunculaceae are a large plant family with a worldwide distribution. This family has been considered as one of the most basal families within the eudicots (Soltis & al., 2005; Simpson, 2006; Heywood & al., 2007) and its crown age has been estimated as ca. 75 Ma (Anderson & al., 2005). The family shows a wide variation in morphological characters, especially in fruit types, and in its floral organization. Several classifications have been proposed for Ranunculaceae based on morphological characters (Hutchinson, 1923; Janchen, 1949; Tamura, 1995), on molecular data (Jensen & al., 1995; Ro & al., 1997), and on a combined molecular and morphological dataset (Wang & al., 2009). From the conventional characters used, chromosome type and base number have been found to be most congruent with the phylogeny of the family as inferred from molecular data (Ro & al., 1997; Wang & al., 2009). Recent molecular studies have given insights into the phylogenetic relationships within this family (Johansson & Jansen, 1993; Jensen & al., 1995; Johansson, 1995; Hoot, 1995; Kosuge & al., 1995; Ro & al., 1997, 1999; Wang & al., 2005, 2009).

The family has been subdivided into three subfamilies and eleven tribes by Tamura (1995). This classification has been based on chromosome base number, carpel and fruit types.

The tribe Ranunculaceae DC., in the subfamily Ranunculoideae Hutch., includes about 650 species and it is distributed in all continents (Tamura, 1995). A number of molecular phylogenetic studies within the Ranunculaceae suggest that this tribe is monophyletic (Hoot, 1995; Hoot & al., 2008; Johansson, 1995, 1998; Ro & al., 1997; Lehnebach & al., 2007; Wang & al., 2009). The tribe has unitegmic ovules as in Anemoneae and Callianthemaceae (sensu Wang & al., 2009), but in Ranunculaceae ovules are ascending (except *Myosurus* which has pendent ovules; Tamura, 1995). Petals in Ranunculaceae have at least one nectary gland near the base. There are only a few worldwide studies on Ranunculaceae (e.g., Candolle, 1824; Prantl, 1887; Tamura, 1993, 1995) and different classifications for its members have been proposed (Table 1). Discrepancies between these classifications are probably due to the ample variation in floral characters, e.g., bisexual or unisexual flowers, petaloid or sepaloid sepals and the presence or absence of petals. Candolle (1817) described Ranunculaceae based on floral features, underground parts and achenes. In his classification, Ranunculaceae comprised four genera: *Myosurus*, *Ranunculus*, *Ceratocephala* and *Ficaria* (Table 1).

Prantl (1887) based the classification of genera on features of fruits and the perianth, and treated *Myosurus*, *Ranunculus*, *Trautvetteria*, and *Oxygraphis* as closely related

Table 1. Summary of classifications of Ranunculeae.

Genera accepted in this study (worldwide)	Whittemore (1997, North America)	Tamura (1995, worldwide)	Prantl (1887, worldwide)	Ovczinnikov (1937, U.S.S.R.)	De Candolle (1824)
<i>Arcteranthis</i> Greene	<i>R. subg. R. sect. Arcteranthis</i>	<i>Arcteranthis</i>	–	–	–
<i>Beckwithia</i> Jeps.	<i>R. subg. Crymodes</i>	<i>R. subg. Crymodes</i>	<i>R. sect. Hypolepium</i>	–	–
<i>Callianthemoides</i> Tamura	–	<i>Callianthemoides</i>	–	–	–
<i>Ceratocephala</i> Moench	<i>R. subg. Ceratocephalus</i>	<i>Ceratocephala</i>	<i>R. sect. Ceratocephalus</i>	<i>Ceratocephala</i>	<i>Ceratocephala</i>
<i>Coptidium</i> (Prantl) Beurl. ex Rydb.	<i>R. subg. Coptidium & Pallasiantha</i>	<i>R. subg. Coptidium & Pallasiantha</i>	<i>R. sect. Marsypadenium</i>	<i>R. subg. Auricomus sect. Coptidium</i>	<i>Ranunculus</i>
<i>Cyrtorhyncha</i> Torr. & A. Gray	<i>R. subg. R. sect. Cyrtorhyncha</i>	<i>Cyrtorhyncha</i>	<i>Oxygraphis?</i>	–	–
<i>Ficaria</i> Guett.	<i>R. subg. Ficaria</i>	<i>R. subg. Ficaria</i>	<i>R. sect. Ficaria</i>	<i>Ficaria</i>	<i>Ficaria</i>
<i>Halerpestes</i> Greene	<i>R. subg. R. sect. Halodes</i>	<i>Halerpestes</i>	<i>Oxygraphis</i>	<i>Halerpestes</i>	<i>Ranunculus</i>
<i>Hamadryas</i> Comm. ex Juss.	–	<i>Hamadryas</i>	–	–	<i>Anemoneae</i>
<i>Krapfia</i> DC.	–	<i>Krapfia</i>	–	–	<i>Ranunculus</i>
<i>Kumlienia</i> E. Greene	<i>R. subg. R. sect. Pseudaphanostemma</i>	<i>Kumlienia</i>	<i>Oxygraphis?</i>	–	–
<i>Laccopetalum</i> Ulbr.	–	<i>Laccopetalum</i>	–	–	–
<i>Myosurus</i> L.	<i>Myosurus</i>	<i>Myosurus</i>	<i>Myosurus</i>	<i>Myosurus</i>	<i>Myosurus</i>
<i>Oxygraphis</i> Bunge	<i>R. subg. Oxygraphis</i>	<i>Oxygraphis</i>	<i>Oxygraphis</i>	<i>Oxygraphis subg. Euxoxygraphis</i>	–
<i>Paroxygraphis</i> W.W. Sm.	–	<i>Paroxygraphis</i>	–	–	–
<i>Peltocalathos</i> Tamura	–	<i>Peltocalathos</i>	–	–	–
<i>Ranunculus</i> L.	<i>Ranunculus</i>	<i>Ranunculus</i>	<i>Ranunculus</i>	<i>Ranunculus</i>	<i>Ranunculus</i>
	–	<i>Aphanostemma</i>	<i>R. sect. Marsypadenium</i>	–	<i>Ranunculus</i>
	<i>R. subg. Batrachium</i>	<i>R. subg. Batrachium</i>	<i>R. sect. Marsypadenium</i>	<i>Batrachium</i>	<i>R. sect. Batrachium</i>
<i>Trautvetteria</i> Fisch. & C.A. Mey.	<i>Trautvetteria</i>	<i>Trautvetteria</i>	<i>Trautvetteria</i>	<i>Trautvetteria</i>	–

genera (Table 1). Although his study had a worldwide coverage, several South American taxa were not included. The most up to date and worldwide classification of the tribe is that by Tamura (1995) based on differences in the structure of achenes. In his classification, 16 genera were included in the tribe (Table 1) and these were grouped into three subtribes; Trautvetteriinae without petals (one genus), Myosurinae with a spur-like projection at the base of the sepals and pendulous ovules (one genus) and Ranunculinae (14 genera).

Delimitation of *Ranunculus* L., the largest genus in Ranunculinae and closely related genera has been a source of debate for centuries. Previous classifications often included the genera *Ficaria*, *Coptidium*, and *Arcteranthis* in *Ranunculus* (Candolle, 1824; Prantl, 1887) or excluded *Batrachium* from *Ranunculus* (Janichen, 1958; Rostrup, 1958; Löve & Löve, 1961; Wang & al., 2009). Tamura (1995) segregated several small genera from *Ranunculus* (Table 1) and used characters from reproductive structures, such as achene and petals to establish generic boundaries. Although a number of studies have assessed the diversity of achenes (Trzaski, 1999), petals (Parkin, 1928), nectary scales (Benson, 1940), karyotypes (Goepfert, 1974), and pollen structure (Santisuk, 1979) within Ranunculeae, none have revealed individual characters diagnostic for delimitation of the genera. In combination some characters are potentially informative for identifying genera (Tamura, 1995), however little insight has been gained from morphological analyses regarding relationships among genera.

A number of molecular investigations of the Ranunculeae and its members are currently available (e.g., Johansson, 1998; Hörandl & al., 2005; Paun & al., 2005; Lehnebach & al., 2007; Hoot & al., 2008; Gehrke & Linder, 2009; Hoffmann & al., 2010). These studies included ca. 200 species covering all sections and subgenera of *Ranunculus* sensu Tamura (1995), with the exception of *R. pinardii* (*R. subg. Gampsoceras*), and *R. sect. Ficariifolius* L. Liou., and have provided a comprehensive phylogenetic framework for the species of *Ranunculus* s.str. These phylogenetic studies have revealed that the water-buttercups, *Batrachium* (= *R. sect. Batrachium*) are nested within *Ranunculus* s.str.; and that

Aphanostemma (= *R. apiifolius*) is a monotypic genus nested within *Ranunculus* s.str. (Hörandl & al., 2005; Paun & al., 2005; Lehnebach & al., 2007). Unfortunately, none of these studies have included all genera of the tribe (as delimited by Tamura, 1995) and phylogenetic relationships between some of the genera are still unknown. The tree topologies of previous molecular studies on the genus *Ranunculus* and allied genera (Johansson, 1998; Hörandl & al., 2005; Paun & al., 2005; Lehnebach & al., 2007; Hoot & al., 2008; Gehrke & Linder, 2009; Hoffman & al., 2010) have revealed the position of *Ficaria* separate from the *Ranunculus* clade and all (except for Johansson, 1998) have supported the inclusion of *Myosurus* within Ranunculeae. The separation of *Coptidium* from a core *Ranunculus* clade is evident in Johansson (1998), Hörandl & al. (2005), Paun & al. (2005), Lehnebach & al. (2007), Gehrke & Linder (2009), and Hoffman & al. (2010). Results of Hörandl & al. (2005) placed *Arcteranthis*, *Callianthemoides*, *Halerpestes*, *Oxygraphis*, and *Peltocalathos* on basal branches and supported Tamura's (1995) classification of separate genera. The analyses of Hoot & al. (2008) suggested an exclusion of *Hamadryas* from the core *Ranunculus* clade, while Lehnebach & al. (2007) accepted *Krapfia* and *Laccopetalum* as sister taxa to *Ranunculus* s.str. However, some authors refrained from final taxonomic conclusions at the generic level because of incomplete sampling of taxa or markers (Johansson, 1998; Hörandl & al., 2005; Hoot & al., 2008). For some taxa of the tribe, molecular data were not available (*Kumlienia hystricula*, *Cyrtorhyncha ranunculina*, *Gampsoceras pinardii*). A comprehensive framework for the classification of this tribe based on morphology and a complete molecular phylogeny has not yet been published.

Ranunculeae have a cosmopolitan, mainly extratropical distribution with *Ranunculus*, being the only genus distributed in all continents. Most of the other genera in the tribe have very restricted distributions and many of the monotypic genera are endemic to one continent, e.g., *Arcteranthis* (northwestern North America), *Cyrtorhyncha* (western North America), *Kumlienia* (western North America), *Krapfia* and *Laccopetalum* (northern Andes in South America) and *Peltocalathos* (southern South Africa). Other genera, such as *Ceratocephala*, *Myosurus*, and *Ficaria* are mainly distributed in the Northern Hemisphere, but seem to have extended their distribution rather recently (Tamura, 1995). Only *Halerpestes*, occurring in Asia, South and North America, and the cosmopolitan genus *Ranunculus* have larger distribution areas. The different taxonomic treatments of genera in regional floras (Table 1) have hampered so far a worldwide classification of the tribe. Most species of this tribe are adapted to temperate and cold climates and are found in mountainous regions of the world.

The main aims of this study were to (1) to investigate the phylogenetic relationships within Ranunculeae using molecular data, (2) to identify morphological synapomorphies and diagnostic characters useful for the classification of genera, and (3) to provide a revised classification of the tribe. Unlike previous studies, we examined morphological characters including type of pollen aperture, achene surface and shape of the nectar. We studied these features within a phylogenetic framework provided by analyses of the internal transcribed spacer region

(ITS) and chloroplast markers (*matK/trnK*, *psbJ-petA*). This comparative approach provided a means to evaluate character evolution terms of its significance for ecology and systematics (Stuessy, 2003).

■ MATERIALS AND METHODS

Plant material. — Fifty-three taxa representing 16 of the 17 genera Tamura (1993, 1995) included in the Ranunculeae were sampled (Table 1). Only the genus *Paroxygraphis* was not included because material was not available. Except for monotypic genera, at least two species for each genus were studied. At least two species were studied from each of the clades and subclades identified for *Ranunculus* s.str. in previous studies (Hörandl & al., 2005; Paun & al., 2005; Emadzade & al., unpub.). We included also *Ranunculus pinardii*, a species which has been described as a distinct monotypic genus, *Gampsoceras* (Steven, 1852), and one representative of *R.* sect. *Ficariifolius* sensu Tamura (*R. cheirophyllus*). *Anemone* and *Isopyrum* were chosen as outgroup taxa. The first one belongs to the tribe Anemoneae, sister to the tribe Ranunculeae (Hoot & al., 2008; Wang & al., 2009) and the second one to the tribe Thalictroideae which is distantly related to Ranunculeae (Hoot & al., 2008; Wang & al., 2009). Voucher information and GenBank accession numbers are provided in Appendix 1.

Morphological characters. — Based on herbarium material and literature data, 33 morphological characters were investigated (Ovczinnikov, 1937; Benson, 1940; Davis, 1960; Goepfert, 1974; Riedl & Nasir, 1990; Iranshahr & al., 1992; Rau, 1993; Tutin & Cook, 1993; Whittimore, 1997; Wang & Gilbert, 2001). We have indicated the character states in Appendix 2, and how they were scored in Appendix 3. Selected characters were mapped for 39 taxa representing genera and sections, using MacClade v.4.0 (Maddison & Maddison, 2000), onto a tree topology inferred using concatenated sequence data.

Surface of the achenes and type of apertures of the pollen grains were studied with a scanning electron microscope (SEM). Samples taken from herbarium specimens were glued to aluminium stubs, and coated with gold (BALZERS Sputter Coater). The samples were viewed and photographed on a SEM, JEOL JSM-6390 at 10 kV at the Faculty Center of Biodiversity, University of Vienna. Pollen aperture types were coded following the terminology of Santisuk (1979). For histological observations, achenes were fixed in alcohol-formalin-acetic acid solution overnight and then dehydrated using ethanol series and embedded in Paraplast. Sections of 10 µm thickness were obtained with the microtome, stained with Toluidine blue and later mounted.

Ranunculeae have a single-seeded, indehiscent dry fruit with a hardened pericarp. Because of numerous definitions and applications of fruit terms in the literature (e.g., achene, utricle, nutlet) their description can be ambiguous. "Achene" here is treated as "*An indehiscent pericarpium, or fruit, with a pericarp contiguous to the seed(s)*" (Spjut, 1994; Simpson, 2006). The space between seed and pericarp in the fruits of Ranunculeae is variable. However, we use the term achene for the fruit of

the Ranunculaceae as do most modern authors (Tamura, 1993, 1995; Simpson, 2006). Ontogenetic studies have shown that nectary organs in Ranunculaceae are derived from stamens (e.g., Erbar & al., 1998). In most genera of Ranunculaceae, tepals have the function of a calyx and the petals. They are called “honey-leaves” (Prantl, 1887) or “nectar-leaves” (Janichen, 1949), and have an insect-attracting function. In this study we apply the commonly used terms sepals and petals for the two whorls of the perianth (Ovczinnikov, 1937; Tamura, 1995; Whittimore, 1997).

DNA extraction, amplification and sequencing. — Total genomic DNA from silica-dried or herbarium material was extracted using a modified CTAB technique (Doyle & Doyle, 1987). The whole internal transcribed spacer (ITS, including ITS1, the 5.8 S rDNA, ITS2) was amplified as a single piece with primers ITS 18sF and ITS 26sR (Gruenstaeudl & al., 2009) or in the case of degraded DNA from poor quality herbarium tissue, in two pieces with additional primers (ITS 5.8sF and ITS 5.8sR) as internal primers (Gruenstaeudl & al., 2009). Sequencing of the *matK/trnK* region was performed according to the protocol described by Paun & al. (2005). Amplification of the non-coding *psbJ-petA* region carried out as a single piece in all samples with using *psbJ* and *petA* primers of Shaw & al. (2007). PCR was performed in 23 μ l reactions containing 20 μ l 1.1 \times Reddy Mix PCR Master Mix (including 2.5 mM MgCl₂; ABgene, Epsom, U.K.), 1 μ l of each primer (10 mmol/L) and 1 μ l template DNA. 1 μ l of 0.4% bovine serum albumin (BSA, Promega, Madison, Wisconsin, U.S.A.) for *matK* and *psbJ-petA*, and in the case of the ITS region, dimethyl sulfoxide (DMSO) was added to reduce problems associated with DNA secondary structure. PCR products were purified using *E. coli* Exonuclease I and Calf Intestine Alkaline Phosphate (CIAP; MBI-Fermentas, St. Leon-Rot, Germany) according to the manufacturer’s instructions. Cycle sequencing was performed using Big Dye™ Terminator v3.1 Ready Reaction Mix (Applied Biosystems, Applied Austria GmbH, Vienna, Austria), using the following cycling conditions: 38 cycles of 10 s at 96°C, 25 s at 50°C, 4 min at 60°C. All DNA regions were sequenced in both directions. The samples were run on a 3130xl Genetic Analyzers capillary sequencer (Applied Biosystems).

Sequence alignment and phylogenetic analysis. — The sequences of all markers were initially aligned using Clustal X (Thompson & al., 1997). Subsequent corrections were carried out manually using BioEdit v.7.0.9.0 (Hall, 1999). Indels were treated as binary characters following the “simple indel coding method” (Simmons & Ochoterena, 2000) using the program SeqState v.1.36 (Müller, 2005). Due to degraded DNA from poor quality herbarium tissue and difficulties in amplification of DNA, we could neither sequence the *matK/trnK* region in *Arcuteranthus* nor the *psbJ-petA* region in *Krapfia* and *Myosurus*. Thus these absent sequences were scored as missing data. The *psbJ-petA* sequence of *Ceratocephala* was extremely highly diverged and could not be aligned to the other species. High relative levels of divergence for *Myosurus* and *Ceratocephala* were also reported in the cpDNA restriction site analyses of Johansson (1998). Since the tree building assumption of similar evolutionary constraint (Lockhart & Steel, 2005) appears violated in the *psbJ-petA* sequences for these taxa we excluded

this region for these species from the analysis. Nuclear and chloroplast sequences were analyzed separately and in combination. A heuristic search for the most parsimonious (MP) trees was performed with PAUP* v.4.0b8 (Swofford, 2002). The analyses involved 1000 replicates with stepwise random taxon addition, tree bisection-reconnection (TBR) and branch swapping saving no more than 10 trees per replicate. All characters were equally weighted and treated as unordered (Fitch, 1971). Internal branch support was estimated using non-parametric bootstrapping (Felsenstein, 1985) with 10,000 bootstrap replicates each with 10 random sequence addition replicates holding maximally 10 trees per replicate, SPR branch swapping, and MulTrees on. Since our phylogenetic reconstructions indicated numerous relationships where bootstrap support was <50%, we were interested to determine whether this was due to conflicting support or absence of phylogenetic signal. Phylogenetic network methods (Huson & Bryant, 2006) provide a means of evaluating the extent to which data exhibits a hierarchical structure. Interestingly, non-hierarchical data structure has been inferred frequently in *Ranunculus* by using split decomposition (Lockhart & al., 2001; Hörandl & al., 2005). However, for larger datasets, the Neighbor Net method often provides better resolution than split decomposition due to the criterion it uses to calculate support for relationships among taxa. Like split decomposition, Neighbor Net also calculates the support for “splits” (relationships) from distances and displays these splits in a graph (i.e., a “splits graph” or “split network”). While split decomposition uses the criterion of “weak compatibility” (Lockhart & al., 2001) in identifying splits, Neighbor-Net uses an algorithm that determines a circular ordering of taxa (i.e., based on the extent of differences between their sequences the taxa are ordered around a circle). The layout on the circle determines what splits occur in the data and can be displayed in a planar graph. The support for each of these splits is then measured using a least-squares method that adjusts the lengths of the splits in the splits graph so as to minimize the difference with the pairwise distances in the original data matrix (Bryant & Moulton, 2004; Huson & Bryant, 2006). Non tree-like splits graphs indicate contradictory support for relationships. Phylogenetic error, hybridization and horizontal gene transfer can all potentially contribute to the non tree-like nature of splits graphs (Bryant & Moulton, 2004). We used the Neighbor Net analysis implemented in SplitsTree4 v. 4.10 (Huson & Bryant, 2006), applying Hamming distances with gaps and ambiguous sites coded as missing data. Bootstrap support for internal splits (which define clusters) was calculated with 1000 replicates.

■ RESULTS

Molecular data. — Total sequence length for the ITS, *matK/trnK* and *psbJ-petA* regions in the 55 taxa are 595–617, 1543–1821 and 461–507 bp, respectively. We used 3416 aligned nucleotide positions in total: 650 bp in the ITS dataset and 2766 bp in the chloroplast dataset.

The analysis of the ITS dataset resulted in 147 most parsimonious trees with a length of 1335 steps (252 parsimony

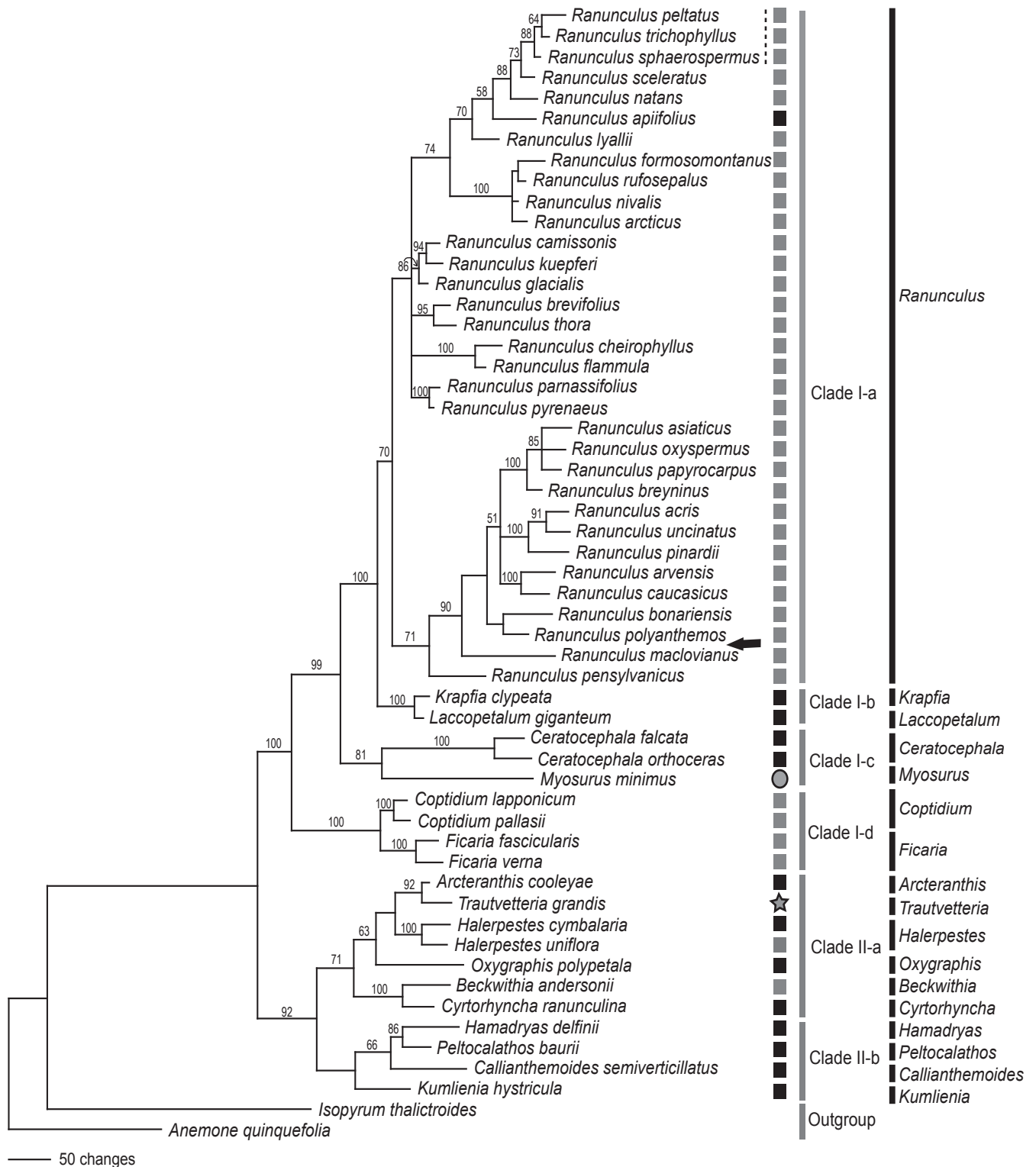


Fig. 1. Strict consensus of 33 most parsimonious trees from the combined ITS, *matK/trnK* and *psbJ-petA* dataset. Generic names correspond to accepted names in this study. Numbers listed above the horizontal lines are bootstrap values $\geq 50\%$. Symbols represent the subtribes used in the Tamura (1995) classification: Circle, Myosurinae; asterisk, Trautvetteriinae; squares, Ranunculinae, whereas black squares are genera, gray squares species of *Ranunculus*. The arrow represents the position of the *Krapfia-Laccopetalum* clade in the topology based on chloroplast markers only. The dashed line indicates the clade corresponding to *R. sect. Batrachium*. The genus names in the right column indicate the finally accepted classification. For further synonyms, see Table 1.

informative characters, consistency index [CI] = 0.49, retention index [RI] = 0.61, rescaled consistency index [RC] = 0.30). In the strict consensus tree (not shown) the *Myosurus-Ceratocephala* clade was found sister to a large clade of taxa with 66% bootstrap support. This large clade contained a polytomy with *Ranunculus*, *Arcteranthis*, *Beckwithia*, *Callianthemoides*, *Coptidium*, *Cyrtorhyncha*, *Ficaria*, *Halerpestes*, *Hamadryas*, *Kumlienia*, *Oxygraphis*, *Peltocalathos* and *Trautvetteria*. *Krapfia* and *Laccopetalum* formed a strongly supported monophyletic group (100% BS) within *Ranunculus*, but their position in the core *Ranunculus* clade was weakly supported. The monotypic South American genus *Aphanostemma* also emerged within the core *Ranunculus* clade (*R. apiifolius*).

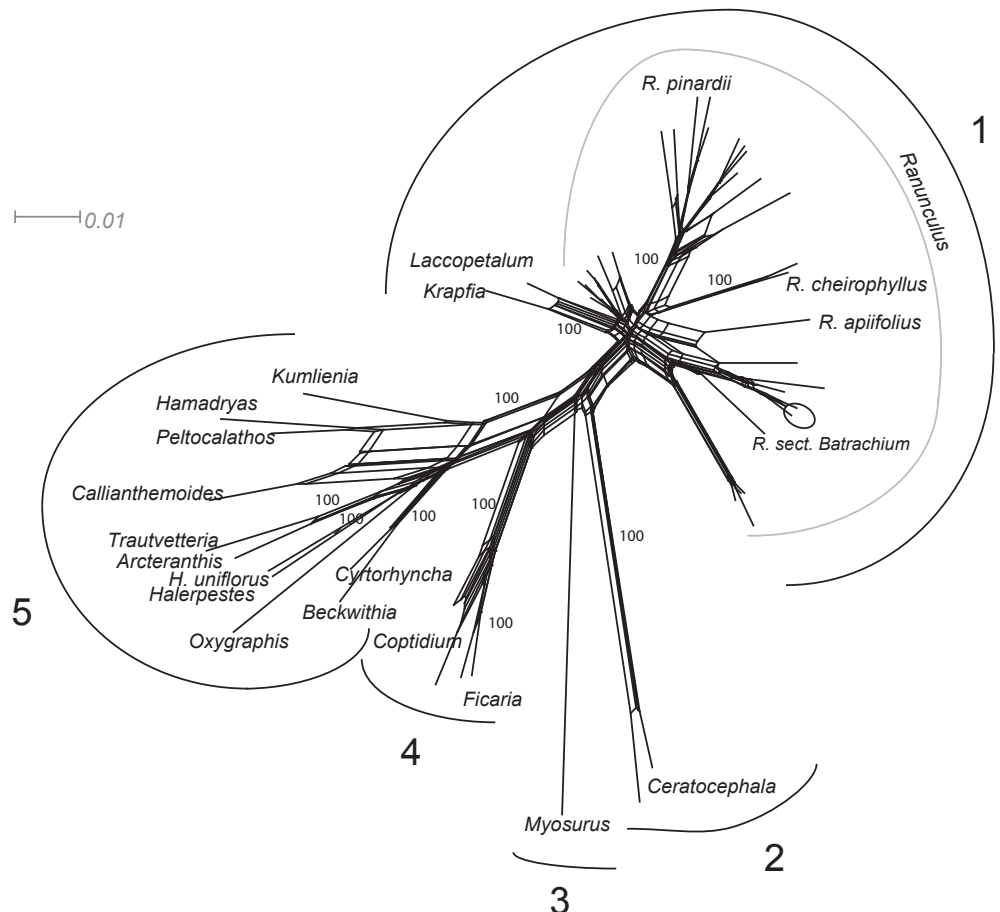
Analysis of the combined chloroplast dataset (*matK/trnK*, *psbJ-petA*) resulted in 60 most parsimonious trees with a length of 2860 steps (787 parsimony informative characters, CI = 0.62, RI = 0.73, RC = 0.46). The strict consensus tree contained two major clades with 100% bootstrap for each clade (not shown). The first clade comprised *Ceratocephala*, *Coptidium*, *Ficaria* and *Myosurus* and was sister to the core *Ranunculus* clade. *R. apiifolius* (*Aphanostemma*) was nested again within the core *Ranunculus* clade. *Krapfia* and *Laccopetalum* formed a monophyletic clade with high bootstrap support which was nested within the core *Ranunculus* clade with a low bootstrap support. The second clade also contained dichotomous split. *Arcteranthis*, *Halerpestes*, *Oxygraphis* and

Trautvetteria formed a monophyletic group which was sister to clade II-b formed by *Callianthemoides*, *Hamadryas*, *Kumlienia* and *Peltocalathos*.

Parsimony analysis of the combined dataset resulted in 33 most parsimonious trees of 4316 steps (1039 parsimony informative characters, CI = 0.56, RI = 0.67, RC = 0.38). This tree showed greater resolution and more well-supported nodes than the trees from the datasets analyzed independently. The topology provided by the combined data is similar to the topology of the chloroplast data (Fig. 1), except for the position of the *Krapfia-Laccopetalum* clade which, in the combined analysis, was found sister in the core *Ranunculus* clade.

The Neighbor Net (NNet) analysis (Fig. 2), in which indels were not considered as informative characters, did not confirm the basic dichotomy of two major clades (clades I and II) found in the parsimony analysis. Instead, NNet identified five strongly supported splits (and clusters) which correspond partly to the well-supported clades in the topology of the combined tree obtained with the parsimony analysis (Fig. 2). The first cluster in the NNet splits graph comprised clade I-a and I-b in Fig. 1 and united the *Krapfia-Laccopetalum* group with *Ranunculus* s.str. Within *Ranunculus* s.str. the nesting of *R. pinardii*, *R. apiifolius*, *R. cheirophyllus* and *R. sect. Batrachium* within *Ranunculus* s.str. in the NNet splits graph is congruent with the results of the parsimony analysis (see clade I-a; Fig. 1). The second cluster in the NNet splits graph comprised only

Fig. 2. Neighbor Net splits graph based on combined ITS, *matK/trnK* and *psbJ-petA* datasets. Clusters correspond to those well-supported clades shown in the topology of the combined tree. Group 1 corresponds to clades I-a plus I-b, groups 2 and 3 are the same as clade I-c and group 4 refers to clade I-d, and group 5 corresponds to clades II-a and II-b. Bootstrap support values = 100 are shown.



Ceratocephala and there was little support evident in the splits graph analysis for a split that linked this genus with *Myosurus* (cluster 3). In contrast, parsimony analysis united these two clusters in clade I-c (Fig. 1). Cluster 4 comprised *Coptidium* and *Ficaria* and this inferred relationship is congruent with clade I-d (Fig. 1). Cluster 5 united the remainder of the Ranunculeae genera as found in the parsimony analyses; however, support for separation of species belonging to clades II-a and II-b within this cluster was less clear.

Morphological data. — From the 33 morphological characters studied, only the structures of the achene surface suggested relationships among taxa congruent with the two main clades of the molecular tree. A sclerenchymatous layer in the pericarp of the achene (e.g., Fig. 3) occurs in all genera of clade I except for *Coptidium*, but is largely missing in clade II (Fig. 5A). The presence of longitudinal, parallel, straight veins on the surface of achenes occurs in most genera of clade II (except for *Beckwithia*) but the venation pattern is specific for genera (Fig. 4A–H): *Kumlienia* (Fig. 4B) and *Oxygraphis* (Fig. 4G) show only one big vein, the other genera (Fig. 4A, C–F, H) have more, but smaller longitudinal veins. The genera of clade I have no veins on the lateral surface (Fig. 4I–P) except for some species of *Ranunculus* s.str. which have irregular anastomosing or strongly curved veins on the lateral surface (Appendix 3).

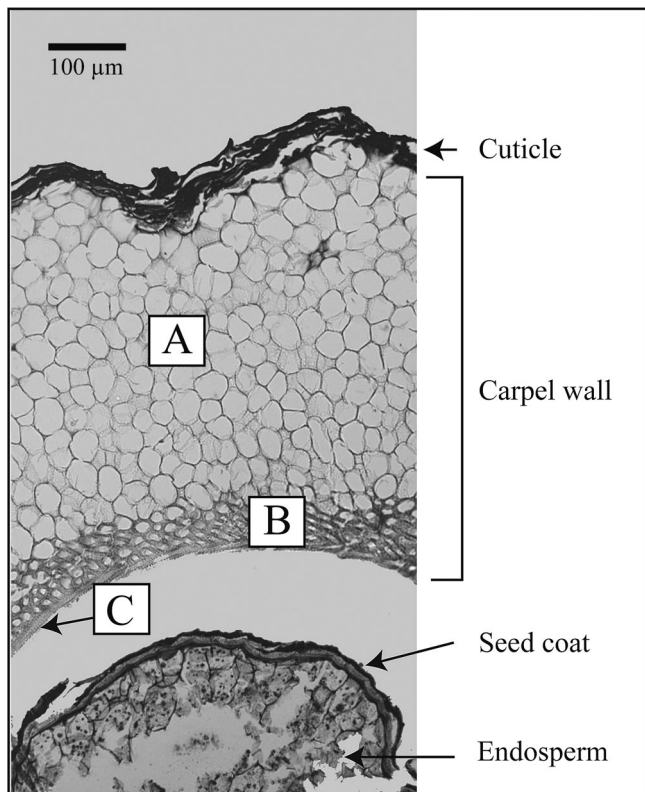


Fig. 3. Cross section of a mature achene of *Ranunculus acaulis* and details of its structure. **A**, parenchymatous cells; **B**, inner part of carpel wall with thick-walled cells (sclerenchymatous layer); **C**, inner epidermal layer of the carpel wall.

Ranunculus species of *R.* sect. *Batrachium* (Fig. 4P) have transversal ridges on the achene surface, which are formed by up-turned edges of elongated sclereid cells (Cook, 1963). *Ranunculus arvensis* (Fig. 4M) and *R. pinardii* (Fig. 4N) have spiny or tuberculate achenes which are formed by the parenchyma layer of the pericarp (Lonay, 1901). These macroscopic surface structures found in *Ranunculus* s.str. have a different anatomical background than the venation patterns.

The microstructure of the pericarp surface is mostly irregular rugose (Fig. 4A–G), with pronounced convex cell surfaces in *Cyrtorhyncha* (Fig. 4H) and a reticulate cell pattern in *Myosurus* (Fig. 4J). Within *Ranunculus* s.str., finely papillate (Fig. 4M), foveolate (Fig. 4O) and rugose (Fig. 4P) microstructures are present.

Palynological studies identified seven types of apertures in the pollen grains (Fig. 5C). Diversity of aperture types was even observed at the species level as well, e.g., in *Beckwithia andersonii*, *Cyrtorhyncha ranunculina*, *Ficaria fascicularis* and *Ranunculus pensylvanicus* (Fig. 5C). Mapping the character states on the phylogenetic tree based on the combined nuclear and chloroplast sequences suggested that the tricolpate type is ancestral in the tribe, but the variation of this character is too high to characterize genera. Only the *Krapfia-Laccopetalum* clade has a consistently pantoporate aperture type.

Morphological characters of the perianth, i.e., presence of petals or the shape of the nectary, are not congruent with the tree topology of the combined nuclear and chloroplast sequences (e.g., Fig. 4B). Other morphological characters are either unique for certain genera (Appendix 3) or show an overall high level of homoplasy.

■ DISCUSSION

Phylogenetic reconstruction and morphology. — Given the low resolution in the ITS topology, we have based the discussion of our results on analyses of the combined nuclear and chloroplast sequence datasets.

The parsimony analysis revealed a strict consensus tree with six main clades, clades I-a, -b, -c, -d and clades II-a, -b (Fig. 1) within the Ranunculeae. This grouping is incongruent with the classification of Tamura (1993, 1995) on subtribal level. Tamura subdivided Ranunculeae into three subtribes, Trautvetteriinae, Myosurinae and Ranunculinae. Under this classification, achenes in the Trautvetteriinae have no sclerenchymatous layer in the pericarp while in Myosurinae it is weakly developed. Ranunculinae on the other hand, have either well-developed sclerenchymatous layers or none at all. Clade I identified in our analysis includes mainly genera with a sclerenchymatous layer except *Coptidium* (Fig. 5A). Clade II includes Trautvetteriinae and the remaining genera of Ranunculinae sensu Tamura (Fig. 5A). Tamura's (1995) concept of subtribes is therefore not supported by the molecular data.

With the exception of some species of *Ranunculus*, the taxa in clade I have no distinct veins on the lateral surface of the achenes. However, the pattern of venation in these species is anastomosed or strongly curved and not longitudinal-parallel,

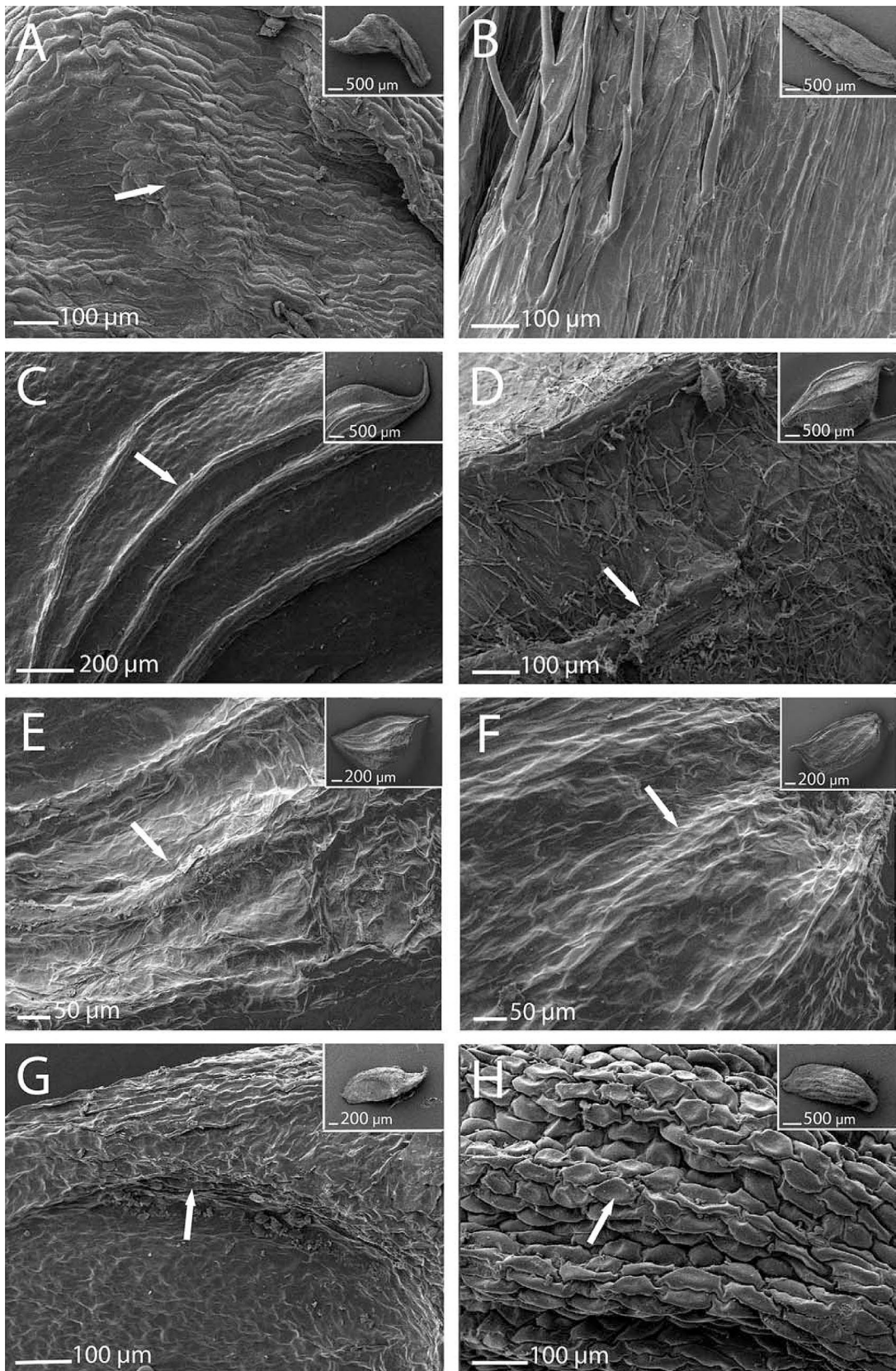
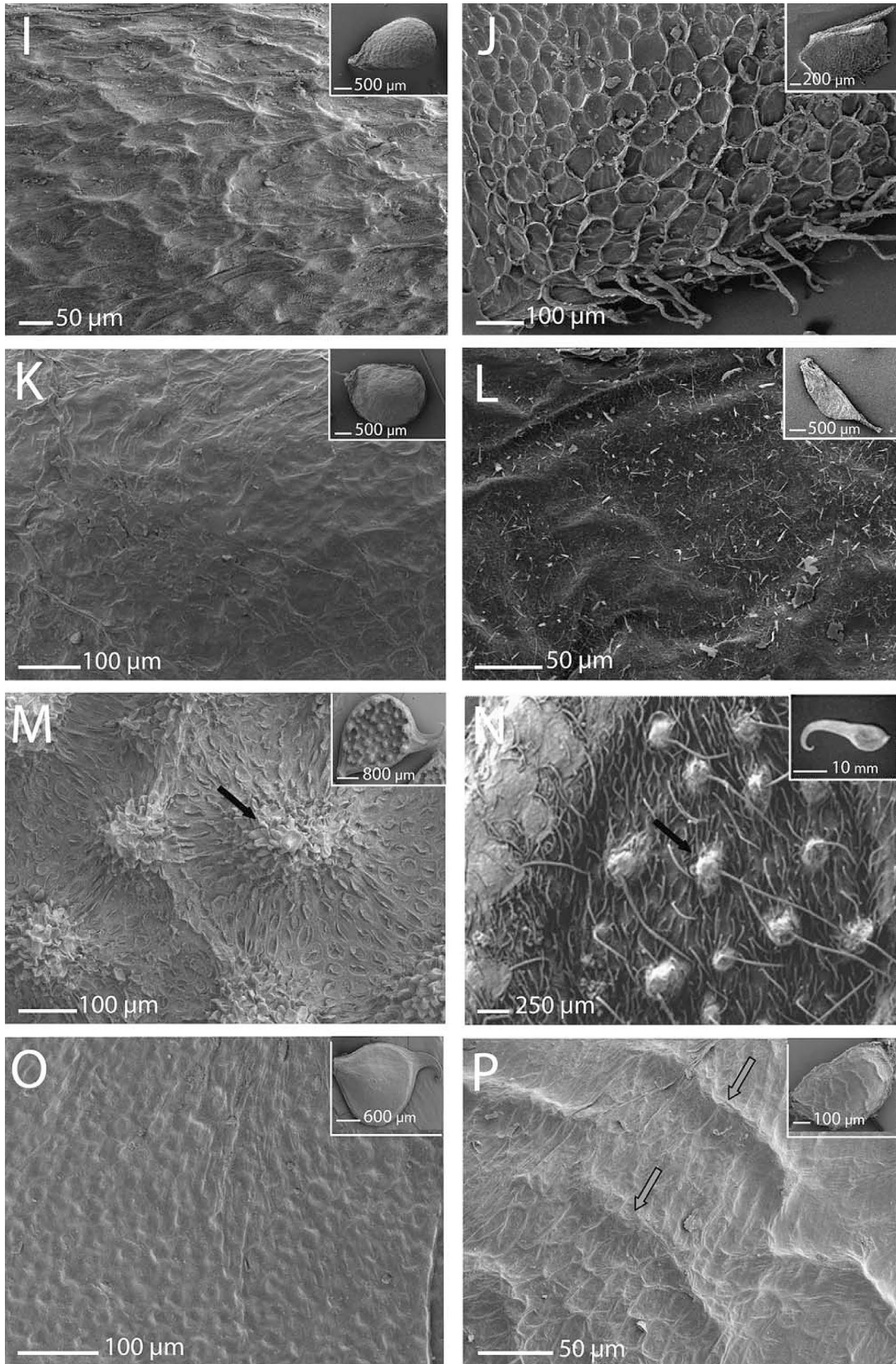


Fig. 4. Morphology of the achene surface of genera in tribe Ranunculeae, SEM micrographs. Small inserts show an overall view of the achene. **A–H**, taxa with longitudinal veins on the lateral surface (white arrows); **I–P**, taxa without longitudinal veins, but sometimes with tubercles (M–N, black arrow) or transversal ridges (P, grey arrow). **A**, *Hamadryas delfinii*; **B**, *Kumlienia hystricula*; **C**, *Arcteranthis cooleyae*; **D**, *Trautvetteria* ▶



► *carolinensis*; **E**, *Halerpestes cymbalaria*; **F**, *H. uniflora*; **G**, *Oxygraphis polypetala*; **H**, *Cyrtorhyncha ranunculina*; **I**, *Ficaria fascicularis*; **J**, *Myosurus minimus*; **K**, *Ranunculus apiifolius*; **L**, *Coptidium pallasii*; **M**, *Ranunculus arvensis*; **N**, *R. pinardii*; **O**, *R. lanuginosus*; **P**, *R. trichophyllus*. The microstructure of the surface of the pericarp is described in the text.

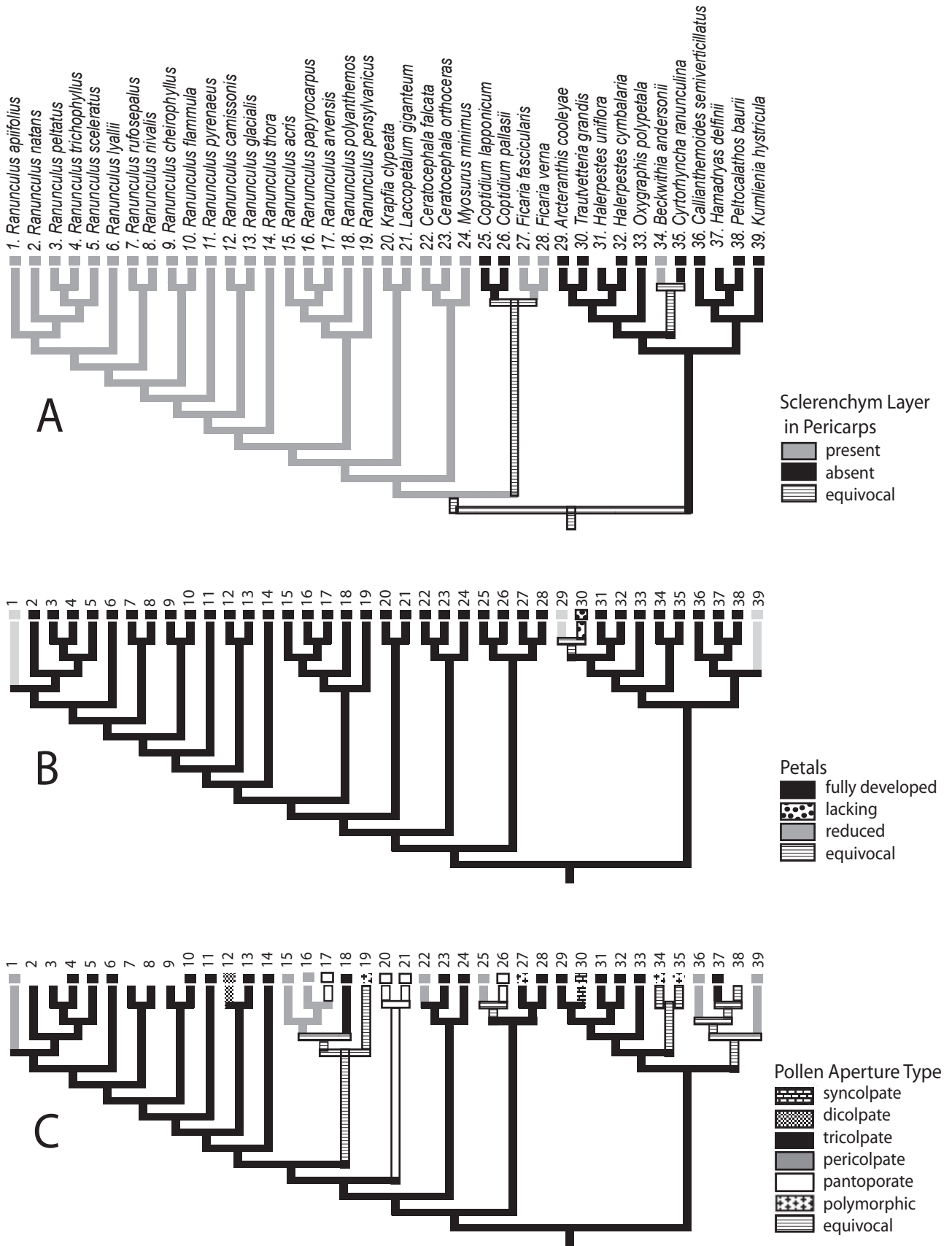


Fig. 5. Optimization of three morphological characters on the tree topology based on the combined ITS, *matK/trnK* and *psbJ-petA* dataset. **A**, sclerenchyma layer in the pericarp; **B**, presence of petals; **C**, aperture type of pollen.

as in the taxa of clade II. In the genera of clade I, veins occur only at the dorsal and ventral edges of the achenes (Lonay, 1901), which may be a derived character (Tamura, 1995). Although the sclerenchyma layer and venation patterns are not completely congruent with the molecular phylogeny, they represent probably the most conservative characters at the generic level. In their specific patterns, they can be used as diagnostic features.

Other macroscopic fruit structures might be best understood as dispersal mechanisms for certain taxa. For instance, *Ranunculus* sect. *Batrachium* has transversal ridges (Fig. 4P) which are formed by sclereid cells inside the pericarp (Cook, 1963). These ridges are breaking zones allowing the passage of water during germination. This feature could potentially be advantageous in aquatic habitats (Cook, 1963). Presence of spiny, tuberculate, and hooked structures of the pericarp such as in *Ranunculus arvensis* (Fig. 4M) and in *R. pinardii* (Fig. 4N) might be interpreted as an adaptation to epizoochory (Müller-Schneider, 1986). In *Ceratocephala* the achenes do not fall apart at maturity and the collective fruit, with its spine-like long beaks, is dispersed as a whole. All these taxa occur in dry areas, where spiny diaspores are an efficient dispersal mechanism via epizoochory.

Neither analyses of molecular nor morphological data revealed a strongly supported basal subdivision of the tribe. The basal dichotomy of the parsimony analysis (clades I and II) is not supported by a strong split in the Neighbor Net analysis, and this division is not supported by the presence/absence of any shared morphological characters. The Neighbor Net analysis does not suggest a strongly hierarchical (bifurcating) structure of the data, but rather indicates a network composed of five major lineages (Fig. 2). These graph features do not provide support for an hypothesis of gradual evolution (Hoot & al., 2008), as might be inferred from a bifurcating tree-topology. Rather they suggest that the main genetic lineages diverged within a relatively short geological time period, most likely within the Eocene (Paun & al., 2005; Hoffmann & al., 2010). The major lineages identified in the Neighbor Net analyses are not supported by morphological features and do not correspond to previous classifications, although as mentioned above, they correspond partly to well-supported clades of the parsimony analysis (Fig. 1). Greater congruence of molecular data and morphological characters occurs in the terminal clades. Presence of a lack of resolution in relationships of the some clades (e.g., clades II-a, II-b) is probably due to rapid ancient radiation, an inference consistent with the shape of the NNet splits graph (Fig. 2).

Clade I-a, *Ranunculus* clade. — The maximum parsimony analysis revealed a well-supported main *Ranunculus* clade including *Aphanostemma* and *R.* subg. *Batrachium* (Fig. 1), consistent with findings from earlier studies (Hörandl & al., 2005; Paun & al., 2005). *Ranunculus pinardii*, which had been previously unstudied, is clearly nested within *Ranunculus*.

Ranunculus apiifolius was described by Persoon in 1806. However, later this species was assigned to a monotypic genus, *Aphanostemma* (Pers.) A. St.-Hil. (1825) in consideration of its small, bilabiate petals. In our tree topology, based on all markers, this species was nested within the core *Ranunculus* clade

as in previous studies (Hörandl & al., 2005; Paun & al., 2005; Lehnebach & al., 2007). A morphology-based cladistic analysis placed *Aphanostemma apiifolius* in a clade with *Ranunculus* as well (Loconte & al., 1995). The very small petals in this species have been assumed to be an ancestral feature in the tribe (Janchen, 1949; Tamura, 1995). However, reduced petals occur in different genera within the tribe several times, e.g., in *Kumlienia hystriculula* and, less pronounced, in *Arcteranthis cooleyae* (Fig. 5B). In Ranunculaceae, the formation of petals is probably controlled by a shared, homologous developmental program that can be rapidly modified by gene expression patterns (Rasmussen & al., 2009). Our character optimization suggests that the presence of petals is ancestral in Ranunculaceae (Fig. 5B). In other genera with reduced petals, the large and colored petaloid sepals have an insect-attracting function. In *R. apiifolius* the sepals are also inconspicuous and the perianth may be secondarily reduced in this annual, ephemeral species. The surface of the achenes is similar to other *Ranunculus* species (Fig. 4K) and lacks longitudinal veins or other prominences on the surface of the achene. Considering the molecular evidence and the presence of only a single autapomorphy, the species should be kept as *Ranunculus apiifolius*.

In all our analyses, species of *R.* sect. *Batrachium* (*R. peltatus*, *R. trichophyllus*, *R. sphaerospermus*) form a distinct clade with high bootstrap support nested in the core *Ranunculus* clade, as sister to other species growing in wet habitats (Figs. 1, 2), e.g., *Ranunculus natans* (Hörandl & al., 2005; Paun & al., 2005; Gehrke & Linder, 2009; Hoffmann & al., 2010; Emadzade & al., unpub.). *Batrachium* was described by Candolle (1817) as a section of *Ranunculus* and elevated to generic status by Gray (1821). This section includes aquatic species having white petals with no starch layer, reduced nectary pits, achenes with transversal ridges on the surface, and often heterophyllous leaves. The two latter characters are putative adaptations to aquatic habitats (Cook, 1963, 1966). Transverse ridges on the surface of the achene, which is one of most characteristic features of this group (Fig. 4L), occur also in some species of *Ranunculus*, e.g., *R. sceleratus*, *R. rivularis* (Cook, 1963). According to these morphological characters and molecular data, the classification of this group of species as a section of *Ranunculus* is supported (Hörandl, in press). All other representatives of sections of *Ranunculus* s.str. sensu Tamura, including *R. cheirophyllus* as a member of *R.* sect. *Ficariifolius*, were nested within *Ranunculus* (Fig. 1). A more comprehensive discussion of sections within *Ranunculus* has been presented in Hörandl & al., 2005 and Hörandl, in press). A formal infrageneric classification of *Ranunculus* will be presented elsewhere (Hörandl & Emadzade, in prep.).

Our study also confirms the position of the former monotypic Central Asian genus *Gampsoceras* within *Ranunculus*. *Gampsoceras pinardii* was first described by Steven (1852) but later classified as a member of the genus *Ranunculus* in the subgenus *Gampsoceras* specifically by Tamura (1991, 1995). *Ranunculus pinardii* is an annual species with conspicuous flat, spiny and tuberculate fruits with very long, apically hooked beaks. The surface structure and the size of achenes resemble achenes of *R. arvensis*, and the length of the beaks

is similar to those of *Ceratocephala* (Fig. 4N). The molecular tree based on the combined sequence data reveals that this taxon is nested within the *Ranunculus* clade, with a strongly supported sister relationship with the perennial species *R. uncinatus* and *R. acris* (Fig. 1). A more comprehensive phylogenetic analysis of *Ranunculus* s.str. places *R. pinardii* together with other species of the Irano-Turanian region (*R. sericeus*, *R. strigillosus*, and *R. constantinopolitanus*; Emadzade & al., subm.). Appendages on the diaspores increase the potential for epizoochorous dispersal (Tackenberg & al., 2006; de Pablos & Peco, 2007).

Clade I-b, *Krapfia-Laccopetalum* clade. — *Krapfia*, comprising eight species, and the monotypic genus *Laccopetalum* are endemic to the central Andes of South America. In all our analyses these two species form a strongly supported clade (Fig. 1, BS: 100). Previous molecular studies confirmed the sister relationship of the two genera (Lehnebach & al., 2007; Hoot & al., 2008). The tree topology based on combined chloroplast markers shows that this clade is nested in the core *Ranunculus* clade with low bootstrap support (its position is indicated in Fig. 1). The analyses based on combined nuclear and chloroplast markers placed this clade sister to the core *Ranunculus* clade without high bootstrap support. The *Krapfia-Laccopetalum* clade shows only 14 substitutions compared to the *Ranunculus* clade based on the combined data. In this case, more markers might be needed to resolve the position of these taxa. The Neighbor Net analysis places the *Krapfia-Laccopetalum* group within the core group of *Ranunculus* and indicates incompatibilities consistent with reticulate evolution in the *Krapfia-Laccopetalum* clade (Fig. 2). Lehnebach & al. (2007), analyzing *matK/trnK*, and Hoot & al. (2008), analyzing *atpB* and *rbcL*, found that the *Krapfia-Laccopetalum* clade was placed outside of the *Ranunculus* clade, but with low bootstrap support. In these studies, species representing the genus *Ranunculus* were not available from all sections, so the position of the *Krapfia-Laccopetalum* clade was less reliable.

Krapfia and *Laccopetalum* have subglobose flowers, concave, thick sepals and petals, fleshy and clavate receptacles with both stamens and carpels attached (androgynophore), and finally a very distinct character, a free zone between the carpellate and the staminate areas. Both genera have numerous (in *Laccopetalum* up to 10,000) small carpels. Petals in *Krapfia* have one to three nectaries, whereas petals in *Laccopetalum* have many (up to 30) nectaries. These two genera can be distinguished by these characters from other taxa in Ranunculeae (Tamura, 1995; Lehnebach & al., 2007). Multiple nectary glands occur in alpine *Ranunculus* species from New Zealand as well, but the position and shape differ from those of *Laccopetalum* (Lehnebach & al., 2007). Previous molecular studies have shown that the species from New Zealand are not related to the *Krapfia-Laccopetalum* clade (Lehnebach & al., 2007). Additional to these characters, our palynological study showed that *Krapfia* and *Laccopetalum* have pantoporate pollen grains which occur in other species of *Ranunculus*, as well (Fig. 5C; Santisuk, 1979). Pantoporate pollen does not occur in any other genus of the tribe except for *Coptidium* (*C. pallasii*). However, pores in *Coptidium* are elongate and represent

an intermediate stage between a pantoporate and pantocolpate pattern. *Laccopetalum giganteum* has a special kind of pericopate pollen which is not observed in other taxa. It has six relatively large pores, whereas other taxa have pollen grains smaller in size and with more than six pores. A morphology-based cladistic analysis has suggested that *Laccopetalum* and *Krapfia* are sister to *Ceratocephala*, but not to *Ranunculus* (Loconte & al., 1995).

We hypothesize that the strong geographical isolation of this clade in the Andes has resulted in the evolution of very distinct morphological characters in these genera. Fleshy sepals and receptacles and also coriaceous leaves could be adaptations to xerophytic conditions (Lehnebach & al., 2007). The molecular data suggest a close relationship of this clade to *Ranunculus* s.str. or even derivation from the core *Ranunculus* clade (Fig. 2). The two genera could be included into *Ranunculus* s.str., as suggested by Janchen (1949). Nevertheless, because of their combination of unique morphological characters, we will maintain these two species as members of separate genera. Future studies should include more species of *Krapfia* to test the phylogenetic placement of *Laccopetalum* and its relationship to *Krapfia*.

Clade I-c, *Ceratocephala-Myosurus* clade. — The position of the *Ceratocephala-Myosurus* clade as sister to the core *Ranunculus* clade has been reported in all previous studies based on plastid and nuclear markers (Paun & al., 2005; Lehnebach & al., 2006; Hoot & al., 2008; Gehrke & Linder, 2009; Hoffmann & al., 2010). In our study, the maximum parsimony tree topology based on the combined dataset reveals that the *Ceratocephala-Myosurus* clade was sister to the *Ranunculus* clade with high bootstrap support (Fig. 1). *Myosurus* and *Ceratocephala* form one clade in the MP analysis, but do not cluster together in the Neighbor Net analysis (Fig. 2, cluster 2 & 3). The result of Neighbor Net analysis shows that these genera not only are separated from the core *Ranunculus* clade, but they are also highly diverged from each other.

The distinctive morphological characters of both *Ceratocephala* and *Myosurus* support their segregation from *Ranunculus*. The achenes of *Ceratocephala* have inflated empty chambers on either sides, an elongated beak, except one endemic species in New Zealand (Garnock-Jones, 1984), and a base chromosome number $x = 7$, which has been reported in only some species of *Ranunculus*. The karyotype of *Ceratocephala*, however, is different from these species (Goepfert, 1974). *Myosurus*, on the other hand, is a distinct small annual, scapose genus, distinguished from other genera of Ranunculeae by spurred sepals, strongly elongated fruits, a strong dorsal ridge on the achene (Fig. 4J) and pendant anatropous ovules. Other members of Ranunculeae have ascending hemitropous ovules (Tamura, 1995). *Myosurus*, which was described by Linnaeus (1753), has never been included in *Ranunculus* and was treated as the single member of Myosurinae by Tamura (1995). Chromosome studies in this taxon showed that chromosomes types are intermediate between the R-Type and the T-Type (Kurita, 1963), although T-type chromosomes have not been reported in Ranunculeae. Two shared morphological characters of *Ceratocephala* and *Myosurus* are the persistence

of hypocotyl and the development of adventitious roots in the transitional zone between the hypocotyl and the primary root (Tamura, 1995). These features are also observed in *R. pinardii* and could play a role in the rapid development of root systems in annual species. Our SEM study shows that *Myosurus* has an unusual reticulate microstructure on the surface of the pericarp (Fig. 4D). This pattern has neither been observed in any other species of *Ranunculus* nor in allied genera.

These distinctive morphological and chromosomal characters, the molecular data and high sequence divergence in the *psbJ-petA* region provide strong support for the exclusion of *Ceratocephala* and *Myosurus* from *Ranunculus* as classified by most European and Asian authors (Ovczinnikov, 1937; Iranshahr & al., 1992; Tutin & Cook, 1993; Hörandl, in press).

Clade I-d, *Ficaria-Coptidium* clade. — All datasets support a close relationship between *Ficaria* and *Coptidium*, which is in agreement with previous molecular studies (Johansson, 1998; Hörandl & al., 2005; Paun & al., 2005; Hoot & al., 2008; Gehrke & Linder, 2009; Hoffmann & al., 2010).

The position of *Ficaria* has always been controversial and it has been considered as a subgenus, a section of *Ranunculus* or a separate genus. Tamura (1995) classified it as a subgenus of *Ranunculus*. However, *Ficaria* has been accepted by many Asian botanists as a separate genus because of its distinct features: three sepals, more than five petals, and stalked but non-beaked achenes (Ovczinnikov, 1937; Iranshahr & al., 1992). Similarly, *Coptidium*, with two species (*C. lapponicus*, *C. pallasii*), is differentiated from other *Ranunculus* species by three sepals and achenes without a sclerenchymatous layer but with two separate parts, the upper part filled with spongy tissue and the lower part containing the seed. This feature probably helps in the dispersal of the seed by water, i.e., hydrochory (Tamura, 1995). *Coptidium* differs also by pocket-like nectary scales from *Ficaria*, which has flap-like nectary scales. In the most recent revision of Ranunculaceae (Tamura, 1995), *C. pallasii* and *C. lapponicus* were classified in subgenera of *Ranunculus* (*Pallasiantha* and *Coptidium* respectively), based on petal color and leaf shape. Both species have four acrocentric and four metacentric pairs of chromosomes per diploid set (Goepfert, 1974). Flovik (1936) reported that *C. lapponicus* has particularly large chromosomes in comparison with other related taxa. This diploid species hybridizes with tetraploid *C. pallasii* and the triploid hybrid (*R. × spitzbergensis*) combines the different chromosomes of the parents (Benson, 1948; Cody & al., 1988). The shared fruit characters, the sister-relationship in the phylogenetic reconstruction and the interspecific hybridization of these taxa support their treatment as a single genus, *Coptidium*.

Furthermore, the Neighbor Net analysis (Fig. 2) and all tree topologies based on nuclear and chloroplast markers (Fig. 1) show a clear separation of the *Ficaria-Coptidium* clade from the core *Ranunculus* clade (there are c. 160 substitutions between the core *Ranunculus* clade and the *Ficaria-Coptidium* clade in the combined dataset). Results by Hoot & al. (2008) also confirm the separation of *Ficaria* (*Ranunculus ficaria* in this paper) from the core *Ranunculus* clade based on *atpB* and *rbcL* markers. In Hoot & al. (2008), *Ficaria* is sister to *Hamadryas*, *Halerpestes* and *Trautvetteria*. In our analysis, *Ficaria*

is more closely related to the *Ceratocephala-Myosurus-Ranunculus* clade than to the *Hamadryas-Halerpestes-Trautvetteria* clade. Based on all the morphological and molecular evidence, we conclude that *Ficaria* and *Coptidium* should be treated as genera and not merged with *Ranunculus*, in accordance with many Eurasian authors (Ovczinnikov, 1937; Iranshahr & al., 1992; Hörandl, in press).

Clade II. — Our combined analysis shows that *Trautvetteria*, *Halerpestes*, *Oxygraphis*, *Arcteranthis*, *Beckwithia* and *Cyrtorhyncha* form one clade (Clade II-a, Fig. 1) and *Hamadryas*, *Peltocalathos*, *Callianthemoides* and *Kumlienia* another one (Clade II-b, Fig. 1). However, this subdivision is only weakly supported (71 BS for clade IIa) and is neither confirmed by Neighbor Net analysis, nor by earlier studies with an incomplete sampling of the tribe. In Hoot & al. (2008), *Oxygraphis* is sister to a clade comprising *Hamadryas*, *Peltocalathos*, and *Callianthemoides*. These two clades form a single strongly supported cluster in the Neighbor Net analysis (Fig. 2, 100% BS). In this analysis the two clades II-a and II-b are basal sister groups that are poorly resolved. Since the taxa in clade II show mostly disjunct geographical distributions, and may have diverged between the Miocene and the early Pliocene (Paun & al., 2005, Emadzade & al., submitted), it is unlikely that the lack of resolution and incompatibilities visualized in the splitsgraph are the result of recent hybridization. A possible explanation is ancient radiation within clade II and a strong morphological divergence of taxa in different geographical areas.

Clade II-a, *Arcteranthis-Beckwithia-Cyrtorhyncha-Halerpestes-Oxygraphis-Trautvetteria* clade. — *Oxygraphis*, a genus of four species, is located on a long branch in clade II-a and similarly so in all analyses (Figs. 1, 2). This taxon, with persistent and enlarged sepals, has been accepted by most taxonomists as a separate genus. Gray (1886) has emphasized that the texture of the carpels of *Oxygraphis* is so distinct that this taxon should be without any doubt excluded from *Ranunculus*. One of the main diagnostic characters of *Oxygraphis* is the persistence of sepals at the fruiting stage, although this character has also been observed in *Paroxygraphis*, *Beckwithia*, and *Ranunculus glacialis* as well. Similar to *O. polypetalata*, all these taxa are distributed in high alpine zones or in the Arctic, and this feature might be explained as a homoplasious adaptive character that protects the fruits from wind or low temperatures in harsh cold climates. There is at least one longitudinal prominence on the surface of the achene. This feature, along with a small triangular beak, can be used to distinguish the achene of *Oxygraphis* from all other genera (Fig. 4G). In the maximum parsimony analysis, *Oxygraphis* represents a highly diverged lineage nested within clade II-a. Its phylogenetic placement within this clade is also supported by the Neighbor Net analysis (Fig. 2).

Halerpestes comprises about ten species and is relatively widespread in the Northern Hemisphere and in South America. The taxonomic status of *Halerpestes* has varied from being considered as a subgenus or section of *Ranunculus* to being included in *Oxygraphis* or treated as a separate genus. Due to the basic karyotype of four acrocentric and four metacentric chromosome pairs, Goepfert (1974) assumed that *Oxygraphis*

has an ancestral status. Ploidy levels in *Halerpestes* vary from diploid to hexaploid. The tree topology based on combined data (Fig. 1) revealed its position in clade II-a, which agrees with the study by Hoot & al. (2008), which reported analyses of *atpB* and *rbcL* data. Our SEM studies on two species show that *Halerpestes* has flap-like nectary scales, in contrast to Tamura (1995), who described them as pocket-like. Variation of the nectary scale has been observed in *Ranunculus* s.str. as well.

Analyses of all molecular datasets suggest that *Halerpestes uniflora* is sister to *Halerpestes cymbalaria* with 100% bootstrap support (Figs. 1, 2). This species was described as *Ranunculus uniflorus*, endemic to the alpine zones of South America. It is a perennial species with entire leaves, three sepals, seven petals, and a high number of carpels (ca. 100). The presence of longitudinal veins on the achenes, which is typical of clade II, is similarly observed on the achenes of *H. uniflora* (Fig. 4F). The SEM study shows that the pattern of veins in this taxon is the same as in *H. cymbalaria* (Fig. 4E). Additionally, this taxon has tricolpate pollen as *H. cymbalaria*. According to these morphological characters, habitat and molecular data, we classify this species as a member of *Halerpestes*.

Trautvetteria has been treated as a single genus in Trautvetteriinae due to its apetalous by Tamura (1967). Tamura (1995) considered this genus as the ancestor of the whole tribe since monochlamydeous flowers have been considered as a primitive condition in the family. However, recent phylogenetic studies revealed the evolution of perianth differentiation for Ranunculales as highly dynamic; the condition of two perianth whorls, with the outer one sepaloid, the inner one tepaloid, is ancestral for Ranunculales, while the presence of petals and sepals is derived (Endress & Doyle, 2009). For the core Ranunculaceae, the ancestral state is that both tepal whorls are petaloid (Endress & Doyle, 2009). In Ranunculaceae the presence of petals is the ancestral state (Fig. 5B). According to the scattered presence of some taxa with more or less reduced petals in the whole tree (*Ranunculus apiifolius*, *Kumlienia*, *Trautvetteria*), it is likely that apetalous flowers are a homoplasious, derived feature in this tribe (Fig. 5B). However, in all analyses *Trautvetteria* is nested in the clade II-a, sister to *Arcteranthis* with high bootstrap support. Previous studies based on *atpB* and *rbcL* provide good support for the close relationship to *Hamadryas*, *Halerpestes*, and *Ficaria* in one clade with 60% BS (Hoot & al., 2008).

Arcteranthis and *Cyrtorhyncha* are monotypic genera endemic to northwestern and western North America, respectively. Based on the combined data, *Arcteranthis* shows a well-supported close relationship with *Trautvetteria* (Fig. 1). The Neighbor Net analysis also confirms this affinity (Fig. 2). *Trautvetteria* and *Arcteranthis* have a similar pattern in the veins on the surface of the achenes but *Trautvetteria* has some thin veins between the main veins which are lacking in *Arcteranthis* (Fig. 4C–D). Analyses of molecular data, reduced petals and petaloid sepals, and a partly shared distribution area in North America strongly suggest a common ancestry of *Arcteranthis* and *Trautvetteria*.

Beckwithia andersonii, which has been classified in *Ranunculus* subg. *Crymodes* by Tamura (1967), is sister to *Cyrtorhyncha* and located in clade II-a in all our analyses (Figs. 1, 2).

This genus is characterized by bladder-like fruitlets and membranaceous pericarps. Due to these characters some authors have described the fruit of this taxon as utricle (Whittemore, 1997). Membranaceous pericarps are observed in *Ranunculus papyrocarpus* as well. A cavity in the fruit could be some kind of adaptation to wind dispersal (Müller-Schneider, 1986).

Achenes of *Cyrtorhyncha* have long triangular hooked beaks and almost parallel longitudinal veins (Fig. 4H) which are unique within the tribe. Although there are no obvious morphological synapomorphic characters shared between *Cyrtorhyncha ranunculina* and *Beckwithia andersonii*, these two taxa form a clade with 100% BS in tree topologies based on combined nuclear and chloroplast data (Figs. 1, 2) and have a similar distribution area.

Clade II-b, *Hamadryas-Peltocalathos-Callianthemoides-Kumlienia* clade. — *Hamadryas* is one of two dioecious genera in Ranunculaceae (in addition to *Paroxygraphis*), and it is endemic to South America. Based on chloroplast data, it forms a clade with *Peltocalathos*, *Callianthemoides* and *Kumlienia*, but without high bootstrap support. This weakly supported and heterogeneous clade comprises four monotypic genera with distinct geographical distributions: *Hamadryas* and *Callianthemoides* are endemic to South America, *Peltocalathos* is endemic to South Africa, and *Kumlienia* is endemic to southwestern North America. All members of this clade have colored sepals. The main diagnostic characters of the members of this clade are: *Hamadryas* is dioecious; *Callianthemoides* has four to seven times pinnately ternate leaves; *Peltocalathos* has peltate, rounded leaves, and *Kumlienia* has small, cup-shaped petals and conspicuous white sepals. Our palynological study shows that *Callianthemoides semiverticillatus* and *Kumlienia hystricula* have pericarpate pollen while *Hamadryas delfinii* has tricolpate pollen. All of the species in this clade have pocket-like nectary scales except *Callianthemoides* which has a thickened nectary with a short scale. Each genus has a distinct shape of achenes. *Kumlienia* and *Peltocalathos* have elongated achenes, hairy in *Kumlienia* (Fig. 4B). Achenes are obovoid in *Callianthemoides* and semiovoid in *Hamadryas*. All of these four genera have distinct venation patterns on the surface of achenes (Fig. 4A–B). The morphological divergence in the clade is not accompanied by a pronounced genetic divergence, as inferred from branch lengths and relationships in the Neighbor Net analysis (Figs. 1, 2). The evolution of distinct morphological features is probably the result of a strong geographical isolation and rapid character evolution in different areas.

■ TAXONOMIC CONCLUSIONS

Parallel, adaptive and convergent evolution of morphological characters has occurred not only in *Ranunculus* and allied genera, but also the other genera of Ranunculaceae (Hoot, 1991; Hoot & al., 1994; Johansson, 1995, 1998; Ro & al., 1999; Lockhart & al., 2001; Hörandl & al., 2005; Paun & al., 2005). In fact, homoplasy of morphological characters has made morphology-based classifications in this tribe difficult. The molecular phylogenetic study provides the basic framework

for an improved classification and a better understanding of character evolution.

Most of the micro- and macromorphological characters studied here show incongruence with the molecular tree (Fig. 5). Our study suggests that fruit characters may be linked to dispersal mechanisms (e.g., achenes with spines, long hooked beaks, swollen fruits). The shape of the pollen apertures also shows parallel evolution. The basic and most common type is tricolpate, which is observed in most of the *Ranunculus* species. Pollen structures have developed in other genera as an adaptation to pollination mechanisms (Proctor & al., 1996; Hesse, 2000; Tanaka & al., 2004). Characters of the perianth are probably based on a shared developmental program, and may be highly dynamic according to activation or de-activation of gene expression patterns (Rasmussen & al., 2009).

Our study confirms a great diversity of morphological characters which have evolved multiple times within the tribe. According to these characters and molecular studies, aggregating all genera of the tribe under *Ranunculus* s.l. would give a very heterogeneous taxon lacking common morphological features. Except for *Myosurus* and *Ceratocephala*, the morphological divergence in the clade is not accompanied by a pronounced genetic divergence, as inferred from branch lengths and genetic relationships as suggested in the Parsimony and Neighbor Net analyses (Figs. 1, 2). Neighbor Net analysis confirmed that the genetic structure of the tribe is not hierarchical, but rather suggests several distinct clusters emerged out of an unresolved backbone phylogeny. Moreover, the two clades I and II each lack diagnostic morphological features. We agree with most authors that morphology is of crucial importance for a delimitation of genera (e.g., Stuessy, 2009 and literature therein), and we prefer to separate genera according to those well-supported clades or branches which can be identified by morphological features. These diagnostic characters can be used for identification. This concept fits largely to Tamura's (1995) narrow circumscription of genera, but avoids a polyphyletic genus *Ranunculus* s.str. by excluding *Ficaria* and *Coptidium*. We do not regard the morphological and genetic divergence of *R. apiifolius* as strong enough for a monotypic genus which would leave *Ranunculus* s.str. as a paraphyletic taxon (see discussion in Hörandl, 2006, 2007, 2010; Stuessy & König, 2008). Moreover, our data support acceptance of several monotypic genera in clade II, because none of the groupings suggested by the molecular data would be accompanied by shared morphological features. The strong geographical isolation of sister taxa over long time periods (e.g., in clade II-b) might have triggered the evolution of distinct, unique features, and further supports a delimitation of genera (e.g., Stuessy, 2009). The monotypic taxa could be relictual survivors of ancient radiations, or alternatively, they may have never diversified.

■ TAXONOMIC IMPLICATIONS

We list here accepted generic names with their types and the most important synonyms, and new combinations. A full synonymy list for each taxon is available in Tamura (1995).

Tribe Ranunculeae DC.

Arcteranthis Greene in Pittonia 3: 190. 1897 – Type: *Ranunculus cooleyae* (Vasey & Rose) Greene in Pittonia 3: 190. 1897 (*Ranunculus cooleyae* Vasey & Rose in Bull. Torrey Bot. Club 19: 239. 1892).

Beckwithia Jeps. in Erythea 6: 97. 1898 – Type: *B. austinae* Jeps. in Erythea 6: 99. 1898.

Callianthemoides Tamura in Acta Phytotax. Geobot. 43: 140. 1992 – Type: *C. semiverticillatus* (Phil.) Tamura in Acta Phytotax. Geobot. 43: 140. 1992 (*Ranunculus semiverticillatus* Phil. in Anales Univ. Chile 1: 60. 1861).

Ceratocephala Moench, Methodus: 218. 1794 – Type: *C. spicata* Moench, Methodus: 218. 1794 (*Ranunculus falcatus* L., Sp. Pl. 1: 556. 1753).

Coptidium (Prantl) Beurl. ex Rydb., Fl. Rocky Mts.: 302. 1917 – Type: *C. lapponicum* (L.) Rydb., Fl. Rocky Mts.: 302. 1917 (*Ranunculus lapponicus* L., Sp. Pl.: 553. 1753).

Cyrtorhyncha Nutt. ex Torr. & A. Gray, Fl. N. Amer. 1: 26. 1838 – Type: *Cyrtorhyncha ranunculina* Nutt. ex Torr. & A. Gray.

Ficaria Guett. in Hist. Acad. Roy. Sci. Mem. Math. Phys. 1750: 355. 1754 – Type: *F. verna* Huds., Fl. Angl.: 214. 1762 (*Ranunculus ficaria* L., Sp. Pl. 1: 550. 1753).

Halerpestes Greene in Pittonia 4: 207. 1900 – Type: *H. cymbalaria* (Pursh) Greene in Pittonia 4: 207. 1900 (*Ranunculus cymbalaria* Pursh, Fl. Amer. Sept. 2: 392. 1814).

According to our results, a new combination is needed for *Halerpestes uniflora*:

Halerpestes uniflora (Phil. ex Reiche) Emadzade, Lehnebach, Lockhart & Hörandl, **comb. nov.** Basionym: *Ranunculus uniflorus* Phil. ex Reiche in Anales Univ. Chile 88: 70. 1894.

Hamadryas Comm. ex Juss., Gen. Pl.: 232. 1789 – Type: *H. magellanica* Lam., Encycl. 3: 67. 1789.

Krapfia DC., Syst. Nat. 1: 228. 1817 – Type: *K. ranunculina* DC., Syst. Nat. 1: 228. 1817.

Kumlienia E. Greene in Bull. Calif. Acad. Sci. 1: 337. 1886 – Type: *K. hystricula* (A. Gray) E. Greene in Bull. Calif. Acad. Sci. 1: 337. 1886.

Laccopetalum Ulbr. in Bot. Jahrb. Syst. 37: 404. 1906 – Type: *L. giganteum* (Wedd.) Ulbr. in Bot. Jahrb. Syst. 37: 404. 1906 (*Ranunculus giganteus* Wedd., Chlor. Andina 2: 304. 1857).

Myosurus L., Sp. Pl. 1: 284. 1753 – Type: *M. minimus* L., Sp. Pl. 1: 284. 1753.

Oxygraphis Bunge, Verz. Altai Pfl. 2: 46. 1836 – Type: *Oxygraphis glacialis* (Fisch. ex DC.) Bunge, Verz. Altai Pfl. 2: 47. 1836 (*Ficaria glacialis* Fisch. ex DC., Prodr. 1: 44. 1824).

Paroxygraphis W.W. Sm. in Rec. Bot. Surv. India 4: 344. 1913 – Type: *Paroxygraphis sikkimensis* W.W. Sm. in Rec. Bot. Surv. India 4: 344. 1913.

Peltocalathos Tamura in Acta Phytotax. Geobot. 43: 139. 1992 – Type: *P. baurii* (MacOwan) Tamura in Acta Phytotax.

Geobot. 43: 140. 1992 (*Ranunculus baurii* MacOwan in J. Linn. Soc. Bot. 18: 390. 1881).

Ranunculus L., Sp. Pl. 1: 548. 1753 – Type: *Ranunculus acris* L., Sp. Pl. 2 1753 (Jarvis, 2007). Incl. *Batrachium* (DC.) Gray 1821; incl. *Aphanostemma* A. St.-Hil. 1825; incl. *Gampsoceras* Steven 1852.

Trautvetteria Fisch. & C.A. Mey., Ind. Sem. Hort. Petrop. 1: 22. 1835 – Type: *T. palmata* (Michx.) Fisch. & C.A. Mey., Ind. Sem. Hort. Petrop. 1: 22. 1835 (*Cimicifuga palmata* Michx., Fl. Bor.-Amer. 1: 316. 1803).

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Appendix 1. Materials used in this study (BG, Botanical garden).

Taxon (synonym); Country; Collector, Collection number, Herbarium; ITS Genbank no.; *matK/TrnK* GenBank no.; *psbJ-petA* GenBank no.

Anemone quinquefolia L.; Connecticut; *Mehrhoff 12602* CONN; GU257978; GU257980; GU257995. *Arcteranthis cooleyae* (Vasey & Rose) Greene (*R. cooleyae*); Canada; *U. Jensen 28432* MPN; AY680201; –; GU258002. *Beckwithia andersonii* (A. Gray) Jeps. (*R. andersonii*); cult. Gothenburg BG; *J.T. Johansson s.n.* GB; AY680197; AY954238; GU258003. *Callianthemoides semiverticillatus* (Philippi) Tamura (*R. semiverticillatus*); Argentina; *C. Lehnebach s.n.* VALD; AY680199; AY954236; Gothenburg, BG; *J.T. Johansson s.n.*; GU258004. *Ceratocephala falcata* (L.) Pers. (*R. falcatus*); Iran; *K.H. Rechinger, Jr. 50857* W; AY680191; AY954229; GU257996. *C. orthoceras* DC. (*R. testiculatus*); Austria; *E. Hörandl 3837* WU; AY680190; AY954230; GU257997. *Coptidium lapponicum* (L.) Rydb. (*R. lapponicus*); Sweden; *J.T. Johansson s.n.*; –; AY680194; AY954234; GU257998. *C. pallasi* (Schlecht.) Tzvelev (*R. pallasi*); Alaska; *R. Elven & al. SUP02-175* O; AY680195; AY954233; GU257999. *Cyrtorhyncha ranunculina* Nutt. ex Torr. & A. Gray. (*R. ranunculinus*); USA; *S. Nunn 1775* RM; GU257973; GU257981; GU258005. *Ficaria fascicularis* K. Koch (*R. kochii*); cult. Gothenburg BG; *J.T. Johansson s.n.* GB; AY680193; AY954231; GU258000. *F. verna* Huds. ssp. *verna* (*R. ficaria* ssp. *bulbilifer*); Sweden; *J.T. Johansson s.n.*; –; AY680192; AY954232; GU258001. *Halerpestes cymbalaria* (Pursh) Greene (*R. cymbalaria*); cult. Rezia BG; *J.T. Johansson 204* LD; AY680196; AY954237; GU258006. *H. uniflora* (Phil. ex. Reiche) Emadzade & al. (*R. uniflorus*); Chile; *C. Lehnebach s.n.* MPN; GU258007; GU258008; Argentina; *M. Weigend 7003* M; GU258007. *Hamadryas delfinii*; Argentina; *P. Schönswetter AR08-20* WU; GU257974; GU257982; GU258011. *Isopyrum thalictroides* L.; Austria; *E. Hörandl 641* WU; GU257977; GU257979; GU258014. *Krapfia clypeata* (Ulbr.) Standl. & J.F. Macbr. (*R. clypeata*); Peru; *Sanchez & al. 11173* F, CPUN, MPN; GU258011; DQ490058; –. *Kumlienia hystricula* (A. Gray) E. Greene; USA; *L. Grant 286* ZT; GU257975; GU257983; GU258008. *Laccopeltium giganteum* Ulbr. (*R. giganteus*); Halle, BG; *J.T. Johansson s.n.*; GU258006; Peru; *Cano & al. 15196* USM; DQ400695; Halle, BG; *J.T. Johansson s.n.*; GU258009. *Myosurus minimus* L.; Genbank; AJ347913; AJ414344; –. *Oxygraphis polypetala* Hook. F. & Thomson; Nepal; ? 1926-3 LI; GU257976; GU257984; GU258012. *Peltocalathos baurii* (McOwan) Tamura (*R. baurii*); South Africa; *L. Mucina 030103/22* WU; AY680200; AY954235; GU258010. *Ranunculus acris* L.; cult. Bonn BG; *J.T. Johansson 194* CONN; AY680167; AY954199; GU258015. *R. apiifolius* Pers. (*Aphanostemma apiifolia*); Chile; *C. Lehnebach s.n.* VALD; AY680092; AY954140; Uruguay; *Lorentz 533* W; GU258016. *R. arvensis* L.; cult. Kiel BG; *J.T. Johansson 180* CONN; AY680177; AY954193; Iran; *Emadzade 109* WU; GU258017. *R. asiaticus* L.; Iran; *Shooshtari 2569* TARI; GU257963; GU257985; GU258018. *R. bonariensis* Poir.; Argentina; *P. Schönswetter AR08-2a* WU; GU257964; GU257986; GU258019. *R. brevifolius* ssp. *brevifolius* Ten.; cult. Gothenburg BG; *J.T. Johansson s.n.* GB; AY680187; AY954212; GU258020. *R. breyninus* Cr. (*R. oreophilus*); Austria (loc. class.); *E. Hörandl 5249* WU; AY680115; AY954172; GU258021. *R. camissonis* Aucl. (*Beckwithia camissonis*); U.S.S.R.; *R. Koropewa s.n.* W; AY680083; AY954218; GU258022. *R. caucasicus* MB.; Georgia; *E. Hörandl 8259* WU; AY680178; AY954192; GU258023. *R. cheirophyllus* Hayata; Taiwan; *E. Hörandl 9550* WU; GU257965; GU257987; GU258024. *R. flammula* L.; cult. Oldenburg BG; *J.T. Johansson 193* CONN; AY680185; AY954204; GU258025. *R. formosomontanus* Ohwi; Taiwan; *E. Hörandl 9548* WU; GU257966; GU257988; GU258026. *R. glacialis* L.; Sweden; *J.T. Johansson s.n.*; –; AY680082; AY954219; GU258027. *R. kuefferi* ssp. *orientalis* W. Huber; Austria; *E. Hörandl 4336* WU; AY680085; AY954213; GU258028. *R. lyallii* Hook. f.; New Zealand; *M.A. Steel 24603* MPN; AF323277; AY954142; *G. Schneeweiss & al.* – WU; GU258029. *R. maclovinianus* Urv.; Chile; *C. Lehnebach s.n.* VALD; AY680158; AY954181; Argentina; *P. Schönswetter AR08-17* WU; GU258030. *R. natans* C.A. Mey.; Russia; *A. Tribsch 9558* WU; AY680113; AY954134; GU258031. *R. nivalis* L.; Sweden; *J.T. Johansson s.n.*; AY680046; AY954123; GU258032. *R. oxyspermus* Willd.; Iran; *Emadzade 100* WU; GU257967; GU257989; GU258033. *R. papyrocarpus* Rech. F., Aell. & Esfand.; Iran; *Tajeddini 110* WU; GU257968; GU257990; GU258034. *R. parnassifolius* ssp. *parnassifolius* L.; France/Spain; *G. Schneeweiss & al. 6509* WU; AY680072; AY954224; GU258035. *R. pedatifidus* J.E. Smith, USA; *R. Orthner 593RM*; GU257969; GU257991; GU258036. *R. peltatus* ssp. *peltatus* Moench (*Batrachium peltatum*); cult. Nantes BG; *J.T. Johansson 206* LD; AY680068; AY954131; GU258037. *R. pensylvanicus* L. f.; U.S.A.; *V. Zila 447002* LI; AY680147; AY954190; GU258038. *R. pinardii* (Stev.) Boiss.; Iran; *Ghahremani 108* WU; GU257970; GU257992; GU258039. *R. polyanthemoides* L.; Austria; *E. Hörandl 5130* WU; AY680121; AY954185; GU258040. *R. pyrenaicus* L.; Spain; *G. Schneeweiss & al. 6498* WU; AY680074; AY954225; GU258041. *R. rufosepalus* Franch.; Pakistan; *A. Millinger 392897* LI; AY680047; AY954121; GU258042. *R. sceleratus* L.; Iran; *Emadzade 112* WU; GU257971; GU257993; GU258043. *R. sphaerospermus* Boiss. & Blanche (*Batrachium sphaerospermum*); Turkey; *G. Dahlgren B87B* LD; AY680066; AY954132; GU258044. *R. thora* L.; cult. Lund BG; *J.T. Johansson 223* LD; AY680188; AY954210; GU258045. *R. trichophyllum* Chaix (*Batrachium trichophyllum*); Greece; *G. Dahlgren B23* LD; AY680067; AY954133; GU258046. *R. uncinatus* D. Don.; USA; *N. Holmgren 5379* ZT; GU257972; GU257994; GU258047. *Trautvetteria grandis* Honda; cult. California BG; *J.T. Johansson 82.1322* –; AY680202; AF007945; GU258013.

Appendix 2. Character list and their corresponding states used in this study. Symbols refer to the bibliographic source used, † Tamura, 1995; ‡ Goepfert, 1974. In the case no plant material or no complete vouchers were available, states were extracted from the literature cited in the materials and methods section.

1 Life form (0) annual–biennial, (1) perennial. **2 Number of flowers** (0) one, (1) more than one. **3 Flower position** (0) terminal inflorescence, (1) axillary in stem leaves, (2) arising from basal rosette. **4 Flower** (0) bisexual, (1) unisexual. **5 Sepals** (0) sepaloid, (1) petaloid. **6 Consistency of petal and sepal** (0) not fleshy, (1) fleshy. **7 Number of sepals** (0) three, (1) four, (2) more than four. **8 Spur in the sepal** (0) absent, (1) present. **9 Petals** (0) present, (1) absent, (2) reduced. **10 Number of petals** (0) less than five, (1) five, (2) more than five, (–) not applicable. **11 Color of petals** (0) yellow, (1) other than yellow, (–) not applicable. **12 Shape of nectary** (0) ridge, (1) flap, (2) pocket, (3) U-form, (4) ring, (5) double scale, (–) not applicable. **13 Number of nectary glands** (0) single, (1) three, (2) more than three. **14 Androecium & gynoecium** (0) not separated (androgynophore), (1) separated. **15 Indumentum of receptacle** (0) glabrous, (1) hairy. **16 Shape of fruit** (length/width ratio) (0) globose (0.5–1.0), (1) ellipsoid (1.0–2.5), (2) elongated (2.5–5), (3) linear (>5). **17 Ovule** (0) not pendulous, (1) pendulous. **18 Connection of achenes** (0) connate, (1) not connate. **19 Veins on achenes surface** (0) absent, (1) present, parallel & straight, (2) present, irregular reticulate & curved. **20 Size of achenes** (in mm) (0) <1.5, (1) 1.5–3.0, (2) 3.0–4.5, (3) >4.5. **21 Shape of achenes** (0) compressed, (1) swollen, (2) swollen with lateral bulges, (3) triangular. **22 Sclerenchyma layer of achenes†** (0) present, (1) absent. **23 Spongy tissue of achenes†** (0) present, (1) absent. **24 Achene surface** (microstructure × 570) (0) irregular rugose, (1) fine papillose, (2) foveolate, (3) reticulate rugose. **25 Achene surface, tubercles or spines** (macrostructure × 5) (0) absent, (1) present. **26 Achene surface, transversely wrinkles** (macrostructure × 5) (0) absent, (1) present. **27 Indumentum of achenes** (0) glabrous, (1) partly hairy, (2) hairy throughout. **28 Margin of achenes** (0) inconspicuous, (1) bordered, (2) winged. **29 Stalk of achenes** (mm) (0) short or missing (up to 0.5 mm), (1) long (>0.5 mm). **30 Beak length** (0) equalling body of achene, (1) shorter than body of achene, (3) missing. **31 Shape of beak** (length/width) (0) >5, (1) <1, (2) 1–5, (3) missing. **32 Basic chromosome number (x)‡** (0) 8, (1) 7. **33 Pollen aperture type** (0) syncolpate, (1) dicolpate, (2) tricolpate, (3) stephanocolpate, (4) periculate, (5) pantoporate.

Appendix 3. Data matrix of 33 morphological characters of Ranunculaceae. Inapplicable characters are coded with “–” and missing data are coded with “?”. Boxes and bold numbers (in the box) indicate diagnostic characters at generic level.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
<i>Arcteranthis cooleyae</i>	0	1	0	0	1	0	1	0	0	1	–	2	0	1	0	1	0
<i>Beckwithia andersonii</i>	1	0	2	0	0	0	1	0	0	1	1	4	0	1	1	0	0
<i>Callianthemoides semiverticillatus</i>	1	1	0	0	0	0	1	0	0	2	0	4	0	1	1	0	0
<i>Ceratocephala falcata</i>	0	0	2	0	0	0	1	0	0	0&1	0	1	0	1	0	1&2	0
<i>Ceratocephala orthoceras</i>	0	0	2	0	0	0	1	0	0	0&1	0	1	0	1	0	1&2	0
<i>Coptidium lapponicum</i>	1	0	1	0	0	0	0	0	0	2	0	2	0	1	0	0	0
<i>Coptidium pallasii</i>	1	0	1	0	0	0	0	0	0	2	1	2	0	1	0	0	0
<i>Cyrtorhyncha ranunculina</i>	1	1	0	0	0	0	1	0	0	2	0	0	0	1	0	0	0
<i>Ficaria fascicularis</i>	1	1	0	0	0	0	0	0	0	2	0	1	0	1	0	0	0
<i>Ficaria verna</i>	1	1	0	0	0	0	0	0	0	2	0	1	0	1	1	0	0
<i>Halerpestes cymbalaria</i>	1	0&1	0&2	0	0	0	2	0	0	1&2	0	2	0	1	1	1	0
<i>H. uniflora</i>	1	0	2	0	0	0	0	0	0	2	0	2	0	1	1	1	0
<i>Hamadryas delfinii</i>	1	0	2	1	0	0	2	0	0	2	0	2	0	1	0	1	0
<i>Krapfia clypeata</i>	1	1	0	0	0	1	2	0	0	2	1	2	0&1	0	0	0	0
<i>Kumlienia hystricula</i>	1	0	2	0	1	0	1	0	2	2	–	2	0	1	0	1	0
<i>Laccopetalum giganteum</i>	1	0	2	0	0	1	1	0	0	1	1	2	2	0	0	0	0
<i>Myosurus minimus</i>	0	0	2	0	0	0	1	1	0	1	0	2	0	1	0	3	1
<i>Oxygraphis polypetala</i>	1	0	2	0	0	0	1	0	0	2	0	0	0	1	0	1	0
<i>Peltocalathos baurii</i>	1	1	0	0	0	0	1	0	0	2	0	2	0	1	1	0	0
<i>Ranunculus acris</i>	1	1	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0
<i>R. apiifolius</i>	0	1	0	0	0	0	1	0	2	1	1	2	0	1	0	1	0
<i>R. arvensis</i>	0	1	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0
<i>R. asiaticus</i>	1	0&1	0	0	0	0	1	0	0	1	0	0&1	0	1	0	2	0
<i>R. bonariensis</i>	1	0	1	0	0	0	0	0	0	0	0	2	0	1	1	0	0
<i>R. brevifolius</i>	1	1	0	0	0	0	1	0	0	1	0	2	0	1	0	0	0
<i>R. breynius</i>	1	1	0	0	0	0	1	0	0	1	0	1	0	1	1	0	0
<i>R. camissonis</i>	1	1	0	0	0	0	1	0	0	1	1	5	0	1	0	0	0
<i>R. caucasicus</i>	1	1	0	0	0	0	1	0	0	1	0	1	0	1	1	0	0
<i>R. cheirophyllus</i>	0	1	1	0	0	0	0	0	0	0	0	2	0	1	0	0	0
<i>R. flammula</i>	1	1	0	0	0	0	1	0	0	1	0	2	0	1	0	0	0
<i>R. formosomontanus</i>	1	1	0	0	0	0	1	0	0	1&2	0	0	0	1	0	0	0
<i>R. glacialis</i>	1	1	0	0	0	0	1	0	0	1	1	5	0	1	0	0	0
<i>R. kuepferi</i>	1	1	0	0	0	0	1	0	0	1	1	5	0	1	1	1	0
<i>R. lyallii</i>	1	1	0	0	0	0	1	0	0	2	1	4	0	1	1	0	0
<i>R. maclovianus</i>	1	0&1	0&2	0	0	0	1	0	0	1	0	1	0	1	0	0	0
<i>R. natans</i>	1	0	1	0	0	0	1	0	0	1	0	2&4	0	1	1	0	0
<i>R. nivalis</i>	1	0&1	0	0	0	0	1	0	0	1	0	2	0	1	0	1	0
<i>R. oxyspermus</i>	1	1	0	0	0	0	1	0	0	1	0	1	0	1	0	1	0
<i>R. papyrocarpus</i>	1	1	0	0	0	0	1	0	0	2	0	2	0	1	0	0	0
<i>R. parnassifolius</i>	1	1	0	0	0	0	1	0	0	1	1	5	0	1	0	0	0
<i>R. pedatifidus</i>	1	1	0	0	0	0	1	0	0	1	0	2	0	1	1	1	0
<i>R. peltatus</i>	0&1	0	1	0	0	0	1	0	0	1	0	3&4	0	1	1	0	0
<i>R. pennsylvanicus</i>	0	1	0	0	0	0	1	0	0	1	0	1	0	1	1	1	0
<i>R. pinardii</i>	0	1	0	0	0	0	1	0	0	1	0	?	?	1	1	0	0
<i>R. polyanthemos</i>	1	1	0	0	0	0	1	0	0	1	0	1	0	1	1	0	0
<i>R. pyrenaicus</i>	1	1	0	0	0	0	1	0	0	1	1	5	0	1	0	0	0
<i>R. rufosepalus</i>	1	0&1	0	0	0	0	1	0	0	1	0	2	0	1	0&1	0	0
<i>R. sceleratus</i>	0	1	0	0	0	0	1	0	0	1&2	0	4	0	1	0	1	0
<i>R. sphaerospermus</i>	0	0	1	0	0	0	1	0	0	1	1	4&5	0	1	1	0	0
<i>R. thora</i>	1	1	0	0	0	0	1	0	0	1	0	2	0	1	0	0	0
<i>R. trichophyllus</i>	0&1	0	1	0	0	0	1	0	0	1	1	3&4	0	1	1	0	0
<i>R. uncinatus</i>	1	1	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0
<i>Trautvetteria grandis</i>	1	1	0	0	1	0	0	0	1	–	–	–	–	1	1	0	0

Appendix 3. Continued.

Taxon	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
<i>Arcteranthus cooleyae</i>	1	1	0	1	1	1	0	0	0	0	1	0	1	0	0	2
<i>Beckwithia andersonii</i>	1	0	3	1	0	1	0&2	0	0	0	0	0	1	1	?	0&2&4
<i>Callianthemoides semiverticillatus</i>	1	1	2	1	1	1	0	0	0	0	0	0	2	2	0	4
<i>Ceratocephala falcata</i>	0	0	3	2	0	1	0	0	0	2	0	0	2	0	1	4
<i>Ceratocephala orthoceras</i>	0	0	3	2	0	1	0	0	0	2	0	0	2	0	1	2
<i>Coptidium lapponicum</i>	1	0	2	1	0	0	0	0	0	0	0	0	1	0	0	4
<i>Coptidium pallasii</i>	1	0	2	1	0	0	0	0	0	0	0	0	1	0	0	5
<i>Cyrtorhyncha ranunculina</i>	1	1	1	0	1	1	0&2	0	0	0	0	0	1	0	0	0&3
<i>Ficaria fascicularis</i>	1	0	2	1	0	1	0	0	0	0	0	1	3	3	0	0&2&4
<i>Ficaria verna</i>	1	0	2	1	0	1	0	0	0	2	0	1	3	3	0	2
<i>Halerpestes cymbalaria</i>	1	1	1	1	1	1	0	0	0	0	0	0	1	2	0	2
<i>H. uniflora</i>	1	1	2	1	?	1	0	0	0	0	0	0	1	2	0	2
<i>Hamadryas delphinii</i>	1	1	2	1	?	1	0	0	0	0	0	0	1	0	?	2
<i>Krapfia clypeata</i>	1	0	0	1	0	1	?	0	0	1	0	0	2	0	?	5
<i>Kumlienia hystricula</i>	1	1	2	1	1	1	0	0	0	1	0	0	1	0	?	4
<i>Laccopetalum giganteum</i>	1	0	0	1	0	1	?	0	0	0	0	0	0	0	?	5
<i>Myosurus minimus</i>	1	0	0	1	0	1	3	0	0	0	2	0	1	0	0	2
<i>Oxygraphis polypetala</i>	1	1	0	0	1	1	0	0	0	0	0	0	1	0	0	2
<i>Peltocalathos baurii</i>	1	1	2	1	1	1	0	0	0	0	1	0	1	0	0	?
<i>Ranunculus acris</i>	1	0	1	0	0	1	2	0	0	0	1	0	1	1	1	4
<i>R. apiifolius</i>	1	0	0	1	0	1	0	0	0	0	1	0	1	1	0	4
<i>R. arvensis</i>	1	0	3	0	0	1	1	1	0	0	1	0	1	0	0	5
<i>R. asiaticus</i>	1	0	1	0	0	1	2	0	0	0	1	0	1	2	0	5
<i>R. bonariensis</i>	1	0	0	1	0	1	2	0	0	0	0	0	1	1	?	?
<i>R. brevifolius</i>	1	3	3	1	0	1	0	0	0	0	0	0	1	0	0	?
<i>R. breyninus</i>	1	0	2	0	0	1	2	0	0	0	1	0	1	0	0	?
<i>R. camissonis</i>	1	3	3	1	0	1	0	0	0	0	1	0	1	0	?	1
<i>R. caucasicus</i>	1	0	2	0	0	1	2	0	0	0	1	0	1	0	0	?
<i>R. cheirophyllus</i>	1	0	1	1	0	1	2	0	0	0	0	0	0	1	0	?
<i>R. flammula</i>	1	0	0	1	0	1	2	0	0	0	1	0	1	0	0	2
<i>R. formosomontanus</i>	1	0	1	1	0	1	2	0	0	0	0	0	1	1	0	?
<i>R. glacialis</i>	1	3	2	1	0	1	0	0	0	0	2	0	0	2	0	2
<i>R. kuepferi</i>	1	3	1	1	0	1	0	0	0	0	0	0	1	0	0	?
<i>R. lyallii</i>	1	0	2	1	0	1	0	0	0	2	1	0	2	0	0	2
<i>R. maclovianus</i>	1	0	1	1	0	1	2	0	0	1	0	0	1	0	?	?
<i>R. natans</i>	1	0	1	1	0	1	0	0	0	0	0	0	1	0	0	?
<i>R. nivalis</i>	1	0	1	1	0	1	0	0	0	0	0	0	1	0	0	?
<i>R. oxyspermus</i>	1	0	2	0	0	1	2	0	0	0	0	0	0	2	0	?
<i>R. papyrocarpus</i>	1	0	2	0	0	1	2	0	0	0	2	0	1	1	?	4
<i>R. parnassifolius</i>	1	3	2	1	0	1	0	0	0	0	0	0	1	0	0	?
<i>R. pedatifidus</i>	1	0	1	1	0	1	2	0	0	2	0	0	1	0	0	?
<i>R. peltatus</i>	1	0	1	1	0	1	0	0	1	0&2	1	0	1	0	0	?
<i>R. pensylvanicus</i>	1	0	1	0	0	1	2	0	0	0	1	0	1	0	0	0&4
<i>R. pinardii</i>	1	0	3	0	0	1	1	1	0	2	1	0	2	2	?	?
<i>R. polyanthemus</i>	1	0	1	0	0	1	2	0	0	0	1	0	1	0	0	2
<i>R. pyrenaeus</i>	1	0	1&2	1	0	1	0	0	0	0	0	0	1	0	0	2
<i>R. rufosepalus</i>	1	0	1	1	0	1	2	0	0	0	0	0	1	0	?	?
<i>R. sceleratus</i>	1	0	0	0	0	1	2	0	0&1	0	0	0	1	1	0	2
<i>R. sphaerospermus</i>	1	0	1	1	0	1	0	0	1	0	1	0	1	0	0	?
<i>R. thora</i>	1	3	2	1	0	1	0	0	0	0	0	0	1	0	0	2
<i>R. trichophyllus</i>	1	0	1	1	0	1	0	0	1	0&2	1	0	1	0	0	2
<i>R. uncinatus</i>	1	0	1	0	0	1	2	0	0	0	1	0	1	0	0	?
<i>Trautvetteria grandis</i>	1	1	1	3	1	1	0	0	0	1	2	0	1	0	0	0