PHYLOGENETIC STUDIES IN THE GENUS SAXIFRAGA (SAXIFRAGACEAE)

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Zhuoxin Zhang

Department of Biology

University of Leicester

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Zhuoxin Zhang

Phylogenetic studies in the genus *Saxifraga* (Saxifragaceae)

Abstract

Saxifraga L., the largest genus (ca. 460 spp.) in the family Saxifragaceae, is widely distributed across Europe and Asia, mainly in the Alps and the Sino-Himalayan area, with extensions in the Rocky Mt / Andean Cordillera south to Tierra del Fuego. The genus displays remarkable morphological variation. Phylogenetic analyses were conducted to test previous classifications. Molecular evidence comprised DNA sequences from the chloroplast gene (*matK*) and nuclear regions (*ITS* and *Duo1*). The sequences were also used in plant identification. In many cases genetic distances between species in terms of DNA sequences were very low, indicating rapid evolution. Morphological evidence, including microscopic characters from pollen nuclear number, exine and leaf venation, was also collected. Phylogenetic trees were produced from the molecular data, the morphological data and from the combined data. Morphological data improved resolution in the molecular trees. The phylogenies were in substantial agreement and suggested that a few taxonomic realignments and new combinations are needed to make the sections and subsections monophyletic. A revised classification of *Saxifraga* primarily to subsection level is presented. The main changes from previous classifications are: (1) genus *Saxifragella* is included as a monotypic section in *Saxifraga*; (2) sect. *Ciliatae* subsect. *Serpyllifoliae* is merged into sect. *Ciliatae* subsect. *Rosulares*; (3) sect. *Ciliatae* subsect. *Cinerascentes* is merged with subsection *Gemmiparae*; (4) section *Porphyrion* is monophyletic only if *S.mutata* and *S. florulenta* (formerly in sect. *Ligulatae*) are included; this transfer also makes sect. *Ligulatae* monophyletic; (5) *S.mutata* and *S.aizoides* (the later formerly in sect. *Xanthizoon*) are closely related and are now placed together in ser. *Xanthizoon* of sect. *Porphyrion*. The geographical distributions of the basal sections of the genus, i.e. *Irregulares, Heterisia, Saxifragella* and *Trachyphyllum* suggest an origin of the genus around the shores of the Pacific Ocean, probably in the Beringian area.

Acknowledgements

I would like to express the deepest appreciation to my supervisor, Dr Richard Gornall. This dissertation would not have been possible without his professional guidance and persistent help. His enthusiasm for Plant Systematics motivated me to choose it as my career. I want to thank my parents for their precious support and deepest care which enable me to study in the University of Leicester. I am grateful to Professor Pat Heslop-Harrison, Dr Trude Schwarzacher, Dr John Bailey and Professor David Twell for kindly providing me experimental support. I thank D.S. Rawat from the G.B. Pant University of Agriculture & Technology, Uttarakhand, India, for providing a new species. My appreciation also extends to my laboratory colleagues. Farah Badakshi, Nauf Alsayaid, Stuart Desjardins, Dr Worku Mhiret, Dr Yifei Liu, Dr Faisal Nouroz and Dr Niaz Ali kindly helped me in the DNA extraction and PCR experiments. Dr Michael Borg, Dr Nadia Taimur, Dr Hoda Khtab, Ugur Sari from the Twell lab provided important information for DUO1 gene and the DAPI staining method. I would like to thank Professor Shilong Chen and Dr Qingbo Gao from the Northwest Plateau Institute of Biology, Xining, China, who provided the opportunities for two valuable field trips in China and very kindly helped me in the trips. I also want to thank Adrian Young who sent me books for *Saxifraga* hybrids and precious seedling plants. I am grateful to Dr David Boufford (Harvard University), Professor Georg Miehe and Sabine Miehe (University of Marburg) for making recently collected specimens available. I am especially grateful to Natalie Allcock and Stefan Hyman from the Electron Microscopy Lab for their work and help with the TEM and SEM photos. In addition, I thank my husband Dan for his understanding for my leaving home in these three years.

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Abbreviations

Herbarium and garden abbreviations

A	Herbarium, Arnold Aboretum, Harvard University, Cambridge, U.S.A.
BM	Herbarium, the Natural History Museum, London, U.K.
CGG	Botanic Garden, Cambridge University, Cambridge, U.K.
E	Herbarium, Royal Botanic Garden, Edinburgh, U.K.
HNWP	Herbarium, Northwest Plateau Institute of Biology, Xining , China
JACA	Herbario, Unidad de Geobotánica, Instituto Pirenaico de Ecología, Jaca, Spain
K	Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey, U.K.
KUN	Herbarium, Kunming Institute of Botany, Academia Sinica, Kunming, China
KUN	Botanic Garden, Kunming Botanical Garden, Academia Sinica, Kunming, China
LTR	Herbarium, University of Leicester, Leicester, U.K.
LTR	Botanic Garden, University of Leicester, Leicester, U.K.
MA	Herbario, Real Jardín Botánico, Plaza de Murillo, Madrid, Spain
MAK	Makino Herbarium, Tokyo Metropolitan University, Tokyo, Japan
MB	Herbarium, Botanik, Philipps-Universität, Marburg, Germany
ÖBG	Ökologisch-Botanischer-Garten, Universität Bayreuth, Bayreuth, Germany
OS	Herbarium, Ohio State University, Columbus, Ohio, U.S.A.
PE	Herbarium, Institute of Botany, Academia Sinica, Beijing, China
RNG	Herbarium, University of Reading, Reading, U.K.
WS	Marion Ownbey Herbarium, Washington State University, Washington, U.S.A.

Other abbreviations

Acro.	Acrodromous
Actin.	Actinodromous
APG	Angiosperm phylogeny group
BS	Bootstrap test
Campto.	Camptodromous
CI	Consistency index
Cili.	Ciliatae
CNI	Close-neighbour-interchange
CTAB	Cetrimonium bromide
Cymb.	Cymbalaria
DAPI	4',6-diamidino-2-phenylindole
DUO1	The male germline-specific R2R3 MYB transcription factor DUO POLLEN1

EDTA	Ethylenediaminetetraacetic acid
Flagell.	Flagellares
G	Gamma distribution
Gemmip.	Gemmiparae
GEP	Gap extension penalty
GOP	Gap opening penalty
GTR	Generalised time reversible
Gymno.	Gymnopera
Hemis.	Hemisphaericae
Hirculo.	Hirculoideae
Holo.	Holophyllae
Irreg.	Irregulares
ITS	Internal transcribed spacer region of 18S-26S nuclear rdna
JC	Jukes cantor
K2	Kimura 2 parameter
Ligu.	Ligulatae
Mesogy.	Mesogyne
MP	Maximum parsimony analysis
NJ	Neighbour-Joining analysis
PCR	The polymerase chain reaction
Porphyr.	Porphyrion
PP	Posterior probability
PSRF	Potential scale reduction factor
PVPP	Polyvinylpolypyrrolidone
RI	Retention index
Rosa.	Rosulares
Sax.	Saxifraga
SBL	The sum of branch lengths
SEM	Scanning electron microscopy
Serpy.	Serpyllifoliae
TE	Tris-Cl and EDTA solution
TEM	Transmission electron microscopy
Trachy.	Trachyphyllum
Tridact.	Tridactylites
Triplin.	Triplinervium
Tris-Cl	Tris (hydroxymethyl) amino methane
Xanth.	Xanthizoon

Chapter One: Introduction

"Come, Watson, come!" he cried. "The game is afoot. Not a word! Into your clothes and come!"

Sherlock Holmes -*The Adventure of The Abbey Grange*

1.1 The family Saxifragaceae

Saxifragaceae are part of the Saxifragales, an order basal to the Eudicots (Fig. 1.1) The family consists of herbs, mainly characterized by vessel elements with simple perforation plates; most species have a set of basal leaves arranged in a rosette of some sort; cauline leaves lack stipules in the ordinary sense, although in many species the proximal margins of the petiole are expanded, sometimes to the point where they become foliar, as in *Boykinia major* (Gornall and Bohm 1984). The floral formula is {K {4-5}, C (0)4--5(6), A 5—10}, G {2(3)}, where curly bracket indicates united structures and parentheses indicate rare states; fruit is a septicidal capsule or follicle (Judd 2007; Soltis 2007). The family is distributed across the temperate and arctic regions of the Northern Hemisphere and extends into South America down the Andes to Tierra del Fuego (Fig. 1.2) (Stevens 2001 onwards).

Figure 1.1. The position of Saxifragales (a) and Saxifragaceae (b) in the Angiosperm Phylogeny Group (APG) III system (Stevens 2001onwards).

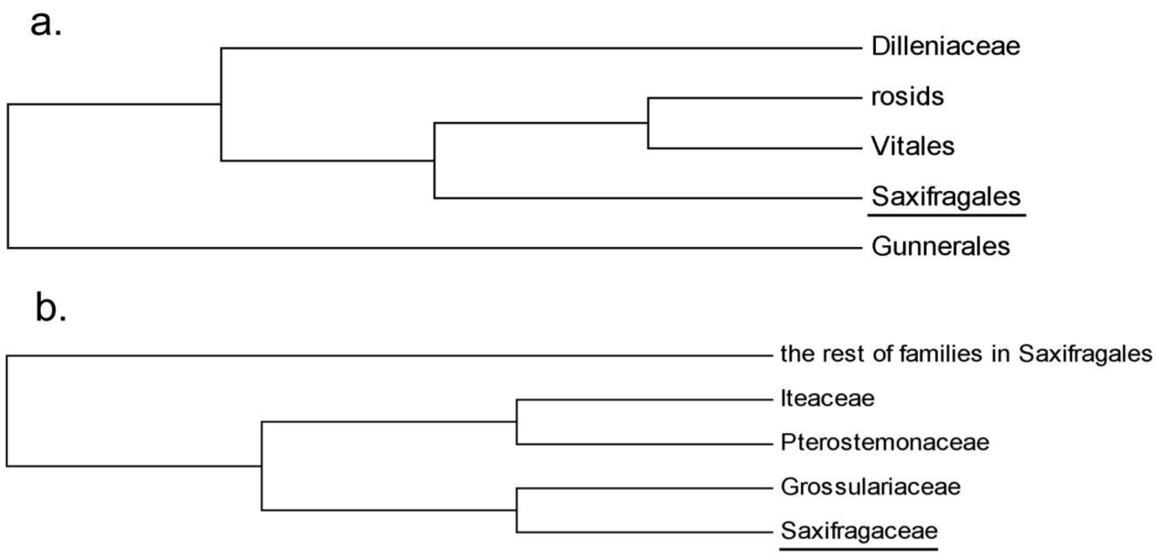
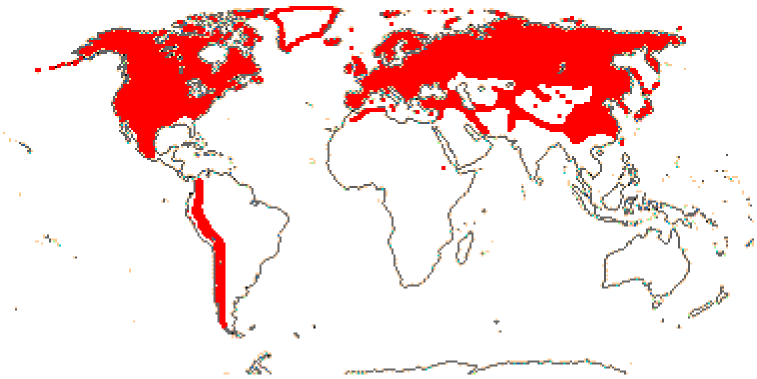
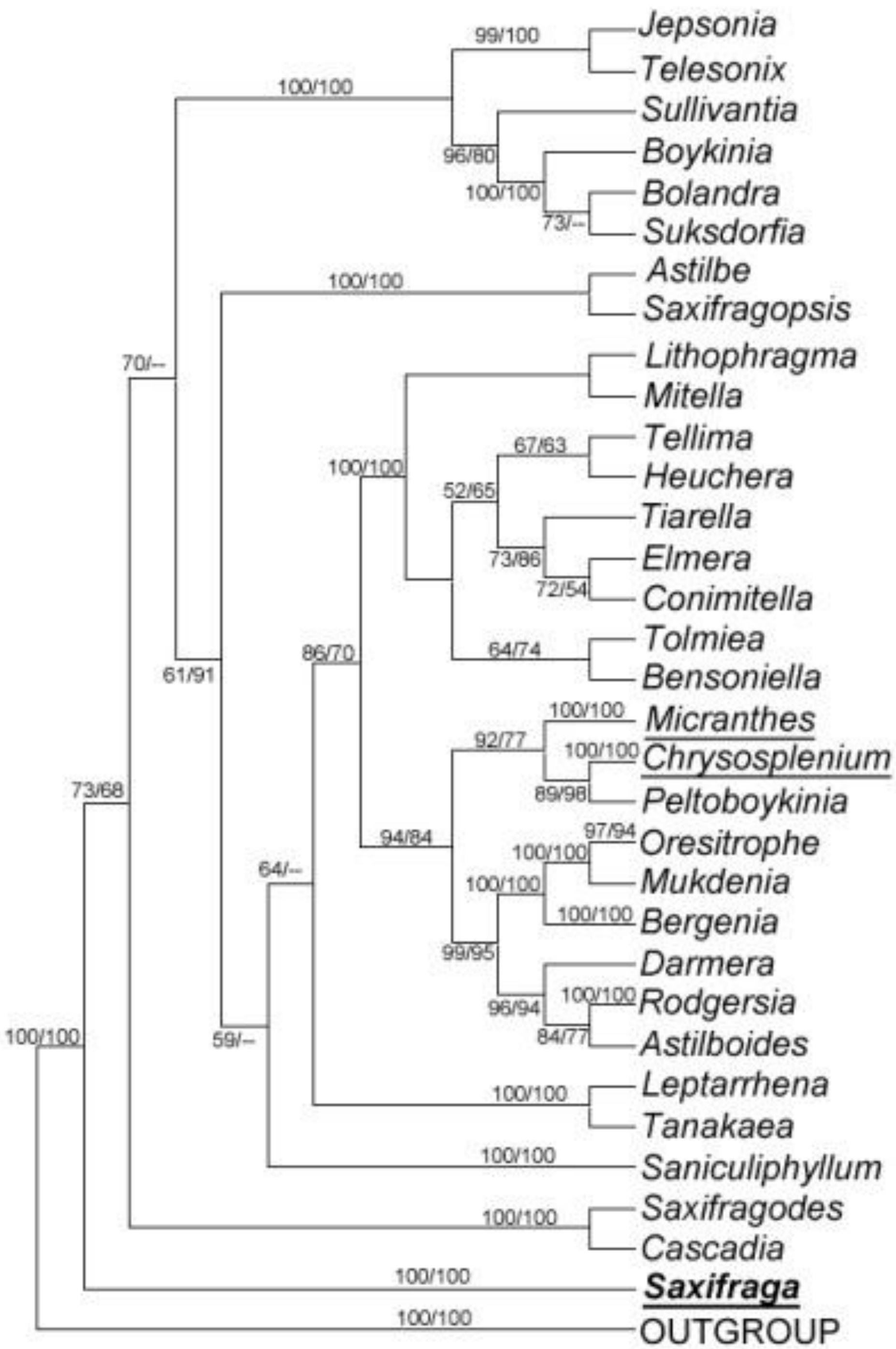


Figure 1.2. Global distribution of the Saxifragaceae, after Stevens (2001 onwards).



The family has around 600 species in 33 genera (Soltis 2007). *Saxifraga* is the largest genus in the family, having ca. 470 species (R.J. Gornall, unpublished, Appendix 1). The rest of the family includes popular garden plants such as *Bergenia*, *Heuchera*, *Tolmiea*, *Mitella*, and *Darmera*. Phylogenetic studies based on the DNA sequences of six regions (rbcL, matK, trnL-trnF, psbA-trnH, the internal transcribed spacer (ITS) region of 18S-26S nuclear rDNA and expansion segments of the 26S rDNA) reveal ten reasonably well-marked clades (Fig. 1.3), comprising *Cascadia*, *Micranthes*, *Peltoboykinia*, *Darmera*, *Heuchera*, *Boykinia*, *Astilbe*, *Leptarrhena*, *Saniculiphyllum*, and *Saxifraga* groups (Soltis 2007; Xiang et al. 2012). The genus *Micranthes* (ca. 75 spp.) belongs to the Heucheroid clade, a separate lineage from *Saxifraga* s. str. It therefore can no longer be regarded as a section of *Saxifraga* (Soltis et al. 2001a), which it once was (Engler and Irmscher 1916/19). The segregation is also supported by morphological evidence, such as ribbed seeds, a reticulate pollen exine and a single integument (Brouillet and Gornall 2007; Ferguson and Webb 1970).

Figure. 1.3. The six-gene phylogeny of the Saxifragaceae, adapted and redrawn from Xiang et al. (2012). RAxML and the maximum parsimony (MP) analysis' bootstrap (BS) support values (>50%) are indicated above or below the branches as RAxML BS / MP BS.



1.2 Saxifraga biology

- *Geographical distribution*

Saxifraga is distributed chiefly in the mountains and temperate areas of Europe and Asia, with a few species occupying a circum-polar distribution and some extending southwards in the Americas down the Rocky Mountain – Andean cordillera to Tierra del Fuego (where the endemic species *S. bicuspidata* grows), the southern tip of South America (Pan et al. 2001; Webb and Gornall 1989). Roughly speaking, half of the ca. 460 species occur in Europe and the other half in China and the Himalaya.

- *Morphological variation*

The genus displays a more or less constant floral morphology, viz. pentamerous, bicarpellate, actinomorphic or zygomorphic flowers, lacking a free hypanthium; petals 5 (rarely 4 or 0), with 10 (rarely 8 or 5) stamens (Webb and Gornall 1989); pollen grains three colpate, with an exine of variable morphology (Hideux and Ferguson 1976); the ovary varies from subsuperior to fully inferior, placentation axile, ovules with two integuments (Webb and Gornall 1989). The fruit is a 2-valved capsule containing numerous tiny seeds (Pan et al. 2001).

In contrast, *Saxifraga* displays vast morphological variation in its vegetative organs. Usually perennial, but sometimes biennial or annual, plants range from compact cushions, sometimes somewhat woody below, through sprawling mats, to tall herbs forming cespitose, rhizomatous clumps, to individuals with single stems; some species produce filiform runners, and others conspicuous leafy buds. Leaves vary correspondingly from large (c.25cm^2), palmately-lobed structures to much smaller (c.1mm^2), needle- or scale-like appendages; in most species they are alternately arranged but in some they are opposite. The inflorescence is a cyme or a solitary flower. The genus is cytologically variable, with sporophytic chromosome numbers ranging from 2n=14 to 2n=198 and c.220 (Webb and Gornall 1989).

- *Habitat preferences*

In terms of habitat, species live in woodland margins, meadows, grassland, tundra, stony and rocky ground, and rock faces, where the competition between plants is low (Pan et al. 2001; Webb and Gornall 1989, Fig. 1.4). Most species prefer well-drained conditions but some, like *S. hirculus*, prefer wet flushes. The preferences for light are variable but most of the species like half-shaded conditions, e.g. woodland margins or glades, or open but north-facing aspects. Most *Saxifraga* are not able to survive prolonged heat and aridity, but they can survive low winter temperatures.

- *Pollination biology*

There are limited records about the pollinators of *Saxifraga* (Proctor and Yeo 1973) and most of them relate to British species. The insects that visit *S. hypnoides* include crane-fly relatives (*Limonia,* family Limoniidae), phorid flies (*Phora*, family Phoridae), sawflies (suborder Symphyta), ichneumon wasps (family Ichneumonidae), gall wasps (Cynipidae and Lamprotatidae) in order Hymenoptera. *Saxifraga oppositifolia* is visited by minute black scavenger flies (*Scatopse*, family Scatopsidae). *Saxifraga aizoides* is visited by blow-flies (family Calliphoridae) and family Ichneumonidae. London Pride (*S. × urbium*) is visited by *Syrphus cf. luniger* (a hover fly). The British families in the group Hymenoptera-Aculeata (true wasps) have been recorded on saxifrage flowers. The insects are attracted to the exposed nectar-producing disc or tissue that is present in *Saxifraga* flowers (Proctor and Yeo 1973).

- *Hybridisation*

Natural hybrids are fairly common where parental ranges overlap (Webb & Gornall 1989). In gardens, numerous cultivars have been made by hybridising species, especially in sections *Saxifraga* (the so-called 'mossy' saxifrages) and *Porphyrion* (the 'Kabschias'). There are also at least two cases of allopolyploidy that have been documented by means of molecular evidence: *S. nathorstii*, of hybrid origin between *S. oppositifolia* and *S. aizoides* (Bocher 1983) and *S. osloensis*, of hybrid origin between *S. adscendens* and *S. tridactylites* (Brochmanet al. 1996).

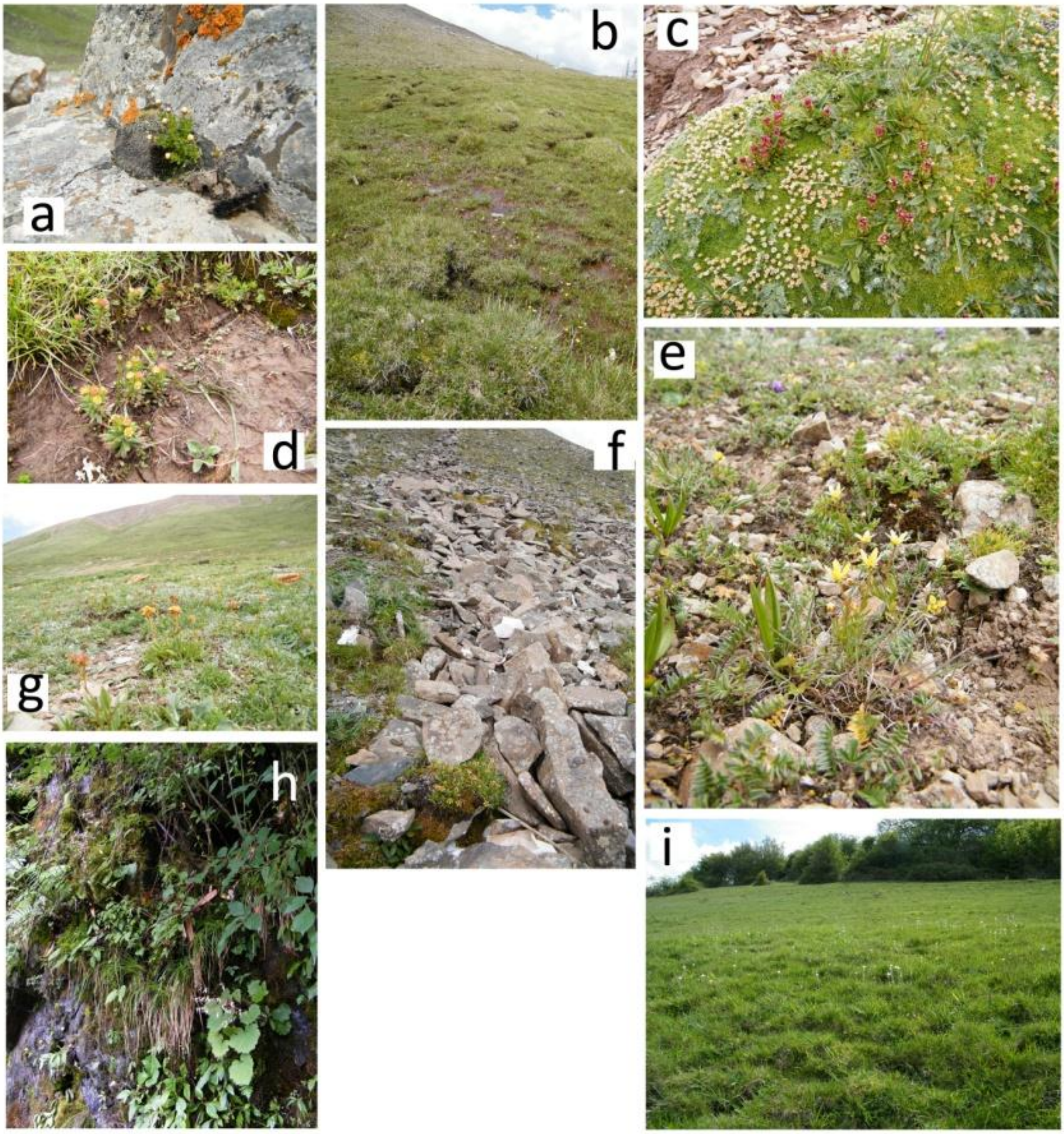
- *Field studies*

Herbarium specimens are the common study material of *Saxifraga*. These two-dimensional objects convey a huge amount of information about *Saxifraga* biology, but not all of it: for example, details of petal colour and spotting are often lost in pressed material, and the habit and branching patterns are difficult to see (plants are fixed). Another thing that is not adequately reflected in a herbarium specimen is the breeding biology of a species: you need to see live plants to observe pollinators and to examine many plants from a population to get a reliable idea about sex expression [some *Saxifraga* species turn out to be dioecious (Chambers 1964, Webb and Gornall 1989)**,** have male and female individuals, like *Ilex aquifolium* L.].

I had two field trips in China to look for *Saxifraga* plants and collect leaves from a range of populations and species for molecular studies. The first trip is in Qinghai Province, north-western China, in 2011. The second one is in Qinghai, Sichuan, and Yunnan Provinces, from north-west to south-west China, in 2012. Field study sites in U.K. include Leicester, Derbyshire Dales National Nature Reserve, and Petland Hills.

Field studies provided not only fresh plant materials but also direct observation of the habitats (Fig. 1.4); information of morphology, sex expression and pollinators were collected.

Figure 1.4. Different habitats occupied by *Saxifraga*: a) rock outcrop; b) damp turf; c) hummock; d) exposed earth; e) scree; f) rock crevices; g) short turf; h) woodland edge; and i) meadow. All photographs taken by me in a-g) Qinghai Province, China; h) Sichuan Province, China and i) Cock Marsh, Berkshire, U.K.



- *Pests and diseases*

Saxifraga has several pests and diseases (Griffiths 1972; Savile 1973; Savile 1975). Two species of *Phytomyza*, in family Agromyzidae (leaf-miner flies), are known as larvae leaf-miners of *Saxifraga*: *P. saxifragae* Hering and *P. aizoon* Hering. The larvae of these two species make linear or expanded channels inside the leaf tissue of S. *rotundifolia* L. and *S. paniculata* Miller, respectively (Griffiths 1972).

Puccinia rust fungi can infect some *Saxifraga* as listed in Table 1.1 (Savile 1973).

Table 1.1. Rust fungi and their *Saxifraga* hosts.

Puccinia rust	*Saxifraga* species
P. pazschkei var. *heterisiae*	*S. mertensiana*
P. heucherae var. *japonica*	*S. cortusifolia*
P. austroberingiana var. *saxifragarum*	*S.fortunei*
P. pazschkei var. *tricuspidata*	*S. tricuspidata*
P. fischeri	*S. flagellaris, S. biflora*
P. saxifragae var. *curtipes*	*S. serpyllifolia*
P. austroberingiana ssp. *austroberingiana* *P. saxifragae* var. *longior* *P. saxifragae* var. *curtipes* x var. *longior*	*S. cernua*
P. saxifragae var. *curtipes, P. saxifragae* var. *longior, P. saxifragae* var. *curtipes* x var. *longior*	*S. rivularis*
P. pazschkei var. *pazschkei*	*S. callosa, S. cotyledon, S. hostii, S. longifolia, S. paniculata.*
P. pazschkei var. *huteri*	*S. mutata*
P. pazschkei var. *jueliana, P. fischeri*	*S. aizoides*
P. pazschkei var. *oppositifoliae, P. fischeri, P. joerstadii*	*S. oppositifolia*
P. saxifragae var. *saxifragae*	*S. rotundifolia, S. granulata, S. cespitosa*

1.3 Uses

Saxifraga can be used in two main areas: herbalism and horticulture.

- *Herbalism*

As one of the relatives of the well-known herbal genus *Bergenia*, it is not surprising that some saxifrages are of medicinal use. The origin of “saxifrage” is from Dioscorides, the Greek herbalist of the 1st century AD, as the Latin root “saxum” means rock, and “frangere” means break (Webb and Gornall 1989). The reason is that Dioscorides believed that the plants could be used to remove urinary calculi (Dioscorides et al. 1959). This idea came from the resemblance between the rhizome bulbils of *Saxifraga granulata* L. and kidney stones (Webb and Gornall 1989), but there is no scientific evidence that they are effective in treating this ailment. However, some of the Chinese species are actually used in herbal medicines. *Saxifraga stolonifera* is commonly used to cure infection of the middle ear; its folk botanical name, *ear plant*, possibly refers to its effect. It is also used as a classical Japanese herbal remedy (WikiProjectPlants 2012). *Saxifraga przewalskii* is an important Tibetan medicine for treating middle ear infections, trauma, bleeding, pain, eczema, vomiting and so on (Chen 2011). Both *S. stolonifera* and *S. przewalskii* have pharmacological activity to inhibit the growth of cancerous cells (Chen 2011; Chen et al. 2008), although the active compounds have not been identified.

- *Horticulture*

Saxifrages are very popular as garden plants due to their prettiness and variable flowers which range from actinomorphic to zygomorphic and from white to yellow, orange, pink or red. An extensive account of the horticultural variation is provided by McGregor (McGregor 2008). Members of all sections, except sect. *Ciliatae*, are relatively easy to grow and are common foliage and flowering plants in European gardens. Many hybrids and cultivars have been bred, especially in sections *Porophyllum* and *Saxifraga* (the latter contains the 'mossy' saxifrages), and are widely used, such as the popular *Saxifraga* x *urbium* (= *S. spathularis* x *umbrosa*) or London Pride, in cultivation since the 17th century (McGregor 2008).

1.4 Review of *Saxifraga* taxonomy

Linnaeus (1753) described 31 species of *Saxifraga* but did not indicate any infra-generic categories. Sometime later, however, the vast diversity of the morphological character states gave rise to different divisions of the genus.

Haworth (1803) was the first to divide *Saxifraga* into sections (Table 1.2). He recognized six containing a total of 49 species. In 1812, Haworth recognized three segregate genera in addition to *Saxifraga* in a partial account of the group (Haworth 1812). In a final monographic work, Haworth (1821) distributed 115 species into 17 segregate genera mainly on the basis of floral characters, including ovary position and shape, petal shape, and style morphology (Table 1.2). Although Haworth's taxonomy was apparently ignored, chiefly by Engler and Irmscher (1916/19) who did not adhere strictly to the principle of priority, his sectional names were rediscovered by Siplivinsky (1982) and three of them (*Irregulares*, *Ligulatae* and *Ciliatae*) replaced later systems' sectional names and are now used in contemporary systems.

Sternberg's (1810, 1822, 1831) treatments and that of Tausch (1823) largely followed Haworth (1803, 1821) except that Sternberg reduced the segregate genera to sectional level, a decision also followed by Tausch. Don (1822) distributed 104 species into five sections (Bergenia, Gymnpera, Leiogyne, Micranthes, Saxifraga). Characters for division were mainly based on details of floral morphology.

Seringe (1830) listed 150 species allocated to eight sections, the latter delimited on the basis of calyx, seed and leaf morphology, and on flower colour. Up until the mid-19th century, the classifications were primarily of European and American material. Very few Asiatic species were known. It was not until the explorations of Joseph Hooker and Abbé Delavay in the late 19th century, and George Forrest and Ernest H. Wilson in the early 20th century, that plants from the Sino-Himalaya were described, principally by Franchet and by Engler and Irmscher. The latter incorporated the new species into a new taxonomic system for the genus (Engle and Irmscher 1916/19).

Indeed, the system produced by Engler and Irmscher (1916/19) has had the greatest influence over the following years, mainly because of the extraordinary detail it went into, including classifying down to subforma level. In <*Das Pflanzenreich – Saxifragaceae:* Saxifraga>, Engler and Irmscher wrote accounts of 302 species and divided the genus *Saxifraga* into 15 sections: *Boraphila, Hirculus, Robertsonia, Miscopetalum, Cymbalaria, Tridactylites, Nephrophyllum, Dactyloides, Trachyphyllum, Xanthizoon, Euaizoonia, Kabschia, Porphyrion*, and *Tetrameridium*; they also divided the sections into subsections and greges, a taxon of no legal standing but equivalent to series (Engler and Irmscher 1916/19). They also studied many hybrids. The work benefitted hugely from the discoveries made in Asia by the European explorers. These resulted in the recognition of more than one hundred new species. The morphological basis of this encyclopaedic work involved consideration of leaf morphology (in particular leaf arrangement and the occurrence of chalk glands), flora morphology (mainly ovary position) and seed morphology (mainly shape). They also made reference to trichome anatomy and aspects of vegetative reproduction, including axillary shoots and bulbils. As a result, a much greater variety of taxonomic evidence was brought to bear on the classification of *Saxifraga*.

Based on Engler & Irmscher's system, Gornall (1987a) presented a revised classification with 15 sections, 19 subsections and 34 series (Table 1.2). This system took into account the extensive collections and descriptions of numerous new Sino-Himalayan species by Harry Smith (1958, 1960). The three main modifications to Engler's system are: a) the merging of section *Kabschia* Engler into the opposite-leaved section *Porphyrion* Tausch; b) new section *Saxifraga* includes sections *Nephrophyllum* (Gaudin) W. D. J. Koch and *Dactyloides* Tausch and section *Mesogyne* Sternberg contains *Saxifraga sibirica* L. and its related species removed from section *Nephrophyllum*; c) section *Boraphila* Engler was split up into four not necessarily related sections: *Micranthes* D.Don, *Merkianae* (Engler & Irmscher) Gornall, *Heterisia* (Rafinesque ex Small) A. M. Johnson, and section *Odontophyllae* Gornall (1987a). The characters used for the main groups are listed in Table 1.3.

The largest section *Ciliatae* (Gornall 1987a) (synonymous with sect. *Hirculus* (Engler and Irmscher 1916/19)), which consists of 179 species with various and distinctive divisions, is poorly studied due to lacking specimens (Webb and Gornall 1989; Zhang et al. 2008). *Ciliatae* are mainly distributed in the Himalayan-Tibetan area (Webb and Gornall 1989) and western China, while 112 species of the section are endemic in China (Zhang et al. 2008). Pan proposed a revised system based on works of Engler and divided *Ciliatae* into four subsections (Hirculoideae, Gemmiparae, Rosulares, and Flagellares), 16 series and four subseries (Pan 1991). However, he removed some species into section *Ligulatae* Haworth, for example *S. lychnitis* and *S. tangutica*.

Molecular studies have given rise to a few changes to the genus classification. The phylogenetic analysis of matK and rbcL DNA sequences (Soltis et al. 1996) suggested that Saxifraga was polyphyletic. This conclusion was confirmed by a six gene analysis (Soltis et al. 2001b). As a consequence, in order to make *Saxifraga* monophyletic, sections *Micranthes* and *Merkianae* were removed and recognised as the independent genus *Micranthes*. Brouillet and Gornall (2007) proposed new combinations for the North American *Saxifraga* species that belong to *Micranthes*, and Akiyama & Gornall (2011) did the same for Asiatic species.

Moreover, the deep level phylogenetic study for the family Saxifragaceae (Soltis et al. 2001a) suggests that genus *Saxifragella*, which contains only *Saxifragella bicuspidata* (Hook.) Engl., is embedded in *Saxifraga*. Zhang et al. (2008) divided sect. Ciliatae into three subsections, *Hirculoideae*, *Rosulares*, and *Gemmiparae* based on ITS sequence analysis for the section. These works are further discussed in Chapter 2.

Table 1.2. A concordance of the main classification systems of *Saxifraga*.

<table>
<tr><th>TYPE SPECIES</th><th>Haworth 1803</th><th>Haworth 1812</th><th>Haworth 1821</th><th>Engler and Irmscher 1916/19</th><th>Gornall 1987a</th></tr>
<tr><td>Saxifraga macrostigma</td><td></td><td></td><td></td><td>sect. Hirculus (Haw.) Tausch gx. Densifoliatae Engl.& Irmsch.</td><td rowspan="5">sect. Ciliatae subsect. Hirculoideae series Hirculoideae (Engl. & Irmsch) Gornall</td></tr>
<tr><td>S. turfosa</td><td></td><td></td><td></td><td>sect. Hirculus (Haw.) Tausch gx. Turfosae Engl.& Irmsch.</td></tr>
<tr><td>S. cardiophylla</td><td></td><td></td><td></td><td>sect. Hirculus (Haw.) Tausch gx. Stellariifoliae Engl.& Irmsch.</td></tr>
<tr><td>S. hirculus</td><td>sect. Ciliatae Haw.</td><td></td><td>Hirculus Haw.</td><td rowspan="2">sect. Hirculus (Haw.) Tausch gx. Hirculoideae Engl.& Irmsch.</td></tr>
<tr><td>S. nigroglandulosa</td><td></td><td></td><td></td></tr>
<tr><td>S. lychnitis</td><td></td><td></td><td></td><td>sect. Hirculus (Haw.) Tausch gx. Lychnitideae Engl.& Irmsch.</td><td>sect. Ciliatae subsect. Hirculoideae series Lychnitidae (Engl. & Irmsch) Gornall</td></tr>
<tr><td>S. nutans</td><td></td><td></td><td></td><td>sect. Hirculus (Haw.) Tausch gx. Nutantes Engl.& Irmsch.</td><td>sect. Ciliatae subsect. Hirculoideae series Nutantes (Engl. & Irmsch) Gornall</td></tr>
<tr><td>S. excellens</td><td></td><td></td><td></td><td></td><td>sect. Ciliatae subsect. Hirculoideae series Cinctae (H. Sm.) Gornall</td></tr>
<tr><td>S. gemmipara</td><td></td><td></td><td></td><td>sect. Hirculus (Haw.) Tausch gx. Gemmiparae Engl.& Irmsch.</td><td>sect. Ciliatae subsect. Gemmiparae series Gemmiparae (Engl. & Irmsch.) Gornall</td></tr>
<tr><td>S. fimbriata</td><td></td><td></td><td></td><td></td><td>sect. Ciliatae subsect. Gemmiparae series Spinulosae (C.B.Clarke) Gornall</td></tr>
<tr><td>S. cinerascens</td><td></td><td></td><td></td><td>sect. Hirculus (Haw.) Tausch gx. Cinerascentes Engl.& Irmsch.</td><td>sect. Ciliatae subsect. Cinerascentes Engl. & Irmsch.</td></tr>
</table>

S. sediformis				sect. Hirculus (Haw.) Tausch gx. Sediformes Engl.& Irmsch.	sect. Ciliatae subsect. Rosulares Gornall
S. serpyllifolia					sect. Ciliatae subsect. Serpyllifoliae Gornall
S. flagellaris				sect. Hirculus (Haw.) Tausch gx. Flagellares Engl.& Irmsch.	sect. Ciliatae subsect. Flagellares (C.B.Clarke) Engl. & Irmsch.
S. hemisphaerica				sect. Hirculus (Haw.) Tausch gx. Hemisphaericae Engl.& Irmsch.	sect. Ciliatae subsect. Hemisphaericae (Engl.& Irmsch.) Gornall
Robertsonia crenata Haw.= S. hirsuta		*Robertsonia* Haw.	*Robertsonia* Haw.	sect. Robertsonia (Haw.) Sternb.	sect. Gymnopera D. Don
S. rotundifolia		*Miscopetalum* Haw.	*Miscopetalum* Haw.	sect. Miscopetalum (Haw.) Sternb.	sect. Cotylea Tausch
S. cymbalaria				sect. Cymbalaria Grisebach	sect. Cymbalaria Grisebach
S. tridactylites			*Tridactylites* Haw.	sect. Tridactylites (Haw.) Grisebach	sect. Saxifraga subsect. Tridactylites (Haw.) Gornall
S. arachnoidea				sect. Nephrophyllum (Gaud.) W.D.J.Koch gx. Arachnoideae Engl. & Irmsch.	sect. Saxifraga subsect. Triplinervium (Gaudin) Gornall series Arachnoideae (Engl. & Irmsch) Gornall
S. irrigua				sect. Nephrophyllum (Gaud.) W.D.J.Koch gx. Irriguae Engl. & Irmsch.	sect. Saxifraga subsect. Triplinervium (Gaudin) Gornall series Aquaticae (Engl.) Pawlowska
S. granulata	sect. *Lobatae* Haw.	*Saxifraga* L.	*Saxifraga* L.	sect. Nephrophyllum (Gaud.) W.D.J.Koch gx. Granulatae Engl. & Irmsch.	sect. Saxifraga subsect. Saxifraga series Saxifraga
S. biternata					sect. Saxifraga subsect. Saxifraga series Biternatae (Engl. & Irmsch.) Gornall
S. sibirica			*Lobaria* Haw.	sect. Nephrophyllum (Gaud.) W.D.J.Koch gx. Sibiricae Engl. & Irmsch.	sect. *Mesogyne* Sternb.
S. tenella				sect. Dactyloides Tausch subsect. Holophyllae (Engl.) Engl. & Irmsch. gx. Tenellae Engl. & Irmsch.	sect. Saxifraga subsect. Holophyllae (Engl.) Engl. & Irmsch. series Tenellae (Engl. & Irmsch.) Pawlowska

S. sedoides				sect. Dactyloides Tausch subsect. Holophyllae (Engl.) Engl. & Irmsch. gx. Sedoideae Engl. & Irmsch.	sect. Saxifraga subsect. Holophyllae (Engl.) Engl. & Irmsch. series Sedoides (Gaudin) Pawlowska
S. muscoides			*Muscaria* Haw.	sect. Dactyloides Tausch subsect. Holophyllae (Engl.) Engl. & Irmsch. gx. Muscoideae Engl. & Irmsch.	sect. Saxifraga subsect. Holophyllae (Engl.) Engl. & Irmsch. series Muscoideae (Engl. & Irmsch.) Pawlowska
S. aphylla				sect. Dactyloides Tausch subsect. Holophyllae (Engl.) Engl. & Irmsch. gx. Aphyllae Engl. & Irmsch.	sect. Saxifraga subsect. Holophyllae (Engl.) Engl. & Irmsch. series Sedoides (Gaudin) Pawlowska
S. androsacea				sect. Dactyloides Tausch subsect. Holophyllae (Engl.) Engl. & Irmsch. gx. Androsaceae Engl. & Irmsch.	sect. Saxifraga subsect. Holophyllae (Engl.) Engl. & Irmsch. series Androsaceae Engl. & Irmsch.
S. glabella				sect. Dactyloides Tausch subsect. Holophyllae (Engl.) Engl. & Irmsch. gx. Glabellae Engl. & Irmsch.	sect. Saxifraga subsect. Holophyllae (Engl.) Engl. & Irmsch. series Glabellae (Engl. & Irmsch.) Pawlowska
S. ajugifolia				sect. Dactyloides Tausch subsect. Eudactyloideae Engl. & Irmsch. gx. Axilliflorae (Willk.) Engl. & Irmsch.	sect. Saxifraga subsect. Triplinervium (Gaudin) Gornall series Axilliflorae (Willk.) Pawlowska
S. aquatica				sect. Dactyloides Tausch subsect. Eudactyloideae Engl. & Irmsch. gx. Aquaticae (Engl.) Engl. & Irmsch.	sect. Saxifraga subsect. Triplinervium (Gaudin) Gornall series Aquaticae (Engl.) Pawlowska
S. ceratophylla				sect. Dactyloides Tausch subsect. Eudactyloideae Engl. & Irmsch. gx. Ceratophyllae (Willk.) Engl. & Irmsch.	sect. Saxifraga subsect. Triplinervium (Gaudin) Gornall series Cetatophyllae (Haw.) Pawlowska
S. geranioides	sect. Stolonifera e Haw.			sect. Dactyloides Tausch subsect. Eudactyloideae Engl. & Irmsch. gx. Ceratophyllae (Willk.) Engl. & Irmsch.	sect. Saxifraga subsect. Triplinervium (Gaudin) Gornall series Cetatophyllae (Haw.) Pawlowska
S. granatensis				sect. Dactyloides Tausch subsect. Eudactyloideae Engl. & Irmsch. gx. Gemmiferae (Willk.) Engl. & Irmsch.	sect. Saxifraga subsect. Triplinervium (Gaudin) Gornall series Gemmiferae (Willk.) Pawlowska
S. cespitosa				sect. Dactyloides Tausch subsect. Eudactyloideae Engl. & Irmsch. gx. Caespitosae (Engl.) Engl. & Irmsch.	sect. Saxifraga subsect. Triplinervium (Gaudin) Gornall series Cespitosae (H.G.L.Rchb.) Pawlowska
S. exarata				sect. Dactyloides Tausch subsect. Eudactyloideae Engl. & Irmsch. gx. Exarato-moschatae Engl. & Irmsch.	

S. aizoides			*Leptasea* Haw.	sect. Xanthizoon Grisebach	sect. Xanthizoon Grisebach
S. aspera			*Ciliaria* Haw.	sect. Trachyphyllum (Gaudin) W.D.J.Koch	sect. Trachyphyllum (Gaudin) W.D.J.Koch
S. crustata				sect. Aizoonia (Tausch) Engl. gx. Crustatae Engl. & Irmsch.	sect. Ligulatae subsect. Aizoonia (Tausch) Schott
S. aizoon				sect. Aizoonia (Tausch) Engl. gx. Peraizooniae Engl. & Irmsch.	
S. cotyledon	sect. Ligulatae Haw.		*Chondrosea* Haw.	sect. Aizoonia (Tausch) Engl. gx. Cotyledoniae Engl. & Irmsch.	
S. florulenta				sect. Aizoonia (Tausch) Engl. gx. Florulentae Engl. & Irmsch.	sect. Ligulatae subsect. Florulentae (Engl. & Irmsch.) Gornall
S. mutata				sect. Aizoonia (Tausch) Engl. gx. Mutatae Engl. & Irmsch.	sect. Ligulatae subsect. Mutatae (Engl. & Irmsch.) Gornall
S. media				sect. Kabschia Engl. gx. Mediae Engl. & Irmsch.	sect. Porphyrion subsect. Engleria (Sundermann) Gornall
S. juniperifolia				sect. Kabschia Engl. gx. Juniperifoliae Engl. & Irmsch.	sect. Porphyrion subsect. Kabschia (Engl.) Rouy & Camus series Juniperifoliae (Engl. & Irmsch.) Gornall
S. kotschyi				sect. Kabschia Engl. gx. Kotschyanae Engl. & Irmsch.	
S. lilacina					sect. Porphyrion subsect. Kabschia (Engl.) Rouy & Camus series Lilacinae Gornall
S. marginata				sect. Kabschia Engl. gx. Marginatae Engl. & Irmsch.	sect. Porphyrion subsect. Kabschia (Engl.) Rouy & Camus series Marginatae (Engl. & Irmsch.) Gornall
S. squarrosa				sect. Kabschia Engl. gx. Squarrosae Engl. & Irmsch.	sect. Porphyrion subsect. Kabschia (Engl.) Rouy & Camus series Squarrosae (Engl. & Irmsch.) Gornall
S. vandellii				sect. Kabschia Engl. gx. Rigidae Engl. & Irmsch.	sect. Porphyrion subsect. Kabschia (Engl.) Rouy & Camus series Rigidae (Engl. & Irmsch.) Gornall

S. aretioides				sect. Kabschia Engl. gx. Aretioideae Engl. & Irmsch.	sect. Porphyrion subsect. Kabschia (Engl.) Rouy & Camus series Aretioideae (Engl. & Irmsch.) Gornall
S. subsessiliflora					sect. Porphyrion subsect. Kabschia (Engl.) Rouy & Camus series Subsessiliflorae Gornall
S. purpurea				sect. Porphyrion Tausch subsect. Purpureae Hayek	sect. Porphyrion subsect. Oppositifoliae series Oppositifoliae (Hayek) Gornall
S. biflora				sect. Porphyrion Tausch subsect. Biflores Hayek	
S. oppositifolia			*Antiphylla* Haw.	sect. Porphyrion Tausch subsect. Oppositifoliae Hayek	
S. nana				sect. Tetrameridium Engl.	sect. Porphyrion subsect. Oppositifoliae series Tetrameridium (Engl.) Gornall
S. sarmentosa	sect. Irregulares Haw.		*Ligularia* Haw.	sect. Diptera (Borkhausen) Sternb.	sect. Irregulares
S. mertensiana.					sect. Heterisia (Raf. ex Small) A.M.Johnson
S. odontophylla Wallich					sect. Odontophyllae Gornall
Micranthes punctata				sect. Boraphila Engl. gx. Punctatae Engl.& Irmsch.	sect. Micranthes subsect. Rotundifoliatae A.M.Johnson
M. nelsoniana					
M. davurica				sect. Boraphila Engl. gx. Davuricae Engl.& Irmsch.	sect. Micranthes subsect. Cuneifoliatae series Melanocentrae (Engl. & Irmsch.) Gornall
M. melanocentra				sect. Boraphila Engl. gx. Melanocentrae Engl.& Irmsch.	
M. nivalis			*Dermasea* Haw.	sect. Boraphila Engl. gx. Nivali-virginienses Engl.& Irmsch.	sect. Micranthes subsect. Micranthes (Haw.) Gornall series Dermasea (Haw.) Gornall
M. unalaschensis					sect. Micranthes subsect. Cuneifoliatae A.M.Johnson
M. birostris Engl. & Irmsch.					sect. Micranthes subsect. Cuneifoliatae series Birostres Gornall
M. pallida					sect. Micranthes subsect. Cuneifoliatae series Astasianthes (Sternb.) Gornall

M. semipubescens Haw.		*Micranthes* Haw.	*Micranthes* Haw.	sect. Boraphila Engl. gx. Integrifoliae Engl. & Irmsch.	sect. Micranthes subsect. Micranthes (Haw.) Gornall series Micranthes (Haw.) Gornall
M. leucanthemifolia Michx			*Spatularia* Haw.	sect. Boraphila Engl. gx. Stellares Engl. & Irmsch.	sect. Micranthes subsect. Stellares (Engl. & Irmsch.) Gornall
M. stellaris				sect. Boraphila Engl. gx. Stellares Engl. & Irmsch.	
M. micranthidifolia			*Aulaxis* Haw.	sect. Boraphila Engl. gx. Stellares Engl. & Irmsch.	sect. Micranthes subsect. Micranthes (Haw.) Gornall series Aulaxis (Haw.) Gornall
M. tolmiei				sect. Boraphila Engl. gx. Intermediae Engl. & Irmsch.	sect. Merkianae (Engl. & Irmsch.) Gornall
M. merkii				sect. Boraphila Engl. gx. Merkianae Engl. & Irmsch.	sect. Merkianae (Engl. & Irmsch.) Gornall
Bergenia crassifolia (L.) Fritsch	sect. Nudicaules Haw.		*Megasea* Haw.	Bergenia	n/a

Table 1.3. The main morphological characters used to diagnose the sections in the Gornall (1987a) system (*Micranthes* excluded).

Section/Subsection	**Main characters**
Irregulares	Reniform leaves containing crystals. Flowers zygomorphic.
Ciliatae	Foliar crystals absent. Petals yellow, usually with proximal calloses.
Ciliatae, subsect. Hirculoideae	At least proximal stem nodes and petiole bases with brown, crisped, villous hairs.
Ciliatae, subsect. Gemmiparae	Leaves often shiny or hairy; leafy buds produced in axils of cauline leaves.
Ciliatae, subsect. Rosulares	Plants forming well-defined basal leaf rosettes.
Ciliatae, subsect. Serpyllifoliae	Plants with leafy shoots, without a well-defined, basal leaf rosette.
Ciliatae, subsect. Flagellares	Rosette leaves producing axillary, filiform stolons.
Ciliatae, subsect. Hemisphaericae	Habit cushion-forming. Basal leaves with a dense distal fringe of eglandular hairs.
Cymbalaria	Annuals or biennials with ascending diffuse stems. Leaves soft and slightly fleshy, palmately lobed or toothed or almost entire.
Porphyrion	Leaves leathery, usually stiff and hard with chalk glands (calcium-secreting pores), usually forming compact rosette cushions.
Porphyrion, subsect. Kabschia	Forming dense cushions. Leaves alternate and hard.
Porphyrion, subsect. Engleria	Leaves alternate. Flowering stem and inflorescence densely glandular hairy. Petals concealed by sepals.
Porphyrion, subsect. Oppositifoliae	Leaves usually opposite.
Ligulatae	Leaves fleshy, leathery, or stony, with chalk glands, linear to spathulate, forming leafy rosettes. Inflorescence a panicle.
Xanthizoon	Leaves fleshy, narrow, entire, with chalk glands no sunken. Petals yellow.
Trachyphyllum	Leaves usually entire, leathery, lacking chalk glands and crystals. Axillary buds often prominent. Petals white to cream.

Gymnopera	Leaves leathery with petiole, without chalk glands, toothed.
Cotylea	Leaves round or reniform, with teeth or slightly lobed. Petals white.
Mesogyne (incl. Odontophyllae)	Rhizomes with tubers, sometimes in leaf axils. Leaves thin, reniform shape with palmate lobs.
Saxifraga	Leaves soft and lobed. With leafy shoots. Petals usually white.
Saxifraga, subsect. Saxifraga	With bulbils in the axils of basal leaves. Leaves ovate to reniform.
Saxifraga, subsect. Triplinervium	Axillary leafy buds on shoots sometimes present. Leaves variously lobed.
Saxifraga, subsect. Holophyllae	Small in size. Leaves entire to slightly 3-lobed.
Saxifraga, subsect. Tridactylites	Annuals or biennials. No bulbils or leafy shoots. Leaves entire to short 3-/5-lobed.

1.5 Aims of project

In the context of *Saxifraga* and its several schemes of classification, although we know that the remodelled genus is monophyletic, none of the various subgenera, sections, subsections and series, etc. has been tested for their phylogenetic ancestry or relationships. This study has the following aims:

1) To gather both morphological and molecular data to establish an estimate of the phylogenetic tree of the genus.
2) To test the different classifications against this tree and to establish the monophyly of the different sections, subsections & series. By doing so it is hoped to understand better the relationships of species, especially in the poorly studied section *Ciliatae*.
3) To produce a revised classification of the genus.
4) To compare the relative merits of molecular vs. morphological data in constructing phylogenetic trees.
5) To contribute molecular data to the plant DNA barcode library, to help with plant identification in the future. In particular, some *Saxifraga* species are narrowly drawn and provision of inter-specific genetic distances may help in understanding these issues better.
6) To shed light on the origin of the genus *Saxifraga*.

Chapter Two: Molecular data

"There is nothing like first-hand evidence."

Sherlock Holmes -*A Study in Scarlet*

2.1 Introduction

The vast morphological diversity across the large numbers of species and the different characters which used to separate and group those species have given rise to different systems of classification (Chapter 1.4). These systems are not only based on morphological / anatomical data but also have yet to be tested for their congruence with an evolutionary tree. Molecular studies can provide an additional, largely independent source of data for the purposes of establishing such a tree, and thereby test classifications and contribute to solving the problem of subdividing the genus *Saxifraga*.

The internal transcribed spacer (ITS) region of 18S-26S nuclear rDNA contains ITS1 and ITS2 internal transcribed spacers and 5.8S coding region. ITS is a major focus of sequencing at the specific and generic level in the angiosperm (Soltis & Sotis 1998). Chloroplast regions, for example, rbcL, trnL-F and matK are also widely used. In chloroplast genome, matK, which encodes a maturase, is one of the rapidly evolving protein-coding regions therefore also used in specific and generic level (Soltis & Sotis 1998). Based on different molecular markers, Angiosperm Phylogeny Group (APG) system for the classification for the families of flowering plant is gradually produced and updated (Stevens 2001 onwards). Recently, a new classification APG III was published (Angiosperm Phylogeny Group 2008).

In the context of the present study, there have been three studies that have used DNA data to examine the taxonomy of Saxifraga. In each case the focus was on only a single section. Thus Conti et al. (1999) studied the phylogenetic relationships in sect. *Ligulatae* using matK and the ITS of nuclear ribosomal DNA; Vargas (2000) studied sect. *Saxifraga* using ITS data; and Zhang et al. (2008) examined sect. *Ciliatae*, also using ITS sequences. So far, however, there has not been an extensive analysis of the genus as a whole, so we have no real idea of whether the infra-generic groups that have been proposed are monophyletic or not. A genus-wide study is necessary because restricted analyses not only may be prone to the effects of missing taxa (taxonomic sampling error), but also may suffer from the confounding effects of long-branch attraction (Judd 2007).

A second problem area to which molecular data can contribute is in taxon identification. Plant barcoding is a relatively new idea, whereby a library of DNA sequences is established that can be used for plant identification and phylogenetic studies (Hollingsworth et al. 2011). ITS shows a high discriminatory power as a barcode marker, and a combination of ITS and any single plastid marker was able to discriminate 69.9–79.1% of 1,757 angiosperm species studied. This compares with only 49.7% discriminatory power with two chloroplast sequences, rbcL + matK (Li et al. 2011). matK is one of the core barcode sequences for seed plants and is widely used

(Hollingsworth et al. 2011). However, owing to sometimes obscure species limits, which may be quite artificial, barcodes accept the application of a broad species concept (i.e. identification to an aggregated species, a group of species for which limits are not clear, or to one of a closely related set of species, a species complex) (Chase et al. 2005).

In terms of identification problems, some *Saxifraga* species, especially in sect. *Ciliatae*, are very difficult to determine because of the very small differences between relatives. It is also questionable whether some recently-described species are truly different from some which have already been recognised. This problem is especially acute in China when the type specimens of long-standing species are housed in distant herbaria, effectively inaccessible to local botanists; in such cases international collaboration, as in the case of writing the *Flora of China* (Pan et al. 2001), is required. By using ITS and matK sequences, it is hoped that some light can be shed on the species concept in *Saxifraga*.

In addition to these two DNA regions, I also examined an allegedly single-copy nuclear gene which is the male germline-specific R2R3 MYB transcription factor DUO POLLEN1 (DUO1). It was the first male germline-specific transcription factor to be identified in plants. It is crucial gene to control the timing of the second round of mitosis which can make trinucleate pollen (Borg et al. 2011). Therefore, it is interesting to see whether its sequences will relate to the tri-nucleate pollen distribution in *Saxifraga* major sections (see Chapter 3). No other genera have been tested their phylogeny by DUO1 gene.

2.2 Plant material

DNA was extracted from silica gel-dried leaves of fresh collected plants or from parts of herbarium specimens. Fresh plants were collected from the wild in China, Iceland, U.K., and from the University of Leicester Botanical Garden (LTR), Cambridge University Botanic Garden (CGG), Kunming Botanical Garden (KBG), and the national collection of *Saxifraga* at Waterperry Gardens, Oxford. Accession details are given in Table 2.1. Herbarium voucher specimens are lodged in herbaria listed in Table 2.1.

Table 2.1. Sample details for all the sequences used in the DNA analyses. Taxonomy follows Gornall (1987a) system. Sequence numbers starting with a 'Z' or 'R' are newly produced by this study; others are from Genbank.

Taxon	Locality	Voucher	Sequence No./Genbank No.		
			ITS	matK	DUO 1
Section *Heterisia*					
S. mertensiana	?	?	--	L34142	--
Section *Irregulares*					
S. epiphylla	China	Hort. KBG	Z123	--	--
S. fortunei (1)	Japan	Soltis 2519 (WS)	AF374821	--	--
S. fortunei (2)	Japan	Soltis 2519 (WS)	AF374822	--	--
S. mengtzeana	China, Yunnan	Hort. SCBG	Z175	--	--
S. rufescens	China, Sichuan, Ya'an, Erlangshan	Chen 20120056-2 (HNWP)	Z124	--	--
S. stolonifera	Unknown	Thomson 19550380 Hort. CGG	Z192	Z192	--
S. stolonifera	Unknown	Hort. LTR	--	--	Z2
S. stolonifera	China, Anhui	HEC- -0209-18	EU592002	--	--
Section Saxifragella					
Saxifraga bicuspidata	Chile	Arroyo et al. 950914	AF374819/ 20	--	--
Section *Trachyphyllum*					
S. aspera	?	Horwood L.39 16.VII.1967 (LTR)	--	--	Z90
S. bronchialis (1)	?	Brunsfeld 3094	AF087602	--	--
S. bronchialis (2)	?	Brunsfeld 3094	AF087632	--	--
S. bryoides	Jugoslavia, sar Planina.	Polunin 11396 (LTR)	--	--	Z91
S. tricuspidata (1)	?	Parker C., s.n.	AF087601	--	--
S. tricuspidata (2)	?	Parker C., s.n.	AF087631	--	--
S. tricuspidata	Canada, Kluane.	Pavlick 83-230 8.VII.1983 (BM)	--	--	Z95
Section *Ciliatae*					
Subsect. *Hirculoideae*					
Series *Hirculoideae*					
S. auriculata	China, Sichuan, Langmusi	Chen *20120022b-3* (HNWP)	--	Z155	--
S. bergenioides	China, Xizang, Medog	Liu654 (PE)	Z137	Z137	Z137
S. egregia	China, Sichuan, Sêrtar	Chen 2003075 (HNWP)	EU158836	--	--
S. egregia	China, Sichuan, Rangtang	Boufford 38981 (A)	--	--	Z13
S. hookeri	China, Xizang, Qüxü	Chen 2002085 (HNWP)	EU158840	--	--
S. hirculus (1)	Norway, circumboreal	Brochmann 93-219 (OS)	AF374823	--	--
S. hirculus (2)	Norway, circumboreal	Brochmann 93-219 (OS)	AF374824	--	--
S. hypericoides	China, Xizang, Qusum,	Chen 2002107 (HNWP)	EU158844	--	--
S. hypericoides	China, Sichuan, Litang, Tuershan	Chen 20120238 (HNWP)	--	--	Z130
S. insolens	China, Yunnan, Lijiang	Chen 2003147 (HNWP)	EU158841	--	--
S. insolens	China, Yunnan, Xiaozhongdian, Bigutianchi	Chen 20120115-1 (HNWP)	--	Z127	Z127
S. isophylla	China, Xizang, Nyingchi	Qinghai-Xizang Exped. 751144 (HNWP)	EU158848	--	--

S. isophylla	China, Xizang, Medog	Liu 632 (PE)	--	Z139	--
S. kingiana	China, Xizang, Nyalam	Xizang Chinese Medicine General Survey Team 1556 (HNWP)	EU158851	--	--
S. macrostigma	China, Deqin, Baimashan pass	Chen20120143-1 (HNWP)	Z173	--	--
S. macrostigma	China, Yunnan, Zhongdian	Boufford 42024 (A)	--	--	Z71
S. montanella	China, Qinghai, Qumalai	Chen 20110061-5 (HNWP)	Z140	Z140	--
S. parva	China, Qinghai, Zhiduo	Chen 20110067-4 (HNWP)	Z141	Z141	--
S. sinomontana	China, Qinghai, Dawu	Chen 2003015 (HNWP)	EU158834	--	--
S. sinomontana	China, Qinghai, Banma	Chen 20110040-1 (HNWP)	--	Z179	--
S. tangutica	China, Qinghai, Dawu	Chen 2003004 (HNWP)	EU158858	--	--
S. tangutica	China, Qinghai, Dari	Chen 20110052-2 (HNWP)	--	--	Z33
S. tangutica	China, Sichuan, Chazhenliangzi Pass	Chen 20120046-1 (HNWP)	Z177	Z177	--
S. tangutica	China, Gansu, Xunhua, Jishishan Pass	Chen 20120010-2 (HNWP)	--	Z166	--
S. tibetica	China, Qinghai, Zhiduo	Chen 20110065-3 (HNWP)	Z143	--	--
Series *Lychnitidae*					
S. pseudohirculus	China, Qinghai, Banma	Chen 20110042-4 (HNWP)	--	Z136	Z136
S. oresbia	China, Sichuan, Litang, Tuershan	Chen 20120239-1 (HNWP)	Z135	Z135	Z135
Series *Nutantes*					
S. nigroglandulifera	China, Sichuan, Daocheng, Guluke	Chen 20120196-4 (HNWP)	Z134	Z134	Z134
S. nigroglandulifera	China, Xizang, Riwoqe	Boufford 32299 (A)	--	--	Z72
Subsect. *Rosulares*					
S. pasumensis	China, Xizang, Qamdo	Chen 2002137 (HNWP)	EU158832	--	--
S. punctulata	China, Xizang, Yadong	Qinghai-Xizang Exped. 75-7344 (HNWP)	EU158854	--	--
S. sanguinea	China, Qinghai, Jiuzhi	Qinghai-Xizang Exped. 71-616 (HNWP)	EU158849	--	--
S. sediformis	China, Yunnan, Lijiang, Mont. Yulong	Chen 20120104-1 (HNWP)	Z152	--	--
S. umbellulata	China, Xizang, Gyaca	Chen 2002118 (HNWP)	EU158833	--	--
S. unguiculata	China, Qinghai, Mt. Dalijia	Chen 2004033 (HNWP)	EU158839	--	--
S. unguiculata	China, Qinghai, Dari	Chen 20110051-5 (HNWP)	--	Z125	--
S. unguiculata	China, Sichuan, Langmusi	Chen 20120022a-2 (HNWP)	--	Z154	--
S. unguiculata	China, Qinghai, Dari	Chen 20110047-3 (HNWP)	--	--	Z50
Subsect. *Serpyllifoliae*					
S. aurantiaca	China, Sichuan, Mt. Zhegu	Chen 2003048 (HNWP)	EU158845	--	--
S. flexilis	China, Yunnan, Zhongdian	Niu 2012001 (KUN)	Z156	Z156	Z156
S. gemmigera var. *gemmigera*	China, Qinghai, Darlag	(HNWP)	EU158856	--	--
S. gemmigera var. *gemmuligera*	China, Qinghai, Dari	Chen 20110036-4 (HNWP)	--	Z157	--
S. minutissima	India, Garhwal	Rawart 2007 (BM)	Z160	--	--

	Himalaya				
S. nanella	China, Xizang, Medog	Liu 439 (PE)	Z158	Z158	--
Subsect. *Hemisphaericae*					
S. hemisphaerica	China	D8529 (MB)	Z117	--	--
S. zhidoensis	China, Qinghai, Yushu	Wang 85-608 (HNWP)	EU158862	--	--
Subsect. *Gemmiparae*					
Series *Gemmiparae*					
S. balfourii	China, Yunnan, Heqing	Chen 2003164 (HNWP)	EU158838	--	--
S. balfourii	China, Yunnan, Lijiang, Mt. Yulong	Chen 2012103-1 (HNWP)	R18	--	--
S. brachypoda	China, Xizang, Lhünzhub	Xizang Chinese Medicine General Survey Team 1926 (HNWP)	EU158855	--	--
S. cinerascens	China, Yunnan, Lijiang, Mt. Yulong	Chen 20120078-1 (HNWP)	Z169	Z169	--
S. gemmipara	China, Yunnan, Dali, Cangshan	Chen 2012061-2 (HNWP)	R25	--	--
S. gemmipara	China, Yunnan, Dali, Cangshan	Chen 2012061-1 (HNWP)	R26	--	--
S. gemmipara	China, Yunnan, Lijiang, Mt. Yulong	Chen 2012073-2 (HNWP)	R21	--	--
S. gemmipara	China, Yunnan, Lijiang, Mt. Yulong	Chen 2012061-2 (HNWP)	R22	--	--
S. gemmipara	China, Yunnan, Lijiang, Mt. Yulong	Chen 2012072-2 (HNWP)	R5	--	--
S. gemmipara	China, Yunnan, Lijiang, Mt. Yulong	Chen20120072-1 (HNWP)	Z145	--	Z145
S. gemmipara	China, Yunnan, Lijiang	Chen 2003142 (HNWP)	EU158859	--	--
S. gemmipara var. viridiflora	China, Sichuan, Liangshan, Huili	Chen 20120059-1 (HNWP)	Z167	Z167	--
S. hispidula	China, Yunnan,Zhongdian	Boufford 35124 (A)	--	--	Z70
S. strigosa	China, Yunnan, Lijiang, Mt. Yulong	Chen 2012074-2 (HNWP)	R19	--	--
S. strigosa	China, Yunnan, Lijiang, Mt. Yulong	Chen 2012074-1 (HNWP)	R20	--	--
S. strigosa	China, Yunnan, Dali	Chen 2003135 (HNWP)	EF369514	--	--
S. strigosa	China, Yunnan, Dali, Cangshan	Chen 20120063-1 (HNWP)	--	Z146	
S. substrigosa	China	D10650 (MB)	Z116	--	--
S. substrigosa	China, Xizang, Medog	Liu 620 (PE)	Z147	--	--
Series *Spinulosae*					
S. filicaulis	China, Yunnan, Dali, Cangshan	Chen 2012062-2 (HNWP)	R23	--	--
S. filicaulis	China, Yunnan, Dali, Cangshan	Chen 2012062-1 (HNWP)	R24	--	--
S. filicaulis	China, Sichuan, Xiangcheng, Yuangenshan	Chen 2012154-2 (HNWP)	R12	--	--
S. filicaulis	China, Yunnan, Lijiang, Mt. Yulong	Chen 2012075-2 (HNWP)	R3	--	--
S. wallichiana	China, Sichuan, Xiangchen, Daxueshan pass	Chen 2012179-1 (HNWP)	R11	--	--
S. wallichiana	China, Sichuan, Xiangchen, Daxueshan pass	Chen 2012179-2 (HNWP)	R10	--	--
S. wallichiana	China, Sichuan, Xiangchen, Yuangenshan	Chen 2012153-2 (HNWP)	R14	--	--
S. wallichiana	China, Sichuan, Xiangchen,	Chen 2012153-1 (HNWP)	R15	--	--

	Yuangenshan				
S. wallichiana	China, Yunnan, Deqin, Baimashan pass	Chen 2012139-1 (HNWP)	R16	--	--
S. wallichiana	China, Sichuan, Litang to Xinlong, Kagong	Chen 2012251-1 (HNWP)	R7	--	--
S. wallichiana	China, Sichuan, Xiangchen, Maxionggou	Chen 2012188-1 (HNWP)	R9	--	--
S. wallichiana	China, Sichuan, Daochen	Chen 20120217-1 (HNWP)	--	Z148	--
S. wallichiana	China, Sichuan, Yajiang	Boufford 35849 (A)	--	--	Z75
S. wallichiana	China, Yunnan, Heqing	Chen 2003166 (HNWP)	EU158847	--	--
S. wardii	China, Xizang, Medog	Liu 440 (PE)	R1	--	--
S. wardii	China, Xizang, Medog	Liu 506 (PE)	Z149	Z149	--
S. wardii	China, Xizang, Nyingchi	Boufford et.al. 30129 (A)	--	Z76	Z76
Subsect. *Flagellares*					
S. consanguinea	China, Daochen, Guluke	Chen 2012197-5 (HNWP)	R4	--	--
S. consanguinea	China, Daochen, Guluke	Chen 20120197-6 (HNWP)	--	--	Z150
S. consanguinea	China, Qinghai, Nangqên	Chen 2002042 (HNWP)	EU158837	--	--
S. flagellaris (1)	?	Murray 11315A	AF087600	--	--
S. flagellaris (2)	?	Murray 11315A	AF087630	--	--
S. microgyna	China, Sichuan, Yajiang	Boufford et.al. 35760 (A)	--	Z69	Z69
S. nangxianensis	China, Xizang, Cona	Qinghai-Xizang Exped. 751492 (HNWP)	EU158850	--	--
Section *Cymbalaria*					
S. cymbalaria (1)	?	Ferguson 1994-04	AF087599	--	--
S. cymbalaria (2)	?	Ferguson 1994-04	AF087629	--	--
S. cymbalaria	UK, Leicester.	Gornall 688 (LTR)	--	--	Z80
S. hederacea	Libya, Wadi Kouf	Davis 50203 (RNG)	AF261182	--	--
S. hederacea	?	Hort. LTR	--	--	Z5
Section *Saxifraga*					
Subsect. *Saxifraga*					
Series *Saxifraga*					
S. bulbifera	Italy, Fiuminata	Conti (MA)	AF261166	--	--
S. carpetana	Spain, Avila, Solosancho	12-97PV (MA)	AF261168	--	--
S. cintrana	Portugal, Sierra de Montejunto	2515PV (MA)	AF261171	--	--
S. dichotoma	Spain, Toledo, Hinojosa de San Vicente	(MA)	AF261174	--	--
S. granulata	U.K., Berkshire, Cock Marsh	?	--	--	Z164
S. granulata (1)	Spain, Ma´laga, Jubrique	3PV97 (MA)	AJ233860	--	--
S. granulata (2)	Spain, Almeria, Sierra de Filabres	?	AF482692	--	--
S. haenseleri	Spain, Albacete, Sierra de Alcaraz,	Nieto 1297 (MA)	AF261180	--	--
S. trabutiana	Spain, Almeria, Sierra de Filabres	?	AF482693	--	--
Series *Biternatae*					
S. biternata	Spain, Mfilaga, Antequera	8-97PV (MA)	AF261163	--	--
S. bourgaeana (1)	Spain, Cfidiz, Grazalema	7-97PV (MA)	AF261164	--	--
S. bourgaeana (2)	Spain, Cfidiz, Grazalema	7-97PV (MA)	AF261165	--	--
S. gemmulosa	Spain, Mfilaga, Sierra	2-97PV (MA)	AF261177	--	--

	Bermeja				
Subsect. *Triplinervium*					
Series *Aquaticae*					
S. aquatica Lapeyr.	Spain, Huesca, Bielsa	34-97PV (MA)	AF261161	--	--
S. latepetiolata (1)	Spain, Cuenca, Ciudad Encantada	(MA)	AF261183	--	--
S. latepetiolata (2)	?	1959-33301 (K)	AF087603	--	--
S. latepetiolata (3)	?	1959-33301 (K)	AF087633	--	--
Series *Arachnoideae*					
S. arachnoidea (1)	Italy, Lombardia, Riva del Garda	(RNG)	AF261162	--	--
S. arachnoidea (2)	?	Horaendl 4564	AF087607	--	--
S. arachnoidea (3)	?	Horaendl 4564	AF087637	--	--
Series *Axilliflorae*					
S. praetermissa	Spain, Cantabria, Picu Uriellu	3250PV (MA)	AF261186	--	--
Series *Ceratophyllae*					
S. babiana	Spain, Leo´n, True´bano	2992PV (MA)	AJ233879	--	--
S. camposii	Spain, Albacete, River Mundo	Vargas et al. 58ML88 (MA)	AJ233863	--	--
S. canaliculata	Spain, Ledn, Barrios de Luna	2991PV (MA)	AF261167	--	--
S. canaliculata	?	Hort. LTR	--	Z174	--
S. canaliculata	Spain, N. Burgos	Webb 19600319 Hort. CGG	Z208	Z208	--
S. cuneata (1)	Spain, Burgos, Humada	Alejandre, 1991(MA)	AJ233881	--	--
S. cuneata (2)	Spain, Soria, River Lobos	2893PV (MA)	AJ233880	--	--
S. fragilis (1)	Spain, Castelldn, Morella	3147PV (MA)	AF261176	--	--
S. fragilis (2)	?	1958-27701 (K)	AF087605	--	--
S. fragilis (3)	?	1958-27701 (K)	AF087635	--	--
S. genesiana	Spain, Barcelona, Sierra del Montseny	A. Bombi (MA)	AF261178	--	--
S. geranioides	Andorra, E1 serrat	C. Aedo 2099 (MA)	AF261192	--	--
S. intricata	Spain, Huesca, Panticosa	27PV97 (MA)	AJ133030	--	--
S. losae	Spain, Navarra, Foz de Lumbier	28-5-1994 (JACA)	AJ133027	--	--
S. maderensis	Portugal, Madeira archipelago, Madeira island	Franquinho and Costa 1992 (MA)	AJ233882	--	--
S. maderensis	?	Hort. CGG	Z186	Z186	--
S. maderensis	Madeira archipelago, Madeira Is.	Hort. LTR	Z213	Z213	--
S. moncayensis	Spain, Guadalajara, Pelegrina	SOPV97 (MA)	AJ133028	--	--
S. pedemontana (1)	?	1959-33303 (K)	AF087606	--	--
S. pedemontana (2)	?	1959-33303 (K)	AF087636	--	--
S. pentadactylis	Andorra, El Serrat	Aedo et al., 11918SC (MA)	AJ233862	--	--
S. pentadactylis subsp. *willkommiana* (1)	Spain, Sistema Iberico, Sierra de Urbion	?	AY354307	--	--
S. pentadactylis subsp. *willkommiana* (2)	Spain, Sistema Iberico, Sierra de Urbion	?	AY354308	--	--
S. portosanctana	Madeira archipelago, Porto Santo Is.	Franquinho and Costa 1992 (MA)	AJ233883	--	--
S. portosanctana	Madeira archipelago, Porto Santo Is.	19770228 (Hort. K)	Z197	--	--
S. trifurcata (1)	Spain, Asturias, Fuejo	2580PV (MA)	AJ233885	--	--

S. trifurcata (2)	Spain, Asturias, Rengos	2579PV (MA)	AJ233884	--	--
S. trifurcata	?	Lausanne 19550034 Hort. CGG	Z203	Z203	
S. trifurcata	?	Hort. LTR	--	Z8	Z8
S. vayredana	Spain, Barcelona, Sierra del Montseny	2896PV (MA)	AJ133029	--	--
Series *Cespitosae*					
S. cebennensis	France, Gorge de Tarn	51-97PV (MA)	AF261169	--	--
S. cespitosa (1)	Canada, Newfoundland	Baker 769 (RNG)	AF261170	--	--
S. cespitosa (2)	?	Brochmann 92-32	AF087604	--	--
S. cespitosa (3)	?	Brochmann 92-32	AF087634	--	--
S. cespitosa	Iceland	*Gornall & Hollingsworth* Hi 9 (E)	Z218	Z218	--
S. cespitosa	Iceland	Gornall & Hollingsworth 661 (LTR)	--	--	Z98
S. exarata	Austria, Glockturm, Plats	D. Go´mez 1994 (JACA)	AJ233861	--	--
S. hariotii	France, Atlantic Pyrenees	(JACA)	AF261181	--	--
S. moschata	Spain, Pyrenees, Huesca, Portalet	25-97PV (MA)	AF261184	--	--
S. nevadensis	Spain, Sierra Nevada,	508846 (MA)	AF261185	--	--
S. pubescens (1)	France, Hautes Pyrenees	(JACA)	AF261187	--	--
S. pubescens (2)	France, Hautes Pyrenees	(JACA)	AF261188	--	--
S. rosacea	Iceland	Gornall & Hollingsworth Hi 85 (E)	Z227	Z227	--
S. rosacea	?	(RNG)	AF261190	--	--
S. rosacea / cespitosa	Iceland	*Gornall & Hollingsworth* Hi 230 (E)	Z222	--	--
S. rosacea / cespitosa	Iceland	*Gornall & Hollingsworth* Hi 181 (E)	Z221	Z221	--
Series *Gemmiferae*					
S. conifera (1)	Spain, Cantabria, Naranjo de Bulnes	900PV95 (MA)	AJ233865	--	--
S. conifera (2)	Spain, Palencia, Velilla del rıo Carrión	C. Navarro et al. CN 1604 (MA)	AJ233864	--	--
S. continentalis	?	Cherngavsky 19900444 Hort. CGG	Z209	Z209	Z209
S. erioblasta	Spain, Granada, Alfacar	23PV97 (MA)	AJ233866	--	--
S. globulifera (1)	Morocco, Azrou	17953SJ (RNG)	AJ233874	--	--
S. globulifera (2)	Morocco, Tazzeka	13784SJ (RNG)	AJ233873	--	--
S. globulifera (3)	Morocco, Fe`s, Ourtzarh	Montserrat and Valde´s, 1994 (RNG)	AJ233872	--	--
S. hypnoides	United Kingdom, South Wales, Powys	Rumsey and Grindley, 1984 (RNG)	AJ233875	--	--
S. reuteriana	Spain, Ma´laga, Archidona	9PV97 (MA)	AJ233877	--	--
S. rigoi	Spain, Jaen, Cazorla	21PV97 (MA)	AJ233878	--	--
Subsect. *Holophyllae*					
Series *Androsaceae*					
S. androsacea	Switzerland, Valais	Castroviejo 11144 (MA)	AF261159	--	--
S. depressa	Italy, Dolomites, Cazione	70-97PV (MA)	AF261173	--	--

Series *Muscoideae*					
S. facchinii	Italy, Dolomites, passo di Sella	73-97PV (MA)	AF261175	--	--
S. muscoides	France, Hautes-Alpes.	Webb 27.VII.1959 (LTR)	--	--	Z108
Series *Sedoideae*					
S. aphylla	Austria, Innsbruck	Gdmez (JACA)	AF261160	--	--
S. sedoides	Italy, Dolomites, Marmolada Massif,	60-97PV (MA)	AF261191*	--	--
S. sedoides	Italy, Reg. Veneto, Prov. Belluno.	Jury 6698 (LTR)	--	--	Z106
Series *Glabellae*					
S. glabella	Greece, Olibos.	Polunin 13799 (LTR)	--	--	Z96
Series *Tenellae*					
Subsect. *Tridactylites*					
S. adscendens	?	?	EF028688	--	--
S. osloensis (1)	?	Brochmann s. n.	AF087608	--	--
S. osloensis (2)	?	Brochmann s. n.	AF087638	--	--
S. tridactylites (1)	?	?	EF028687	--	--
S. tridactylites (2)	?	?	EF028686	--	--
Section *Cotylea*					
S. rotundifolia (1)	?	BBG 267-94-80-10	AF087598	--	--
S. rotundifolia (2)	?	BBG 267-94-80-10	AF087628	--	--
S. rotundifolia	Italy, Majella, Bocca di Valle, Lama Dei Peligni	19990320 Hort. CGG	--	Z189	
S. rotundifolia	?	Hort. LTR	--	Z7	Z7
Section *Mesogyne*					
S. cernua	China, Gansu, Zhangye	Huang 91-3119 (HNWP)	EU158861	--	--
S. cernua	Iceland	*Gornall & Hollingsworth* Hi 182 (E)	Z226	Z226	--
S. cernua	Sweden, Lakiattakka	Polunin 15610 (LTR)	--	--	Z94
S. cernua	?	?	--	L34140	--
S. granulifera	China, Yunnan, Zhongdian	Niu2012002 (KUN)	Z159	Z159	Z159
S. rivularis	Iceland	*Gornall & Hollingsworth* Hi 222 (E)	Z224	Z224	--
Section *Gymnopera*					
S. cuneifolia (1)	?	ÖBG 76-1520	AF087595	--	--
S. cuneifolia (2)	?	ÖBG 76-1520	AF087625	--	--
S. cuneifolia var. capillipes	France, Alpes-Maritimes, nr. Tende	Webb 19740001 Hort. CGG	--	Z207	--
S. hirsuta (1)		NBG 78-1339	AF087597	--	--
S. hirsuta (2)		NBG 78-1339	AF087627	--	--
S. hirsuta	Spain, Picos de Europa	Victor 20090707 Hort. CGG	Z185	Z185	--
S. hirsuta	?	Hort. LTR	--	Z11	--
S. spathularis (1)	Spain, Pontevedra, Vilasobroso	Vargas et al., 21ML88 (MA)	AJ233858	--	--
S. spathularis (2)	?	BBG 001-91-75-10	AF087596	--	--
S. spathularis (3)	?	BBG 001-91-75-10	AF087626	--	--
S. umbrosa	?	20110353 Hort. CGG	--	Z193	--
Section *Ligulatae*					
Subsect. *Aizoonia*					
S. callosa	Italy, MS, Campocecina	Pisa 19911254 Hort. CGG	Z184	Z184	--
S. callosa ssp. catalaunica	?	Bland 20080322 Hort. CGG	Z205	Z205	--
S. callosa (1)		BBG 137-08-86-10	AF087581	--	--
S. callosa (2)		BBG 137-08-86-10	AF087611	--	--
S. callosa var. australis	France, S. France, Lantosque Valley	Beckett 19800500 Hort. CGG	Z190	Z190	--

S. cochlearis	France, Maritime Alps, Notre Dames des Fontaines	Bland 20080337 Hort. CGG	Z182	Z182	Z182
S. cochlearis	France, Maritime Alps, Vallee de Cairos	Hunter 19740141 Hort. CGG	Z199	Z199	--
S. cochlearis (1)	?	Conti, Martini 33A 7/6/92	AF087580	AF133133	--
S. cochlearis (2)	?	Conti, Martini 33A 7/6/92	AF087610	--	--
S. cotyledon	Norway, Aurlandsdalen	Bland 20080343 Hort. CGG	Z195	Z195	--
S. cotyledon	France, Pyrenees, Gloriette Barrage	Roberts 20080344 Hort. CGG	Z188	Z188	--
S. cotyledon (1)	?	BBG 214-14-81-10	AF087584	--	--
S. cotyledon (2)	?	BBG 214-14-81-10	AF087614	--	--
S. crustata (1)	?	ÖBG 70-97	AF087583	--	--
S. crustata (2)	?	ÖBG 70-97	AF087613	--	--
S. crustata	Italy, Dolomites, Gorge Caprille	Bland 20080350 Hort. CGG	Z196	Z196	--
S. hostii (1)		BBG 091-17-86-10	AF087579	AF133132	--
S. hostii (2)		BBG 091-17-86-10	AF087609	--	--
S. hostii	Germany, Kärnten, Wolayer See	Vienna 19930134 Hort. CGG	Z183	Z183	--
S. hostii ssp. rhaetica	Italy, Italian Alps, East of Breno	Bland 20080357 Hort. CGG	Z187	Z187	--
S. longifolia (1)	Spain	NBG 86-21	AF087585	AF133134	--
S. longifolia (2)	Spain	NBG 86-21	AF087615	--	--
S. paniculata	France, Vercoros south of Grenoble	Cobb 20080361 Hort. CGG	Z181	Z181	--
S. paniculata	Austria, Salzburg, Mont. Untersberg	S6 (LTR)	Z214	Z214	--
S. paniculata	?	Hort. LTR	--	Z3	--
S. paniculata ssp. cartilaginea	?	Bland 20080397 Hort. CGG	Z200	Z200	--
S. paniculata	Italy, Miniera Valley	Bland 20080371 Hort. CGG	Z201	Z201	--
S. paniculata (1)	?	UBC BG 23633-208	AF087586	--	--
S. paniculata (2)	?	UBC BG 23633-208	AF087616	--	--
S. valdensis (1)		UBC BG 23830-208	AF087582	--	--
S. valdensis (2)		UBC BG 23830-208	AF087612	--	--
Subsect. *Mutatae*					
S. mutata (1)	?	Gornall, s.n.	AF087593	AF133138	--
S. mutata (2)	?	Gornall, s.n.	AF087623	--	--
Subsect. *Florulentae*					
S. florulenta (1)	?	Conti, Martini F5 7/8/92	AF087591	AF133137	--
S. florulenta (2)	?	Conti, Martini F5 7/8/92	AF087621	--	--
Section *Xanthizoon*					
S. aizoides (1)	?	Brochmann 92-78-1	AF087594	--	--
S. aizoides (2)	?	Brochmann 92-78-1	AF087624	--	--
S. aizoides (3)	Switzerland, the Prealps	?	AF504547	--	--
S. aizoides	Austria, Salzburg, Mont. Untersberg	S1 (LTR)	Z215	Z215	--
Section *Porphyrion*					
Subsect. *Kabschia*					
Series *Juniperifoliae*					
S. sancta	?	Finnis 19620359 Hort. CGG	Z211	Z211	Z211
Series *Marginatae*					
S. afghanica	China, Qinghai, Nêngqên	Wang 82-0016 (HNWP)	EU158853	--	--
S. marginata	?	Finnis 1965014615	Z210	Z210	Z210

		Hort. CGG			
S. marginata (1)	?	UBC BG 20875-246	AF087589	--	--
S. marginata (2)	?	UBC BG 20875-246	AF087619	--	--
S. scardica	Greece, Mt. Chelmos, Akhaia	Chater 13045 Hort. CGG	Z202	Z202	--
Series *Squarrosae*					
S. caesia (1)	?	G. Pellegrino, A. Cozie, 18/10/94	AF087588	AF133136	--
S. caesia (2)	?	G. Pellegrino, A. Cozie, 18/10/94	AF087618	--	--
S. caesia	Austria, Salzburg, Mont. Untersberg	S5 (LTR)	--	--	Z216
S. squarrosa (1)	?	Gornall, s. n.	AF087587	AF133135	--
S. squarrosa (2)	?	Gornall, s. n.	AF087617	--	--
Series *Tetrameridium*					
S. pulvinaria	China, Xingjiang, Taxkorgan,	(HNWP)	EU158860	--	--
Subsect. *Engleria*					
S. federici-augusti ssp. *grisebachii*	?	20120343 Hort. CGG	Z194	Z194	Z194
S. sempervivum (1)	?	1984-3743 (K)	AF087590	--	--
S. sempervivum (2)	?	1984-3743 (K)	AF087620	--	--
S. sempervivum (2)	?	Hort. CGG	--	Z191	Z191
S. stribrnyi	?	Savory 1958044931 Hort. CGG	Z206	Z206	--
Subsect. *Oppositifoliae*					
Series *Oppositifoliae*					
S. biflora All.	Switzerland, the Alps	?	AF504546	--	--
S. oppositifolia (1)	U.S.A., Tanana Uplands, Alaska	?	AF502089	--	--
S. oppositifolia (2)	Spain: Cantabrian range, Cantabria		AY357945	--	--
S. oppositifolia	?	?	--	L34143	--
S. oppositifolia	Iceland.	Gornall & Hollingsworth 654 (LTR)	--	--	Z87
ID query species					
S. cf. *aurantiaca*	China, Sichuan, Mt. Zhegu	Chen 20110047-4 (HNWP)	--	Z126	--
S. cf. *aurantiaca*	China, Sichuan, Mt. Zhegu	Chen20120047-1 (HNWP)	Z178	--	--
Diversifolia sp.	China, Sichuan, Xiangchen, Daxueshan	Chen20120181-1 (HNWP)	Z161	--	--
S. cf. *implicans*	China, Yunnan, Lijiang, Mont. Yulong	Chen20120080-2 (HNWP)	Z170	Z170	--
× *landaueri*	Waterperry Gard.	Hort. LTR	Z115	--	--
S. cf. *micans*	Nepal, Tarapkholo Dolpo	Hort. Waterperry Garden	Z114	--	--
Rufolanatae sp.	China, Yunnan, Dali	Chen 2003129 (HNWP)	EU158843	--	--
SEP22	Pakistan, Baturq Glazier, Giljit	Hort. Waterperry Garden	Z111	--	--
Outgroup species					
Chrysosplenium album	Japan, Tochigi Pref., Jakko Falls	*Soltis J2534* (?)	--	AF297635	--
Chrysosplenium hydrocotylifolium	China, Yunnan	Lei 20090402 (KUN)	JN102226	--	--
Chrysosplenium lanuginosum var. gracile	China, Hubei	Gan 1912 (KUN)	JN102227	--	--
Chrysosplenium tetrandrum	?	?	--	L34121	--
Itea ilicifolia	China	86.0414 Hort. University of California Botanic	AY138058	--	--

		Garden, Berkeley			
Itea virginica	U.S.	Ware 9401 (WS)*	--	AF274618	--
Itea yunnanensis	China, Yunnan	Xiang 444(KUN)	JN102242	--	--
Micranthes aprica	?	?	--	AF114179	
Micranthes atrata	China, Qinghai, Huzhu	Wu 3057 (HNWP)	EU158852	--	--
Micranthes bryophora	?	?	--	AF114180	--
Micranthes californica	?	?	--	AF114172	--
Micranthes calycina	?	Murry & Kelso 11,309 (?)	--	AF115492	--
Micranthes divaricata	China, Sichuan, Daochen	Chen 20120219-3 (HNWP)	Z122	Z122	--
Micranthes ferruginea	?	?	--	L34141	--
Micranthes foliolosa	?	Murry 7058 (?)	--	AF115494	--
Micranthes fusca	?	Soltis 2524 (?)	--	AF115490	--
Micranthes hieraciifolia	?	Borchmann 92-4-8	--	AF115485	--
Micranthes howellii	?	?	--	AF114178	--
Micranthes integrifolia	?	?	--	L20131	--
Micranthes lyallii (1)	?	Soltis & Soltis 223 (?)	--	AF115487	--
Micranthes lyallii (2)	U. S. A., Oregon, Josephine Co., Illinois River Rd	Schenk 586 (WS)	--	GU226267	--
Micranthes melanocentra	China, Qinghai, Dawu	Chen 2003001 (HNWP)	EU158846	--	--
Micranthes melanocentra	China, Qinghai, Banma	Chen 20110044-1 (HNWP)	Z115	--	--
Micranthes melanocentra	China, Sichuan, Xiaojin	Boufford 38466 (A)	--	--	Z27
Micranthes melanocentra var. *rubiflora*	China, Sichuan, Daochen, Guluke	Chen 20120192-5 (HNWP)	--	Z165	--
Micranthes nidifica	?	?	--	AF114182	--
Micranthes nivalis	?	Murray 11,350 (?)	--	AF115491	--
Micranthes oregana	?	?	--	AF114181	--
Micranthes occidentalis	?	?	--	AF114177	
Micranthes pallida	China, Xizang, Mainling,	Qinghai-Xizang Exped. 75-1328 (HNWP)	EU158863	--	--
Micranthes pensylvanica	?	?	--	AF114175	--
Micranthes punctata	?	?	--	L34144	--
Micranthes reflexa	?	Murray & Kelso 11,308 (?)	--	AF115486	--
Micranthes rhomboidea	?	?	--	AF114176	--
Micranthes rufidula	?	?	--	AF114183	--
Micranthes spicata	?	?	--	AF114173	--
Micranthes stellaris (L.) (1)	Pacific Northwest of North America	Horandl 2703 (WS)	AF374827	--	--
Micranthes stellaris (L.) (2)	Pacific Northwest of North America	Horandl 2703 (WS)	AF374828	--	--

Micranthes stellaris	?	Horandl 2703 (?)	--	AF115493	--
Micranthes stellaris	Austria, Salzburg, Mt. Untersberg	S3 (LTR)	Z217	--	--
Micranthes texana	?	?	--	AF114174	--
Micranthes tolmiei	?	32167 (WS)	--	AF115484	--
Micranthes virginiensis	?	75-449-5 (ÖBG)	--	AF115488	--
Ribes alpestre	?	Zhang DC-07zx-0721 (KUN)	JF978471	--	--
Ribes maximowiczianum	China, Yunnan	Ying et al 1601 (KUN)	--	JN102200	--
Ribes nigrum	?	?	AJ297579	--	--
Ribes soulieanum	China, Yunnan	Ying & Dong 0434 (KUN)	--	JN102202	--

'*' means an inconsistency between the Genbank database and the original paper. Herbarium and garden acronyms follow Holmgren et al. (1990) and Heywood et al. (1990), respectively, and full names are given in the Abbreviation (Page xii).

'?' stands for data not applicable. These information cannot be found in the literatures where the sequences published with.

2.3 DNA methods

2.3.1 DNA sequences

Previously reported sequences of ITS and matK were accessed via Genbank, derived from the following studies: (Chapman and Burke 2007; Conti et al. 1999; Fenton et al. 2000; Garcia-Maroto et al. 2003 ; Johnson and Soltis 1995; Li et al. 2011; Mort and Soltis 1999; Schultheis and Donoghue 2004; Soltis et al. 1996; Soltis et al. 2001a; Vargas et al. 1999; Vargas 2000; Vargas 2001; Vargas 2003; Xiang et al. 2012; Zhang et al. 2008). 199 sequences were newly produced in this study. Altogether 409 sequences were used, comprising 257 ITS, 110 matK, and 42 DUO1.

For the ITS region, 88 sequences were newly produced. As many taxa had already been studied, sampling strategy focused on increasing representation at subsection and series level, especially in section *Ciliatae*. Attention was also paid to sampling at the intra-specific level, in order to address population divergence and speciation questions. Some unidentified species and species with unusual morphology were also included to shed light on their identification or taxonomic position. For example, *S. gemmipara* var. *viridiflora* (a newly discovered and undescribed variant) and a collection labelled *S*. cf. *aurantiaca* were compared with *S. gemmipara* and *S. aurantiaca / unguiculata*, respectively. A recently described species, *S. minutissama* (Gornall et al. 2012) (Chapter 6), which has only five stamens (almost uniquely in the genus), was also studied to test its putative position in sect. *Ciliatae* subsect. *Serpyllifoliae.* The taxonomic scope at subsection level of ITS sampling is shown in Table 2.1.

69 new accessions for matK analysis were selected for a broader sampling. Combined with Genbank data, there are representatives of ten sections (Table 2.1). The sampling strategy was to improve intra-sectional representation and to match taxa used in the ITS analysis (ideally the same individual). Technical problems limited the number of sequences eventually obtained. Especially for herbarium specimen samples, DNA was commonly degraded to fragments <500bp and contained more phenolics and other inhibitors thus the polymerase chain reaction (PCR) gave no result. However, for some sections, e.g. sect. *Trachyphyllum*, fresh material is very difficult to get.

42 DUO1 sequences representing nine sections of the genus *Saxifraga* are reported for the first time here. Samples include several species in sect.

Ciliatae, subsect. *Hirculoideae* because I wanted to find out whether DUO1 gene phylogeny, the gene controlling the appearance of bi- or tri-nucleate cells in pollen, would correspond in any way with the unique, tri-nucleate pollen distribution in subsect. *Hirculoideae* (see Chapter 3, Zhang and Gornall 2011).

2.3.2 DNA extraction

The DNA extraction protocol was modified from the lab protocol of the Royal Botanic Garden Edinburgh, which is in turn derived from the Cetrimonium bromide (CTAB) method (Doyle and Doyle 1990). Processes were as follows:

- 2% CTAB buffer was made from the following contents:

  20gm CTAB

  40ml ethylenediaminetetraacetic acid (EDTA) stock (0.5M)

  100ml tris (hydroxymethyl) amino methane (Tris-Cl) pH 8.0 stock (1M)

  280ml NaCl stock (5M)

  and made up to 1 Litre with distilled water, and adjusted to pH 7.5 - 8.0, and autoclaved. TE buffer was made from 1ml 1M Tris-Cl, 0.2ml 0.5M EDTA and made up to 1 Litre with distilled water, and adjusted to pH 8.0, and autoclaved.
- 20-60 mg dry leaves or 150-250 mg fresh leaves were placed in a 1.5 ml centrifuge tube containing ~10 glass beads (2 mm, Merck) and a pinch of polyvinylpolypyrrolidone (PVPP).
- The tube with fresh leaves was frozen in liquid nitrogen and then placed in a dental homogenizer (Silamat Plus, Vivadent) and processed at high speed for 15 s × 3 times to finely grind the leaves to powder. Fresh leaves were put back into liquid nitrogen between each homogenisation to ensure

the tissue was stiff enough to grind; then the pressure was released from the tube.

- 1.5 ml 2% CTAB buffer + 3 μl β-mercaptoethanol per tube were pre-heated in a 65 °C water bath in a fume cupboard. The solution was added to the tube of leaf tissue and mixed well with a tip-fused glass pipette or glass stick. The mixture was then incubated in a 65 °C water bath for 30 minutes. The sample was mixed by inverting the tube every 10 minutes during incubation.
- The tube was removed and cooled to room temperature. The contents were decanted to a 2 ml tube, 700 ml of Chloroform:Isoamyl alcohol (24:1) added, and the tube placed on an orbital shaker (Intelli-mixer RM-2L, ELMI) to process the reversing mode for 20 minutes. Then it was centrifuged for 10 minutes at 13,000 rpm.
- The supernatant was transferred to a clean 2 ml tube and the previous step repeated.
- The supernatant was transferred to a clean 1.5 ml tube. Ice cold isopropanol (volume = 2/3 of the supernatant) was added. The tube was inverted very gently for 2-3 times and then left at -20 °C overnight or longer.
- The tube was then centrifuged for 10 minutes at 13,000 rpm, and the solution discarded to leave only the bottom pellet. 1000 ml wash buffer (70% ethanol) was added. The pellet was released from the bottom by vigorously agitating. The tube was left at least 30 minute or overnight at 4 °C. This step was repeated.

- The tube was centrifuged for 5 minutes at 13,000 rpm, and the solution drained away to leave only the pellet. The tube was opened and inverted on a clean tissue paper in a 50 °C heated block for ~10 minutes until the pellet was more or less dry.
- 50-100 µl TE buffer was added to dissolve the pellet. The DNA was incubated at room temperature for 3-4 hours until it completely dissolved.
- 1 µl RNAse was added and mixed well, followed by incubation at 37 °C for 1.5 h.
- DNA quality and concentration was checked using a Nanodrop machine.

2.3.3 DNA amplification and sequencing

DNA amplification followed the methods described in previous studies (Conti et al. 1999; Soltis et al. 2001a). A few modifications were made as described below. Primers were produced by Sigma-Aldrich Co. U.K. ITS and matK amplifying primer sequences follows literatures (White et al. 1990, Conti et al. 1999, Cuenoud et al. 2002, Fig. 2.1). The suitable melting temperatures for all PCR amplifying primer pairs were empirically tested and chosen with gradient temperature PCR reactions (Table 2.2).

Table 2.2. Primer name and sequence with primer pairs melting temperature and product size of different DNA regions.

Primer name	Primer sequence	Melting Temperature	Product Size (bp)
ITS			
N18L18F C26AR	CTTGCGACGTCGAAAGAA GTTTCTTTTCCTCCGCT	48°C	840
ITS1			
ITS1F ITS2R	TCCGTAGGTGAACCTGCGGAAGGATCATTG GCTACGTTCTTCATCGATGC	50°C	366
ITS2			
ITS3F ITS4R	GCATCGATGAAGAACGTAGC TCCTCCGCTTATTGATATGC	50°C	421
matK			
trnk710F 1326R	GTATCGCACTATGTWTCATTTGA TCTAGCACACGAAAGTCGAAGT	62°C	1326
matKZ1R	ATTAGAGGAATAATTGGAAC	55°C	1060 (with trnk710F)
DUO1			
putDUO1-F1 putDUO1-R1	TGGAGCTCBATTCGRTCCAAAGG TTCTTVACRTCRTTRTCRGTYCTKCC	50°C	434
Z5F Z2R1	CTTGTCGCCTTCGTTGGGTC TTCTTAACATCGTTGTCGGTTCTGCC	50°C	282
Z4F Z2R2	GCTTCGTTGGGTCAATAAGCTTCGC GTTGTCGGTTCTGCCTTGTAAATATG	45°C	272

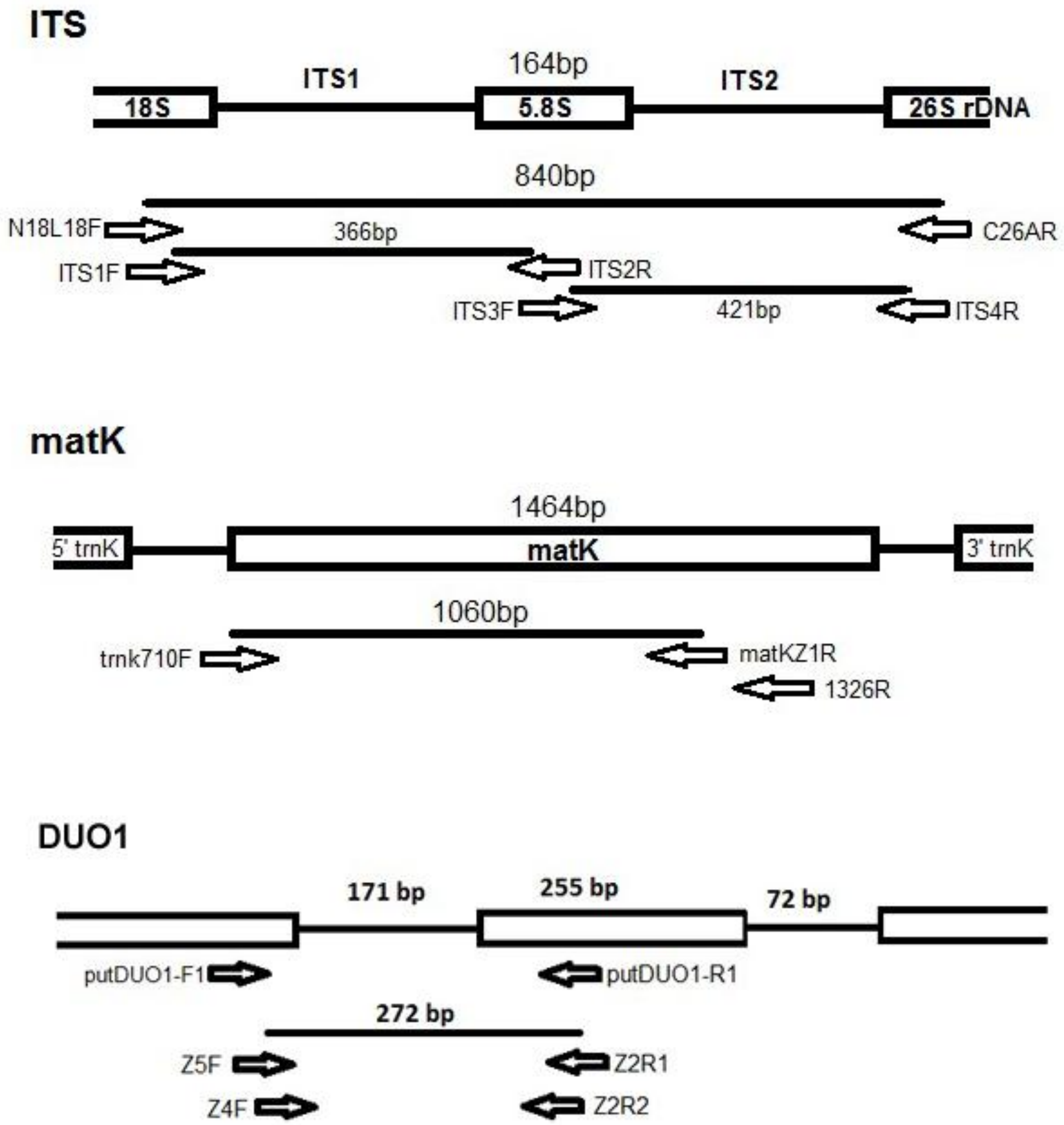
KAPA PCR Kit (KAPA Biosystems) was used for most of the PCR reactions. ITS amplification was conducted by using primers N18L18F and C26AR (Conti et al. 1999) and temperature cycle, repeated 30 times, 1 minute at 94°C, 45 s at 48°C, and 2 minutes at 72°C. Samples with degraded DNA would only produce ITS1 and ITS2 separately, e.g. *S. minutissama*. The KAPA 3G Plant

PCR Kit (KAPA Biosystems) worked well with crude plant DNA that contained more phenolic. The primer pairs for ITS1 and ITS2 are ITS1F + ITS2R and ITS3F + ITS4R (White et al. 1990), respectively. They share the same thermo cycle, repeated 40 times, viz. 25 s at 95°C, 15 s at 50°C, and 30 s at 72°C.

The primers used to amplify matK were trnk710F (Conti et al. 1999) and 1326R (Cuenoud et al. 2002). The temperature cycle repeated 30 times: 1.5 minutes at 94°C, 2 minutes at 62°C, and 2 minutes at 72°C.

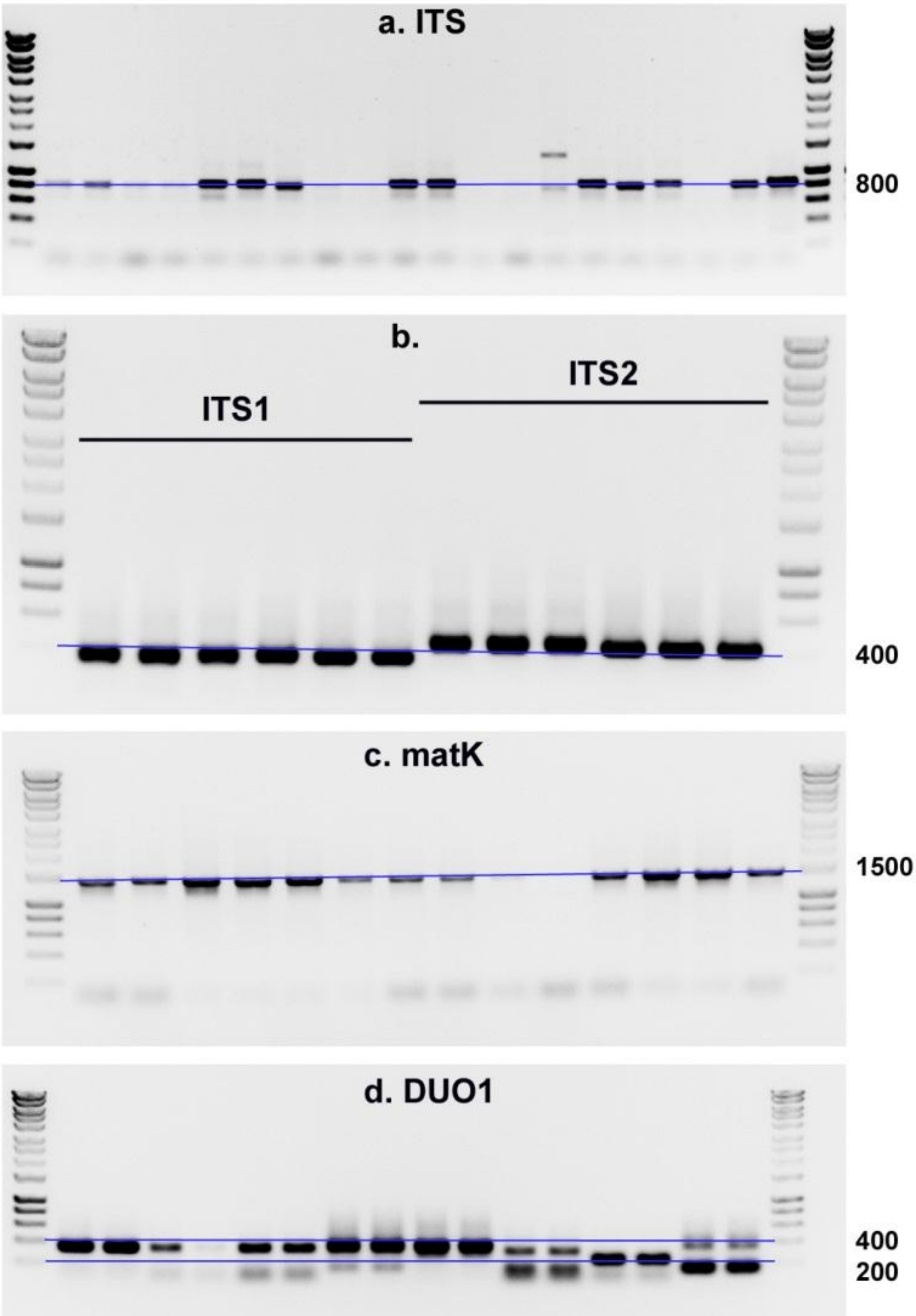
DUO1 had not been studied in the genus *Saxifraga* in the past; the general sequence for the region was unknown. The amplification strategy was to apply "nested" primers. First, by using the degenerate primers putDUO1-F1 and putDUO1-R1 (D. Twell, unpublished data), the first intron was amplified correctly in *S. tangutica, S. egregia* and *S. hederacea* with an overall sequence pair divergence of 0.413. The first and second PCR primer pairs were then designed in the conserved areas (Fig. 2.1). The first PCR was processed with primer pair Z5F + Z2R1 and the thermo cycle, 1 minute at 94°C, 50 s at 50°C, and 40s at 72°C, repeated 30 times. Then 0.25-0.4 μl of the first PCR product was used as the template of the second PCR, which was processed with primers Z4F + Z2R2 and temperature cycle, repeated 35 times, 1 minute at 94°C, 50s at 45°C, and 40 s at 72°C.

Figure 2.1. The primer positions for ITS, ITS1, ITS2, matK and DUO1. The rectangles are exons. Arrows indicate primers.



The DNA was purified with NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel). DNA quality was checked on 1%~0.8% agarose gel before PCR purification. If the PCR product had multiple bands, the band with expected length was cut out (Fig. 2.2) and then the DNA was isolated from the gel with the purification kit following the manufacturer's instructions.

Figure 2.2. The agarose gel electrophoresis pictures for ITS, ITS1, ITS2, matK and DUO1 PCR product with HyperLadder1 (Bioline). a) ITS bands ca. 800bp; b) ITS1 and ITS2 bands both about 400bp; c) matK bands ca. 1500bp; d) DUO1 bands ca. 300-400bp.



Sanger sequencing was processed by Source Bioscience plc, U.K. The sequencing primers for ITS, ITS1 and ITS2 were same as their amplifying primers; primers for matK were trnk710F + matKZ1R; primers for DUO1 were Z4F + Z2R2.

2.3.4 Alignment and phylogenetic analyses

Sequence alignment was performed with MEGA5 (Tamura et al. 2011). Its AlnExplorer enable viewing sequences, aligning with ClustalW or Muscle methods, and manual editing.

ITS sequences are difficult to align due to its variability. Following the method of Conti (Conti et al. 1999), ITS was initially aligned using ClustalW with parameters Gap Opening Penalty (GOP) 20 for Pairwise Alignment and 15 for Multiple Alignment, Gap Extension Penalty (GEP) 6.66 for Pairwise and 5 for Multiple Alignment; DNA Weight Matrix was set as ClustalW (1.6) with Transition Weight 0.1. But after adjusting the parameters, the choosing of DNA Weight Matrix IUB produced a better alignment which showed higher BS support in the neighbour-joining tree. Then the alignment was checked and modified manually. The ambiguous sites were kept. The 5.8S region was deleted from the new sequences as some Genbank ITS sequences from the previous studies have no 5.8S, which means the accessions are either ITS1 or ITS2 (Fig. 2.1). Therefore, the sequence for ITS in this study only contained the ITS1 and ITS2 area which are much more informative than the conserved 5.8S coding area. Some Genbank ITS1 or ITS2 accessions for the same species were combined to produce a complete ITS sequence (ITS1+ITS2).

These sequences were given their accession numbers (Table 2.1, after species name), for example, *S. cochlearies* 1,2 and *S. arachnoidea* 2,3; otherwise, a sequence number from this study is given, like *S. crustata* Z196 (the numbers always begin with 'Z' or 'R').

matK sequences were easily aligned with ClustalW with default setting GOP 15 for Pairwise and Multiple Alignment, GEP 6.66 for Pairwise and Multiple Alignment, and DNA Weight Matrix ClustalW (1.6) with Transition Weight 0.5.

The ITS and matK sequences were combined for simultaneous analysis. Some samples where the matK and ITS sequences were not from the same collection were marked. But most samples in the combined data set have same sample numbers (Table 2.1).

The alignment for DUO1 was performed with Muscle method because ClustalW could not properly align the common area (the sequences are too divergent from each other). The parameters were GOP = -417, GEP = 0, Max Memory in MB = 2407, Max Interations = 8, Clustering Method = UPGMB, Min Diag Length = 24.

Tests of the homogeneity of substitution patterns between sequences and of the best DNA substitution model for NJ and Bayesian analyses were calculated for each dataset. ITS, matK, and their combined data are homogeneous but DUO1 is not.

Neighbour-joining (NJ) and Maximum Parsimony (MP) trees were produced for ITS, matK, DUO1, and ITS+matK data in MEGA5. The NJ bootstrap consensus trees were inferred from 1000 replicates. The evolutionary distances were computed using the Maximum Composite Likelihood substitution method/model (Tamura et al. 2004) and are in the units of the number of base substitutions per site. Rates among sites were set uniform and the pattern among lineages was homogeneous for all except DUO1 which was heterogeneous. Pairwise deletion was selected as Gap/Missing data treatment so that those gaps that are involved in the comparison were saved by computing a distance for each pair of sequences (Kumar et al. 1994). The best-fit models are different with Maximum Composite Likelihood model but it was applied in NJ analysis since it is the sum of their log-likelihoods for all pairwise distances in the matrix. It relates to the phylogenetic relationships among the sequences. In practice, it was clear that the NJ tree produced by Maximum Composite Likelihood model had a better structure and BS support than that produced by the calculated best-fit models. Tamura et al. (2004) also found that it had no disadvantage in comparison with independent estimation (models) of each pairwise distance. The mean divergence between *Saxifraga* sequences were calculated for ITS and matK trees.

The MP trees were conducted with parameters as follows: BS test = 1000 replications, Substitution Type = Nucleotide, Gap/Missing Data Treatment = Partial Deletion, Sit Coverage Cut-off = 90% (matK) or 95% (ITS, DUO1, and matK+ITS data), MP Search Method = Close-Neighbour-Interchange (CNI) on Random Trees, No. of Initial Trees (random addition) = 10 and MP Search

Level = 2. The parameter setting was selected, for having better BS supports on the MP tree, from a few modified parameter sets. A bootstrap consensus tree was conducted with cut-off BS value 50% (branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed) for each data set.

The posterior probability (PP) values were conducted with MrBayes version 3.2.0 (Ronquist et al. 2012). The evolutionary model is GTR+G (Generalised time reversible and Gamma distribution) for matK and matK+ITS data and K2+G (Kimura 2 parameter and Gamma). For matK and combined data, I used the default prior probability density Dirichlet (all values are 1.0) for both Revmatpr and Statefreqpr. For ITS, the prior set is fixed (equal) for Statefreqpr and beta (1,1) for Tratiopr. Each analysis ran two simultaneous independent analyses and computed diagnostics every 1000th generation with three heated and one cold chains, discarded the first 25 % samples from the cold chain, and sample the chain every 100th generation. The average standard deviation of split frequencies was lower than 0.01, as a good indication of convergence, after 2×10^6 generations, except ITS data lower than 0.028. The Potential Scale Reduction Factor (PSRF) for all data set is close to 1.0 which shows they are good samples for the posterior probability distribution. The PP values from the clade credibility tree were plotted into the MP trees for each analysis where topologies were the same.

2.3.5 Outgroup selection

Outgroup species were chosen from related genera in the family (*Chrysosplenium* and *Micranthes*) and from genera in two families immediately basal to Saxifragaceae, viz. *Ribes* (Grossulariaceae) and *Itea* (Iteaceae), respectively (Fig. 1.1 and 1.3).

2.4 Results

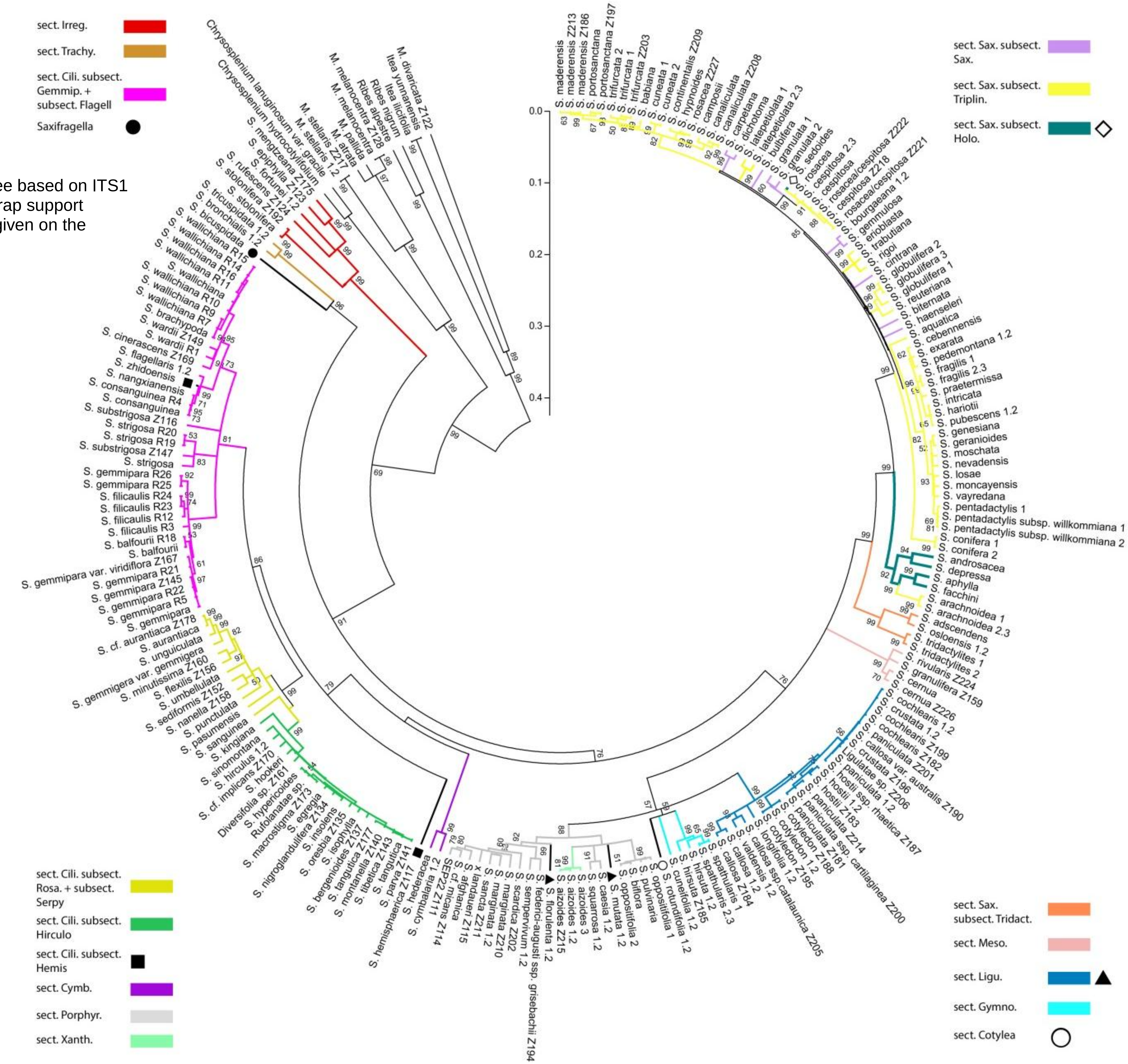
2.4.1 ITS sequences: neighbour joining analysis

The sum of branch lengths (SBL) = 7.06. The analysis involved 222 nucleotide sequences. There were a total of 609 positions (=aligned length) in the final dataset. The overall mean distance between examined species in *Saxifraga* is 0.25 (Fig. 2.3).

The tree was rooted with *Itea*. *Micranthes divaricata* Z122 is not supposed be sister to *Itea*. The other outgroup taxa are in a position corresponding to previous studies (Soltis et al. 2001a; Stevens 2001 onwards), i.e. *Ribes* is sister to the family Saxifragaceae; *Micranthes* is sister to *Chrysosplenium*; and these two genera together are sister to a monophyletic *Saxifraga*. The topology of the outgroup branching is the same in the ITS MP tree.

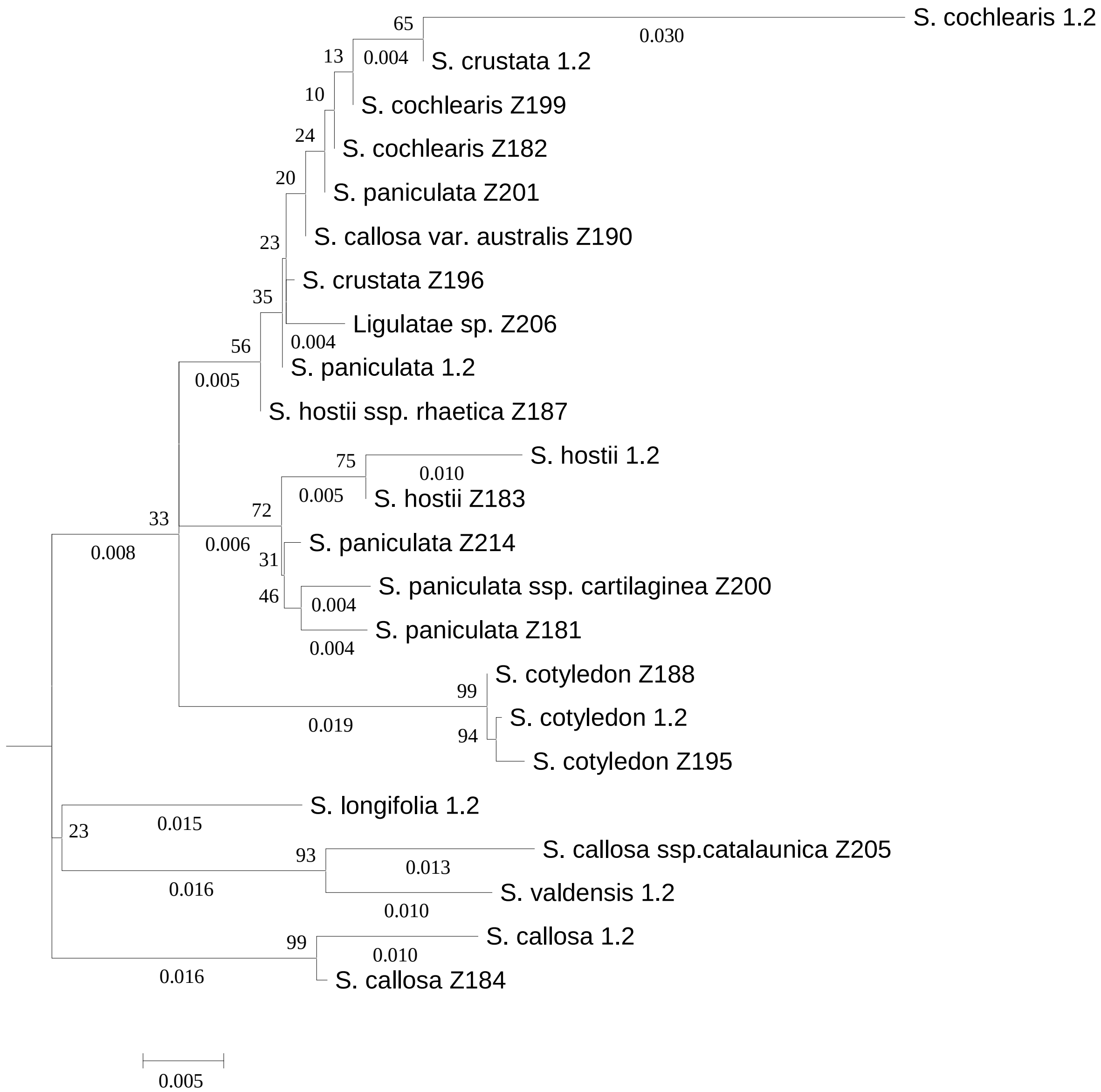
The topology of main clades (*Irregulares*, *Trachyphyllum*, *Ciliatae*, *Cymbalaria*, *Porphyrion*, *Ligulatae*, *Mesogyne*, and *Saxifraga*) are similar to the MP tree and >80% BS supported. Irregulares clade is most distinctive from the other saxifrages, ca. 0.1 distance away. I isolated some important subtrees for detail examination.

Figure 2.3 Neighbour-Joining tree based on ITS1 and ITS2 sequence data. Bootstrap support percentages for each clade are given on the subtending internode.



In sect. *Ligulatae* clade, the distance between *S. cochlearies* 1,2 and *S. hostii* ssp. *rhaetica* Z187 is ca. 0.04. This very distinctive *S. cochlearis* is separated from *S. cochlearis* Z199 and Z182. Populations of *S. crustata*, *S. paniculata*, and *S. callosa* show a degree of interweaving. However, the three populations of *S. cotyledon* are together and only 0.0025 distance between them (Fig. 2.4).

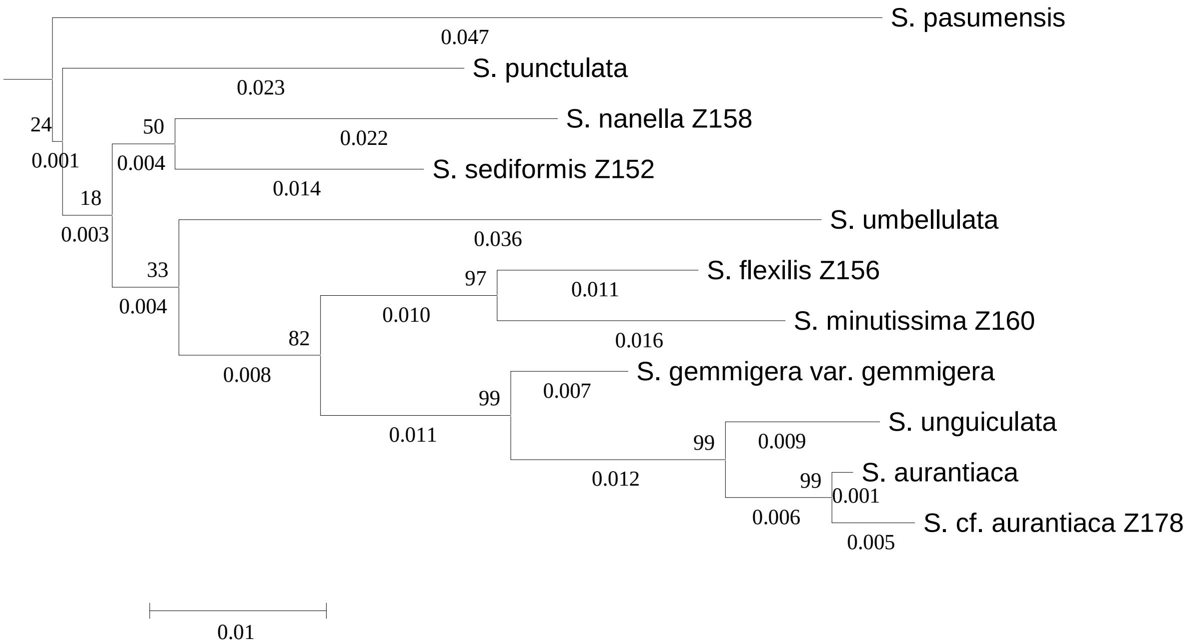
Figure 2.4. Neighbour-joining tree based on ITS1+2 sequences of sect. *Ligulatae.* Numbers above branches are % bootstrap supports, below are genetic distances.



The differences between species in the sect. *Ciliatae* subsect. *Rosulares* & *Serpyllifoliae* clade are small. The distance between *S. cf. aurantiaca* Z178 and *S. sediformis* Z152 is 0.028. *S. minutissima* and *S. flexilis* are very close (0.005 away). Species from subsect. *Rosulares* (*S. pasumensis*, *S. punctulata*, *S. sediformis*, *S. umbellulata*, and *S. unguiculata*) is mixed together with subsect.

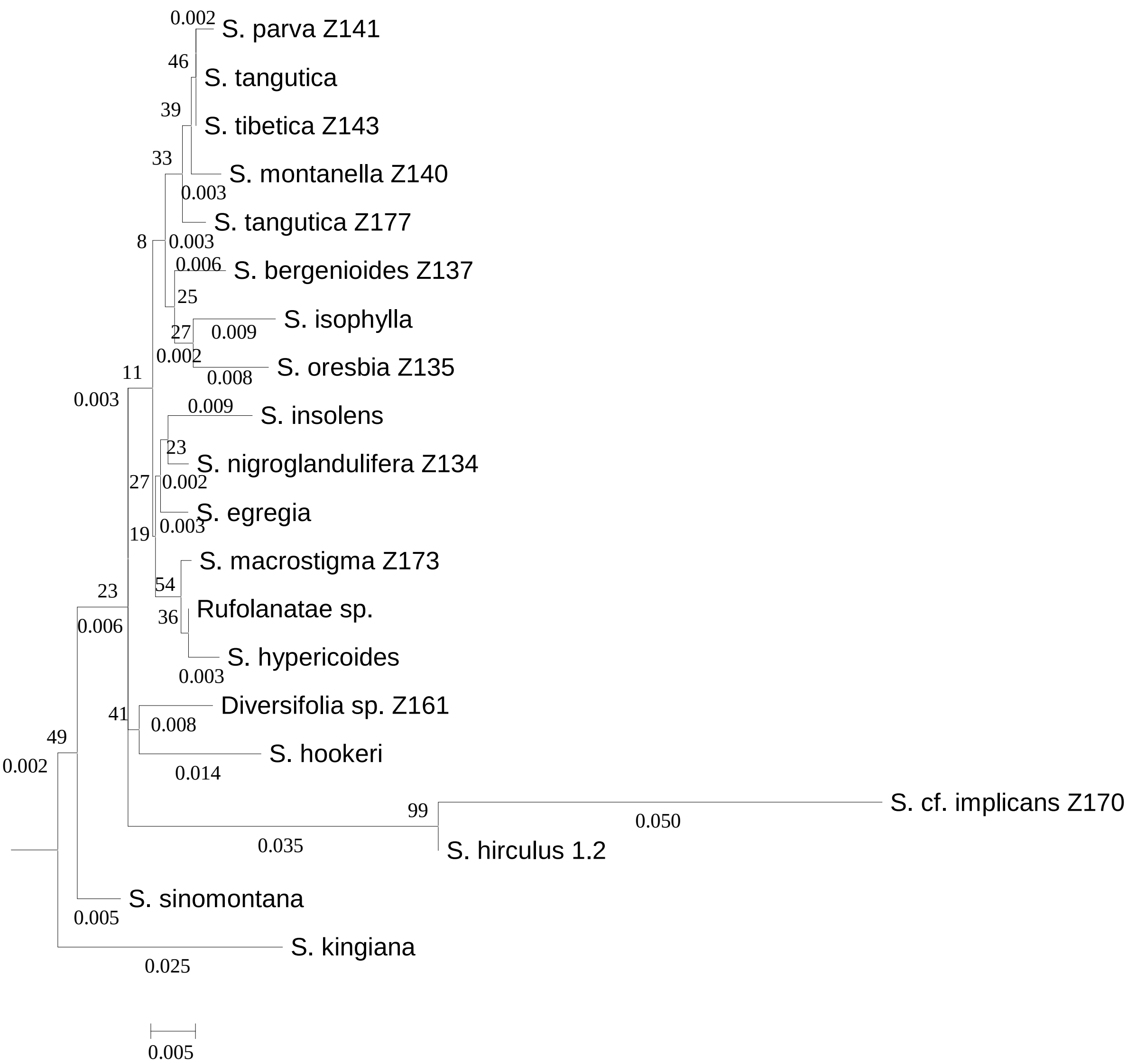
Serpyllifoliae species (*S. nanella*, *S. flexilis*, *S. minutissima*, *S. gemmigera*, and *S. aurantiaca*) and have similar distances. *S. sanguinea* is not in this clade but sister to clade *Hirculoideae* though morphologically it is also a *Rosulares* (Fig. 2.5).

Figure 2.5. Neighbour-joining tree based on ITS1+2 sequences of sect. *Ciliatae*, subsect. *Rosulares* + *Serpyllifoliae.* Numbers above branches are % bootstrap supports, below are genetic distances.



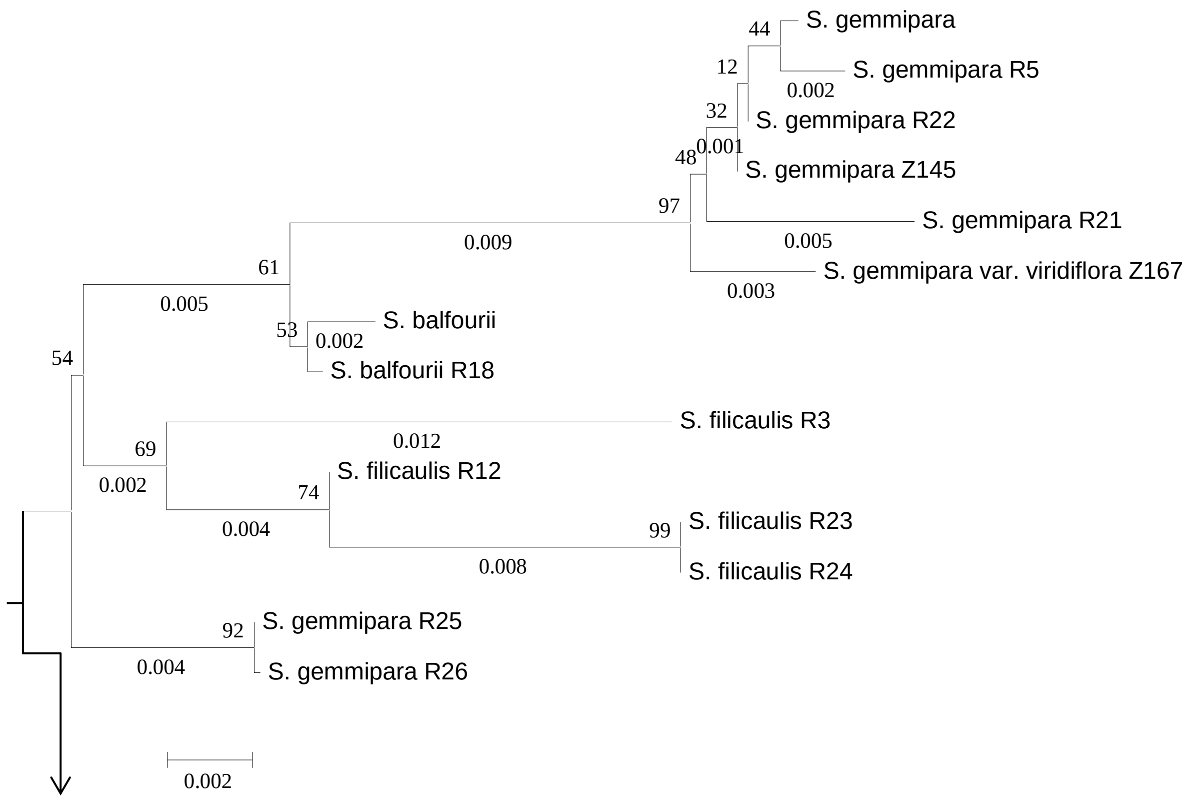
The differences within the sect. *Ciliatae* subsect. *Hirculoideae* clade are very small, except for *S.cf. implicans* Z170, which is 0.086 distance away from *S. sinomontana*. The second distinctive accession is *S. hirculus* 1,2, a collection from Norway, while the other species in this clade are from China. *S. tangutica* Z177, possibly better identified as *S. przewalskii*, is different from *S. tangutica*. It is noticeable that *S. tangutica* and *S. tibetica* Z143 have no difference (Fig. 2.6).

Figure 2.6. Neighbour-joining tree based on ITS1+2 sequences of sect. *Ciliatae* subsect. *Hirculoideae.* Whole numbers are % bootstrap supports, decimals are genetic distances.



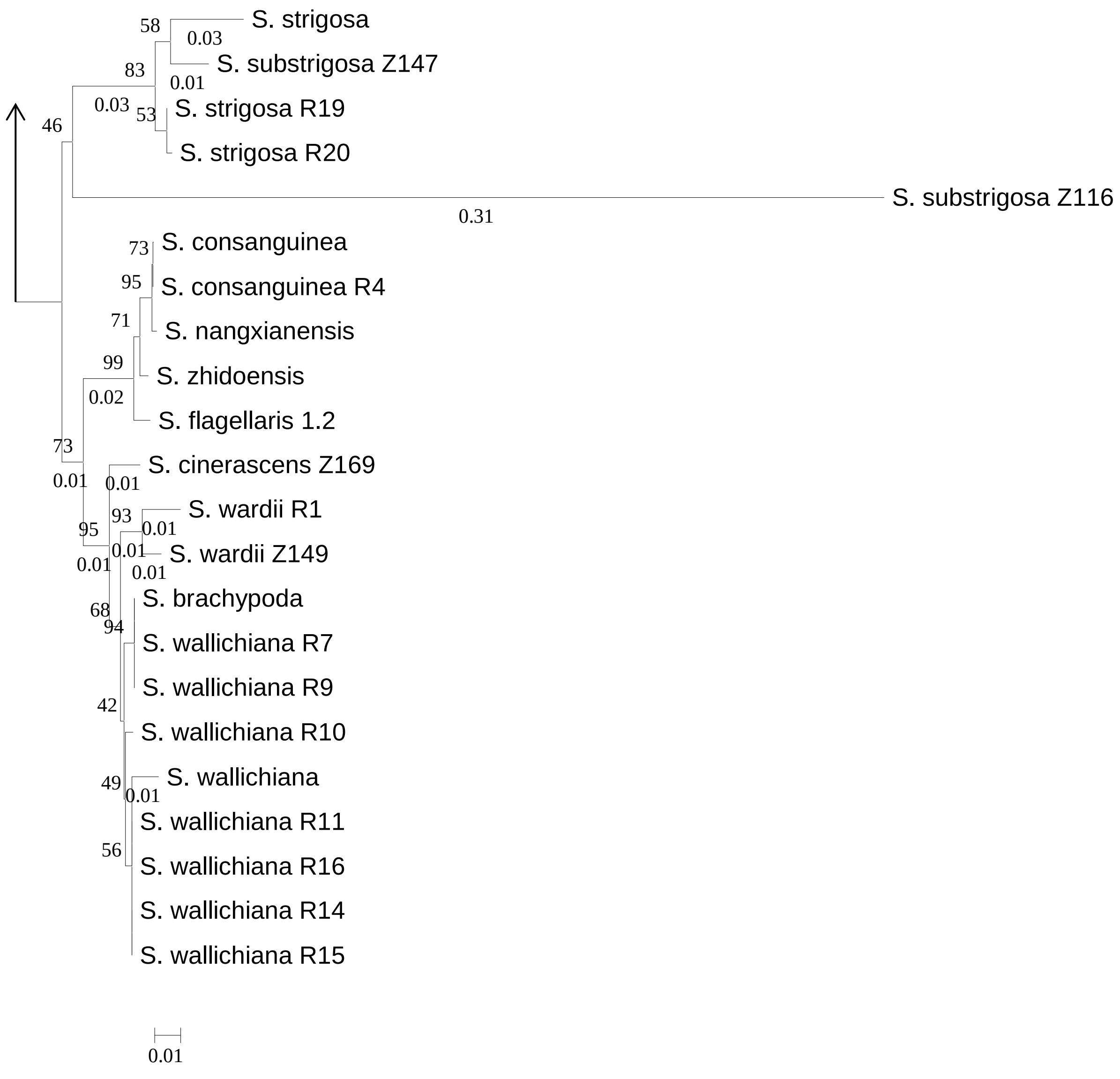
The subclade containing sects. *Gemmiparae* and *Flagellares* is presented in two parts owing to its length (Fig. 2.7, 2.8). It is interesting that two *S. gemmipara* plants, R25 and R26, which are from one population, are very distinct from the other *S. gemmipara*; the distance between the two groups is 0.12. The unpublished new variety *S. gemmipara* var. *viridiflora* clusters at the end of a relatively long branch with the other *S. gemmipara* plants and is not very different from them.

Figure 2.7. Neighbour-joining tree based on ITS1+2 sequences of sect. *Ciliatae*, subsect. *Gemmiparae + Flagellares.* (Part 1). Whole numbers are % bootstrap supports, decimals are genetic distances.



S. substrigosa Z116 is dramatically different (0.28) from the other *S. substrigosa* population, comparing to the genetic distances of rest of the species in this NJ subtree for subsect. Gemmiparae and Flagellares. The species from subsect. *Flagellares* are very similar to each other and nested in the *Gemmiparae* group; *S. zhidoensis*, a subsect. *Hemisphaericae* species, is clustered into the *Flagellares* (also in the MP tree). Populations of *S. wallichiana* have few differences from each other: distances are less than 0.01. However, *S. brachypoda* is very similar to *S. wallichiana* populations.

Figure 2.8. Neighbour-joining tree based on ITS1+2 sequences of sect. *Ciliatae*, subsect. *Gemmiparae + Flagellares.* (Part 2). Whole numbers are % bootstrap supports, decimals are genetic distances.



2.4.2 ITS sequences: maximum parsimony analysis

The analysis involved 222 nucleotide sequences. There were a total of 444 positions in the final dataset. The parsimony analysis found 421 trees of length 2613, CI = 0.35, RI = 0.87. The tree produced by Bayesian analysis was almost identical to the parsimony tree (Fig. 2.9).

The outgroup taxa have a similar topology to the NJ tree (Fig. 2.3) which is corresponding to the previous studies but *M. divaricata* Z122 still does not nest in the *Micranthes* clade. Sect. *Irregulares* is the ancestral clade for *Saxifraga* and well supported by 99% BS and 100% PP. Sect *Trachyphyllum* is well supported (99% BS, 100% PP) but sister to *S. bicuspidata* with weak support (58% BS, 98% PP).

Sect. *Ciliatae* has a strong PP value (100%) but weak BS value (57%). The subsect. *Hemisphaericae* is unresolved. Three main clades can be clearly identified: one containing subsects. *Gemmiparae* and *Flagellares* as well as *S. cinerascens*, the sole member of subsect. *Cinerascentes*; one with subsects. *Rosulares* and *Serpyllifoliae*, and one containing subsect. *Hirculoideae*. It is interesting to see that the first two clades contain more than one subsection. Moreover, *S. zhidoensis*, morphologically a member of subsect. *Hemisphaericae*, is deeply nested in the *Gemmiparae* + *Flagellares* clade alongside species of subsect. *Flagellares* (99% BS, 100% PP), far away from the other *Hemisphaericae* species, *S. hemisphaerica* (Fig. 2.9). This will be discussed later. *S. gemmipara* and its unpublished variety cluster together but two samples, R25 and R26, from one population, are separated from the other *S. gemmipara* by two *S. balfourii* populations. Also, two populations of *S. wallichiana* (R7, R9) are separated from the others. But populations of *S. filicaulis*, *S. substrigosa*, and *S. strigosa* form individual clades with good support. The species from subsect. *Rosulares* and *Serpyllifoliae* are intermixed and reciprocally monophyletic clades for each subsection were not recovered (partly because of the unresolved topology of many species). Especially well supported is a clade containing *S. aurantiaca* (in *Serpyllifoliae*), *S. unguiculata* (in *Rosulares*), and *S. gemmigera* (in *Serpyllifoliae*) (95% BS, 100% PP). Furthermore *S. sediformis* (in *Rosulares*) and *S. nanella* (in *Serpyllifoliae*) form a clade with a PP value 100% in the Bayesien tree, but the BS support for the clade is less than 50% in the MP tree (Fig. 2.9, where the cross is). The newly described *S. minutissama* is confirmed as belonging to the Rosulares-Serpyllifoliae clade, in particular closely related to *S. flexilis* (BS/PP = 97%/100%). Subsect. *Hirculoideae* form a clade which is very well supported (BS/PP = 99%/100%) but the constituent species are mostly unresolved. This corresponds to the the NJ tree (Fig. 2.3) where genetic distances inside the clade are short.

The sect. *Ciliatae* clade is sister to a sect. *Cymbalaria* clade (99% BS, 100% PP), and *Cymbalaria* is sister to the rest of the genus (80% BS, 100% PP). In the latter clade, sect. *Ligulatae*, sect. *Mesogyne*, sect. *Porophyrion*, sect. *Gymnopera* and sect. *Saxifraga* form well-supported individual clades. It is noticeable that not only two species of *Ligulatae* (*S. mutate* and *S. florulenta*) but also the single species of sect. *Xanthizoon* (three populations of *S. aizoides*) are nested in the sect. *Porophyrion* clade. Within the sect. *Saxifraga* clade, subsect. *Tridactylites* is monophyletic and well-supported (92% BS, 100% PP) but the other three subsections are polyphyletic. Most populations from the same species clustered together, except *S. sedoides* which clustered with *S. rosacea* and a *S. cespitosa* population and an unidentified *S. rosacea/cespitosa* accession.

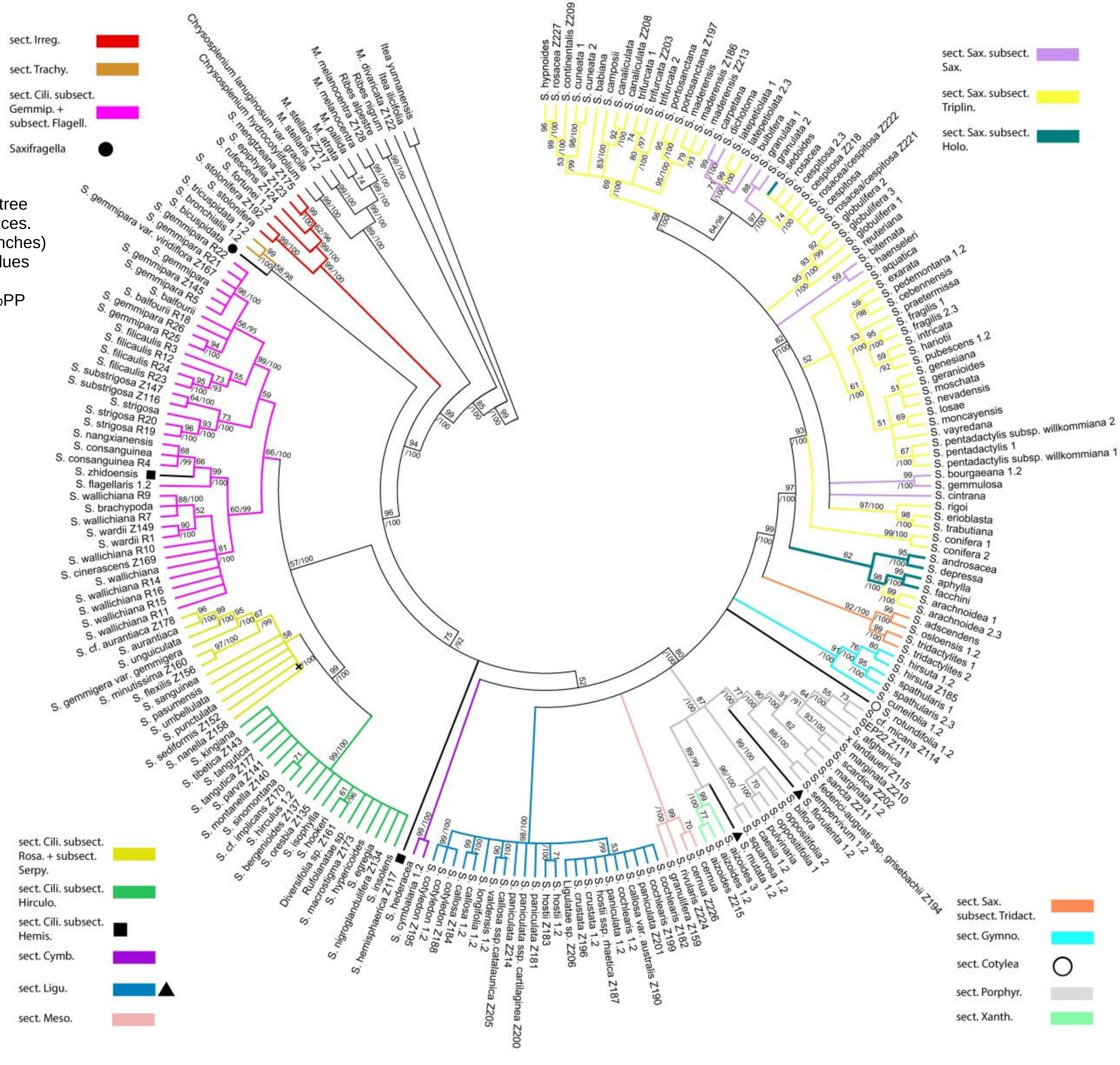
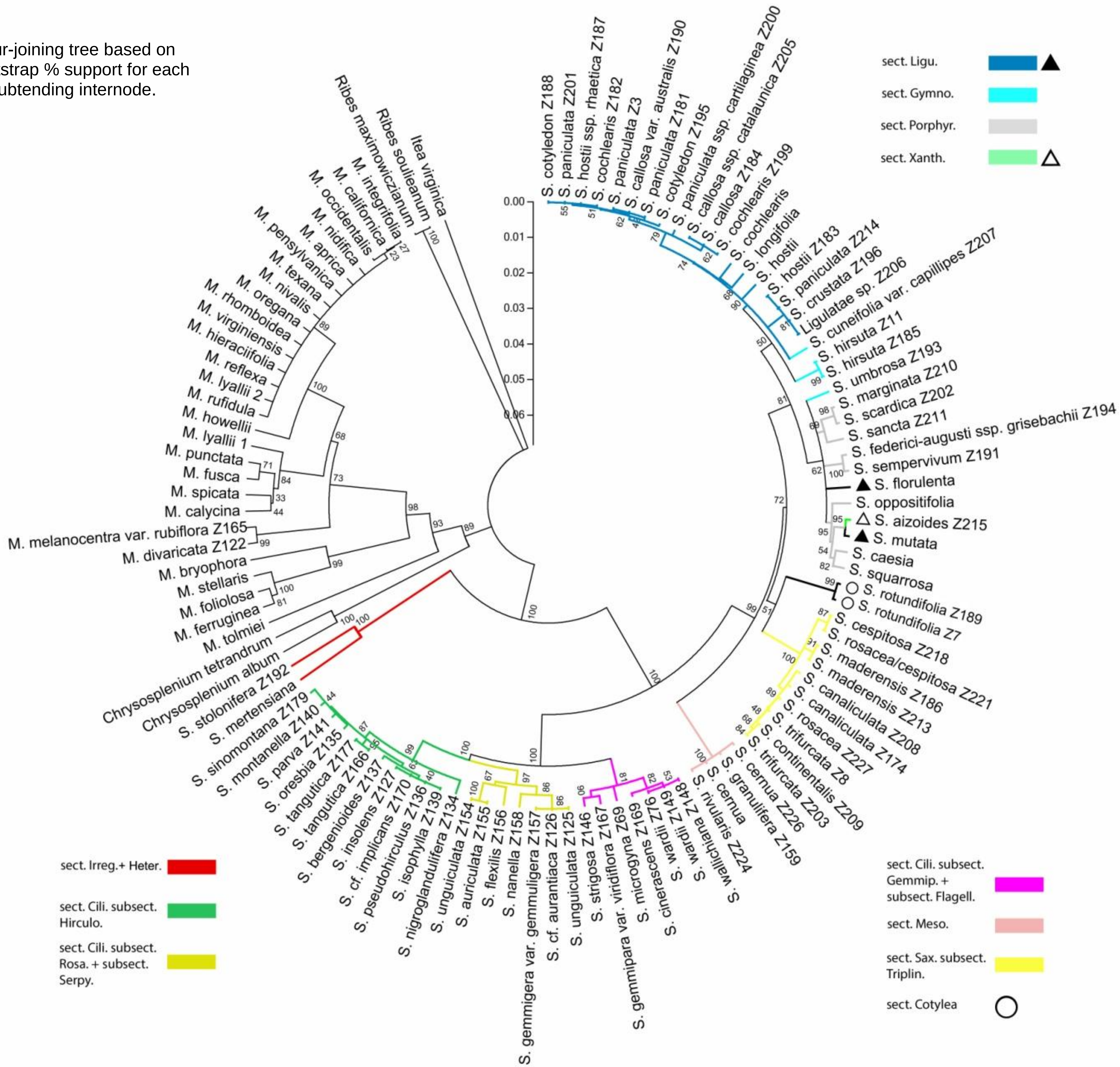


Figure 2.9 Maximum parsimony tree based on ITS1 and ITS2 sequences. Bootstrap % support (above branches) and posterior probability (PP) values (below branches or after "/"). "×" indicates a branch with 100%PP in the Bayesian tree.

2.4.3 matK sequences: neighbour joining analysis

The analysis involved 111 nucleotide sequences (Fig. 2.10). There were a total of 1107 positions in the final dataset, SBL = 0.8975. The overall mean distance for *Saxifraga* is 0.04, which is much smaller than that of ITS. The topology is similar to its MP tree (Fig. 2.11).

Figure 2.10. Neighbour-joining tree based on matK sequences. Bootstrap % support for each clade is given on the subtending internode.



2.4.4 matK sequences: maximum parsimony analysis

The analysis involved 111 nucleotide sequences, 1033 positions, a total of 246 trees, CI = 0.69, RI = 0.94 (Fig. 2.11).

The topology is similar to the ITS MP tree (Fig. 2.9) except for several differences: a) *Chysosplenium* clade did not cluster with *Micranthes* but with *Saxifraga*; b) *S. mertensiana* (sect. *Heterisia*) is included and clustered with *S. stolonifera* (sect. *Irregulares*) with good support (99% BS, 100% PP); c) *Gemmiparae* clades and *Flagellares* clade are not resolved, but *S. cinerascens* (subsect. Cinerascentes) is nested in subsect. *Gemmiparae*; d) sect. *Mesogyne* clade is ancestral to all the rest (except the previous clades); e) *S. rotundifolia* (sect. *Cotylea*) clustered with the sect. *Saxifraga* clade, while in the ITS tree the position of *S. rotundifolia* is unresolved; f) the sect. *Gymmopera* clade is polyphyletic – *S. cuneifolia* and *S. hirsute* join with sect. *Ligulatae*, but *S. umbrosa* clustered with sect. *Porphyrion*. Together these three sections joined into one clade with BS support 77%.

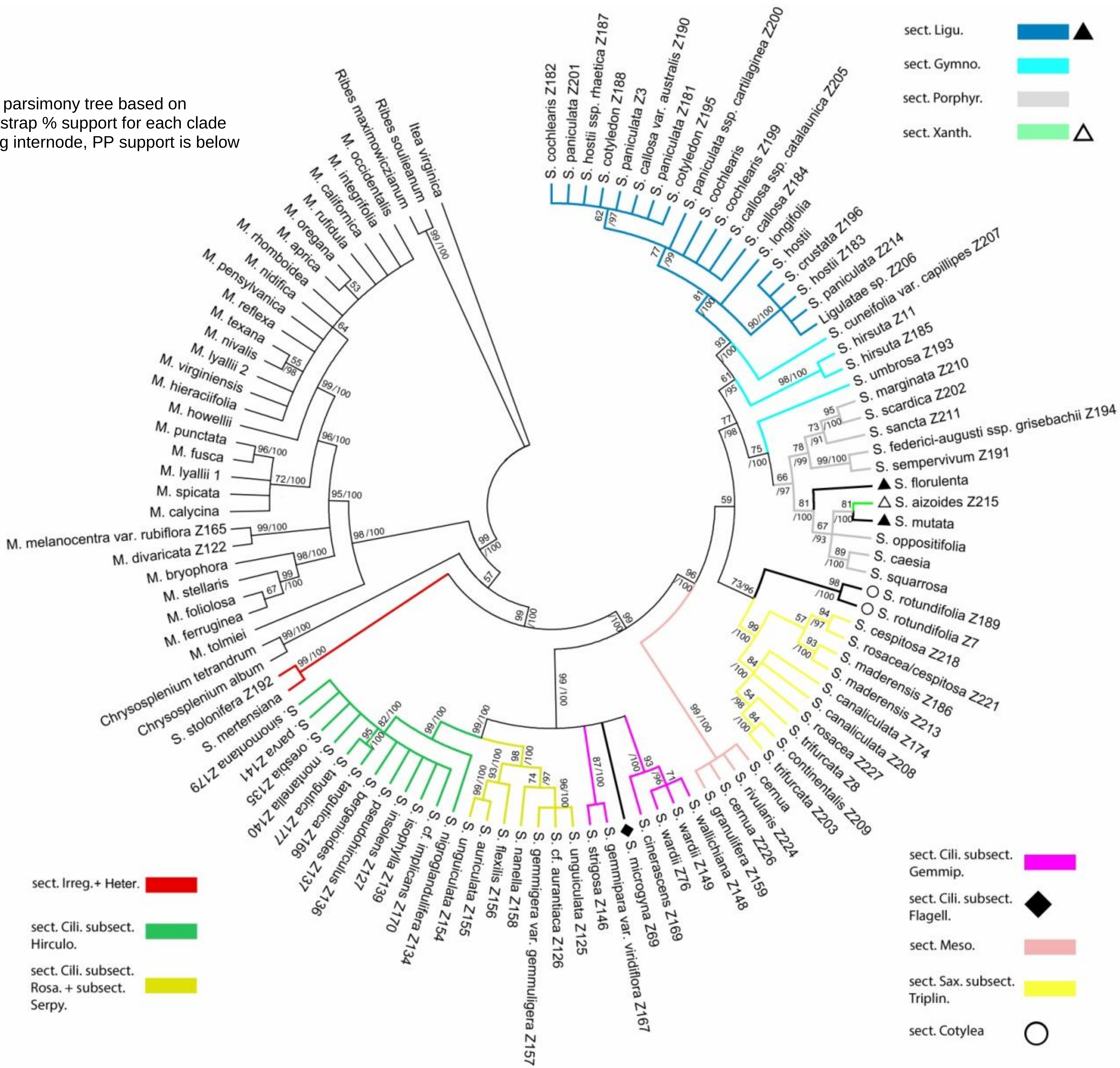


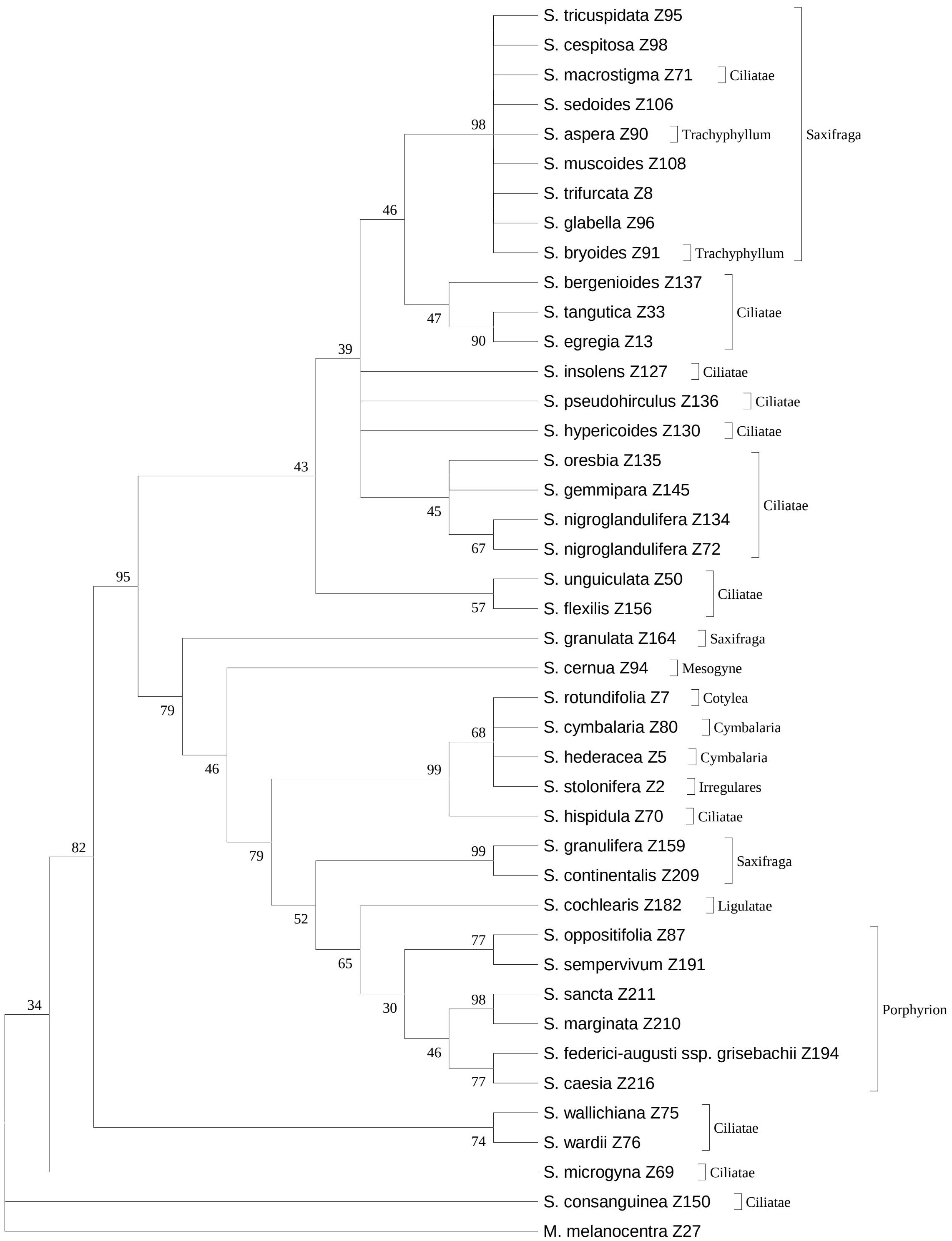
Figure 2.11. Maximum parsimony tree based on matK sequences. Bootstrap % support for each clade is above the subtending internode, PP support is below or after “/”.

2.4.5 DUO1 sequences: maximum parsimony analysis

The analysis involved 42 nucleotide sequences, 132 positions, a total of 635 trees; CI = 0.72, RI = 0.90. Branches corresponding to partitions reproduced in fewer than 30% bootstrap replicates are collapsed (Fig. 2.12).

The topology of the tree failed to match any of the molecular trees and nor did it agree in several places with morphological data. For example, species of sect. *Ciliatae* were scattered through the tree, and this was despite the fact that the primers were designed based on a species of *Ciliatae*. Therefore data from DUO1 was not included into the combined analysis with ITS and matK data. The possible reasons why DUO1 produced these peculiar results will be discussed later.

Figure 2.12. Maximum parsimony tree based on DUO1 sequences. Bootstrap % support is given above branches.



2.4.6 Combined ITS + matK sequences: neighbour-joining analysis

The analysis involved 65 nucleotide sequences and 1669 positions; SBL=1.42. The divergence between related species are relatively small in sect. *Ligulateae* clade and sect. *Ciliate* subsect. *Hirculoideae* clade (Fig. 2.13). The topology is similar to the MP tree (Fig. 2.14).

Figure 2.13. Neighbour-joining tree based on combined ITS1+2 and matK sequences. Bootstrap % support for each clade is above the subtending internode.

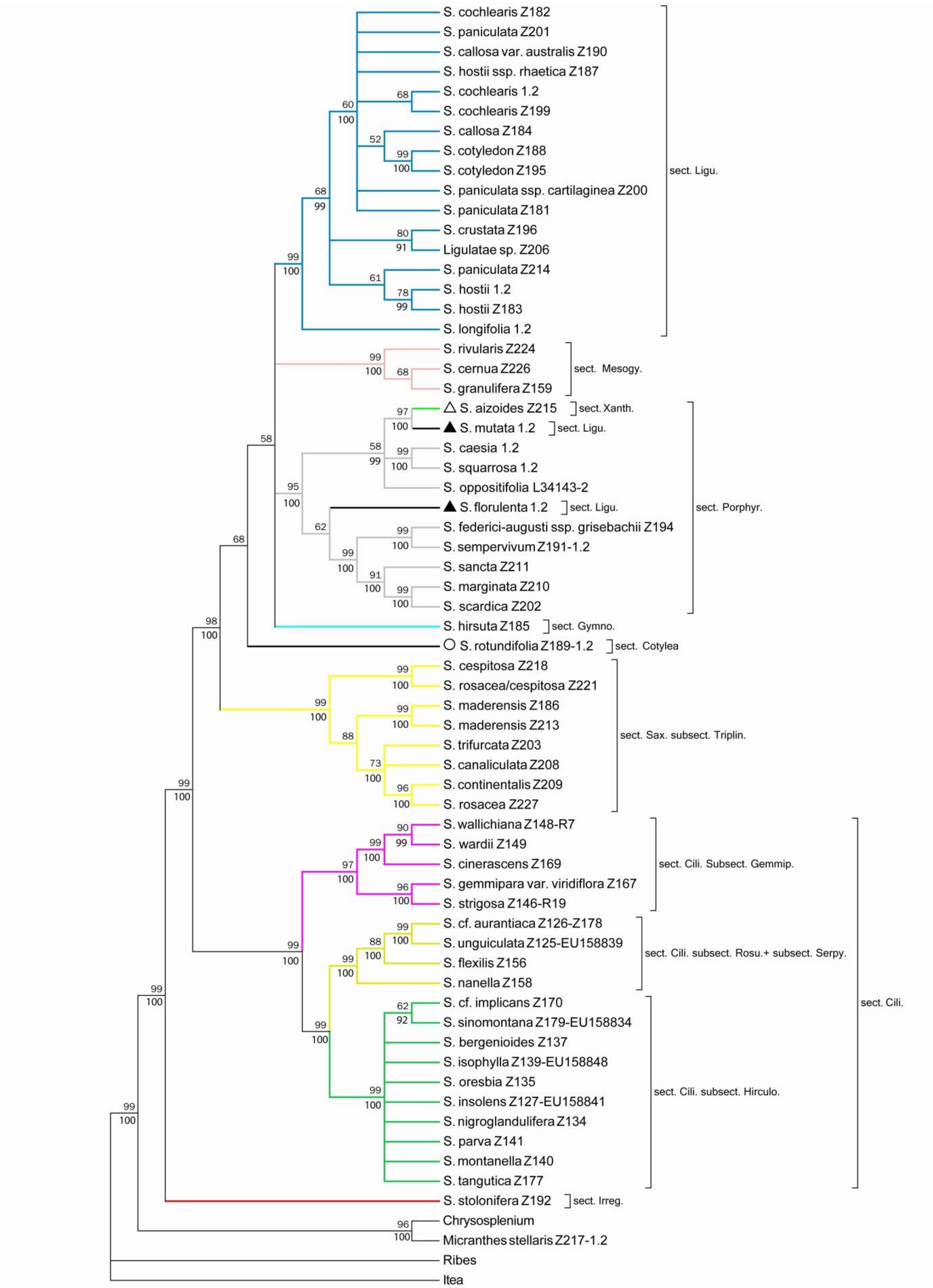
2.4.7 Combined ITS + matK sequences: maximum parsimony analysis

The analysis involved 65 nucleotide sequences and 1478 positions. A total of 40 trees were produced: CI = 0.62, RI = 0.84 (Fig. 2.14).

The outgroup topology is the same as the ITS MP tree (Fig. 2.9) which corresponded to the six-gene analysis of (Soltis et al. 2001a). Main clades are well-supported. The BS value for the *Saxifraga* clade is the same as that of matK (99%) but higher than ITS (94%). Several main clades showed increased BS support: 1) sect. *Ciliatae* clade BS 99%, equal to the matK tree (Fig. 2.11) but much improved over the ITS tree (55%); 2) sect. *Ciliatae* subsect. *Rosulares* + *Serpyllifoliae* clade BS value is the highest of all three analyses (combined 99%, matK 98%, ITS 58%); 3) the sect. *Porphyrion* clade is better supported (95%BS) in the combined analysis compared with either ITS (87%) or matK (66%); 4) the bootstrap support (99%) for sect. *Ligulatae* clade is similar to ITS (98%) but higher than matK BS (81%).

The main differences between the combined MP tree (Fig. 2.14) and the ITS (Fig. 2.9) or matK (Fig. 2.11) trees came from the two incongruences between the ITS and matK trees. *S. rotundifolia* (sect. *Cotylea*), instead of being unresolved (ITS tree) or sister to sect. *Saxifraga* clade (matK tree), is close to sect. *Saxifraga* but sister to the sect. *Ligulatae* -- G*ymnopera* clade. The other discrepancy involved sect. *Gymnopera*, where *S. hirsuta* (sect. *Gymnopera*) is unresolved like in ITS tree, although in matK it linked with the sect. *Ligulatae* clade. Also, the position of sect. *Mesogyne*, *Ligulatae*, and *Porphyrion* are unresolved like in ITS tree, not following the matK topology.

Figure 2.14. Maximum parsimony tree based on combined ITS1+2 and matK sequences. Bootstrap % support for each clade is above the subtending internode, PP support is below.



2.5 Discussion

2.5.1 Problems in DNA analysis

The *Saxifraga mertensiana* (sect. *Heterisia*) ITS sequence, whose Genbank accession number is AY231367, was found to be a probable mistake. It nested deeply in sect. *Saxifraga* while both matK and morphological characters indicated that it should be closely related to sect. *Irregulares*. The ITS tree made from Zhang, et al. (2008) also shows this mistake as they used this sequence. The mistake maybe a misidentification or sample contamination. The sequence was omitted from my analyses.

Micranthes divaricata Z122 is another sample found in a peculiar position in the ITS trees (Fig. 2.9) but the identification of the material is correct and there is no source of contamination. It is sister to genus *Itea* but all *Itea* sequences were from Genbank and there was no *Itea* DNA available for sequencing in this study. The reason for this result is unknown.

Strict consensus tree and bootstrap consensus trees are different. I used bootstrap consensus trees because empirically they are more strict than the strict consensus tree. Bootstrap consensus trees are the majority rule consensus trees (which contain all clusters occurring in at least half the trees) with BS support and I also collapsed the clades with less than 50% BS support. Strict consensus trees contain all clusters occurring in all the trees but a shared cluster may be due to a small majority of data without any test of robustness. The MP bootstrap consensus trees in the paper show identical topologies with the strict consensus trees but tend to collapse near the tips.

The unexpected DUO1 sequences (Fig. 2.12) may due to the design of primers. Sequences were difficult to align and the heterogeneity of the data shows that regions not from the DUO1 gene were amplified. This means that not only the degenerate primers but also the specially designed nested primers failed to product the required product. This result is difficult to explain. Although the degenerate primers were initially designed for cDNA, DUO1 is a single-copy gene and functionally important: the exon area should be conserved. However, when the same primer was used to amplify *Magnolia* genomic DNA, it too produced multiple products (Twell lab, unpublished data). It seems, regrettably, that DUO1 is not an ideal single- copy nuclear marker for molecular phylogenetic analysis.

2.5.2 Congruence between ITS and matK trees

The congruence between ITS and matK sequences has been studied previously (Conti et al. 1999; Soltis et al. 2001b). The conclusion is that ITS and matK data for *Saxifraga* can be combined. The increased bootstrap support of the combined tree (Fig. 2.14) is a strong argument in favour of combining the sequences. Although they show differences in the position of the sect. *Mesogyne*, sect. *Cotylea*, sect. *Gymmopera*, sect. *Ligulatae* and sect. *Porphyrion* clades, this is mainly due to non-resolution at this taxonomic level in the ITS tree (Fig. 2.9). The comparison between the two analyses shows that matK performs (Fig. 2.11) better at section level, but ITS is more variable (larger NJ tree mean distance).

2.5.3 Comparison with previous studies

The results of this study can be compared to those of Conti, et al. (1999), Soltis, et al. (2001), and Zhang, et al. (2008). My combined tree (Fig. 2.14) was very similar to that produced by Conti et al. (1999), except that mine showed somewhat greater resolution and higher bootstrap values in certain critical places, probably owing to the inclusion of more sequences.

Soltis et al. (2001) presented one of four shortest trees from parsimony analysis of a combined cpDNA (rbcL, matK, trnL-trnF, psbA-trnH) and nuclear data set (ITS and expansion segments of the 26S rDNA) including all the main sections except sect. *Ligulatae*). The combined MP tree from my study (Fig. 2.14) does not include *S. bicuspidata*, sect. *Trachyphyllum* or sect. *Cymbalaria*; nevertheless the topology is similar to that of Soltis' tree. The missing sections are present in the ITS MP tree of this study and in the same position as in Soltis' tree. Sect. *Mesogyne*, *Gymnopera*, and *Porphyrion* clade is unresolved in the combined MP tree of this study, unlike Soltis' tree where only a single species per section was included.

The ITS MP tree (Fig. 2.9) from this study is much more extensive taxonomically than the ITS tree produced by Zhang, et al. (2008). The latter study focused on sect. *Ciliatae* and omitted sect. *Ligulatae*. The topology in sect. *Ciliatae* is almost the same in both trees but *S. zhidoensis* (subsect. *Hemisphaericae*) is nested into subsect. *Flagellares* clade and *S. hemisphaerica* (subsect. *Hemisphaericae*) is ancestral to all the other *Ciliatae* species. This may be a result of sample contamination of *S. zhidoensis* because the voucher specimen is correctly identified. *S. zhidoensis* does not produce runners, unlike all species of sect. *Flagellares* which do. The position of subsect. *Hemisphaericae* requires further study. The relationships of the other species are similar between my study and that of Zhang et al. (2008), except *Micranthes* and sect. *Mesogyne*. It seems that ITS sequences for the main sections are quite constant at the section level.

2.5.4 Phylogenetic relationships

The ITS MP tree (Fig. 2.9) shows that *S. bicuspidata* is sister to sect. *Trachyphyllum* species (*S. tricuspidata* and *S. bronchialis*) but with low support (58% BS, 98% PP). This topology is not shown in the six-gene tree from Soltis, et al. (2001) where the taxa are shown as successive derivatives from the main lineage. *S. bicuspidata* is a Fuegian species while S. *tricuspidata* and S. *bronchialis* are from the Pacific coast of North America. More intensive taxonomic sampling is needed to clarify the situation.

As suggested by Zhang, et al. (2008), subsections *Gemmiparae* and *Flagellares* in section *Ciliatae* have a close relationship and the former is paraphyletic to the latter the ITS MP tree (Fig. 2.9). The morphological characters, however, insist that *Flagellares*, with long and slender runners, is distinct from *Gemmiparae*, which usually has axillary leafy buds but no runners. Also, the matK analysis does not confirm this combination. More DNA regions and a better taxonomic sampling of sect. Flagellares are needed to establish their relationship.

In the ITS (Fig. 2.9) and matK (Fig. 2.11) MP trees of sect. *Ciliatae*, species from subsection *Rosulares* and *Serpyllifoliae* cluster together and cannot be assigned to separate groups. Moreover, they have similar genetic distances in the ITS NJ tree (Fig. 2.5). It seems that Engler and Irmscher's (1916/19) original approach, which was to combine the two sections, is the best one after all (Zhang et al. 2008).

The relationships within the sect. *Porphyrion* clade also require comment. *S. florulenta* (sect. *Ligulatae*, subsect. *Florulentae*), *S. mutata* (sect. *Ligulatae*, subsect. *Mutatae*), and *S. aizoides* (sect. *Xanthizoon*) all belong in this clade. The BS support from ITS (98%), matK (81%) and the combined analysis (99%) agreed with the conclusion of Conti et al. (1999) that sect. *Ligulatae* should exclude *S. florulenta* and *S. mutata*. Taxonomic changes are therefore necessary (Chapter 6). Although Conti et al. (1999) thought that the BS support (91%) for the combination is "low", in this study, the BS support (95%) is sufficient to combine sect. *Porphyrion*, subsect. *Florulentae*, subsect. *Mutatae*, and sect. *Xanthizoon* together and make an extended *Porphyrion*. *S. aizoides* forms a natural hybrid with *S. oppositifolia* (sect. *Porphyrion*) which has undergone polyploidy to make a new amphidiploid species, *S. nathorstii* (Dusen) Hayek. *S. aizoides* also forms natural hybrids with *S. mutata* and shares a similar floral morphology (ligulate petals and star shaped flowers) to that species. It is perhaps no surprise that they appear as sister species in all three trees (Fig. 2.9, 2.11 and 2.14).

In the matK MP tree (Fig. 2.11), all lime-secreting groups (sect. *Ligulatae*, *Porphyrion* and *Xanthizoon*) clustered together, along with species from sect. *Gymnopera* which secretes water but not lime. But the topology is not supported in the ITS (Fig. 2.9) and combined analysis (Fig. 2.14). Sect. *Gymnopera* is polyphyletic in the matK analysis. It partly (*S. cuneifolia, S. hirsuta*) clusters with sect. *Ligulatae*, and partly (*S. umbrosa*) with the sect. *Porphyrion* clade, and together these three sections are joined into one clade (BS 77%). This result is surprising because *Gymnopera* is monophyletic in the ITS tree, where it shows no close relationship with either *Ligulatae* or *Porphyrion*. Morphologically, the leaf shapes of *S. cuneifolia* (spathulate) and *S. hirsuta* (orbiculate) are unlike the *Ligulatae* (ligulate) ones. It is the same for *S. umbrosa* and sect. *Porphyrion*. More studies are needed to understand where sect. *Gymnopera* properly belongs.

2.5.5 Species identification and definition

Species are well distinguished when all the individuals cluster into a monophyletic clade (Li et al. 2011). But it is not always the case in *Saxifraga*. The monophyletic species (with more than one accession) are *S. stolonifera* (ITS), *S. filicaulis* (ITS), *S. substrigosa* (ITS), *S. strigosa* (ITS), *S. tanguitica* (matK), *S. cotyledon* (ITS and combined), *S. cernua* (ITS), *S. aizoides* (ITS), *S. spathularis* (ITS), *S. hirsuta* (ITS and matK), *S. rotundifolia* (matK), *S. tridactylites* (ITS), *S. arachnoidea* (ITS), *S. conifer* (ITS), *S. pentadactylis* (ITS), *S. fragilis* (ITS), *S. globulifera* (ITS), *S. granulate* (ITS), *S. latepetiolata* (ITS), *S. maderensis* (ITS and matK), *S. protosanctana* (ITS), *S. canaliculata* (ITS), and *S. cuneata* (ITS). Several species, however, formed polyphyletic groups:

1) In the ITS MP tree, two populations of *S. wallichiana* (R7, R9) are separated from the others. It is shown in the ITS NJ tree (Fig. 2.8) that *S. brachypoda* is identical to *S. wallichiana* R7 and the R9 populations. All populations of *S. wallichiana* including *S. brachypoda* have little genetic difference (less than 0.01% genetic distance).

2) *S. gemmipara* was separated into two groups in both the ITS NJ (Fig. 2.7) and MP trees (Fig. 2.9). The *S. gemmipara* R25/26 population from Yunnan, Cangshan is very distinct from all the other populations examined, viz. those from Yunnan, Lijiang and *S. gemmipara* var, *viridiflora* Z167 from Sichuan, Liangshan.

3) Populations of *S. crustata*, *S. paniculata*, and *S. callosa* are intermixed in the ITS NJ tree sect. *Ligulatae* clade (Fig. 2.4). *S. cochlearis* 1,2 is very distinct from *S. cochlearis* Z199 and Z182 and close to *S. hostii* ssp. *rhaetica* Z187 (only ca. 0.04% distance away). *S. callosa* var. *australis* is separate from other *S. callosa*.

4) Two species of sect. *Porphyrion*, *S. oppositifolia* and *S. marginata*, in the ITS MP tree are polyphyletic (Fig. 2.9).

5) In sect. Saxifraga, *S. trifurcata* sequences are clustered together in the ITS (Fig. 2.9) but not in the matK (Fig. 2.11) MP tree, where they are separated by *S. continentalis*.

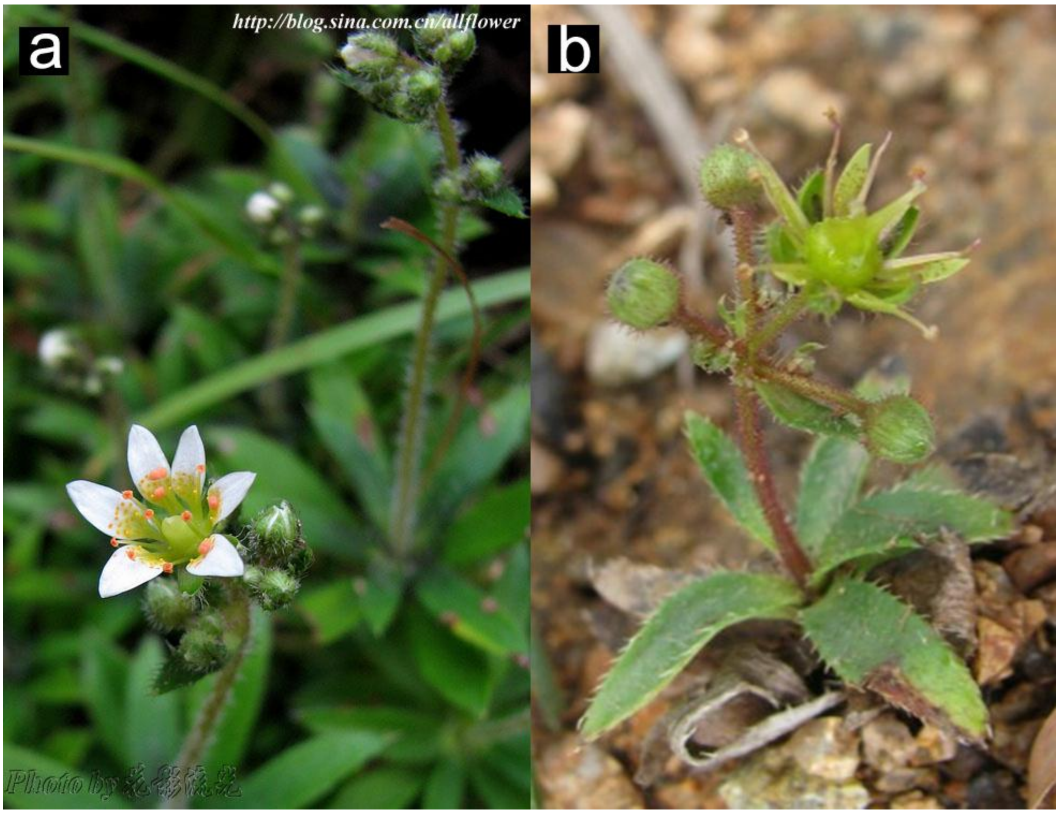
6) *S. rosacea* (sect. *Saxifraga*) is polyphyletic in both ITS (Fig. 2.9) and matK (Fig. 2.11) trees. Population Z227 is sister to *S. hypnoides*.

The possible treatments for these apparently non-monophyletic species could be to: a) dividing the species into more than one species; b) adopt a broader species concept but with infraspecific taxa; c) check the polyphyly with more markers and considering hybridisation events or even technical problems. For cases 1) and 2) in the list above, treatments a) or b) may be appropriate because of the small level of diversity. But cases 3), 4) and 5) will need more DNA studies because of the possibility of hybridisation between species or even sections. *S. continentalis* Z209 (chromosome number 2n=26, 52) is close to *S. hypnoides* (2n=26, 52 from the British Isles and 48 from Iceland) in the ITS MP tree (Fig. 2.9) but it is sister to *S. trifurcata* Z203 (2n=28) in the matK tree (Fig. 2.11). Therefore, more DNA sequences are needed, especially using single-copy nuclear genes, and more related species should be included. Case 6) requires further investigation because *S. rosacea* is part of complex of species that is still poorly understood. The complex includes *S. rosacea*, *S. cespitosa*, *S. hypnoides* and *S. continentalis*. But when one accession is separate from the other accessions of the same species, like *S. callosa* var. *australis*, the specimen identification should be checked. I copy this identification from its original label in University of Cambridge Botanic Garden and I examined the identification following the leaf morphological characters described by Webb and Gornall (1989). Further examination is required.

However, the current trees are already able to answer some species identification questions. *Ligulatae* sp. Z206 from the Cambridge Botanic Garden was mislabelled, the identification was proved wrong by checking with the descriptions in Webb and Gornall (1989), as *S. stribrnyi* but the combined MP tree (Fig. 2.14) indicates that it is *S. crustata* or a closely related species. In the ITS MP tree (Fig. 2.9), *S. cf. aurantiaca* Z178 is shown to be *S. aurantiaca* and is different from *S. unguiculata* which Z178 was identified as.

S. gemmipara var. *viridiflora* Z167 is surely part of *S. gemmipara*, and may not even be a variety. Its genetic distance is in the average of the main *S. gemmipara* clade (Fig. 2.7). Further flowering specimens are needed (the voucher is vegetative) to augment the only other material we have, which is a photograph (Fig. 2.15) before any conclusions can be drawn.

Figure 2.15. a) *S. gemmipara*; b) the unpublished variety *S. gemmipara* var. *viridiflora*, name after its green petals. Picture (a) taken by Lin Zhao and (b) taken by Zhenguo Dong.



S. epiphylla and *S. mengtzana* are related and the ITS NJ distance between them is 0.013. The distance is large comparing to that between the two populations of *S. stolonifera*. *S. epiphylla* was formerly known as *S. aculeata* I.B. Balfour, a synonym of *S. mengtzeana*. This study shows that it is appropriate to treat *S. epiphylla* as a separate species.

Genetic distances cannot be used to define species in *Saxifraga* in this study. There are morphologically very distinctive species that have almost no genetic differences in the DNA sequence studies, e.g. *S. minutissima* Z160 (very distinctive with five stamens and no petals) is very close (0.005% away) to *S.*

flexilis in the ITS NJ tree (Fig. 2.5). *S. tangutica* and *S. tibetica* Z143 have no differences (Fig. 2.6). This phenomenon is also reported by Zhang, et al. (2008) and considered as an example of rapid evolution in the alpine habitat. On the other hand, the polyphyly of some species raises questions about whether the species are artificial and whether the limited collections of populations can give a true picture of the species. For example, adding *S. gemmipara* R25/26 ruined the monophyly of *S. gemmipara* (Fig. 2.9). When intra-specific gene flow among populations is low, the populations are more differentiated from one another and are less likely to show taxon-specific markers (Hollingsworth et al. 2011), which means a broad sampling is needed to cover most populations.

If we do not require species-level accuracy in DNA identification, the current trees can give directions of their putative positions. *S. cf. micans* Z114 and SEP111 are two unidentified accessions from sect. *Porphyrion* subsect. *Kabschia*. From the ITS MP tree (Fig. 2.9), it is clear that they are in ser. *Marginatae* and close to *S. afghanica*. *S. rosacea/cespitosa* Z222, Z221 are either *S. rosacea* or *S. cespitosa*. They did cluster with the latter two species in the ITS MP tree but the detailed position is unresolved. The answer will be shown when adding in more data (genes) to the species.

A hybrid is included in the ITS trees (Fig. 2.9) and something interesting is shown. *S.* × *landaueri* Z115 is a triple hybrid between [*S. burseriana* (subsect. *Kabschia*) x *S. stribrnyi* (subsect. *Engleria*)] x *S. marginata* (subsect. *Kabschia*). In the tree, Z115 is in subsect. *Kabschia* but not in subsect. *Engleria*. Is this because of the two of the parents are from subsect. *Kabschia*?

Chapter Three: Pollen data

'It has long been an axiom of mine that the little things are infinitely the most important.'

Sherlock Holmes

-A Case of Identity

3.1 Introduction

This chapter contains two sections, dealing with pollen nucleus number and with pollen exine anatomy, respectively. The former has been published and is presented here in its printed form. The latter has been submitted.

It has long been known that the angiosperm microspore contains a wealth of taxonomically valuable information (Blackmore et al. 2007). Section one describes the distribution of bi- vs. trinucleate pollen grains in *Saxifraga*; infrageneric variation in this character is most unusual in the angiosperms and it was therefore considered to be a potentially valuable source of evidence. In section two the study of Ferguson and Webb (1970) on exine anatomy in *Saxifraga* was considerably extended by sampling many more species and by conducting an extensive series of detailed studies using the transmission electron microscope.

3.2 Pollen morphology

3.2.1 Pollen nucleus number

Contribution: experiments were conducted and manuscript was written by Z-X. Zhang; the study was designed and manuscript corrected by R.J. Gornall.

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Systematic significance of pollen nucleus number in the genus *Saxifraga* (Saxifragaceae)

Z-X. Zhang and R.J. Gornall*

Biology Dept., University of Leicester, Leicester, LE1 7RH, U.K.

*Author for correspondence: phone: +44(0)116 252 3394, fax: +44(0)116 252 3330, e-mail: rjg@le.ac.uk

ABSTRACT

Pollen cell number was studied in 124 species of *Saxifraga* and six species of *Micranthes*. Binucleate pollen is present in all the six species of *Micranthes* studied and in 96 species of *Saxifraga*. Trinucleate pollen occurs in 28 species of *Saxifraga* and is confined to section *Ciliatae* subsection *Hirculoideae*, where it is a putative synapomorphy, and to two species of subsection *Serpyllifoliae*. Trinucleate pollen does not characterise the whole of section *Ciliatae* as has been thought. The state has probably evolved at least twice, and possibly three times, in the genus. One new nomenclatural combination is made in *Micranthes*.

Key words: Saxifraga; Saxifragaceae; pollen cell number; trinucleate pollen; taxonomy.

INTRODUCTION

In angiosperms, each functional microspore undergoes a first mitotic division to form a bicellular male gametophyte, consisting of a large vegetative cell and a smaller generative cell lying free in the cytoplasm of the former, all inside the spore wall. In most angiosperms, the pollen (spore wall containing the male gametophyte) is shed at this two-celled stage. In others, however, the generative cell divides prior to the dehiscence of the anther to form two sperm cells, and the pollen is consequently shed in the three-celled condition. The taxonomic distribution of these two states is of considerable significance (Schürhoff 1926; Brewbaker 1967): not only does the character exhibit considerable conservatism, with entire families being characterised by a single state, but also the tricellular condition appears to have been derived, irreversibly, from the bicellular condition on numerous occasions, a situation that has become known as the Schürhoff-Brewbaker Law (Webster and Rupert 1973).

Given this situation, the study of polymorphic taxa becomes of great interest. In a recent survey of the distribution of pollen types (Haught 2007), plotted onto the APG II phylogenetic classification, 181/273 angiosperm families were bicellular, 52/273 were tricellular and 44/273 were polymorphic. One of the polymorphic families is the Saxifragaceae. Early studies of the family reported binucleate pollen in the genera *Astilbe* and *Bergenia* (Webb 1902; Mauritzon 1933). The discovery, however, of trinucleate pollen in *Saxifraga diversifolia* by Saxena (1964) indicated that the family was one of the few that are polymorphic for this character. It soon became clear that the genus *Saxifraga* itself may be polymorphic when Rau & Sharma (1967 [1968]) reported the binucleate state in *S. flagellaris*, and implied the same for three other species (*S. diversifolia*, *S. sibirica* and *S. pallida*). Kaplan (1981) extended the information on *Saxifraga* by recording binucleate pollen in *S. arachnoidea*, *S. petraea*, *S. tridactylites*, *S.*

fortunei, *S. stolonifera*, *S. californica* and *S. stellaris*, but trinucleate (and even 5-nucleate!) pollen in *S. hirculus*.

Saxifraga is the largest genus in the Saxifragaceae (Soltis et al. 2001a). In the most recent classification (Gornall 1987a), the genus is divided into 15 sections, with numerous subsections and series. DNA sequences from four chloroplast and two nuclear regions, however, have shown that *Saxifraga* is monophyletic only if sections *Micranthes* and *Merkianae* are separated as an independent genus (Soltis et al. 1996). Removal of their ca 75 species still leaves *Saxifraga* with more than 450 species, consisting of mostly perennial, insect-pollinated herbs. They are distributed mainly in the montane areas of Europe and the Sino-Himalayan region, but also in the arctic and extending down the Rocky Mt – Andean cordillera to southern S. America (Webb and Gornall 1989; Pan et al. 2001). On the basis of the cumulative data on pollen cell number, Kaplan (1981) claimed that trinucleate pollen uniquely characterises *Saxifraga* section *Ciliatae*, a major group of some 210 species, nearly half the genus; and this was despite the fact that, of the only three species from that section studied (*S.diversifolia*, *S.hirculus* and *S.flagellaris*) the first has conflicting reports in the literature and the last was reported to be binucleate. Nevertheless, this claim has now become an accepted fact in the literature (Webb and Gornall 1989). The aim of our study, therefore, was to survey a much greater number of species from the entire genus to discover more precisely the taxonomic distribution of bi- vs trinucleate pollen.

MATERIALS AND METHOD

Pollen was obtained chiefly from recently collected herbarium specimens and fresh material (Table 3.1). Where possible, more than one collection per species was examined, in order to test for infra-specific variation. In total, results were obtained from 124 species representing 38 of the 43 infra-generic taxa recognised by Gornall (1987a), together with six species from the genus *Micranthes* (Table 3.1). Species from some groups were not included owing to lack of material fresh enough for successful pollen staining.

To ensure only mature pollen was collected, we sampled from anthers that were dehiscent and had pollen emerging. Between three and seven anthers were gathered from each accession: the samples were sometimes from a single individual plant and sometimes from more than one; occasionally, owing to the paucity of material, pollen from different accessions of the same species was mixed. Anthers were placed in ependorff tubes and fixed in 150 µl 3:1 ethanol : glacial acetic acid at 4 °C for 22-24 hours. The tubes were then centrifuged at 4,000 rcf for 2 minutes and the fixative pipetted off. 200 µl of 70% ethanol were then added. For DAPI staining, tubes were centrifuged again, the ethanol pipetted off, and 50 µl 10 µg/ml DAPI (4',6-diamidino-2-phenylindole) solution (Coleman and Goff 1985) added to each tube. Tubes were then centrifuged for 1 minute and drops of the solution containing pollen and anthers were pipetted onto a clean microscope slide. Anthers were dissected thoroughly using needles under a dissecting microscope, releasing more pollen. Finally a cover slip was added and sealed with nail varnish. Slides were examined immediately with a UV-fluorescent microscope under oil-immersion using a ×40 objective lens. Under these conditions pollen nuclei fluoresce blue because DAPI binds strongly to DNA and is excited by ultraviolet light. Sometimes, auto-fluorescence of the pollen surface impeded observation of the nuclei. However, by applying some pressure to the cover-slip, the microspore wall could be sloughed off to reveal the microgametophyte directly (Coleman and Goff 1985) (Fig. 3.1a).

Herbarium specimens older than about 20 years typically showed high levels of autofluorescence from the microspore wall and opaque cytoplasm which made accurate observation impossible. Specimens collected in the past 10 years, however, gave good results.

RESULTS

In *Saxifraga*, bicellular pollen has a smaller, brighter generative nucleus, adjacent to a larger but darker vegetative nucleus; usually the generative nucleus is spherical (Fig. 3.1b), ellipsoidal (Fig. 3.1c), and eventually crescentic (Fig. 3.1d), while the vegetative one is spherical (Fig. 3.1c) or spindleform (Fig. 3.1d). In contrast, tri-nucleate pollen has two small, spherical, bright sperm nuclei and one big, spherical or ellipsoidal, dark vegetative nucleus. The sperm nuclei are always arranged one on each side of the vegetative nucleus like the ears of a “Mickey Mouse” (Fig. 3.1a, e).

Figure 3.1. Fluorescence micrographs showing the different types of pollen nucleus in *Saxifraga*. (a): *S. diversifolia var. soulieana*, trinucleate pollen; (b): *S. densifoliata*, binucleate pollen; (c): *S. gyalana*, binucleate pollen; (d): *S. cespitosa*, binucleate pollen; (e): *S. rivularis*, trinucleate pollen (in an otherwise binucleate species). The white arrows point to the generative nucleus or sperm cells and black arrows to the vegetative cell. Bar = 20µm.

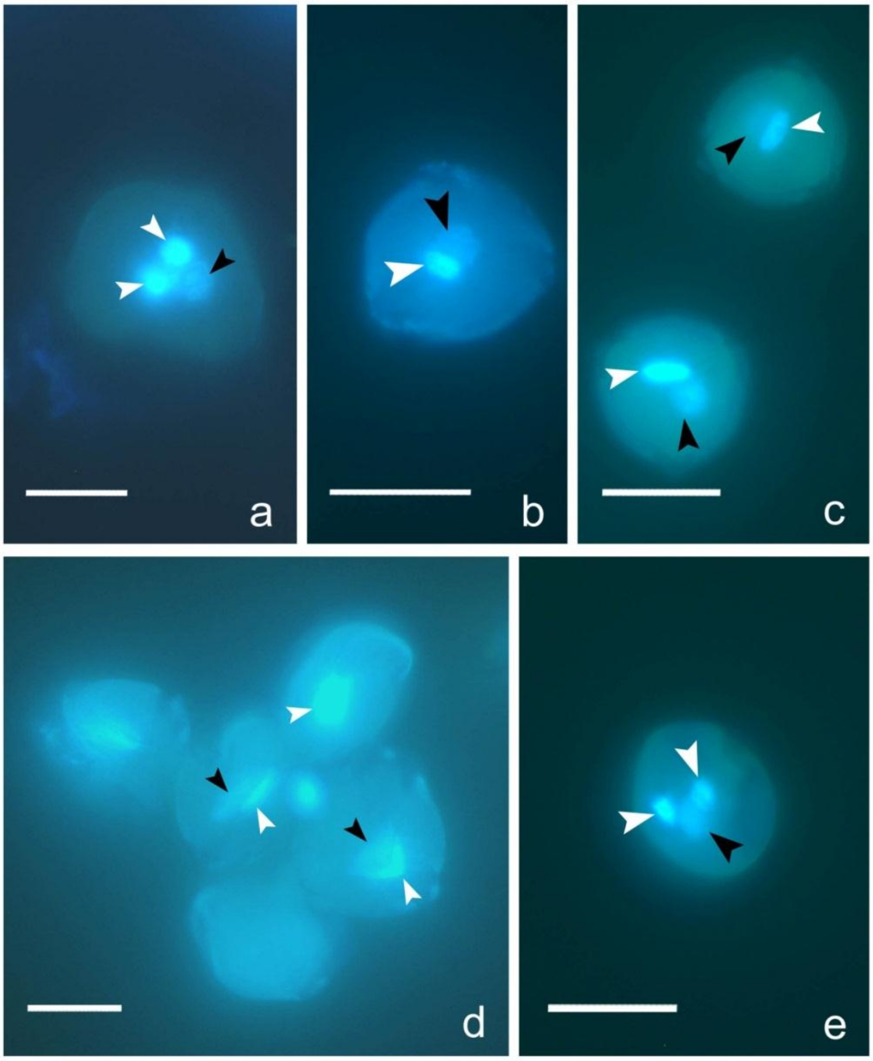


Table 3.1 shows the pollen cell number in 124 species of *Saxifraga* belonging to 38 infra-generic groups, as well as in six species of the genus *Micranthes*. Binucleate pollen is the only type present in the species of *Micranthes* that we studied. It is also the most frequent pollen type in the genus *Saxifraga*, in which 96 out of the 124 studied species (77.4%) are binucleate, and 28 (22.6%) are trinucleate (Table 3.1). The trinucleate species are confined to section *Ciliatae*. Within section *Ciliatae*, all of the 26 species of subsection *Hirculoideae* studied by us are trinucleate, and so are two (*S. engleriana* and *S. jacquemontiana*) of the species studied of subsection *Serpyllifoliae*; the remaining species are binucleate (Table 3.1).

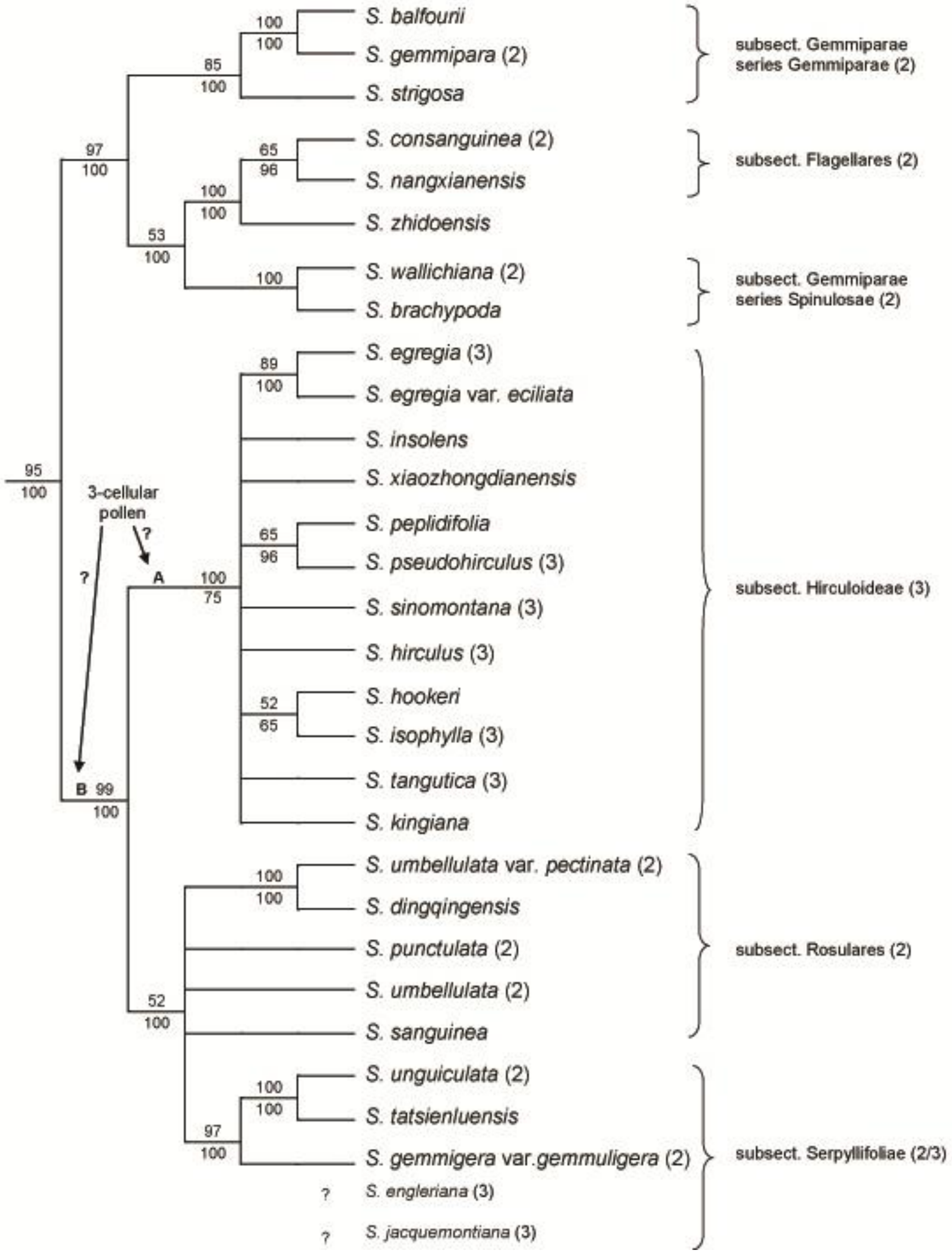
Five species showed at least one polymorphic collection, viz. *S. pasumensis*, *S. rivularis*, *S. cebennensis*, *S. caveana* and *S. pseudohirculus*. In the first three cases (all binucleate species), one or two of the accessions produced a low to moderate frequency of trinucleate grains (*S. pasumensis* 3.7%, *S. rivularis* 67.4%, *S. cebennensis* 21.1%), disturbing an otherwise binucleate picture. In the fourth case, the otherwise trinucleate *S. pseudohirculus* produced a low frequency (0.7%) of binucleate grains in one of its accessions. And in the fifth case, *S. caveana*, we counted 30 binucleate and 15 trinucleate grains but most of the pollen in the sample could not be scored because of autofluorescence. The generative nucleus in many of the binucleate grains was markedly elongated, suggesting it was about to undergo mitosis.

DISCUSSION

Taxonomic and evolutionary significance of pollen cell number

The data clearly show that trinucleate pollen is indeed restricted to section *Ciliatae*, but it does not occur throughout the section as was proposed by Kaplan (1981). Instead it is present only in all those species studied belonging to subsection *Hirculoideae,* and in two species from subsection *Serpyllifoliae.* An evolutionary perspective can be provided by optimising the pollen nucleus number on a DNA-based phylogeny. Two phylogenies are available: a six ‘gene’ tree produced by Soltis et al. (2001a) and an ITS tree produced by Zhang et al. (2008). The two trees are similar in most respects so we chose the latter because it contains more species and, importantly, focuses on section *Ciliatae* (Fig. 3.2). In particular, it confirms morphological evidence (Engler and Irmscher 1916/19; Webb and Gornall 1989) that section *Ciliatae* is monophyletic; furthermore it also suggests that so are subsections *Hirculoideae* and subsections *Rosulares* and *Serpyllifoliae* when the last two are treated as a pair (Fig. 3.2). These findings have implications for our understanding of how many times tricellularity has evolved in *Saxifraga*, as we shall discuss.

Figure 3.2. Distribution of bi- and trinucleate pollen plotted on a phylogeny of *Saxifraga* section *Ciliatae*, taken from the genus-wide survey by Zhang *et al*. (2008). Numbers above and below the nodes are bootstrap values and Bayesian posterior probabilities respectively. Numbers in parentheses adjacent to taxon names are the pollen nucleus number; numbers after infra-generic taxa are based on the cumulative observations in Table 3.1. Assignment of species to subsections and series follows Gornall (1987a) rather than Zhang *et al*. (2008) who combined subsections *Gemmiparae* and *Flagellares* under the name *Gemmiparae*, and also united subsections *Rosulares* and *Serpyllifoliae* under the name *Rosulares*. Note that *S. engleriana* and *S. jacquemontiana* were not studied by Zhang et al. (2008) and their phylogenetic position is unknown, although they are here assigned to subsection *Serpyllifoliae.*



Subsection *Hirculoideae* is diagnosed by the presence of rufous, crisped, eglandular hairs in the proximal leaf axils of the constituent species. This character state is likely to be a synapomorphy for the subsection as is suggested by the phylogeny in Fig. 3.2. We are therefore confident that we have assigned species to subsection *Hirculoideae* correctly in Table 3.1. Consequently, we conclude that a) the subsection appears to be characterised by trinucleate pollen (Table 3.1); b) trinucleate pollen represents a derived mutation, consistent with the Schürhoff-Brewbaker Law (Fig. 3.2); and c) this feature is therefore another synapomorphy for the subsection.

The occurrence of trinucleate pollen in two species (*S. jacquemontiana* and *S. engleriana*) assigned to subsection *Serpyllifoliae* is interesting and raises some questions. Firstly, could the taxonomy be wrong and these two species actually reside in subsection *Hirculoideae*? Unfortunately, neither were included in the study of Zhang et al. (2008), but here we argue that, since they lack axillary rufous hairs and instead have the characters (viz. long prostrate leafy shoots developing from basal axillary buds) of those species that belong to subsection *Serpyllifoliae*, they really do belong there rather than in subsection *Hirculoideae*. Secondly, if that is so, and since subsections *Rosulares* and *Serpyllifoliae* as a pair appear to be sister to subsection *Hirculoideae* (Fig. 3.2), then where on the phylogeny do we place the mutation that led to tricellularity? It is most likely to be at either node A or node B in Fig. 3.2. If it is at node A, then tricellularity has probably evolved at least twice in *Saxifraga*: once in subsect. *Hirculoideae* and once or twice in subsect. *Serpyllifoliae* (i.e. in *S. jacquemontiana* and *S. engleriana*, depending on their relationship). If it is at node B, then only one origin is implied and the binucleate condition in subsections *Rosulares* and *Serpyllifoliae* represents one or more reversals. We consider this to be unlikely given that there is no established case of such a situation occurring anywhere in the angiosperms (Haught 2007). A proper evaluation, however, can only be made once more species from this subsection are studied and a more extensive phylogeny produced.

Infra-specific variation

The presence of both bicellular and tricellular pollen at anther dehiscence in the same species is considered uncommon, but it has been reported by Webster and Rupert (1973) in the Euphorbiaceae, by Grayum (1986) in the Araceae, and by Lora et al. (2009) in *Annona cherimola*. To our knowledge, all reported cases of infra-specific polymorphism appear to involve single plants: no inter-individual polymorphism has been recorded. The situation in *Saxifraga* is the same. We suggest that the cause is likely to be the result of developmental plasticity and open to environmental influence. Evidence for developmental plasticity comes from observations made on old flowers of bicellular species that have been in bloom for an extended period, showing that they may produce a small number of tricellular pollen grains (Wunderlich 1936; Webster and Rupert 1973). This is the case in the present study, where pollen samples taken from older flowers of *S. pasumensis*, *S. cebennensis* and *S. rivularis* (Fig. 3.1e) contained variable numbers of tricellular grains. Poddubnaja-Arnoldi (1936) suggested that pollen cell number could also be altered environmentally, a fact demonstrated by Lora et al. (2009) who showed that elevated temperatures could advance the timing of the second mitosis in *Annona* *cherimola*, resulting in increasing proportions of trinucelate grains in what is an otherwise binucleate species. There appear to be no recorded examples of tricellular species that produce small numbers of binucleate pollen grains but, in our study, we found such a situation in two accessions of *S. pseudohirculus* and one of *S. caveana*. Possible explanations include: premature dehydration arresting the pollen in the bicellular condition; pollen from an immature anther ruptured in the herbarium press; or some other aspect of developmental plasticity (Brewbaker 1967).

Adaptive significance

The adaptive advantage of trinucelate over binucleate pollen has been related to its higher germination rate, allowing faster fertilisation. Knox (1984) thought this might be of significance in water- and wind-pollinated species, in which tricellularity is especially common. Lora et al. (2009) suggested that rapid pollen germination could also be an advantage at higher temperatures which accelerate not only female development but also the whole reproductive process. In the context of the genus *Saxifraga*, most species grow in montane habitats, sometimes at very high elevations (2700-4500m or more in the Sino-Himalaya) or else at high latitudes. In these places the growing season is relatively short, which might favour rapid reproduction and therefore tricellular pollen – but then why does it not characterise other Sino-Himalayan groups or the alpine European ones? And why is it not a feature of high elevation floras generally? A possible explanation is that tricellularity might even be maladaptive under these conditions. Not much has been said in the literature about the disadvantage of tricellular pollen: its higher rate of germination is linked to a higher respiration rate and a shorter life. The short life-span would appear to be maladaptive in situations where the pollen transfer process, from anthesis to pollination, is likely to take a long time. If insect pollinators were in short supply at any time during the flowering season (not unlikely given the ever-changing weather conditions at high elevations), tricellular pollen could die before it reached a receptive stigma. Lora et al. (2009) proposed that polymorphic individuals might benefit from a bet-hedging approach because both long- and short-lived pollen is produced. It is of course possible that in the case of *Saxifraga*, the switch to complete tricellularity has no adaptive significance, but was a neutral mutation that occurred in the ancestor of subsection *Hirculoideae*, for example, and was simply passed on to its descendants. Much more needs to be discovered about the life-histories of the species in section *Ciliatae* before we can begin to understand the nature, adaptive or otherwise, of tricellular pollen in this group.

Acknowledgements

We are grateful to David Boufford (Harvard University), Georg & Sabine Miehe (University of Marburg) and the curators of BM, E and LTR for making recently collected specimens available. We thank David Twell, Trude Schwarzacher, John Bailey, Nadia Taimur and Hoda Khtab for help and advice with the DAPI protocol.

Table 3.1. The distribution of pollen cell number in the genera *Micranthes* and *Saxifraga* {classification following Gornall (1987a) but with *Micranthes* separated}. Polymorphic collections include the numbers of each type of pollen grain observed. Specimens are italicized; those collected by D. Boufford *et al*., Harvard University, are prefixed 'B'; all others have the collector's surname in full. Locations of the herbaria in which the collections may be found are indicated by the standard acronyms.

Taxon	Pollen cell no.	Collection or literature reference
Micranthes		
M. stellaris (L.) Galasso, Banfi &Soldano	2	Kaplan (1981)
M. californica (Greene) Small	2	Kaplan (1981)
M. divaricata (Engl. & Irmsch.) Losinsk.	2	*B 36023* (HUH)
M. lumpuensis (Engl.) Losinsk.	2	*B 36646* (HUH); *B 36741* (HUH); *SAE631*
M. melanocentra (Franch.) Losinsk.	2	*B 31935* (HUH); *B 31757* (HUH); *B 31405* (HUH); *B 31104* (HUH); *B 32312* (HUH); *B 34079* (HUH); *B 33738* (HUH); *B 33331* (HUH); *B 33332* (HUH); *B 36756* (HUH); *B 34819* (HUH); *B 36633* (HUH)
M. pallida (Wall. ex Ser.) Losinsk. *var. pallida*	2	*B 32727* (HUH)
M. pallida var. yunnanensis (Franchet) Gornall & H. Ohba [1]	2	*B 35180* (HUH)
Saxifraga		
Section *Ciliatae*		
Subsect. *Hirculoideae*		
Series *Hirculoideae*		
S. auriculata Engl. & Irmsch.	3	*B 40445* (HUH)
S. bergenioides Marquand	3	*Miehe & Wündisch 94-100-7* (LTR)
S. caveana W.W. Sm.	3	*Miehe 00-303-12 2:3:x = 30:15:55*
S. congestiflora Engl. & Irmsch.	3	*B 36119* (HUH); *B 36024* (HUH); *B 37121* (HUH)
S. cordigera Hook.f. & Thoms.	3	*Miehe 00-303-13* (LTR); *Miehe 00-303-02* (LTR)

[1] *Micranthes pallida* (Wallich ex Séringe) Losinskaja var. *yunnanensis* (Franchet) Gornall & H. Ohba, comb. nov. Basionym: *Saxifraga micrantha* Edgeworth var. *yunnanensis* Franchet, J. Bot. (Morot) 10: 263. 1896. TYPE: China. Yunnan: "in rupibus montis Tsang-chan supra Tali [Dali], prope cacumina," 4 Aug 1884, *J. M. Delavay 68* (lectotype here designated, P, specimen with original field label and description of habitat and flower color; isolectotypes K, P, four specimens).

S. diversifolia Wall. ex Ser.	3	Saxena (1964); *Lyon 11107* (E)
S. diversifolia var. soulieana Engl. & Irmsch. (= *S. egregia* Engl.)	3	*B 36705* (HUH); *B 36706* (HUH); *B 37061* (HUH); *B 37369* (HUH); *B 34623* (HUH); *B 33929* (HUH); *B 34382* (HUH); *B 34244* (HUH); *B 35748* (HUH); *B 32199* (HUH); *B 32321* (HUH); *B 32358* (HUH); *B 32521* (HUH); *B 31877* (HUH)
S. giraldiana Engl.	3	*B 38592* (HUH)
S. heleonastes H. Sm.	3	*B 40083* (HUH)
S. hirculus L.	3	Kaplan (1981); *Gornall 672bi* (LTR); *Gornall 651* (LTR)
S. hypericoides Franch.	3	*Kingdon Ward 12274* (BM)
S. implicans H. Sm.	3	*Dickoré 4719* (LTR)
S. isophylla H. Sm.	3	*B 30140* (HUH)
S. latiflora Hook.f. & Thoms.	3	*Miehe 00-421-04* (LTR)
S. linearifolia Engl. & Irmsch.	3	*B 36197* (HUH); *B 32722* (HUH)
S. macrostigma Franch.	3	*B 42024* (HUH)
S. montanella H. Sm.	3	*Ho et al. 2830*
S. saginoides Hook.f. & Thoms.	3	*Lyon 11152* (E)
S. sinomontana J-T. Pan & Gornall	3	*B 36742* (HUH); *B 36619* (HUH); *B 36605* (HUH); *B 36580* (HUH); *B 36855* (HUH); *B 37352* (HUH); *B 34837* (HUH); *B 31934* (HUH)
S. tangutica Engl.	3	*B 34068* (HUH); *B 31128* (HUH)
S. tibetica Losinsk.	3	*B 29453* (HUH); *B 33687* (HUH)
Saxifraga sp.	3	*B 33365* (HUH)
Series *Lychnitidae*		
S. litangensis Engl.	3	*B 36012* (HUH); *B 38591* (HUH)
S. lychnitis Hook.f. & Thoms.	3	*Dickoré 3819*
S. oresbia Anthony	3	*B 36195* (HUH); *B 37414* (HUH)
S. pseudohirculus Engl.	3	*B 34401* (HUH); *B 36581* (HUH); *B 36594* (HUH); *B 36704* (HUH); *B 34826* (HUH); *B 36754* (HUH); *B 37086* (HUH); *B 34485* (HUH); *B 34412* (HUH); *B 34224* (HUH); *B 32490* (HUH); *B 31781* (HUH); *B 41813* 2:3=2:300 (HUH)
Series *Nutantes*		
S. nigroglandulifera Balakr.	3	*B 37355* (HUH); *B 35747* (HUH); *B 32299* (HUH)
Subsect. *Rosulares*		
S. brunneopunctata H. Sm.	2	*Dickoré 3430* (LTR)
S. gyalana Marquand & Airy-Shaw	2	*B 29783* (HUH); *B 31869* (HUH); *B 32489* (HUH)
S. heterotricha Marquand & Airy-Shaw	2	*Dickoré 11867* (LTR)
S. lhasana H. Sm.	2	*B 30955* (HUH)
S. pasumensis Marquand & Airy-Shaw	2	*B 32403* (HUH); *B 32211* (HUH); *B 39069* 2:3=210:8 (HUH)
S. punctulata Engl.	2	*B 31089* (HUH)
S. signata Engl. & Irmsch.	2	*B 31235* (HUH); *B 32123* (HUH); *B 33667* (HUH); *D5115*
S. umbellulata Hook.f. & Thoms.	2	*Lyon 11013* (E) + *Lyon 11158* (E)
S. vilmoriniana Engl. & Irmsch.	2	*B 35727* (HUH); *B 33364* (HUH); *B*

		36102 (HUH); *B 36019* (HUH); *B 36147* (HUH); *B 37253* (HUH)
Subsect. *Serpyllifoliae*		
S. aurantiaca Franch.	2	*B 35831* (HUH)
S. densifoliata Engl. & Irmsch.	2	*B 36005* (HUH); *B 33197* (HUH)
S. engleriana H. Sm.	3	*Miehe 00-248-15* (LTR)
S. flexilis W.W. Sm.	2	*B 34162* (HUH)
S. gemmigera Engl.	2	*B 39212* (HUH)
S. glacialis H. Sm. var. *rubra* Anthony	2	*B 31827* (HUH)
S. jacquemontiana Decne.	3	*Dickoré 5301* (LTR)
S. nanella Engl. & Irmsch.	2	*B 33550* (HUH); *B 34102* (HUH)
S. stella-aurea Hook.f. & Thoms.	2	*Lyon 11233* (E)
S. unguiculata Engl.	2	*B 29743* (HUH); *B 31481* (HUH); *B 31316* (HUH); *B 32458* (HUH); *B 32198* (HUH); *B 32088* (HUH); *B 41077* (HUH)
Subsect. *Hemisphaericae*		
S. hemisphaerica Hook.f. & Thoms.	2	*Dickoré 8529* (LTR)
Subsect. *Gemmiparae*		
Series *Gemmiparae*		
S. gemmipara Franch.	2	*Dickoré 14547* (LTR)
S. hispidula D. Don	2	*B 35124* (HUH)
S. substrigosa J-T. Pan	2	*Dickoré 4944* (LTR)
Series *Spinulosae*		
S. filicaulis Wall. ex Ser.	2	*Dickoré 10581* (LTR)
S. wallichiana Sternb.	2	*B 35849* (HUH); *B 37076* (HUH)
S. wardii W.W. Sm.	2	*B 30129* (HUH)
Subsect. *Flagellares*		
S. brunonis Wall. ex Ser.	2	*Miehe 00-298-01* (LTR)
S. consanguinea W.W. Sm.	2	*B 32197* (HUH); *B 32306* (HUH); *B 31479* (HUH); *B 34066* (HUH)
S. flagellaris Willd.	2	Rau & Sharma (1967)
S. microgyna Engl. & Irmsch.	2	*B 35760* (HUH)
S. mucronulatoides J-T. Pan	2	*Dickoré 10225* (LTR)
S. pilifera Hook.f. & Thoms.	2	*Dickoré 10023* (LTR); *Miehe & Wündisch 94-105-28* (LTR)
Section *Cymbalaria*		
S. cymbalaria L.	2	*Gornall 688* (LTR)
S. sibthorpii Boiss.	2	*Polunin 12960* (LTR)
Section *Irregulares*		
S. fortunei Hook.f.	2	Kaplan (1981)
S. imparilis I.B. Balf.	2	*B 32626* (HUH); *B 32626* (HUH)
S. mengtzeana Engl. & Irmsch.	2	*B 35811* (HUH)
S. rufescens I.B. Balf.	2	*B 33395* (HUH); *B 35120* (HUH)
S. stolonifera W. Curtis	2	Kaplan (1981); hort.LTR
Section *Porphyrion*		
Subsect. *Kabschia*		

Series *Aretioideae*		
S. aretioides Lapeyr.	2	*Polunin 9437* (LTR)
S. ferdinandi-coburgii Kellerer & Sünd.	2	Hort.LTR
Series *Juniperifoliae*		
S. juniperifolia Adams	2	*Polunin 10732A* (LTR); *Polunin 10674* (LTR)
Series *Marginatae*		
S. marginata Sternb.	2	Hort.LTR
S. marginata var. rocheliana (Sternb.) Engl. & Irmsch.	2	Hort.LTR
S. spruneri Boiss.	2	*Polunin 10274* (LTR); *Polunin 11068* (LTR); *Polunin 11138* (LTR)
S. wendelboi Schönbeck-Temesy	2	Hort.LTR
Series *Squarrosae*		
S. caesia L.	2	*Jury et al. 6937a* (LTR); *Jury et al. 7068* (LTR)
S. squarrosa Sieber	2	*Horwood L.82* (LTR); *Halliday 104/63* (LTR)
Series *Rigidae*		
S. burseriana L.	2	Hort.LTR
Subsect. *Engleria*		
S. media Gouan	2	*Polunin 9165* (LTR); *Webb 6.v.1959* (LTR)
S. sempervivum C. Koch	2	*Polunin & Chater 13091* (LTR); *Polunin & Chater 13038* (LTR)
Subsect. *Oppositifoliae*		
Series *Oppositifoliae*		
S. biflora All.	2	*Jury et al. 6312* (LTR)
S. oppositifolia L.	2	*Primavesi 18.vi.1988* (LTR); *Fraser-Jenkins 2635* (LTR); *Stace 9.v.1961* (LTR)
Section *Ligulatae*		
Subsect. *Aizoonia*		
S. callosa Sm.	2	*Stace vii.1990* (LTR)
S. cotyledon L.	2	Hort.LTR; *Polunin 12421* (LTR)
S. hostii Tausch	2	Hort.LTR
S. longifolia Lapeyr.	2	*P.& J.M. Montserrat 1289/78* (LTR)
S. paniculata Mill.	2	Hort.LTR
S. paniculata 'Correvoniana '	2	Hort.LTR
Subsect. *Mutatae*		
S. mutata L.	2	*Khek vii.1904* (LTR)
Section *Xanthizoon*		
S. aizoides L.	2	*Primavesi 18.vi.1988* (LTR); *Polunin 11418* (LTR); *Jury et al. 6934* (LTR)
Section *Trachyphyllum*		
S. aspera L.	2	*Jury et al. 7089* (LTR)
S. bryoides L.	2	*Polunin 11396* (LTR)

Section *Gymnopera*		
S. cuneifolia L.	2	Hort.LTR
S. hirsuta L.	2	Hort.LTR
S. spathularis Brot.	2	Hort.LTR
S. umbrosa L.	2	Hort.LTR
Section *Cotylea*		
S. rotundifolia L.	2	Hort.LTR *(2 accessions)*
S. taygetea Boiss. & Heldr.	2	*Polunin 15215* (LTR); *Polunin 13485* (LTR)
Section *Mesogyne*		
S. carpatica Sternb.	2	*Chater 5096* (LTR); *Raciborski 868* (LTR)
S. cernua L.	2	*Gornall 666* (LTR); *Gornall 671* (LTR)
S. granulifera H. Sm.	2	*B 32169* (HUH); *B 39870 or 41869* (HUH)
S. rivularis L.	2	*Gornall 646* (LTR); *Gornall 665 +670 +675* 2:3=14:29
Section *Saxifraga*		
Subsect. *Saxifraga*		
Series *Saxifraga*		
S. bulbifera L.	2	*Stace & Cotton 416* (LTR)
S. granulata L.	2	*Gornall 687* (LTR); *Gornall 690* (LTR)
Series *Biternatae*		
S. biternata Boiss.	2	*Gibbs et al. 1403.69* (LTR); *Smythies 264* (LTR)
S. bourgaeana Boiss. & Reut.	2	*Stace v.2003* (LTR); *Molesworth-Allen 8767* (LTR)
S. gemmulosa Boiss.	2	*Molesworth-Allen 8608* (LTR); *Molesworth-Allen 7105* (LTR)
Subsect. *Triplinervium*		
Series *Aquaticae*		
S. aquatica Lapeyr.	2	*Polunin 9514* (LTR); *Horwood L.52* (LTR)*; Burges vi.1957* (LTR)
S. latepetiolata Willk.	2	*Prentice 76/61* (LTR)
Series *Arachnoideae*		
S. arachnoidea Sternb.	2	Kaplan (1981); *Jury et al. 6941* (LTR)
S. petraea L.	2	Kaplan (1981)
Series *Axilliflorae*		
S. wahlenbergii J. Ball	2	*Chater 5130* (LTR); *Chater 5047* (LTR)
S. praetermissa D.A. Webb	2	*Leedal 1975* (LTR), *Groves vii.1963* (LTR)
Series *Ceratophyllae*		
S. pentadactylis Lapeyr.	2	*Smythies 1279* (LTR)
S. trifurcata Schrad.	2	*Polunin 9191* (LTR); *Gornall 689* (LTR)
Series *Cespitosae*		
S. cebennensis Rouy & Camus	2	*Webb 8.vi.1967* (LTR); Hort.LTR 2:3=30:8
S. cespitosa L.	2	*Gornall 629* (LTR); *Gornall 642* (LTR); *Gornall 661* (LTR)

S. pubescens Pourr.	2	Hort.LTR; *Leedal C5* (LTR)
S. rosacea Moench	2	*Gornall 674a* (LTR); *Gornall 668* (LTR)
Series *Gemmiferae*		
S. globulifera Desf.	2	*Dubuis 16.vi.1978* (LTR); *Molesworth-Allen 6212* (LTR)
S. hypnoides L.	2	*Gornall 676* (LTR); *Gornall 659* (LTR); *Gornall 667* (LTR); Hort.LTR
Subsect. *Holophyllae*		
Series *Androsaceae*		
S. androsacea L.	2	*Stace vii.1971* (LTR); *Horwood L.21* (LTR)
S. depressa Sternb.	2	*Jury et al. 6760* (LTR)
Series *Muscoideae*		
S. muscoides All.	2	*Horwood L.21* (LTR)
Series *Sedoideae*		
S. aphylla Sternb.	2	*Halliday 122/59* (LTR)
S. sedoides L.	2	*Jury et al. 6698* (LTR)
Series *Glabellae*		
S. glabella Bertol.	2	*Polunin 13799* (LTR); *Akeroyd 74* (LTR)
Subsect. *Tridactylites*		
S. adscendens L.	2	*Polunin 15603A* (LTR); *Polunin 12154b* (LTR); *Polunin 12316* (LTR)
S. tridactylites L.	2	Kaplan (1981); *Primavesi 9.v.1985* (LTR); *Fraser-Jenkins 11* (LTR)

3.2.2 Pollen exine

Contribution: experiments were conducted and manuscript was written by Zhuoxin Zhang; the study was designed and manuscript was modified by Richard J. Gornall.

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The Morphology and Anatomy of Pollen Exines in *Saxifraga* (Saxifragaceae)

Zhuoxin Zhang & Richard J. Gornall*
Department of Biology, University of Leicester, U.K.

*Author for correspondence: phone: +44(0)116 252 3394, fax: +44(0)116 252 3330, e-mail: rjg@le.ac.uk

Abstract

Details of exine morphology (SEM studies of 132 species) and anatomy (TEM studies of 25 species) are reported. The variation observed was compared with that found in the earlier survey by Ferguson & Webb. Their four main types are confirmed, but we suggest some modifications to their subtypes and the addition of three new ones. Taxonomic conclusions include the following:

1) A novel Type 2.3, in which the single tectum is punctured with numerous micro-capillaries, is a putative synapomorphy for sects. *Heterisia* and *Irregulares*.

2) At least three species from sect. *Ciliatae*, *S. lychnitis*, *S. oresbia* from ser. *Lychnitidae*, and *S. nigroglandulifera* from ser. *Nutantes*, have the novel Type 5. Species from the rest of the section are uniformly of Type 3, with the variation not permitting further subdivision.

3) Section *Saxifraga* has the Type 2.1, with a single tectum, Type 4.3, with a double tectum, and Type 2.2, which is to some extent intermediate. The origin of Types 2.1 and 2.2 from Type 4.3 is suggested.

4) Within sect. *Saxifraga* subsect. *Triplinervium*, *S. wahlenbergii* is unique in possessing pollen with a secondary tectum (Type 4.3), resembling the exine of *S. androsacea* in subsect. *Holophyllae*. We suggest transferring *S. wahlenbergii* to this subsection.

5) Himalayan species with opposite rather than alternate leaves that were formerly assigned to sect. *Porphyrion* subsect. *Oppositifoliae*, have exines with supratectal processes similar to those in subsect. *Kabschia*; in this respect they are unlike the European species with opposite leaves, which lack these processes.

6) Among the species with chalk-glands, exines lacking supratectal processes are restricted to European species, viz. those in sect. *Porphyrion* subsects. *Engleria* and *Oppositifoliae*, and *S. florulenta*, offering morphological support for the transfer of the latter species to sect. *Porphyrion*.

7) *Saxifragella* is recognised as a new section of the genus.

Keywords. Exine; *Saxifraga*; *Saxifragella*; Saxifragaceae; Palynology; Systematics.

Introduction

Saxifraga is the largest genus (about 470 species in 15 sections) in the family Saxifragaceae sensu stricto, where it appears to be sister to the other c. 30 genera, including *Micranthes*, a former section of *Saxifraga* (Soltis et al. 1996; Soltis et al. 2001a). *Saxifraga* is distributed chiefly in the mountains of Europe and Asia, with a few species occupying a circum-polar distribution and some extending southwards in the Americas down the Rocky Mt – Andean cordillera to Tierra del Fuego.

Early studies of pollen morphology within *Saxifraga* focused on Scandinavian species and revealed variation between species (Erdtman 1952; Faegri and Iverson 1964). A number of studies have examined very limited numbers of *Saxifraga* species as part of much wider taxonomic surveys of the Saxifragaceae sensu lato (Agababyan 1961; Pastre and Pons 1973 ; Wakabayashi 1970), but they came to no important conclusions about the infra-generic classification of *Saxifraga* itself. Moore (1969) used pollen morphology to support the separation of the genera *Saxifraga*, *Saxifragella* and *Saxifragodes*.

The first systematic palynological survey of the genus was conducted by Ferguson & Webb (1970) who reported the results of an extensive study of 105 species, with an emphasis on European taxa and including 17 that are now placed in *Micranthes*. They found four main types and a number of subtypes based chiefly on the morphology (particularly surface pattern) and anatomy of the exine, but also in part on grain shape and size. They concluded that, in most cases, the types and subtypes supported the infra-generic classification of Engler & Irmscher (1916/19). Later, Hideux and Ferguson (1976) investigated the stereostructure of the exine in Saxifragaceae sensu lato, but did not add any further species of *Saxifraga*. Kaplan (1981) extended the previous studies mainly by investigating the developmental events involved in the production of Type 4 pollen in *Saxifraga sieversiana*. He also added five more species to the total.

Two floristic studies, one of 26 *Saxifraga* species from north-western Europe (Verbeek-Reuvers 1977) and one of ten from Pakistan (Perveen and Qaiser 2009), reported observations made using the light microscope and by scanning electron microscopy (SEM). The emphasis was on identification rather than taxonomy, and the observations generally agreed with those of Ferguson & Webb (1970).

Previous studies have sampled only relatively sparingly the Sino-Himalayan species in sects. *Ciliatae* and *Porphyrion,* which together contain more than half of all species in the genus. Furthermore, Ferguson & Webb (1970) found it "surprising" that Type 2 pollen occurs in section *Irregulares*, which is distributed in eastern Asia, and in section *Saxifraga*, which occurs chiefly in Europe; in the phylogenetic tree of Soltis et al. (2001a) these sections are not closely related, and so it is rather unexpected that they have the same pollen type. Further study of the pollen in these two sections is therefore needed.

The aim of the present study is to extend the survey of exine structure in the genus to include more species from the Sino-Himalayan region, chiefly in sects. *Ciliatae* and *Porphyrion*. In addition we report on a detailed transmission electron microscopy (TEM) examination of 25 species, extending the sample of five studied by Ferguson & Webb (1970). In this respect, we focus particularly on species from sections *Irregulares* and *Saxifraga*. The results are combined with the data already provided by Ferguson & Webb (1970), Verbeek-Reuvers (1977) and Kaplan (1981) to shed more light on infra-generic taxonomy and on the evolution of the exine in the genus.

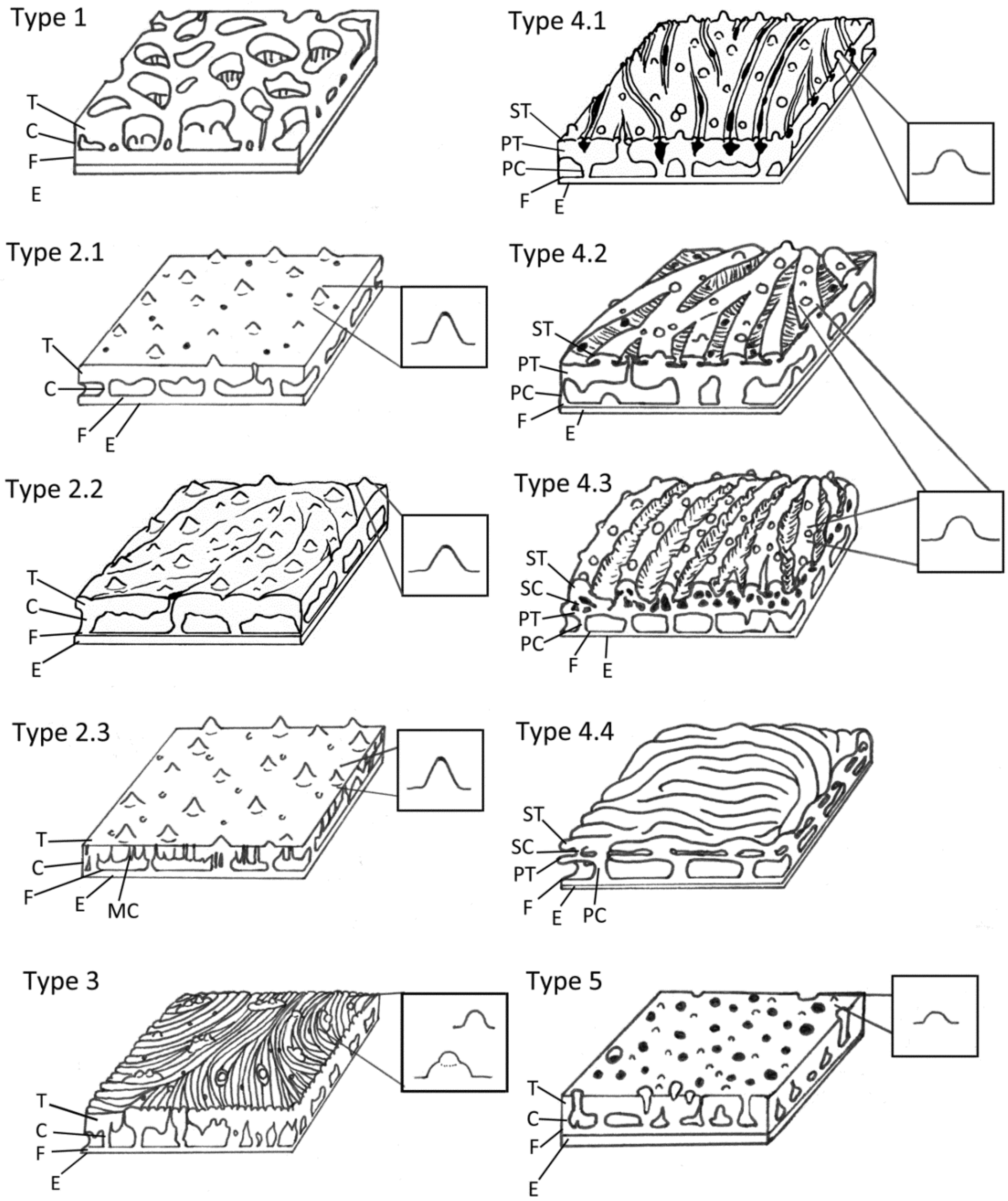
Materials and methods

Anthers of 141 *Saxifraga* species were studied. Most were collected from herbarium sheets housed in Harvard University-Arnold Arboretum (A), Natural History Museum, London (BM), the Royal Botanic Garden Edinburgh (E), the Northwest Plateau Institute of Biology, Xining (HNWP), the Royal Botanic Gardens Kew (K), the University of Leicester (LTR), and the University of Marburg (MB). A few species were collected fresh from University of Leicester Botanical Garden (hort. LTR). Collection details are listed in Table 3.2.

Pollen surface morphology of 132 *Saxifraga* species were studied by SEM and the exine anatomy of 25 species was studied by TEM. With the addition of reports from the literature, data for a total of 207 species are presented (Table 3.2). We have excluded data relating to the genus *Micranthes* because it is now established that this genus belongs outside *Saxifraga* (Soltis et al. 1996; Soltis et al. 2001a).

For the SEM study, dry pollen was released from anthers, mounted on a stub, gold-coated, and observed under a Hitachi S3000H PC scanning electron microscope. For the TEM study, anthers were acetolysed according to the method of Erdtman (1969) and prepared for microscopy following Glauert & Lewis (1998). Samples were examined on a JEOL 1400 TEM machine with an accelerating voltage of 80kV. The terminology used in this study follows Erdtman (1969) and Punt et al. (2007).

Figure 3.3. A illustration of exine stereostructure drawn from existed SEM and TEM images (Ferguson and Webb 1970; Hideux and Ferguson 1976) for each pattern. Supratectal elements were enlarged in separated squares to show details of the size and shape. E=Endexine, F=Foot layer, C=Columellae, T=Tectum, MC=Micro-capillaries, PC=Primary Columellae, PT=Primary Tectum, SC= Secondary Columellae, ST=Secondary Tectum.



Results

Pollen exine morphology

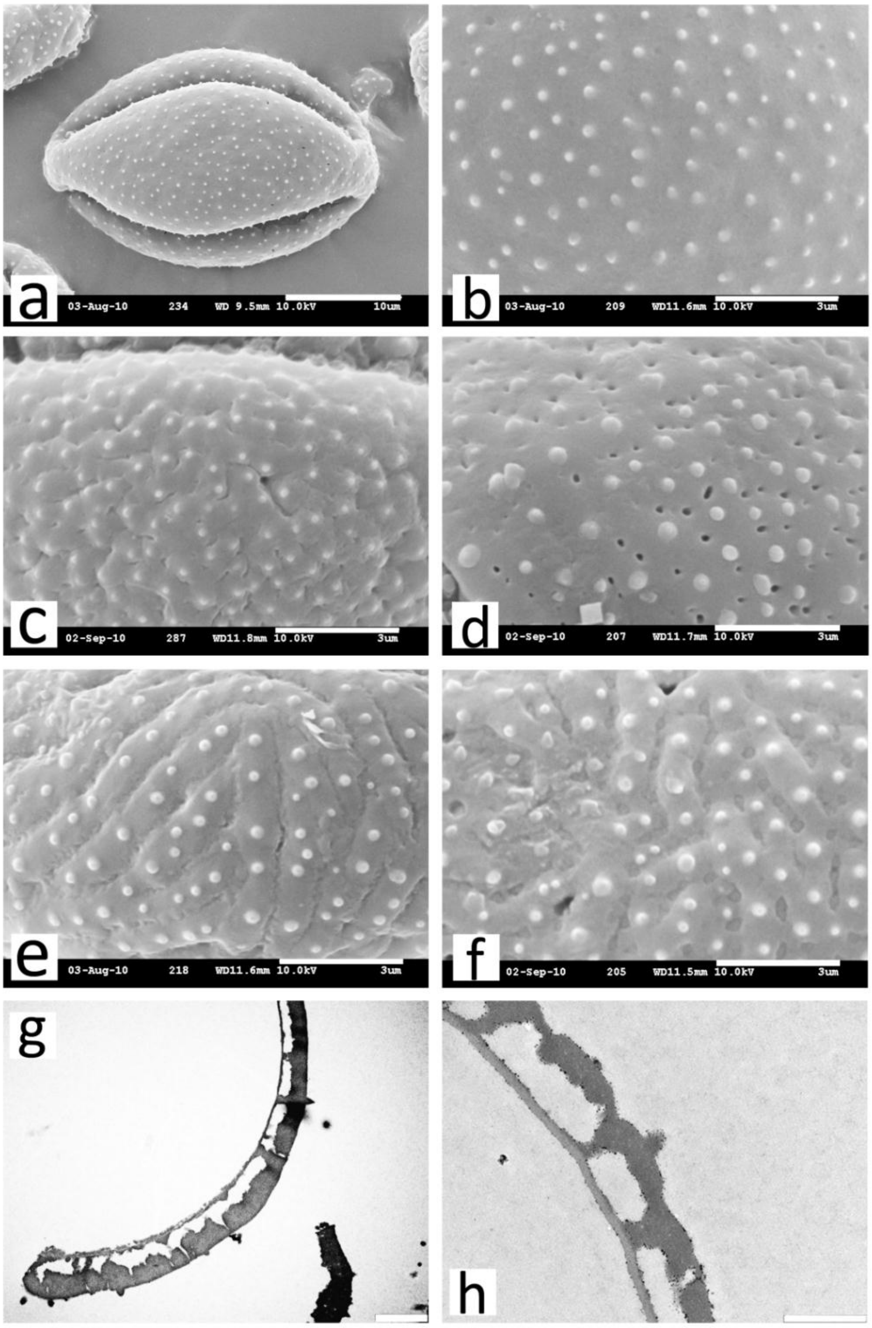
Key features of pollen exine structure are illustrated in Fig. 3.3 and summarised in Table 3.3. Characters that were recorded chiefly from SEM images are: surface pattern, surface perforations, and the occurrence and morphology of supratectal processes. Those recorded from TEM images are the details of exine anatomy, including the relative sizes of the different layers (Table 3.4). We have interpreted structures and patterns seen in SEM images in the light of observations made by TEM. But since the number of species examined by means of TEM is far fewer than that studied by SEM (Table 3.2), we are aware of the problem of making inferences about exine structure in the latter. However, in none of the cases where both sources of evidence were available, were our inferences confounded.

One of the most striking features of exine anatomy in *Saxifraga* is the reduced nature of the nexine (= foot-layer plus endexine) in all species examined. The ratio of primary sexine (= columellae plus tectum) to nexine is consistently at least 2, and often much higher (Table 3.4). In some cases the lighter-staining endexine could not be distinguished, in others it was the darker-staining foot-layer that was indiscernable, and in yet others the nexine was so thin and the staining differential so weak it was impossible to tell which layer predominated.

The main variation in exine structure relates to the tectum. This may be single-layered or double-layered. Whether a secondary tectum is present or not is often clear from SEM preparations in which the different layers are visible, but sometimes TEM observation is essential. Pollen surface pattern is due to the continuity and disposition of the outer tectal elements. It ranges from complete to variously perforate and, in this respect, one particular condition deserves mention: observable primarily in TEM preparations, the tectum is evenly punctured with radially orientated micro-capillaries or fissures, 0.03–0.15 µm in diameter. These are sometimes scarcely visible in SEM and are much smaller than perforations or pits, which are typically 0.09–0.3 (-0.6) µm in diameter. The outer tectum surface itself may be smooth, irregularly uneven, or finely or coarsely striate. The coarse striations (lirae) may run parallel to each other or interweave and anastomose; their morphology varies from long, worm-like structures, to much shorter, irregularly disposed elements. Supratectal processes comprise a) verrucae, which are small, low papillae typically less than 0.1 µm tall x ca 0.08–0.2 µm in diameter; and b) larger processes, which tend to be taller than 0.1 µm and ca (0.12) 0.2–0.6 µm in diameter. The latter can be divided into three kinds according to their shape: those with an acute apex (spinules), those with an obtuse apex (granules), and those with an obtuse apex and an expanded or elongated base (Fig. 3.3).

The exine variation patterns found in this study can be classified into nine types as described below. The taxonomic occurrence of each is shown in Table 3.2.

Figure 3.4. Type 2.1 (a, b, c, d, g) and Type 2.2 (e, f, h) pollens. a) *S. cebennensis*, whole grain, scale bar 10 µm; b) *S. pentadactylis*, smooth exine with spinulose supra-tectal processes, scale bar 3 µm; c) *S. carpetana*, exine with uneven surface, scale bar 3 µm; d) *S. praetermissa*, smooth exine with perforations, scale bar 3 µm; e) *S. aphylla*, exine with lirae-like sculpturing, scale bar 3 µm; f) *S. rosacea*, exine with lirae-like sculpturing, scale bar 3 µm; g) *S. praetermissa*, scale bar 2 µm; h) *S. aphylla*, scale bar 1 µm.



Type 2.1

Tectum single, micro-capillaries absent. Surface smooth to uneven and complete to sparsely perforate (more densely so in *S. praetermissa*). Supratectal processes spinulose (Fig. 3.4 a-d&g).

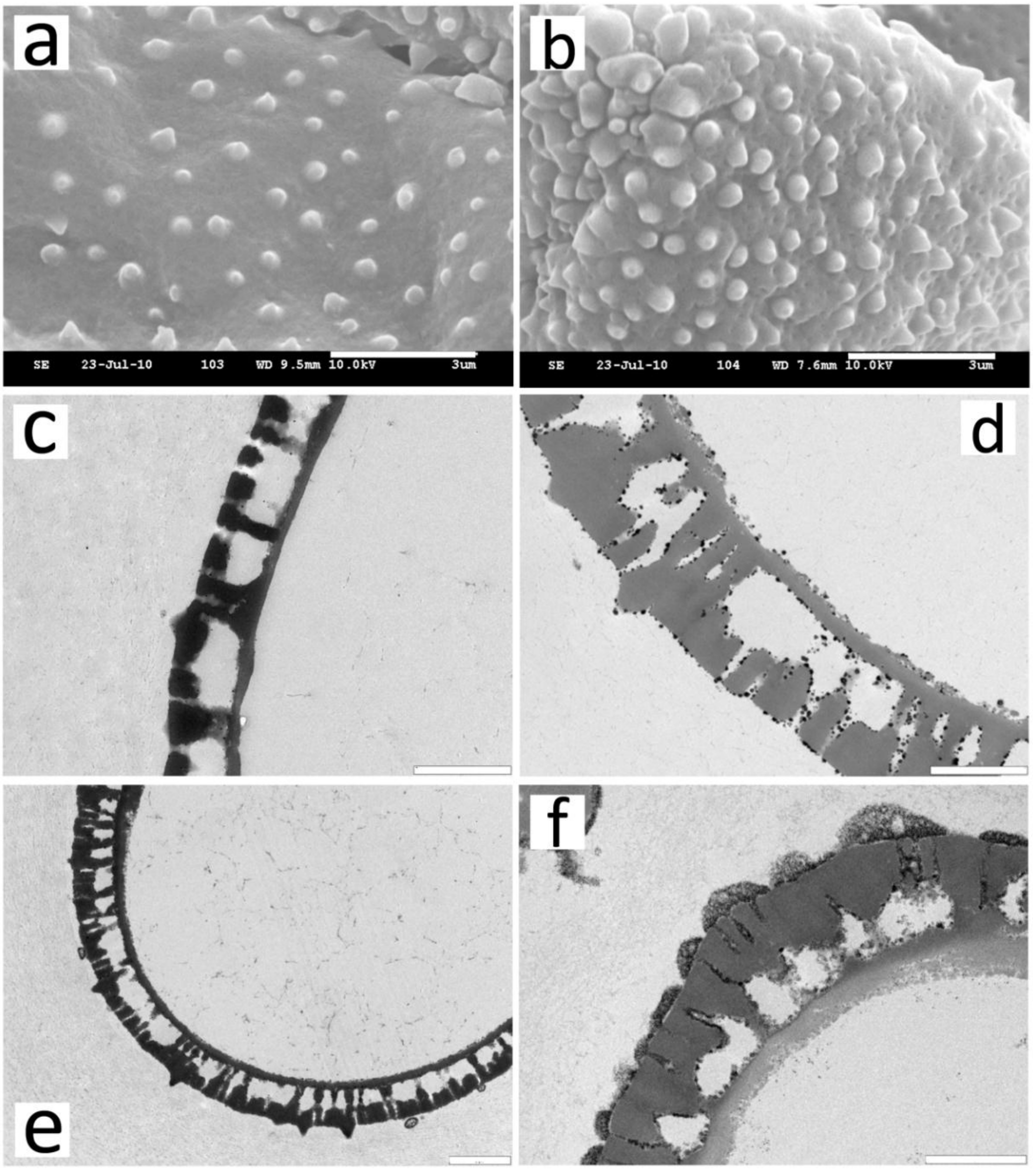
Type 2.2

Tectum single, micro-capillaries absent. Surface sculpted with lirae-like bands, set in low relief, complete or sparsely perforate. Supratectal processes spinulose (Fig. 3.4 efh).

Type 2.3

Tectum single, with numerous fissures or micro-capillaries that run radially between the outer and inner surfaces in all species studied (Fig. 3.5). These are much narrower than the perforations typical of other exine types. Surface smooth (*S. imparilis*) or shallowly pitted (*S. mertensiana*, *S. fortunei* and *S. rufescens*). Supratectal processes spinulose, sometimes in dense array.

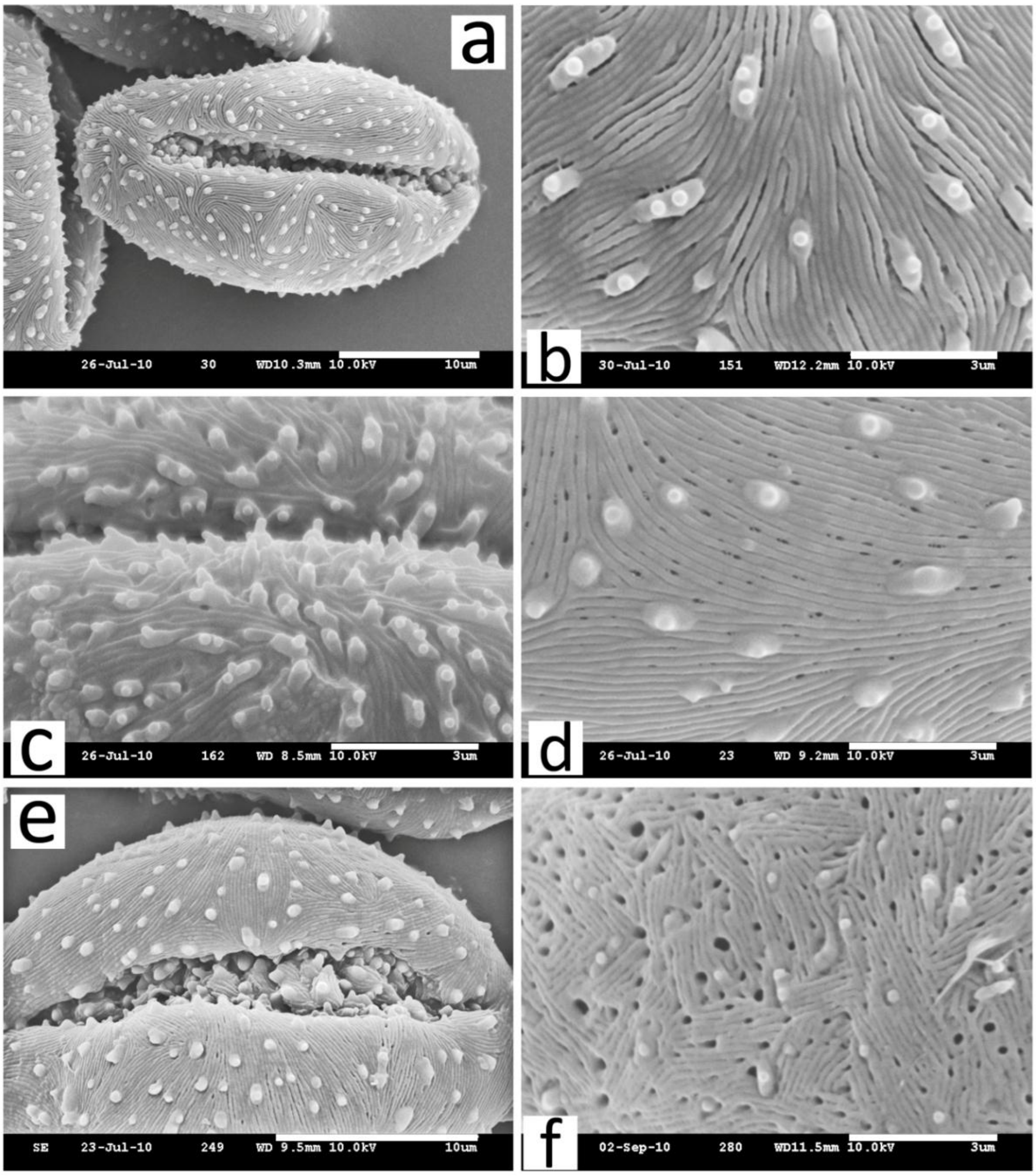
Figure 3.5. Type 2.3 exine. a) *S. imparilis*, exine surface, scale bar 3 µm; b) *S. rufescens*, exine surface showing pits, scale bar 3 µm; c) *S. mertensiana*, TEM of exine in cross-section, showing radial capillaries, scale bar 1 µm; d) *S. imparilis*, scale bar 1 µm; e) *S. stolonifera*, scale bar 2 µm; f) *S. fortunei*, TEM of exine in cross-section, showing radial capillaries, scale bar 1 µm.



Type 3

Tectum single, perforate to complete, without micro-capillaries (see illustration of TEM preparation of *S. brunonis* in Ferguson & Webb (Ferguson and Webb 1970). Surface with closely-set, narrow lirae (0.15–0.25 µm wide) giving a finely striate appearance (Fig. 3.6). In most species studied, the lirae usually run parallel to one another, but in at least *S. excellens* they have a more irregular disposition (Fig. 3.6 f). Supratectal processes granular, wider than the lirae and often straddling at least two, apices rounded, bases expanded/elongated or not, if expanded or elongated then sometimes shared by 2–5 granules (Fig. 3.6 b).

Figure 3.6. SEM pictures of Type 3 exines. a) *S. tibetica*, scale bar 10 µm; b) *S. hemisphaerica*, exine with supra-tectal processes showing an expanded base, scale bar 3 µm; c) *S. mucronulatoides*, scale bar 3 µm; d) *S. punctulata*, exine showing small perforations, scale bar 3 µm; e) *S. giraldiana*, exine showing supra-tectal processes, some with an expanded base and some without, scale bar 10 µm; f) *S. excellens*, showing perforations and a uniquely irregular lirae pattern, scale bar 3 µm.



Type 4.1

Tectum two-layered. Inner, primary tectum perforate. Secondary columellae short. Outer, secondary tectum composed of long, coarse lirae with distinctive marginal ridges, appearing like teddy-bear heads in TEM cross-section; orientation more or less parallel; striae infrequent, slit-like (Fig. 3.7 a, 3.8 a). Supratectal processes granular.

Type 4.2

Tectum two-layered. Inner, primary tectum perforate. Secondary columellae very short. Outer secondary tectum composed of long, coarse lirae, without marginal ridges; orientation more or less parallel to interweaving and anastomosing; striae width variable, but mostly narrower than the lirae (Fig. 3.7 b, 3.8 b). Supratectal processes granular.

Type 4.3

Tectum two-layered. Inner, primary tectum perforate to perforate-reticulate. Secondary columellae tall. Outer, secondary tectum composed of coarse lirae, without marginal ridges; length variable depending on species, the longer ones more or less parallel to interweaving and anastomosing, the shorter ones irregularly disposed, interwoven and anastomosed; striae width variable, wider or narrower than the lirae (Fig. 3.7 c-h, 3.8 c-h). Supratectal processes granular.

Figure 3.7. SEM pictures of Type 4.1 (a), Type 4.2 (b) and Type 4.3 (c-h) pollens. a) *S. taygetea*, scale bar 3 µm; b) *S. granulifera*, scale bar 3 µm; c) *S. rupicola*, scale bar 3 µm; d) *S. cinerea*, showing papillary-like lirae, scale bar 3 µm; e) *S. longifolia*, scale bar 10 µm; f) *S. cymbalaria*, scale bar 3 µm; g) *S. androsacea*, scale bar 3 µm; h) *S. wahlenbegii*, scale bar 10 µm.

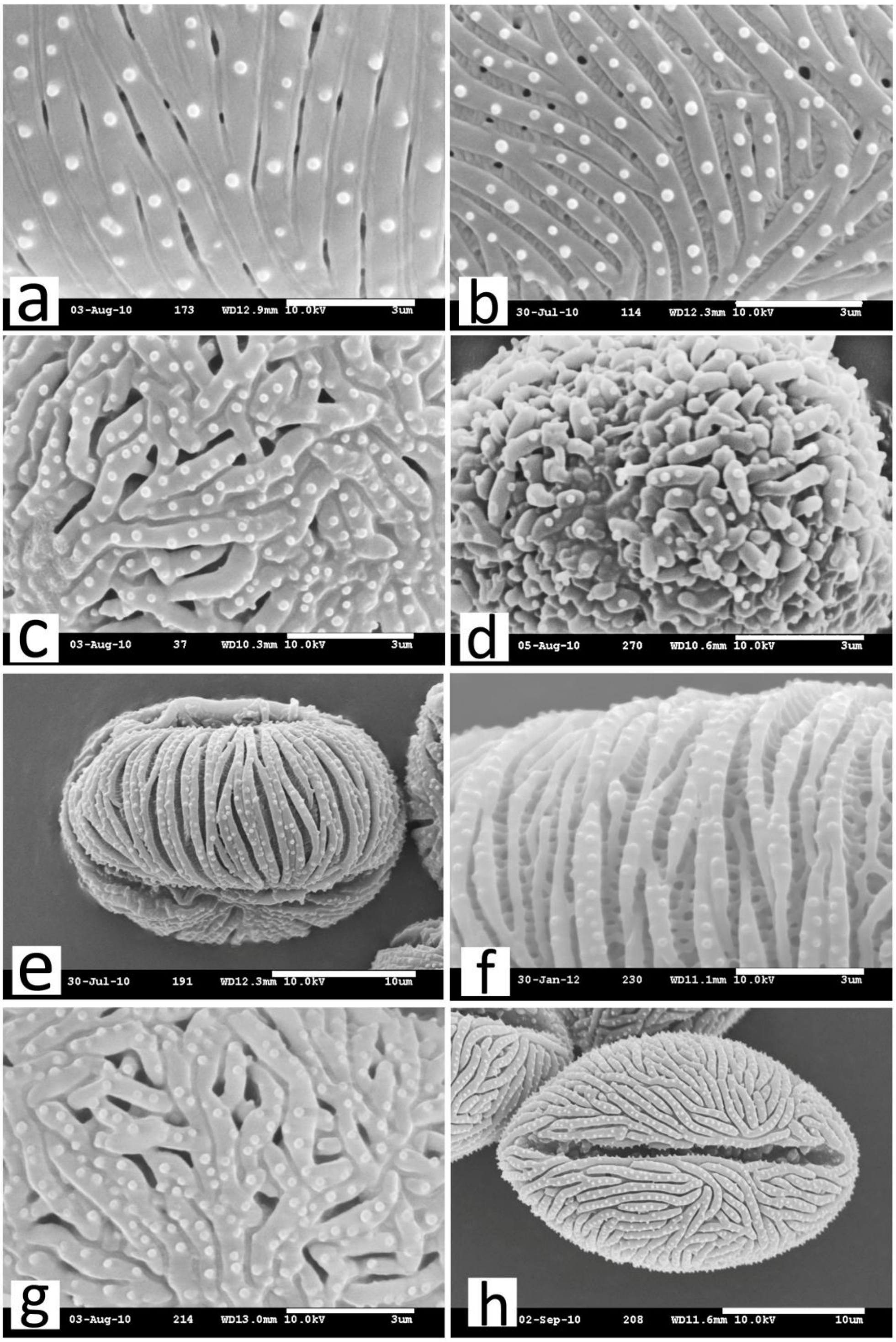
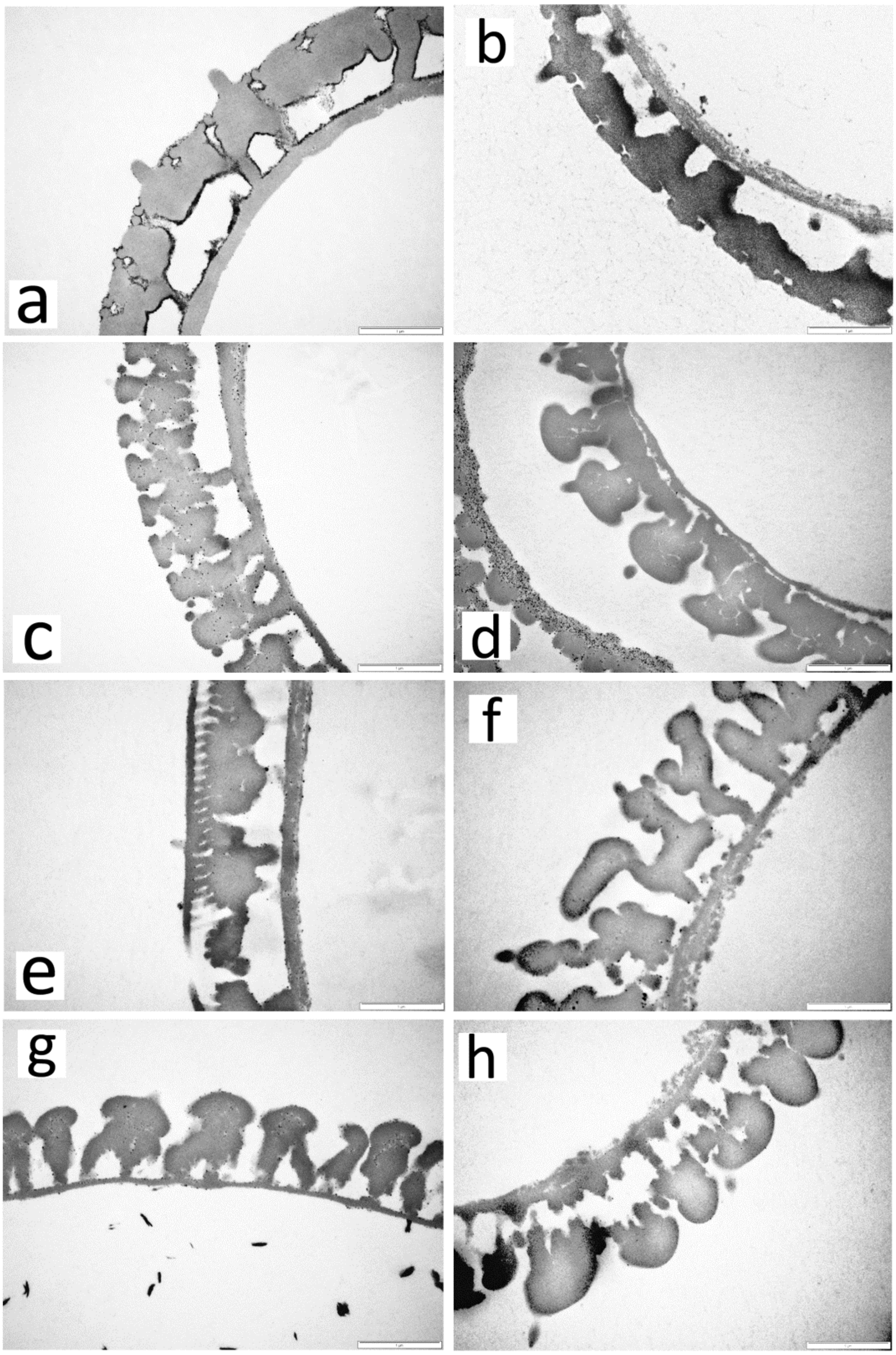


Figure 3.8. TEM picutres of Type 4.1 (a), Type 4.2 (b) and Type 4.3 (c-h) pollens. Scale bar 1 µm. a) *S. granulifera*; b) *S. hirsuta*; c) *S. mutata*; d) *S. rupicola*; e) *S. rotundifolia*; f) *S. androsacea*, showing discontinuous primary tectum; g) *S. wahlenbegii*, showing discontinuous primary tectum; h) *S. tridactylites*, showing obscure secondary columellae.



Type 4.4

Tectum two-layered. Inner, primary tectum complete. Secondary columellae tall. Outer, secondary tectum composed of coarse lirae, of variable length, without marginal ridges; orientation more or less parallel to interweaving and anastomosing; striae of variable width. Supratectal processes largely absent, occasionally sparsely verrucose (Fig. 3.9).

Type 5

Tectum single, perforate-reticulate, composed of a network of muri that anastomose regularly to form numerous small lumina, ca 0.2–0.5 µm in diameter. Sometimes the muri form a more irregular pattern, becoming rugulate-perforate, as in *S. lychnitis* and some collections of *S. oresbia*. Supratectal processes verrucose (Fig. 3.10).

Figure 3.9. Type 4.4 pollen. a) *S. corymbosa*, scale bar 10 µm; b) *S. corymbosa*, scale bar 3 µm; c) *S. media*, scale bar 3 µm; d) *S. media*, showing verrucae, scale bar 5 µm; e) *S. federici-augusti*, scale bar 3 µm; f) *S. oppositifolia*, showing continuous primary tectum, scale bar 2 µm; g) *S. oppositifolia*, scale bar 1 µm; h) *S. oppositifolia*, showing secondary columellae, scale bar 1 µm.

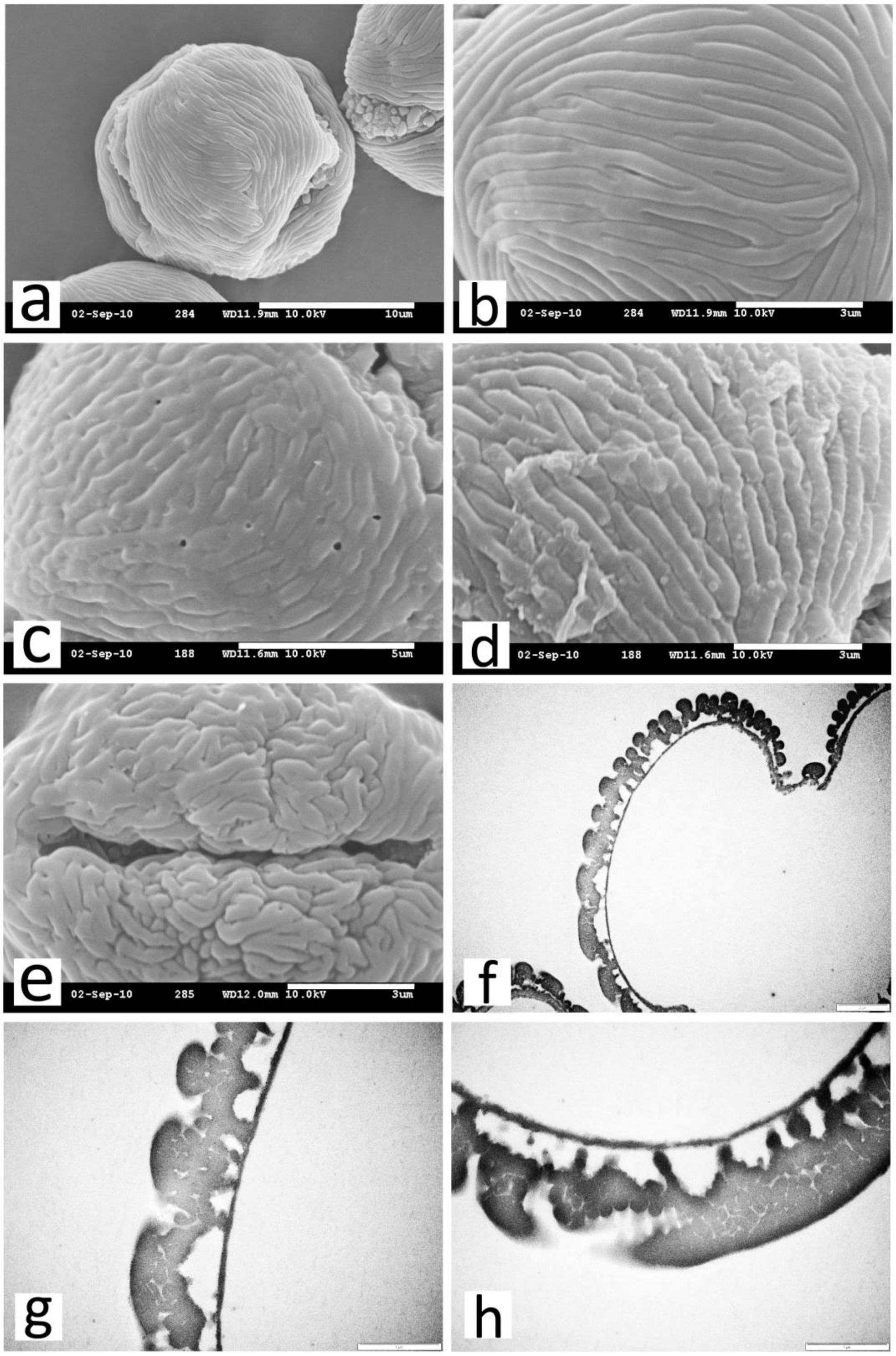
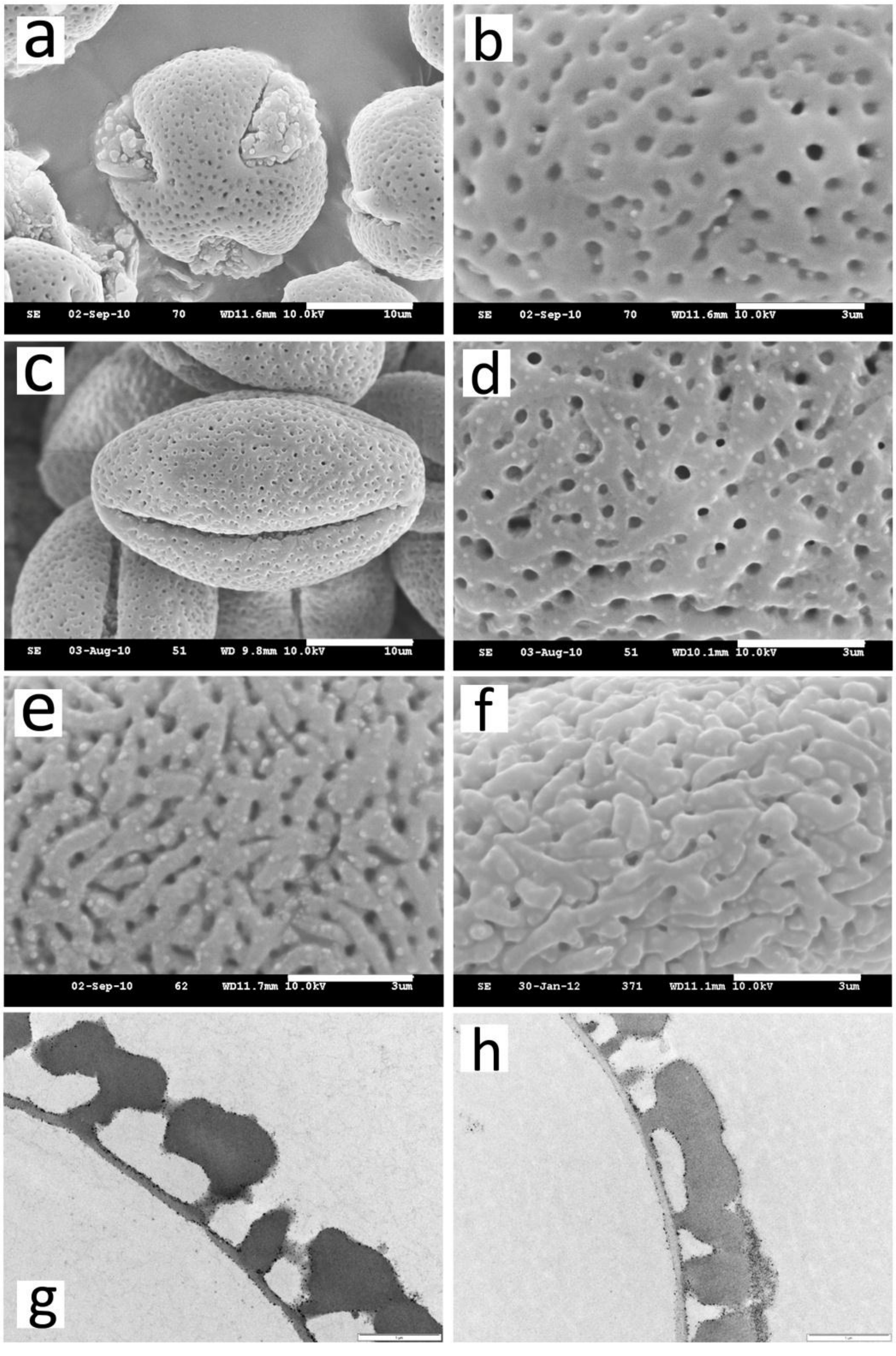


Figure 3.10. SEM (a-f) and TEM (g, h) picutres of Type 5 exines. a) *S. nigroglandulifera*, whole grain, scale bar 10 µm; b) *S. nigroglandulifera*, equatorial view of exine, scale bar 3 µm; c) *S. oresbia*, equatorial view of exine, scale bar 10 µm; d) *S. oresbia*, equatorial view of exine, scale bar 3 µm; e) *S. oresbia*, showing rugulate exine surface, scale bar 3 µm; f) *S. lychnitis*, showing perforate-rugulate exine surface, scale bar 3 µm; g) *S. oresbia*, showing perforate tectum, scale bar 1 µm; h) *S. nigroglandulifera*, scale bar 1 µm.



Discussion

Exine structure and the pollen types of Ferguson and Webb

The results of our survey largely conform to the four exine surface types recognised by Ferguson & Webb (1970), as summarised in Table 3.3. Nevertheless, some of their observations require extension and modification, including the addition of new types and subtypes, as described below.

Ferguson & Webb's Type 1, characterised by a single-layered, reticulate tectum without supra-tectal processes, does not occur in *Saxifraga*, but is widespread among the group of genera sister to *Saxifraga*, the Heucheroid clade, to which *Micranthes* (formerly a section of *Saxifraga*) belongs. In our study, Type 5 is similar in some respects to the reticulate-perforate exines of Ferguson & Webb's Type 1. It occurs in *Saxifraga lychnitis*, *S. oresbia* and *S. nigroglandulifera*, where the tectum varies from perforate-reticulate (with small lumina) to perforate-rugulate. It differs from Ferguson & Webb's Type 1 in that supratectal processes are present (as verrucae) and the nexine is much thinner (Table 3.4).

Type 2 pollen of Ferguson & Webb is characterised by a single tectum that is complete or more or less perforate and topped with spinulose supratectal processes. We found variation that warrants the recognition of three subtypes (Fig. 3.4, 3.5). In the first (Type 2.1) the tectum surface corresponds to Ferguson & Webb's original description, above. In the second (Type 2.2), the surface sculpturing is undulate, consisting of wide, lirae-like bands set in low relief but bearing the spinulose supratectal processes characteristic of Type 2.1

pollen. Our TEM studies show the presence of a single-layered tectum only, but the surface similarity to some Type 4 variants is obvious, a point we shall return to later. The third variant (Type 2.3) is similar to Type 2.1, except that the tectum is punctured throughout with numerous, very narrow (much narrower than the perforations in Type 2.1), radially-arranged micro-capillaries.

Our Type 3 corresponds to Type 3 of Ferguson & Webb. Although there is a certain amount of variation in the orientation of the fine lirae (Fig. 3.6), it is of a continuous nature and not amenable to division into subcategories.

One of the most widespread types in *Saxifraga* is Type 4, whose chief characteristic is the presence of a secondary tectum. This equates to our Types 4.1—4.4. Ferguson & Webb (1970) divided the variation into eight subtypes, based largely on exine features, viz. striae width and lirae height, width and orientation (parallel, anastomosing, or interweaving), and the occurrence of supratectal granules; they also used pollen size and shape in their subdivision of the type. Thus their Type 4 subtypes are better described as pollen types rather than strictly exine types. Their pollen Type 4a corresponds to our exine Type 4.1, characterised by short secondary columellae and slit-like striae (Fig. 3.7 a). Our observations also make it clear that, in addition, the lirae are bounded by ridges along the margin, a feature not seen elsewhere. Pollen Type 4b (exine Type 4.2) was distinguished from 4a partly by its smaller overall grain size but also by its “less distinct” exine pattern (1970). Our observations show that exine Type 4.2 differs chiefly from 4.1 in lacking the marginal ridges and in having longer striae (not slit-like) (Fig. 3.7 b).

According to Ferguson & Webb (1970), their pollen Types 4c and 4d possess "high lirae" (which we interpret as meaning relatively tall secondary columellae), that were said to run parallel to one another (Type 4c) or which anastomose and interweave (Type 4d). Our more extensive survey showed that the boundary between these two subtypes is difficult to maintain: the variation is considerable and involves not only subtle and continuous differences in lirae orientation but also differences in the lengths of the lirae themselves, from long continuous 'worms' to much shorter, irrregularly disposed structures, as in e.g. *Saxifraga cinerea* (Fig. 3.7 d). The patterns extend beyond the descriptions in Ferguson & Webb and defy easy classification. We propose, therefore, to merge Types 4c and 4d and designate them as exine Type 4.3. Verbeek-Reuvers (1997) came to the same conclusion for similar reasons. Type 4.3 differs from Type 4.2 mainly in its taller secondary columellae.

Pollen Type 4h of Ferguson & Webb (1970), found only in sect. *Cymbalaria*, is very similar to Type 4d (=4.3), it was said to differ in having "very high" lirae that were "coarsely and regularly verrucose" rather than granular. However, we found that both in our preparations (Fig. 3.7 f) and in the pictures provided by ((Ferguson and Webb 1970): Plate 7a, b), the "coarse verrucae" of Type 4h cannot be distinguished from the granules of exine Type 4.3 {(Ferguson and Webb 1970): plate 4a, b}. Although the height of the secondary columellae is on average greater than in most other exine types, there is overlap (Table 3.4). The taller lirae may simply be a function of the large pollen grains, the largest in the genus according to Ferguson & Webb. As far as the exine is concerned, therefore, we propose to classify it as Type 4.3.

The remaining three of Ferguson & Webb's pollen Types, viz. 4e, 4f and 4g, correspond to our exine Type 4.4. They share the absence of supratectal processes from the secondary tectum. The only difference between them in exine morphology is that the lirae were said to be "low" in Type 4g but "high" in Types 4e and 4f (Ferguson and Webb 1970). Comparison of the height of the secondary columellae in the TEM photograph provided by Ferguson & Webb with others in their paper and with our own preparations, however, shows that no real distinction can be made (Table 3.4). Otherwise the main difference between the three pollen subtypes lies in the shape of the grains: spheroidal (4e), markedly prolate (4f) or oblong (4g). Our observations of this character, however, show that it is quite variable, and difficult to apply consistently.

Taxonomic inferences

The following discussion is largely based on the classification proposed by Gornall (1987a), itself a modification of that of Engler & Irmscher (1916/19).

Micranthes

Formerly treated as *Saxifraga* sect. *Micranthes*, this taxon was elevated to generic status largely on molecular evidence (Soltis et al. 1996; Soltis et al. 2001a), although there are morphological characters that can also be used in support (Brouillet and Gornall 2007). Not least of these is pollen morphology, where the reticulate exine (Type 1 pollen) indicates a link with the Heucheroid clade of Saxifragaceae, where such a pattern is common (Hideux and Ferguson

1976). One possible further character is the possession by the pollen wall in *Micranthes* of a thick foot-layer and a thick endexine ((Ferguson and Webb 1970): plate 1J), which combined (the nexine) appriximately equal the thickness of the columella plus tectum (the sexine). This contrasts with the situation in *Saxifraga* where the nexine is very thin, half, or less, the thickness of the sexine (Table 3.4). (In some species the differentiation of endexine from foot-layer is obscure, and so we have recorded measurements of nexine thickness.) Whether the thick nexine is universally present in members of the Heucheroid clade remains to be demonstrated, although data that we have from observations of *Tellima grandiflora* and of *Lithophragma maxima*, indicate that it is present in at least these two species (Gornall, unpublished) as well as in *Micranthes*.

Saxifraga* sects. *Heterisia* and *Irregulares

Previously, sections *Irregulares, Heterisia* and *Saxifraga* had been described as having the same Type 2 (Ferguson and Webb 1970; Kaplan 1981). The former authors considered the similarity between sect. *Irregulares* and *Saxifraga* (*Heterisia* not studied) to be surprising, given the considerable morphological differences. Our TEM observations showed that sections *Heterisia* (1/1 sp. studied) and *Irregulares* (4/12 spp. studied) are characterised uniquely by the new Type 2.3, in which the tectum is punctured radially by numerous micro-capillaries (Fig. 3.5). The micro-capillaries occur more densely than in even the most perforate of the otherwise similar Type 2.1 species (*S. praetermissa*, Fig. 3.4 dg). The sharing of Type 2.3 between sects. *Irregulares* and *Heterisia* is strong anatomical evidence for a sister relationship, as indicated in the six-gene phylogeny of Soltis (2001a). Furthermore, our demonstration the the pollen in species of sect. *Saxifraga* lacks the micro-capillaries suggests that the exine subtypes in this section have a different origin.

Saxifraga* sect. *Saxifragella

Moore (1969) studied the pollen of *Saxifraga bicuspidata*, comparing it with that in *Saxifragodes albowiana* (F. Kurtz) D.M. Moore and *Saxifraga magellanica* Poir., related species from the same geographical area (southern S.America). His SEM images show that *S. bicuspidata* has a coarsely lirate tectum lacking supratectal processes, matching Type 4.4. This exine morphology occurs otherwise only in sect. *Porphyrion* subsects. *Engleria* and *Oppositifoliae*, and in *S. florulenta* (sect. *Aizoonia*, subsect. *Florulentae*), none of which is very closely related to *S. bicuspidata*. This suggests that the loss of supra-tectal processes has occurred at least twice.

Saxifraga bicuspidata for a long time was separated from *Saxifraga* as the monotypic genus *Saxifragella* on account of its haplostemonous androecium (Moore 1969). But then the six-gene phylogeny produced by Soltis et al. (2001a) showed that it is nested as a distinct lineage within *Saxifraga*. We here recognise it as a new, independent section of that genus.

Saxifraga section *Saxifragella* (Engler) Gornall & Zhang, comb. nov.

Basionym: *Saxifragella* Engler, in Engler & Prantl., Nat. Pflanzenfam. 3(2a): 61. 1891.

TYPE: *Saxifraga bicuspidata* Hook. f., Bot. Antarct. Voy. I. (Fl. Antarct.). 2: 281, t. 97. 1846 [ante 2 Mar 1846].

Saxifraga* sect. *Trachyphyllum

Species in sect. *Trachyphyllum* (3/10 spp. studied) are uniformly of Type 4.3. This pattern is common in the genus and is shared by five other sections, viz. *Saxifraga*, *Gymnopera, Ligutatae, Xanthizoon* and *Porphyrion*.

Saxifraga* sect. *Ciliatae

Nearly all the studied species of sect. *Ciliatae* (74/218 spp.) have Type 3 exines, a morphology found nowhere else in the genus. Despite some variation in the orientation and length of the fine lirae and in the morphology of the supratectal granules, it has not proved possible to recognise any exine subtypes (Fig. 3.6). Variation is continuous and occurs largely at the species level. Thus, with one exception, no comments can be made on the infra-sectional taxonomy.

The exception concerns three species: *S. nigroglandulifera* (ser. *Nutantes*), *S. lychnitis* and *S. oresbia* (both ser. *Lychnitidae*), which unusually have the novel Type 5, in which the tectum is perforate-reticulate or perforate-rugulate, with verrucose supratectal processes. The previous record of Type 3 pollen in *S. nigroglandulosa* by Ferguson & Webb (1970) is puzzling. We studied not only the same specimen as they did, viz. *Stainton et al. 2303* (BM), but also four other different collections. Our observations show that the species consistently has a Type 5 exine (Table 3.2, Fig. 3.10). The sharing of Type 5 provides evidence for a close relationship between ser. *Lychnitidae* and ser. *Nutantes*. The unusual exine type is a surprise because in other important respects the species concerned sit firmly in sect. *Ciliatae* subsect. *Hirculoideae*, sharing the yellow petals of the section and the trinucleate pollen and nodal, eglandular, crisped-rufous hairs, defining features of the subsection (Zhang and Gornall 2011).

Saxifraga* sect. *Cymbalaria

Species of sect. *Cymbalaria* (2/4 spp. studied) have Type 4.3 exines (Fig. 3.7 f). Ferguson & Webb (1970) designated the pollen as Type 4h mainly on account of its size, the largest among the species they surveyed.

Saxifraga* sect. *Saxifraga

Sect. *Saxifraga* (42/84 spp. studied) has been divided into four subsections (*Holophyllae, Tridactylites*, *Saxifraga* and *Triplinervium*) and numerous series. In subsection *Holophyllae* the tectum is either single but with an undulating surface of lirae-like bands (Type 2.2), or is a double structure with a secondary tectum of coarse lirae (Type 4.3). A double tectum also characterises subsect. *Tridactylites* (Type 4.3), although the secondary columellae are sometimes obscure (Fig. 3.8 h). A close relationship between these two subsections is implied.

With a few exceptions, species from the last two subsections, *Saxifraga* and *Triplinervium*, have a Type 2.1 exine, viz. a single tectum with a smooth to uneven surface and bearing spinules. The exceptional species are *S. petraea* and *S. rosacea* (both in subsect. *Triplinervium*) which have Type 2.2; and *S. wahlenbergii*, also in subsect. *Triplinervium*, which has Type 4.3. TEM studies suggest a closer relationship between *S. wahlenbergii* and species of subsect. *Holophyllae* series *Androsaceae* because in *S. wahlenbergii* the exine structure is identical to that of *S. androsacea*. In particular, they share a discontinuous primary tectum and tall secondary columellae (Fig. 3.8 fg). *S. wahlenbergii* also has short, prostrate shoots with clustered rosettes and terminal flowering stems like those of *S. depressa* from series *Androsaceae*. Wherever it properly belongs, *S. wahlenbergii* is an odd species in that it has bladdery trichomes that are not seen anywhere else in the genus (Gornall 1986).

Saxifraga* sect. *Cotylea

Species of sect. *Cotylea* (3/3 spp. studied) all have Type 4.1, an exine structure characterised by short secondary columellae supporting lirae with marginal ridges, unique in the genus (Fig. 3.7 a, 3.8 a).

Saxifraga* sect. *Mesogyne

In section *Mesogyne* (4/12 spp. studied) the exine structure is Type 4.2, characterised by a secondary tectum composed of granular lirae supported by short secondary columellae (Fig. 3.7 b, 3.8 b). There is, however, some variation. In *S. granulifera* the lirae are strikingly flattened and the striae are wide, but in other species, e.g. *S. carpatica*, the lirae are more convex and the striae narrower.

Saxifraga* sect. *Odontophyllae

The illustrations of the only two species in this section, *S. sieversiana* Sternb. and *S. odontophylla* Wall. & Sternb. (= *S. asarifolia* Wall. ex Hook.f. & Thoms.), provided by Kaplan (1981), suggest that the secondary columellae are tall, and therefore the exine corresponds to Type 4.3.

Formerly included in sect. *Micranthes* by Engler & Irmscher (1916/19), and then in their own section by Gornall (1987a), one of the species, *S. asarifolia,* was treated as belonging to sect. *Mesogyne* in the recent account of the genus in the *Flora of Nepal* (Akiyama and Gornall 2011). Whether the difference in the height of the secondary columellae is important remains to be seen.

Saxifraga sect. *Gymnopera*

In sect. *Gymnopera* (4/4 spp. studied), the pollen is of Type 4.3, which is also found in sects. *Trachyphyllum*, *Odontophyllae*, some members of sect. *Saxifraga* and in members of the two sections that have chalk-glands (lime-secreting hydathodes) on their leaf margins, sects. *Ligulatae* and *Porphyrion*. Support for a relationship of sect. *Gymnopera* specifically with sect. *Ligulatae* subsect. *Aizoonia* comes from a shared chromosome number of 2n=28, and one putative hybrid, *S*. x *andrewsii* (Webb and Gornall 1989). DNA sequence data also indicate a relationship in this direction (Conti et al. 1999). Verbeek-Reuvers (1977) suggested that the pollen of species of sect. *Gymnopera* had characteristically parallel lirae, but our observations and the illustrations in Verbeek-Reuvers (1977) and Ferguson & Webb (1970) suggest that the species show no clear difference from others in which the lirae interweave and anastomose.

Saxifraga sect. *Ligulatae*

Section *Ligulatae* (10/10 spp. studied) is divided into three subsections. Most species belong to subsect. *Aizoonia*; the other two subsections have one species each (*S. mutata* in subsect. *Mutatae*; and *S. florulenta* in subsect. *Florulentae*). The first two subsections have Type 4.3 pollen, whereas the latter has Type 4.4, which lacks supratectal processes. We are not surprised that *S. florulenta* is different because, unusually for the family, it has a gynoecium of three, rather than two, carpels. Its flesh-coloured petals also set it apart from members of subsect. *Aizoonia*. Other species that lack supra-tectal processes are those in sect. *Porphyrion* subsects. *Engleria* and *Oppositifoliae*, and a position in this section may be warranted. Convincing support for this idea comes from the ITS and matK sequence data of Conti et al. (1999). The taxonomic position of *S. mutata* is more likely to be with *S. aizoides* (sect. *Xanthizoon*) as discussed in the next section.

Saxifraga* sect. *Xanthizoon

The only species in sect. *Xanthizoon* is *S. aizoides*. Study shows that it has Type 4.3 pollen. The fact that the species has chalk-glands suggests a relationship with species of sects. *Ligulatae* and *Porphyrion*, which also share this pollen subtype. In particular, there appears to be a strong relationship with *S. mutata*: floral morphology is very similar; there is a convincing hybrid (S. x hausmannii) (Webb and Gornall 1989); and both ITS and matK sequence data indicate a sister relationship (Conti et al. 1999).

Saxifraga* sect. *Porphyrion

Section *Porphyrion* (56/104 spp. studied) is a large group centred primarily in the Sino-Himalaya but with a significant secondary centre of diversity in the European mountains. It is divided into three subsections: *Kabschia*, *Engleria* and *Oppositifolia*e. Species of subsect. *Kabschia* have exines of Type 4.3, and those of subsections *Engleria* and *Oppositifoliae* have Type 4.4. A group of Himalayan species with opposite leaves was assigned by Gornall (1987a) to subsect. *Oppositifoliae*, but SEM observations of their exine show that they have the same morphology, Type 4.3, as is seen in subsect. *Kabschia*. The species studied are *S. alpigena*, *S. georgei, S. monantha*, *S. pulvinaria*, *S. subternata*, *S. vacillans* and *S. nana*. The fact that some of these can make synthetic hybrids with other members of subsect. *Kabschia* but not with those of subsect. *Oppositifoliae* (McGregor 2008), offers further support for a position in the former rather than the latter.

Two other Himalayan species of interest are *S. chionophila* and *S. rupicola.* These have a corolla more or less equalling the calyx, rather than being much longer, and in this respect they are somewhat similar to species of subsect. *Engleria*, in which the campanulate calyx exceeds the corolla. Exine morphology, however, shows that these two Himalayan species have Type 4.3 exines, as in subsect. *Kabschia*, and therefore are best assigned there. This means that subsect. *Engleria* is an entirely European taxon.

Unfortunately, exine variation does not shed any light on how the species-rich subsect. *Kabschia* may be subdivided, as all studied species, from both Europe and the Sino-Himalayan region, have the same exine subtype.

Evolutionary trends in exine morphology

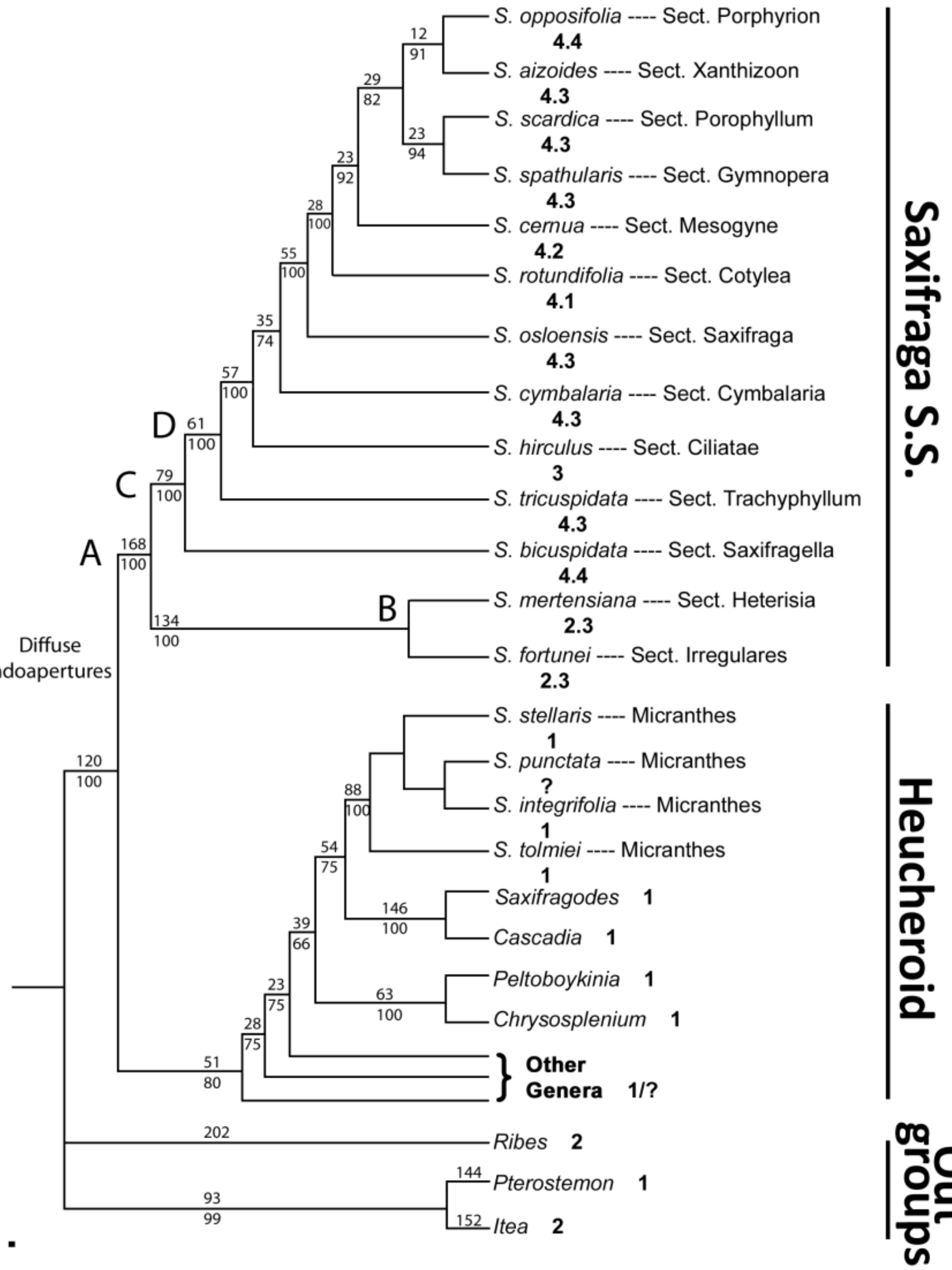
It is difficult to argue convincingly about evolutionary trends in pollen exine morphology and anatomy, particularly when we are largely ignorant of the adaptive significance of the various features and the consequent exposure to natural selection. The exine as a whole plays an important mechanical role in regulating its shape when dry and after hydration, its fit to a pollinator, its fit to a receptive stigma, and in the biochemical regulation of inter-specific and self-incompatibility (Heslop-Harrison 1979; Knox 1984; Zavada 1984). Nevertheless, the questions posed by Ferguson & Webb (1970) about the adaptive significance of individual features and their genetic control are still largely unanswered. The only relevant study in the context of *Saxifraga* is by Ferguson (1972) who showed the additive inheritance of supratectal processes in *S. nathorstii*, the allotetraploid hybrid between the granule-bearing *S. aizoides* and the granule-free *S. oppositifolia* (Böcher 1983).

Hideux & Ferguson (1976) arranged the different exine structures into degrees of evolutionary advancement. The primary level is a reticulate or perforate pattern without further structure or ornamentation (Type 1). The intermediate level is a partial or complete tectum with supratectal processes (Types 2.1—2.3, 5). The most derived level is a complete tectum with horizontal and vertical structures (Types 3 and 4.1—4.4). Within the Saxifragacaeae, *Saxifraga* was regarded as highly derived. Comparison of palynological data provided by Hideux & Ferguson (1976) with the phylogenetic tree of Soltis et al. (2001a) and Conti et al. (1999) (Fig. 3.11) shows that potential synapomorphies for the genus include diffuse endoapertures.

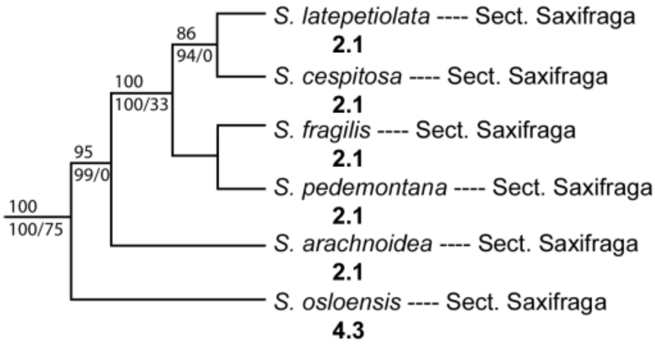
That a reticulate exine might represent the ancestral state in the Saxifragaceae was supported by developmental evidence provided by Kaplan (1981). He showed that the formation of the secondary tectum in *S. sieversiana* (Type 4.3) was preceded by an initial developmental stage whereby a reticulate tectal network formed on the primary columellae. Following the narrowing of the lumina, a secondary structure of lirae with granules was superimposed on the reticulate primary tectum. This ontology led Kaplan (1981) to suggest that Type 4 was derived from Type 1. He also went on to speculate on a close relationship between Types 2 and 4 chiefly because they occur in closely related species, and also suggested that Types 3 and 4 might have evolved in parallel from a common ancestor with a reticulate exine.

It is possible that the secondary tectum of Type 4 pollen had multiple origins (Fig. 3.11), though a more parsimonious explanation is that it originated only once, at node C. If so, then it means that there have been two losses, in the *S. hirculus* (sect. *Ciliatae*) and *S. osloensis* (sect. *Saxifraga*) lineages, respectively. Thus the complete, single tectum (Types 2.1 and 2.2) found in most studied species of sect. *Saxifraga* may be derived by merger or loss of a secondary tectum. Three arguments may be offered in support of this view.

Figure 3.11. Phylogenetic trees of *Saxifraga*. a) Redrawn from the 6-gene analysis by Soltis, et. al. (2001a), percentages below branches are bootstrap values and numbers above are base substitutions; b) redrawn from combined analysis of *matK* and ITS data set by Conti, et.al. (1999), the number above the branches are bootstrap values and the ones below are base substitutions. Where data available, especially for Heucheroid clade (Gornall and Bohm 1984; Hideux and Ferguson 1976; Kaplan 1981; Moore 1969), the exine types of the taxon were given below or after the names. Sections and genus of the species were indicated (Gornall 1987a). The diffuse endoapetures are plotted on the Saxifraga S.S. clade. Four nodes are labelled as A, B, C, and D respectively.


S. oppositifolia ---- Sect. Porphyrion
4.4
S. aizoides ---- Sect. Xanthizoon
4.3
S. scardica ---- Sect. Porophyllum
4.3
S. spathularis ---- Sect. Gymnopera
4.3
S. cernua ---- Sect. Mesogyne
4.2
S. rotundifolia ---- Sect. Cotylea
4.1
S. osloensis ---- Sect. Saxifraga
4.3
S. cymbalaria ---- Sect. Cymbalaria
4.3
S. hirculus ---- Sect. Ciliatae
3
S. tricuspidata ---- Sect. Trachyphyllum
4.3
S. bicuspidata ---- Sect. Saxifragella
4.4
S. mertensiana ---- Sect. Heterisia
2.3
S. fortunei ---- Sect. Irregulares
2.3
S. stellaris ---- Micranthes
1
S. punctata ---- Micranthes
?
S. integrifolia ---- Micranthes
1
S. tolmiei ---- Micranthes
1
Saxifragodes 1
Cascadia 1
Peltoboykinia 1
Chrysosplenium 1
Other Genera 1/?
Ribes 2
Pterostemon 1
Itea 2
Saxifraga S.S.
Heucheroid
Out groups
Diffuse endoapertures
A
B
C
D
12
91
29
82
23
94
23
92
28
100
55
100
35
74
57
100
61
100
79
100
168
100
134
100
120
100
88
100
54
75
146
100
39
66
63
100
23
75
28
75
51
80
202
144
93
99
152

1.


S. latepetiolata ---- Sect. Saxifraga
2.1
S. cespitosa ---- Sect. Saxifraga
2.1
S. fragilis ---- Sect. Saxifraga
2.1
S. pedemontana ---- Sect. Saxifraga
2.1
S. arachnoidea ---- Sect. Saxifraga
2.1
S. osloensis ---- Sect. Saxifraga
4.3
86
94/0
100
100/33
95
99/0
100
100/75

2.

Firstly, there is a series of intermediates between a double tectum and a smooth, single tectum that occurs among closely related species. Thus within sect. *Saxifraga*, a secondary tectum occurs in species of subsect. *Tridactylites* and in some species of subsect. *Holophyllae*. A single tectum, but with lirae-like sculpturing (Type 2.2, e.g. in *S. muscoides* and *S. aphylla*), strongly suggestive of Type 4 exines, is found in many species of subsect. *Holophyllae* as well as in a few in subsect. *Triplinervium*, e.g. *S. rosacea.* The sequence continues in the latter subsection where some species with Type 2.1 exines have markedly uneven surfaces (e.g. *S. bulbifera* and *S. aquatica*) and others are smooth (e.g. *S. pedemontana* and *S. dichotoma*).

The second piece of evidence for thinking the secondary tectum has been merged or lost comes from plotting the exine types onto the phylogenetic trees (Fig. 3.11 subtree 2) produced from molecular analysis (Conti et al. 1999). This shows that the ancestral state is a double tectum (Type 4.3 exine) and that a single tectum (Type 2.1) is derived from it.

Thirdly, in *S. tridactylites*, a member of subsect. *Tridactylites*, the secondary columellae are often obscure. This suggests that the putative loss may involve only the secondary columellae and that the single tectum seen in TEM sections is in fact a composite structure made up of the merged primary and secondary tecta. The end result of this evolutionary trend could be the smooth, single tectum (Type 2.1) found in many species from sect. *Saxifraga* subsects. *Saxifraga* and *Triplinervium*, with no sign of any former secondary structure.

Similarly, the uniquely finely striate, but single, tectum (Type 3) in sect. *Ciliatae* may be a reduced structure. This then brings us to the question of the evolutionary status of the single tectum in the basal lineage of *Saxifraga*, which comprises sects. *Heterisia* and *Irregulares*. Is it a composite structure or ancestrally simple? Outgroup analysis including *Ribes* and *Itea* suggests that it is ancestrally simple. However, the pollen of both these genera is highly derived and may not be representative of the basal groups.

The origin of the other major component of exine morphology, the occurrence of supratectal processes, is also difficult to pin down. By plotting the character on the phylogenetic trees (Conti et al. 1999; Soltis et al. 2001a) (Fig. 3.11 subtree 1), it is not clear whether the presence of supratectal processes is ancestral in *Saxifraga* (at node A) or whether it has evolved twice (at nodes B and D). Whichever the case, it does seem likely that their absence in *S. florulenta* and sect. *Porphyrion* subsects. *Engleria* and *Oppositifoliae* reflect one or more losses.

Note added for thesis: Due to only small differnces in the topology of the six gene phylogenetic tree (ref) used in this paper is similar to the ITS maximum parsimony tree in Chapter 2, phylogenetic discussion of pollen exine morphology would not change. The 4.4 exine of *S. florulenta* is a morphological evidence of the close relationship of this species and subsection *Oppositifolia* (Type 4.4) of sect. *Porphyrion* in the matK MP tree.

Acknowledgements

We thank David Boufford (Harvard University), Georg & Sabine Miehe (University of Marburg) and the curators of BM, E, K, HNWP, and LTR for making specimens available. We are especially grateful to Natalie Allcock and Stefan Hyman from the Electron Microscopy Lab for their work and help with the TEM and SEM photos.

Table 3.2. Collection details and exine types for the species of *Saxifraga* that have been studied. Classification is based on Gornall (Gornall 1987a). Exine types with an asterisk were the subject of TEM study in this work; those with "t" are from Ferguson & Webb (Ferguson and Webb 1970). Specimens are italicized. Locations of the herbaria in which the collections may be found are indicated by the standard acronyms.

Taxon	Exine type	Collection and location of voucher; or literature reference
Section *Heterisia*		
S. mertensiana Bongard	2.3*	*Hall 152* (BM)
Section *Irregulares*		
S. fortunei Hook.f.	2.3*	Hort.LTR; Hideux (1976)
S. imparilis I.B. Balf.	2.3*	*Boufford et al. 32626* (A); *Boufford et al. 32626* (A)
S. rufescens I.B. Balf.	2.3*	*Boufford et al. 33395* (A); *Boufford et al. 35278* (A)
S. stolonifera W. Curtis	2.3*	Hort.LTR; Ferguson & Webb (1970)
Section Saxifragella		
Saxifraga bicuspidata Hook. f.	4.4	Moore (1969); Hideux (1976)
Section *Trachyphyllum*		
S. aspera L.	4.3*	*Jury et al. 7089* (LTR); Ferguson & Webb (1970)
S. bryoides L.	4.3	Ferguson & Webb (1970)
S. tricuspidata Rottb.	4.3	*Hanfgarn 42* (BM)
Section *Ciliatae*		
Subsect. *Hirculoideae*		
Series *Hirculoideae*		
S. auriculata Engl. & Irmsch.	3	*Boufford et al. 40445* (A)
S. bergenioides Marquand	3	Ferguson & Webb (1970)
S. caveana W.W. Sm.	3	*Miehe 00-303-12* (MB)
S. congestiflora Engl. & Irmsch.	3	*Boufford et al. 36119* (A)
S. diffusicallosa C.Y. Wu	3	*Ludlow 6124* (BM)
S. diversifolia Wall. ex Ser.	3	Ferguson & Webb (1970)
S. giraldiana Engl.	3	*Boufford et al. 38592* (A)
S. hookeri Engl.& Irmsch.	3	Ferguson & Webb (1970)
S. heleonastes H. Sm.	3	*Boufford et al. 40083* (A)
S. hirculus L.	3	Ferguson & Webb (1970); Verbeek-Reuvers (1977)
S. hypericoides Franch.	3	*Kingdon Ward 12274* (BM)
S. implicans H. Sm.	3	*Dickoré 4719* (MB)
S. isophylla H. Sm.	3	*Boufford et al. 30140* (A)
S. latiflora Hook.f. & Thoms.	3	Ferguson & Webb (1970)
S. linearifolia Engl. & Irmsch.	3	*Boufford et al. 36197* (A)
S. macrostigma Franch.	3	*Boufford et al. 42024* (A)
S. montanella H. Sm.	3	*Ho et al. 2830* (E)
S. parva Hemsl.	3	*Chen.S.L. 20110058* (HNWP)
S. saginoides Hook.f. & Thoms.	3	*Lyon 11152* (E)
S. sinomontana J-T. Pan & Gornall	3	*Boufford et al. 34837* (A)
S. tangutica Engl.	3	Ferguson & Webb (1970)
S. tibetica Losinsk.	3	*Boufford et al. 29453* (A)
Series *Cinctae*		
S. excellens H. Sm.	3	*Stainton 7473* (BM)

Series *Lychnitidae*		
S. litangensis Engl.	3	*Boufford et al. 38591* (A)
S. lychnitis Hook.f. & Thoms.	5	*Ludlow & Sherriff 8972* (BM); Ferguson & Webb (1970)(3)
S. oresbia Anthony	5*	*Boufford et al. 36195* (A); *Boufford et al. 37414* (A)
S. pseudohirculus Engl.	3	*Boufford et al. 36581* (A); *Boufford et al. 34485* (A)
Series *Nutantes*		
S. nigroglandulifera Balakr.	5*	*Stainton et al. 2303* (BM); *McLaren N155* (BM); *Forrest 6599* (BM); *Rock 24634* (BM); *Boufford et al. 32299* (A); Ferguson & Webb (1970)
Subsect. *Rosulares*		
S. brunneopunctata H. Sm.	3	Ferguson & Webb (1970)
S. candelabrum Franch.	3	*Forrest 13036* (BM)
S. gyalana Marquand & Airy-Shaw	3	Ferguson & Webb (1970)
S. heterotricha Marquand & Airy-Shaw	3	*Dickoré 11867* (MB)
S. lhasana H. Sm.	3	Ferguson & Webb (1970)
S. muricola Marquand & Airy-Shaw	3	Ferguson & Webb (1970)
S. pasumensis Marquand & Airy-Shaw	3	Ferguson & Webb (1970)
S. punctulata Engl.	3	*Boufford et al. 31089* (A)
S. sanguinea Franch.	3	*Pratt 605* (K)
S. signata Engl. & Irmsch.	3	*Boufford et al. 33667* (A)
S. signatella Marquand	3	Ferguson & Webb (1970)
S. umbellulata Hook.f. & Thoms.	3	*Lyon 11013* (E)
S. unguiculata Engl.	3	*Boufford et al. 31481* (A)
S. vilmoriniana Engl. & Irmsch.	3	*Boufford et al. 36019* (A)
Subsect. *Serpyllifoliae*		
S. aurantiaca Franch.	3	Ferguson & Webb (1970)
S. brevicaulis H. Sm.	3	*Ludlow & Sherriff 8959* (BM)
S. contraria H. Sm.	3	*Ludlow & Sherriff 11017* (BM)
S. densifoliata Engl. & Irmsch.	3	*Boufford et al. 33197* (A)
S. engleriana H. Sm.	3	*Kingdon-Ward 11926* (BM)
S. flexilis W.W. Sm.	3	*Boufford et al. 34162* (A)
S. gemmigera Engl.	3	*Boufford et al. 39212* (A)
S. glacialis H. Sm. var. *rubra* Anthony	3	*Boufford et al. 31827* (A)
S. jacquemontiana Decne.	3	*Dickoré 5301* (MB)
S. llonakhensis W.W.Sm.	3	*Ludlow & Sherriff 11017* (E)
S. minutissima D.S.Rawat	3	*Rawat*, 24.viii.2005 (BM)
S. nanella Engl. & Irmsch.	3	*Boufford et al. 34102* (A)
S. stella-aurea Hook.f. & Thoms.	3	*Lyon 11233* (E)
S. tsarongensis Anthony	3	Ferguson & Webb (1970)
Subsect. *Hemisphaericae*		
S. hemisphaerica Hook.f. & Thoms.	3	*Dickoré 8529* (MB)
S. williamsii H. Sm.	3	*Stainton 2049* (BM); Kaplan (1981)
Subsect. *Gemmiparae*		
Series *Gemmiparae*		
S. balfourii Engl.& Irmsch.	3	*Forrest 23194* (BM)
S. brachypoda D. Don	3	Ferguson & Webb (1970)
S. cinerascens Engl.& Irmsch.	3	*Forrest 15172* (BM)
S. gemmipara Franch.	3	*Dickoré 14547* (MB)
S. hispidula D. Don	3	*Boufford et al. 35124* (A)
S. macrostigmatoides Engl.	3	*Smith 11168* (BM)
S. substrigosa J-T. Pan	3	*Dickoré 4944* (MB)

Series *Spinulosae*		
S. filicaulis Wall. ex Ser.	3	Ferguson & Webb (1970)
S. wallichiana Sternb.	3	*Boufford et al. 35849* (A)
Subsect. *Flagellares*		
S. brunonis Wall. ex Ser.	3[t]	Ferguson & Webb (1970)
S. consanguinea W.W. Sm.	3	*Boufford et al. 32197* (A)
S. flagellaris Willd.	3	Ferguson & Webb (1970)
S. microgyna Engl. & Irmsch.	3	*Boufford et al. 35760* (A)
S. mucronulatoides J-T. Pan	3	*Dickoré 10225* (MB)
S. pilifera Hook.f. & Thoms.	3	*Miehe & Wündisch 94-105-28* (MB)
Section *Cymbalaria*		
S. cymbalaria L.	4.3[t]	Hort.LTR ; Ferguson & Webb (1970); Hideux (1976)
S. sibthorpii Boiss.	4.3	*Polunin 12960* (LTR); Ferguson & Webb (1970)
Section *Saxifraga*		
Subsect. *Saxifraga*		
Series *Saxifraga*		
S. bulbifera L.	2.1	*Tutin 6161* (LTR); Ferguson & Webb (1970)
S. carpetana Boiss. & Reut.	2.1	*Stace,* 22.v.1981 (LTR)
S. cintrana Willk.	2.?	Ferguson & Webb (1970)
S. dichotoma Willd. in Sternb.	2.1	*Brummitt et al. 5804* (LTR); Ferguson & Webb (1970)
S. granulata L.	2.1	Ferguson & Webb (1970); Hideux (1976)
Series *Biternatae*		
S. biternata Boiss.	2.1	*Smythies 264* (LTR)
S. bourgaeana Boiss. & Reut.	2.1	*Allen 8767* (LTR); Ferguson & Webb (1970)
S. gemmulosa Boiss.	2.1	*Molesworth-Allen 7105* (LTR)
Subsect. *Triplinervium*		
Series *Aquaticae*		
S. aquatica Lapeyr.	2.1	*Horwood*, 25.vii.1965 (LTR); Ferguson & Webb (1970)
S. latepetiolata Willk.	2.1	Ferguson & Webb (1970)
Series *Arachnoideae*		
S. arachnoidea Sternb.	2.1	Ferguson & Webb (1970)
S. berica (Beguinot) D.A. Webb	2.?	Ferguson & Webb (1970)
S. paradoxa Sternb.	2.1	Ferguson & Webb (1970)
S. petraea L.	2.2	Ferguson & Webb (1970)
Series *Axilliflorae*		
S. praetermissa D.A. Webb	2.1*	*Leedal,* 1975 (LTR)
S. wahlenbergii J. Ball	4.3*	*Jasiewicz,* 7.iv.1957 (LTR)
Series *Ceratophyllae*		
S. camposii Boiss. & Reut.	2.1	*Polunin 9283* (LTR); Ferguson & Webb (1970)
S. fragilis Schrank	2.1	*Webb*, 2.vi.1967 (LTR); Ferguson & Webb (1970)
S. geranioides L.	2.1	Ferguson & Webb (1970)
S. intricata Lapeyr.	2.1	*Neyraut*, vii.1903 (LTR); *Polunin 9426* (LTR); Ferguson & Webb (1970)
S. pedemontana All.	2.1[t]	*Polunin 10924* (LTR); Ferguson & Webb (1970); Hideux (1976)
S. pentadactylis Lapeyr.	2.1	*Smythies 1279* (LTR)
S. trifurcata Schrad.	2.1	Ferguson & Webb (1970)
S. vayredana Luizet	2.1	*Smythies 283* (LTR); Ferguson & Webb (1970)
Series *Cespitosae*		
S. cebennensis Rouy & Camus	2.1	Hort.LTR
S. cespitosa L.	2.1*	*Gornall 629* (LTR); Verbeek-Reuvers

		(1977)
S. magellanica Poir.	2.1	Moore (1969)
S. moschata Wulf.	2.1(~2.2)	*Horwood*, 17.vii.1965 (LTR); Ferguson & Webb (1970); Verbeek-Reuvers (1977)
S. pubescens Pourr.	2.1	Hort.LTR
S. rosacea Moench	2.2*	*Gornall 674.1* (LTR)
Series *Gemmiferae*		
S. continentalis (Engl. & Irmsch.) D.A.Webb	2.1(~2.2)	*Tutin 287* (LTR); Ferguson & Webb (1970)
S. globulifera Desf.	2.1	*Molesworth-Allen 6212* (LTR)
S. hypnoides L.	2.1	Hort.LTR
Subsect. *Holophyllae*		
Series *Androsaceae*		
S. androsacea L.	4.3*	*Horwood*, 16.vii.1967 (LTR); Verbeek-Reuvers (1977)
S. depressa Sternb.	4.3	*Jury et al. 6760* (LTR)
S. presolanensis Engl.	4.3	*Webb 12.vii.1959* (LTR)
Series *Muscoideae*		
S. muscoides All.	2.2*	*Horwood*, 6.vii.1967 (LTR)
Series *Sedoideae*		
S. aphylla Sternb.	2.2*	*Halliday 122/59* (LTR)
S. sedoides L.	2.2	*Webb*, 26.vii.1963 (LTR); Ferguson & Webb (1970)
Series *Glabellae*		
S. glabella Bertol.	4.3*	*Polunin 13799* (LTR); *Akeroyd 74* (LTR); Ferguson & Webb (1970)
Series *Tenellae*		
S. tenella Wulf.	2.2	*Marchesetti*, v.1890 (LTR); Ferguson & Webb (1970)
Subsect. *Tridactylites*		
S. adscendens L.	4.3	Ferguson & Webb (1970); Verbeek-Reuvers (1977)
S. osloensis Knaben	4.3	Ferguson & Webb (1970)
S. tridactylites L.	4.3*	*Primavesi*, 9.v.1985 (LTR); *Fraser-Jenkins 11* (LTR); Ferguson & Webb (1970)(4.2)
Section *Cotylea*		
S. chrysospleniifolia Boiss.	4.1	Ferguson & Webb (1970)
S. rotundifolia L.	4.1*	Hort.LTR ; Ferguson & Webb (1970); Verbeek-Reuvers (1977)
S. taygetea Boiss. & Heldr.	4.1	*Polunin 15215* (LTR)
Section *Mesogyne*		
S. carpatica Sternb.	4.2	*Raciborski 868* (LTR)
S. cernua L.	4.2	Ferguson & Webb (1970); Verbeek-Reuvers (1977)
S. granulifera H. Sm.	4.2*	*Boufford et al. 32169* (A)
S. rivularis L.	4.2	Ferguson & Webb (1970)
Section *Odontophyllae*		
S. sieversiana Sternb.	4.3	Kaplan (1981)
S. odontophylla Wall. ex Sternb.	4.3	Kaplan (1981)
Section *Gymnopera*		
S. cuneifolia L.	4.3	Ferguson & Webb (1970)
S. hirsuta L.	4.3*	Hort.LTR ; Ferguson & Webb (1970); Verbeek-Reuvers (1977)
S. spathularis Brot.	4.3	Ferguson & Webb (1970)
S. umbrosa L.	4.3	Ferguson & Webb (1970)
Section *Ligulatae*		
Subsect. *Aizoonia*		

S. callosa Sm.	4.3	Ferguson & Webb (1970)
S. cochlearis Rchb.	4.3*	*Jolinon 566* (LTR)
S. cotyledon L.	4.3	Ferguson & Webb (1970)
S. crustata Vest	4.3	*Poldini 10279* (LTR)
S. hostii Tausch	4.3	Ferguson & Webb (1970)
S. longifolia Lapeyr.	4.3	*P.& J.M. Montserrat 1289/78* (LTR)
S. paniculata Mill.	4.3	Ferguson & Webb (1970)
S. valdensis DC.	4.3	Ferguson & Webb (1970)
Subsect. *Mutatae*		
S. mutata L.	4.3*	*Polunin 12421*(LTR); Ferguson & Webb (1970)
Subsect. *Florulentae*		
S. florulenta Moretti	4.4	Ferguson & Webb (1970); Hideux (1976)
Section *Xanthizoon*		
S. aizoides L.	4.3*	*Primavesi*, 18.vi.1988 (LTR); *Polunin 11418* (LTR); *Jury et al. 6934* (LTR); Ferguson & Webb (1970); Ferguson (1972); Verbeek-Reuvers (1977)
Section *Porphyrion*		
Subsect. *Kabschia*		
Series *Aretioideae*		
S. aretioides Lapeyr.	4.3	Ferguson & Webb (1970)
S. buceras H. Sm.	4.3	Kaplan (1981)
S. ferdinandi-coburgii Kellerer & Sünd.	4.3	Ferguson & Webb (1970)
S. kongboensis H. Sm.	4.3	*Ludlow et al. 13631* (BM)
S. nambulana H. Sm.	4.3	*Ludlow et al. 13850* (BM)
S. sherriffii H. Sm.	4.3	*Ludlow et al. 18972* (BM)
S. thiantha H. Sm.	4.3	*Ludlow & Sherriff 3210* (BM)
Series *Juniperifoliae*		
S. juniperifolia Adams	4.3	Ferguson & Webb (1970)
Series *Lilacinae*		
S. decora H. Sm.	4.3	*Hanbury-Tracy 154* (BM)
S. likiangensis Franch.	4.3	*Kingdon-Ward 11618* (BM)
S. ludlowii H. Sm.	4.3	*Ludlow et al. 13968* (BM)
Series *Marginatae*		
S. afghanica Aitch. & Hemsl.	4.3	*Dobremez 2279* (BM)
S. andersonii Engl.	4.3	*Polunin 386* (BM)
S. cinerea H. Sm.	4.3	*Lowndes 916* (BM)
S. lamarum H. Sm.	4.3	*Ludlow & Sherriff 9475* (BM)
S. marginata Sternb.	4.3	Ferguson & Webb (1970)
S. micans H. Sm.	4.3	*Dobremez 154* (BM)
S. ramulosa Wall. ex Ser.	4.3	Ferguson & Webb (1970)
S. rhodopetala H. Sm.	4.3	*Polunin et al. 2196* (BM)
S. scardica Griseb.	4.3	*Polunin 13045; Polunin 11126* (LTR)
S. spruneri Boiss.	4.3	*Polunin 10274* (LTR)
S. stolitzkae Duthie ex Engl.& Irmsch.	4.3	*Duthie 2892* (BM)
S. wendelboi Schönbeck-Temesy	4.3	Hort.LTR
Series *Squarrosae*		
S. caesia L.	4.3	Ferguson & Webb (1970)
S. squarrosa Sieber	4.3	Ferguson & Webb (1970)
Series *Subsessiliflorae*		
S. hypostoma H. Sm.	4.3	Ferguson & Webb (1970)
S. lolaensis H. Sm.	4.3	*Ludlow et al. 3775* (BM)
S. lowndesii H. Sm.	4.3	*Lowndes 958* (BM) ; Kaplan (1981)
S. matta-florida H. Sm.	4.3	*Ludlow et al. 20632.1* (BM)

S. mira H. Sm.	4.3	*Polunin, et al. 1094* (BM)
S. roylei H. Sm.	4.3	*Lyon 91* (BM)
S. saxorum H. Sm.	4.3	*Kingdon-Ward 13723* (BM)
S. staintonii H. Sm.	4.3	*Stainton Sylkes & Williams 7276* (BM)
Series *Rigidae*		
S. burseriana L.	4.3[t]	Ferguson & Webb (1970); Hideux (1976)
S. diapensioides Bell.	4.3	Ferguson & Webb (1970)
S. tombeanensis Boiss. ex Engl.	4.3	*Porta*, 13.v.1867 (LTR)
S. vandellii Sternb.	4.3	Ferguson & Webb (1970)
Series *Tetrameridium*		
S. alpigena H. Sm.	4.3	*Lowndes 959* (BM)
S. georgei Anthony	4.3	Ferguson & Webb (1970)
S. monantha H. Sm.	4.3	*Ludlow & Sherriff 1584* (BM)
S. nana Engl.	4.3	*Long et al.1213* (E)
S. pulvinaria H. Sm.	4.3	*Jyson 68* (BM)
S. subternata H. Sm.	4.3	*Ludlow et al. 4593* (BM)
S. vacillans H. Sm.	4.3	*Ludlow et al. 16352* (BM)
Subsect. *Engleria*		
S. chionophila Franch.	4.3	*Delavay*, 9.vii.1889 (P)
S. corymbosa Boiss.	4.4	*Webb,* 20.vii.1966 (LTR)
S. federici-augusti Biasol.	4.4	*Bierbach V. 1904* (LTR)
S. media Gouan	4.4	*Polunin 9165* (LTR); Ferguson & Webb (1970)
S. porophylla Bertol.	4.4	Ferguson & Webb (1970)
S. rupicola Franch.	4.3*	*Delavay*, 9.vii.1889 (P); *Delavay 285* (P)
S. sempervivum C. Koch	4.4	Ferguson & Webb (1970)
S. stribrnyi (Velen.) Podpera	4.4[t]	Ferguson & Webb (1970)
Subsect. *Oppositifoliae*		
Series *Oppositifoliae*		
S. biflora All.	4.4	Ferguson & Webb (1970)
S. nathorstii (Dusen) Hayek	4.4	Ferguson (1972)
S. oppositifolia L.	4.4*	*Primavesi,* 18.vi.1988 (LTR); *Fraser-Jenkins 2635* (LTR); *Stace,* 9.v.1961 (LTR); Ferguson & Webb (1970); Hideux (1976); Ferguson (1972); Verbeek-Reuvers (1977)
S. retusa Gouan	4.4	Ferguson & Webb (1970)

Table 3.3. Summary of exine types recognised in this study, with the corresponding pollen types of Ferguson & Webb (1970).

Exine type	F&W type	Lirae & striae	Tectum structure	Tectum perforation	Secondary columellae	Supra-tectal processes
1	1	n/a	single	reticulate	absent	absent
2.1	2	absent	single	perforate or complete	absent	spinules
2.2	2	lirae coarse and ribbon-like, striae ill-defined	single	perforate or complete	absent	spinules
2.3	n/a	absent	single	micro-capillaries	absent	spinules
3	3	lirae & striae fine	single	perforate or complete	absent	granules, most with base expanded
4.1	4a	lirae coarse and marginal ridges present, striae slit-like	double	primary tectum perforate	<0.1 µm	granules
4.2	4b	lirae coarse and marginal ridges absent, striae of variable width	double	primary tectum perforate	<0.1 µm	granules
4.3	4c & d	lirae coarse and marginal ridges absent, striae of variable width	double	primary tectum perforate	>0.1 µm	granules
4.4	4e, f & g	lirae coarse and marginal ridges absent, striae of variable width	double	?	>0.1 µm	absent or sparse verrucae
5	n/a	n/a	single	perforate-reticulate or perforate-rugulate	absent	verrucae

Table 3.4. Comparison of TEM observations on species representative of the different exine types. Data for species with an asterisk comes from Ferguson & Webb (1970).

Exine type	Species	exine thickness	endexine	foot-layer	nexine	1y columellae	1y tectum	1y tectum + columellae (1y sexine)	1y sexine : nexine	2y columellae	2y tectum	supratectal processes
1	Micranthes clusii*	1.05	0.20	0.30	0.50	0.10	0.45	0.55	1.1	n/a	n/a	n/a
2.3	Saxifraga mertensiana	0.96-1.33	0.10-0.13	0.03-0.06	0.13-0.18	0.33-0.38	0.25-0.45	0.59-0.81	3.6-5.0	n/a	n/a	0.25-0.35
2.3	Saxifraga stolonifera	1.54-1.83	0.13-0.26	0.01-0.06	0.14-0.30	0.48-0.63	0.44-0.60	0.98-1.16	3.3-7.6	n/a	n/a	0.26-0.50
2.3	Saxifraga rufescens	1.11-1.80	0.11	0.04	0.13-0.21	0.34-0.81	0.40-0.51	0.74-1.31	5.9-7.6	n/a	n/a	0.15-0.50
2.3	Saxifraga imparilis	1.36-1.61	0.09-0.19	0.02-0.04	0.11-0.23	0.46-0.74	0.41-0.50	0.90-1.15	5.0-9.9	n/a	n/a	0.20-0.43
2.3	Saxifraga fortunei	1.37-1.90	0.13-0.25	0.01-0.06	0.17-0.30	0.48-0.69	0.53-0.63	1.00-1.24	3.8-7.5	n/a	n/a	0.09-0.40
4.3	Saxifraga aspera	1.20-1.86	0.06-0.32	0.03-0.05	0.09-0.37	0.22-0.54	0.23-0.69	0.51-0.98	2.0-11.0	0.20-0.31	0.20-0.30	0.09-0.18
2.4	Saxifraga oresbia	1.35-1.69	0.09-0.13	0.04-0.07	0.14-0.19	0.48-0.69	0.63-0.89	1.10-1.44	7.1-9.3	n/a	n/a	0.09-0.11
2.4	Saxifraga nigroglandulifera	1.38-1.56	0.10-0.13	0.03-0.05	0.13-0.16	0.50-0.65	0.63-0.81	1.15-1.33	7.7-9.6	n/a	n/a	0.06-0.11
3	Saxifraga brunonis*	1.33-1.83	~0	0.17	0.17	0.66	0.66	1.32	7.8	n/a	n/a	
4.4	Saxifraga cymbalaria*	1.94-2.24	~0	0.15	0.15	0.53-0.60	ca 0.45	1.79-2.09	11.9-13.9	0.37-0.60	0.30-0.38	ca 0.10
2.2	Saxifraga aphylla	1.15-1.25	0.11-0.13	0.02-0.06	0.13-0.19	0.33-0.42	0.38-0.50	0.75-0.88	4.7-6.0	n/a	n/a	0.19-0.31
2.2	Saxifraga muscoides	0.86-1.31	0.05-0.11	0.03	0.08-0.14	0.25-0.54	0.38-0.50	0.63-1.04	6.9-9.7	n/a	n/a	0.13-0.25
4.3	Saxifraga androsacea	1.80-2.39	?	?	0.09-0.35	0.26-0.38	0.38-0.63	0.70-0.93	2.6-8.1	0.30-0.88	0.36-0.50	0.10-0.25
4.3	Saxifraga glabella	0.84-1.31	?	?	0.06-0.11	0.18-0.23	0.20-0.31	0.38-0.53	3.4-7.8	0.10-0.20	0.20-0.30	0.05-0.19
4.3	Saxifraga tridactylites	1.10-1.66	0.17	0.06	0.06-0.23	0.25-0.45	0.20-0.35	0.51-0.80	2.7-8.2	0.09-0.16	0.22-0.36	0.09-0.29
2.1	Saxifraga praetermissa	1.20-1.78	?	?	0.07-	0.39-0.63	0.55-0.74	0.94-1.36	8.0-14.9	n/a	n/a	0.19-

					0.16							0.31
2.1	Saxifraga cespitosa	1.32-1.79	0.06-0.08	0.02-0.04	0.09-0.10	0.44-0.63	0.63-0.69	1.06-1.31	11.3-13.6	n/a	n/a	0.16-0.38
2.1	Saxifraga pedmontana ssp. prostii*	1.25-1.54	~0	0.21	0.21	0.42	0.42-0.56	0.84-0.98	4.0-4.7	n/a	n/a	?
2.2	Saxifraga rosacea	1.25-1.38	0.08-0.19	0.03-0.05	0.10-0.24	0.40-0.50	0.46-0.58	0.90-1.00	3.8-9.0	n/a	n/a	0.19-0.28
4.3	Saxifraga wahlenbergii	1.23-1.57	?	?	0.06-0.16	0.11-0.28	0.31-0.44	0.44-0.63	3.8-10.0	0.20-0.30	0.28-0.40	0.11-0.19
4.1	Saxifraga rotundifolia	1.27-1.79	0.13	0.06	0.13-0.19	0.40-0.60	0.44-0.49	0.84-1.09	4.5-8.7	0.03-0.08	0.10-0.16	0.11-0.38
4.2	Saxifraga granulifera	1.44-1.86	0.09-0.15	0.01-0.04	0.10-0.19	0.40-0.63	0.35-0.56	0.80-1.13	5.3-8.4	0.01-0.04	0.10-0.15	0.20-0.40
4.3	Saxifraga hirsuta	1.48-1.91	0.17	0.05	0.10-0.23	0.31-0.70	0.38-0.53	0.83-1.08	4.4-9.5	0.20-0.40	0.08-0.12	0.06-0.24
4.3	Saxifraga cochlearis	1.44-1.74	0.05-0.06	0.09-0.10	0.06-0.16	0.25-0.29	0.29-0.50	0.56-0.75	3.5-12.0	0.30-0.45	0.25-0.40	0.13-0.24
4.3	Saxifraga mutata	1.05-1.49	0.03	0.14	0.08-0.16	0.25-0.38	0.25-0.53	0.50-0.86	5.0-8.5	0.12-0.25	0.12-0.18	0.06-0.14
4.5	Saxifraga oppositifolia	1.04-1.47	?	?	0.08-0.20	0.18-0.38	0.30-0.50	0.48-0.75	2.4-7.7	0.12-0.30	0.24-0.36	0.00(-0.05)
4.3	Saxifraga aizoides	1.38-1.57	?	?	0.06-0.19	0.30-0.46	0.25-0.44	0.56-0.90	3.7-11.1	0.20-0.40	0.20-0.30	0.09-0.15
4.3	Saxifraga rupicola	1.26-1.71	?	?	0.05-0.10	0.09-0.19	0.25-0.46	0.35-0.65	6.2-9.3	0.30-0.42	0.30-0.40	0.13-0.25
4.3	Saxifraga burseriana*								2.4			
4.5	Saxifraga stribrnyi*	1.35-1.46	0.2	0.1	0.31	0.31-0.42	0.21	0.52-0.62	2	0.21-0.26	0.16-0.21	?

Chapter Four: Leaf venation data

4.1 Introduction

Characters from leaf venation were once considered difficult to use for taxonomic purposes, owing to problems with their description. However, there are now several classifications of leaf architectural structure, reviewed by Pole (1991) and Roth-Nebelsick et al. (2001). The most widely used and comprehensive classification was produced by Hickey (1973, 1979) after a broad survey. He deconstructed leaf architecture into several elements, viz. shape, marginal configuration, venation, and gland structure and position. In addition to basic venation types, he also focused on vein order and the point of origin. The Hickey classification was developed further in the *Manual of leaf architecture* (Ellis et al. 2009).

Venation has been shown to be an important character for specifying and grouping plants (Walls 2011). Patterns of veins can be used as fingerprints to characterize plant taxa (Corner 1968). Many leaf traits are phylogenetically conserved and have a significant impact on leaf form-function relations (Walls 2011). Studies show that the origin of venation diversity is a consequence of the positive feedback regulation between auxin and its efflux carrier as determined by the various leaf shapes and expansion patterns (Dengler and Kang 2001; Fujita and Mochizuki 2006).

The vein patterns in the genus *Saxifraga* have not been studied before. In this study, species from the main *Saxifraga* sections and subsections were selected to cover the range of venation patterns in the genus. The aim was to test whether leaf venation contains any systematic information and, if so, at what taxonomic level.

4.2 Materials and methods

Leaves from one *Micranthes* and 156 *Saxifraga* species were collected from herbarium sheets or fresh plants (Appendix 2).

Venation patterns were investigated following a modification of the method used by Payne (1969). One to several leaves, with petioles if not sessile, were collected into petri dishes and submerged in 10% NaOH at room temperature until they became translucent. This step took one to three weeks, depending on species. Then the leaves were washes twice with water and bleached in 50% commercial bleach for 10 minutes. After another two washes, the leaves were dehydrated for 10 minutes in 50% and then 75% ethanol. Leaves were then stained with 1% Safranin O in 95% ethanol for 20 minutes. Stain was washed off with absolute ethanol. The leaves were submerged in 1:1 absolute ethanol : Histo-clear, and then mounted in DPX resin between glass slides and cover slips. Photos of venation patterns were taken at a range of magnifications with a camera attached to a disserting microscope.

4.3 Results

Following to the terminology of Hickey (1973), there are three main venation types in the genus *Saxifraga*: acrodromous, actinodromous and camptodromous. Pictures of some species were selected to show the main types in *Saxifraga* (Fig. 4.1-11). Drawings for types are given for a clearer view (Fig. 4.12-14).

Acrodromous venation in *Saxifraga* is the type in which three or more primary (or strongly developed secondary) veins run in convergent arches and join at one point in the apex (Fig. 4.1, 4.12). A variant of this type with more primary veins which increase the size of the network (Fig. 4.1: 3, 4, 8) was also encountered and is referred to as campylodromous venation, but the difference was sometimes difficult to recognise.

Actinodromous venation is the palmate type with three or more primary veins diverging from one point into different teeth or lobes and terminating in hydathodes but do not reach the margins (Fig. 4.2-7, 4.13). In one variant (palinactinodromous), there are more points of origin and more primary veins (Fig. 4.2: 2-3, 7; 4.4; 4.13), although again the distinction was sometimes unclear.

Camptodromous venation is basically pinnate, but where the veins do not end at the margin (Fig. 4.8-10, 4.14). The brochidodromous variant has secondary (or 3rd) veins joining together near to the margin (Fig. 4.14). The eucamptodromous variant has free-ending, simple secondary veins that do not form loops (Fig. 4.14). The cladodromous variant has branched secondary veins ending freely (Fig. 4.14).

Three other venation characters were recorded: marginal ultimate venation, vein diameter, and the presence of foliar sclereids. The marginal veins can be closed (Fig. 4.1), open (Fig. 4.9: 4) or intermediate (Fig. 4.8: 5), depending on the degree of joining near to the margin. The behaviour of vein diameter over their course is an interesting finding in this study. In the pictures, the veins are thickened in parts (Fig. 4.5: 3-4) or over the whole network (Fig. 4.8: 6). The expansion is caused by dark-stained elongated cells in the vascular sheath (Fig. 4.11: 4). Foliar sclereids occur in a few species. Where they occur, they are parallel, linear, dark-stained cells clustered at the base of the leaf (Fig. 4.11: 1-3).

Figure 4.1. Pictures of acrodromous vein. 1-2: S. gyalana; 3: S. wallichiana; 4: S. diversifolia; 5: S. caveana; 6: S. pasumensis; 7: S. tenella; 8: S. giraldiana.

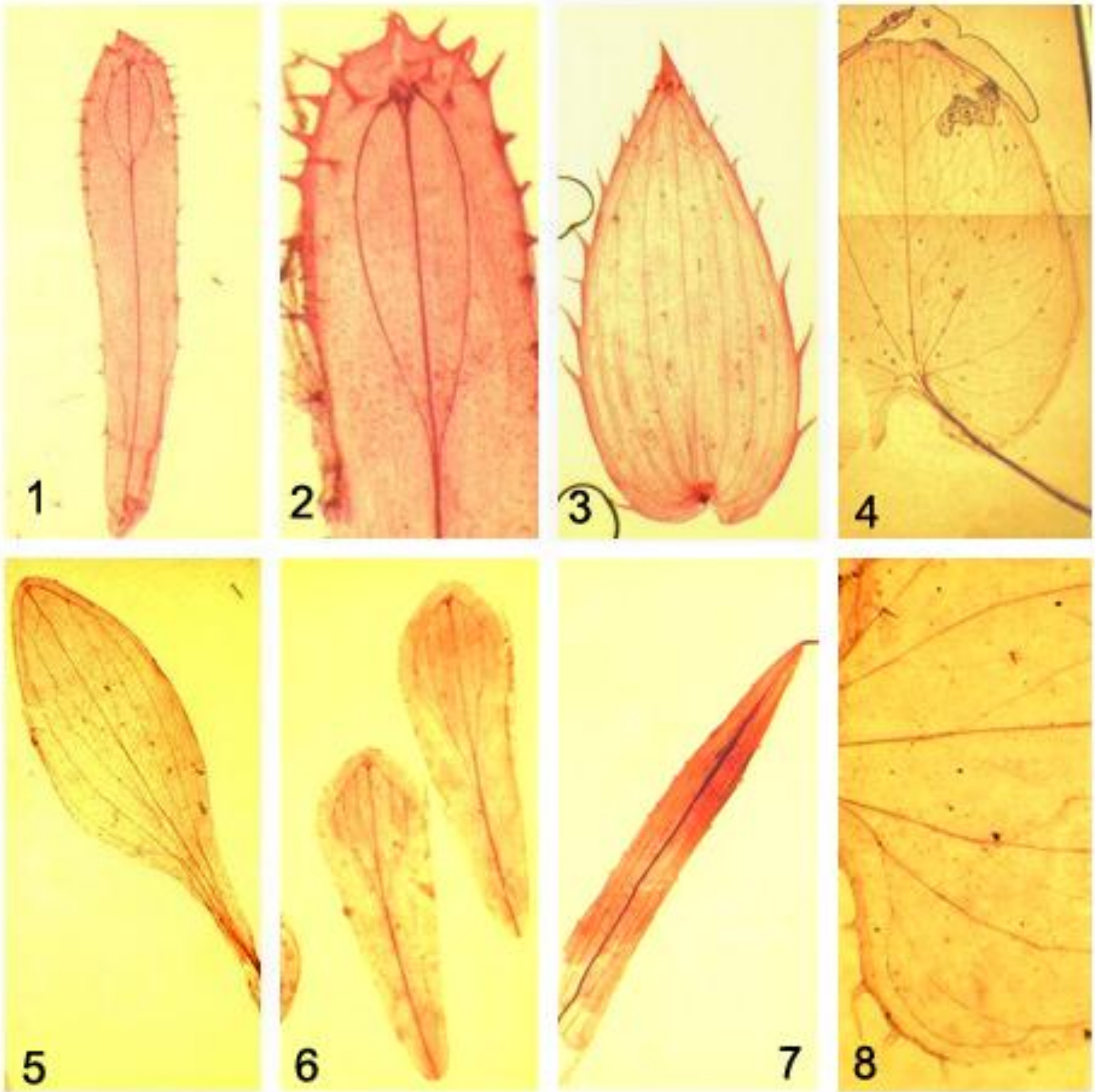


Figure 4.2. Pictures of actinodromous venation. 1: S. hispidula; 2-3: S. imparilis; 4-5: S. granulifera; 6: S. cuneifolia; 7: S. granulata; 8: S. taygetea.

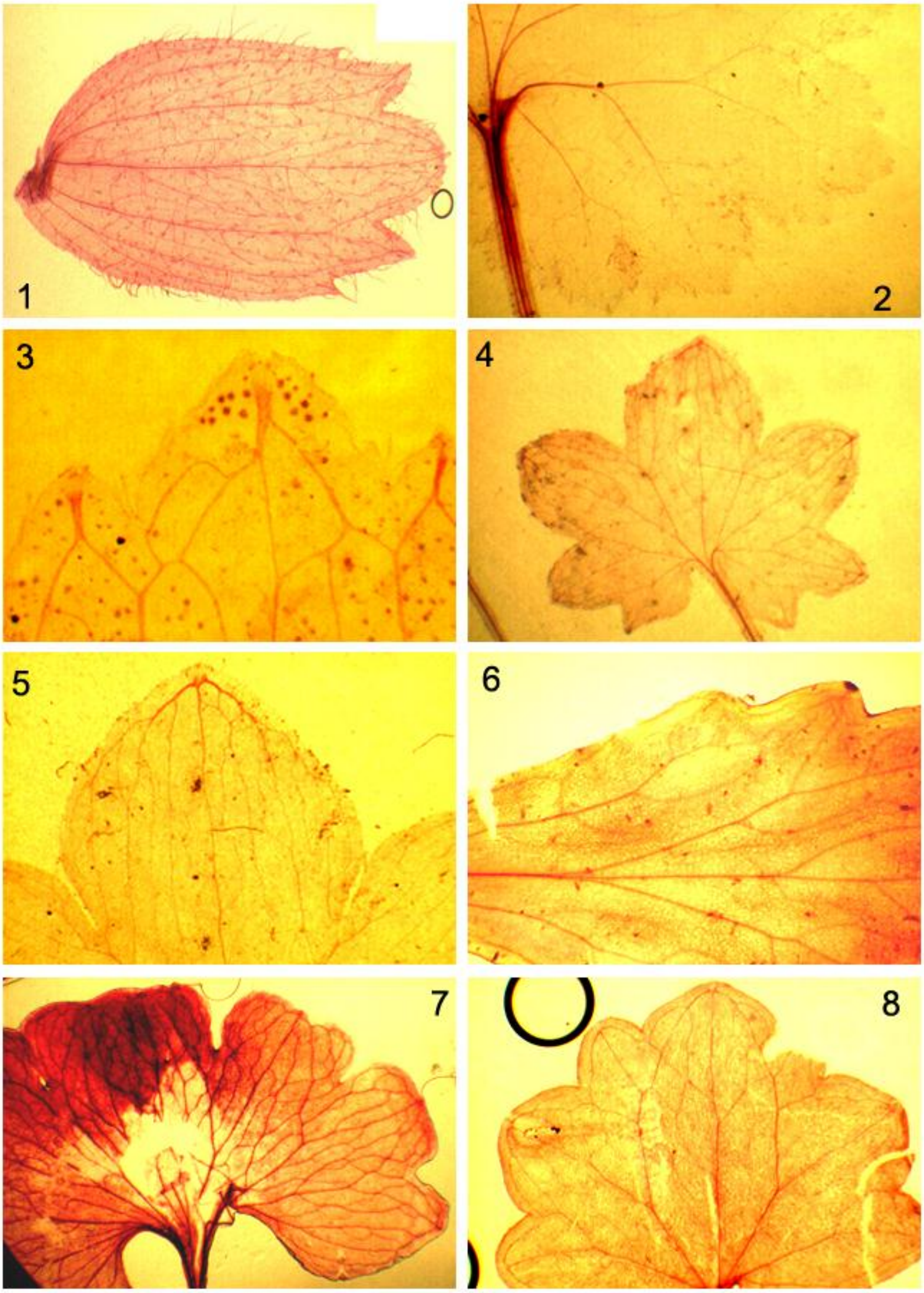


Figure 4.3. Pictures of different S. cernua venation. Matured leaves (1, 3 and 4) are actinodromous but the young one (2) is acrodromous.

Figure 4.4. Pictures of actinodromous venation. 1-2: S. epiphylla; 3-4: S. carpetana; 5-8: S. fortune.

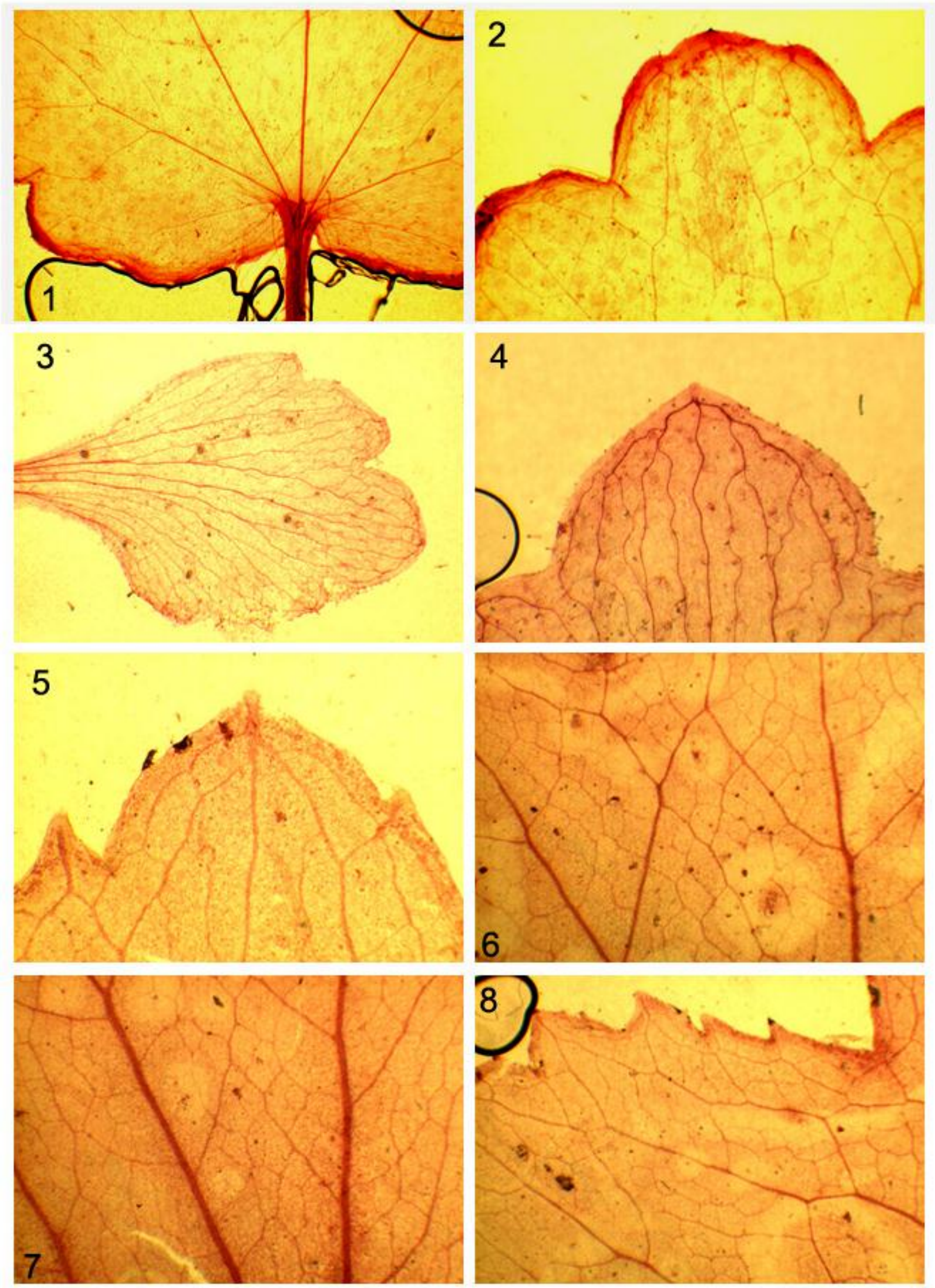


Figure 4.5. Pictures of actinodromous venation. 1-2: S. gemmulosa; 3-4 S. bourgaeana; 5: S. hypnoides var. muscosa; 6: S. rosacea; 7: S. cespitosa; 8: S. pentadactylis.

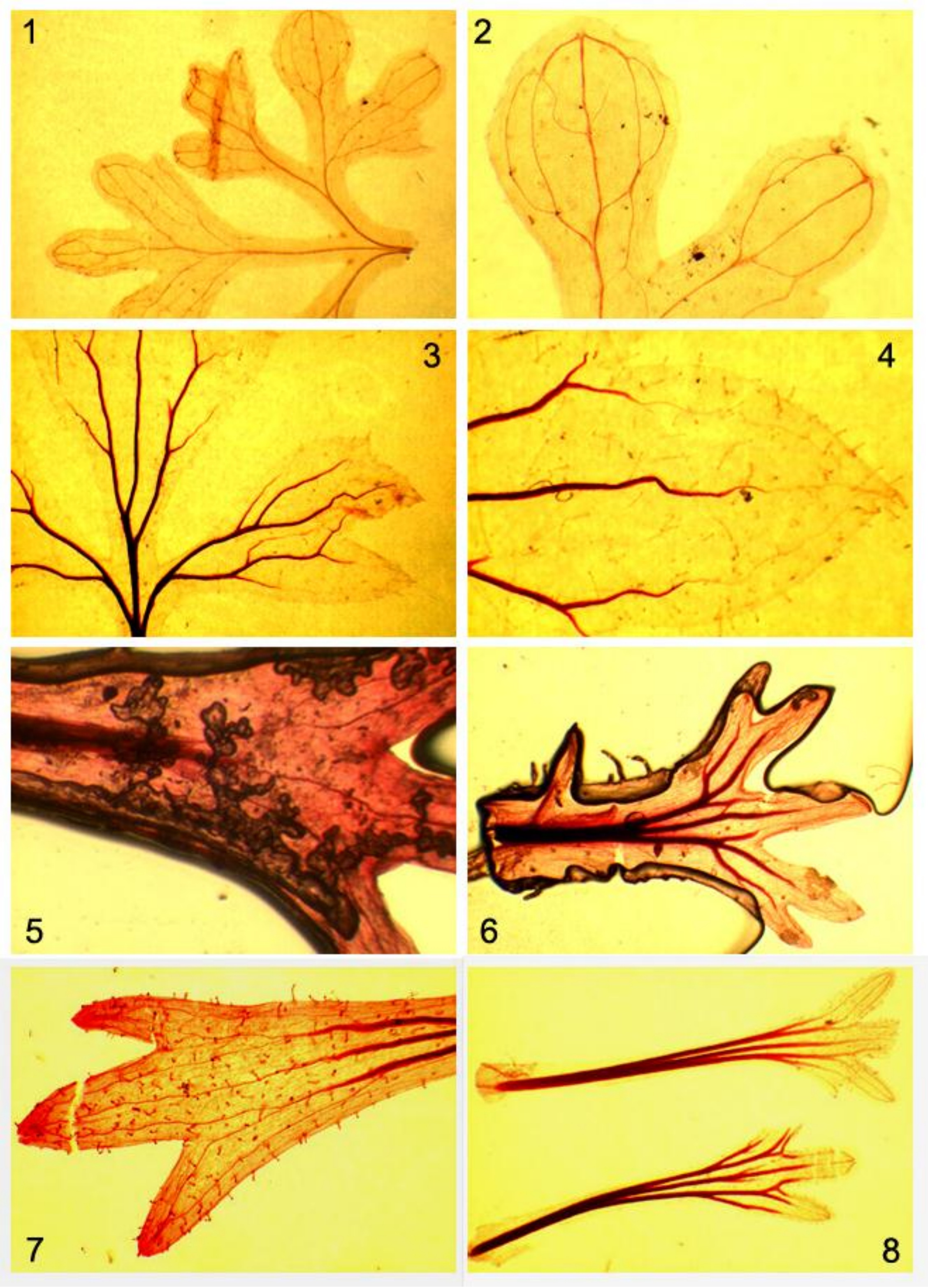


Figure 4.6. Pictures of actinodromous venation. 1-2: S. aquatica; 3: S. aphylla; 4: S. trifurcata; 5: S. reuteriana; 6: S. nevadensis; 7: S. canaliculata; 8: S. cuneata.

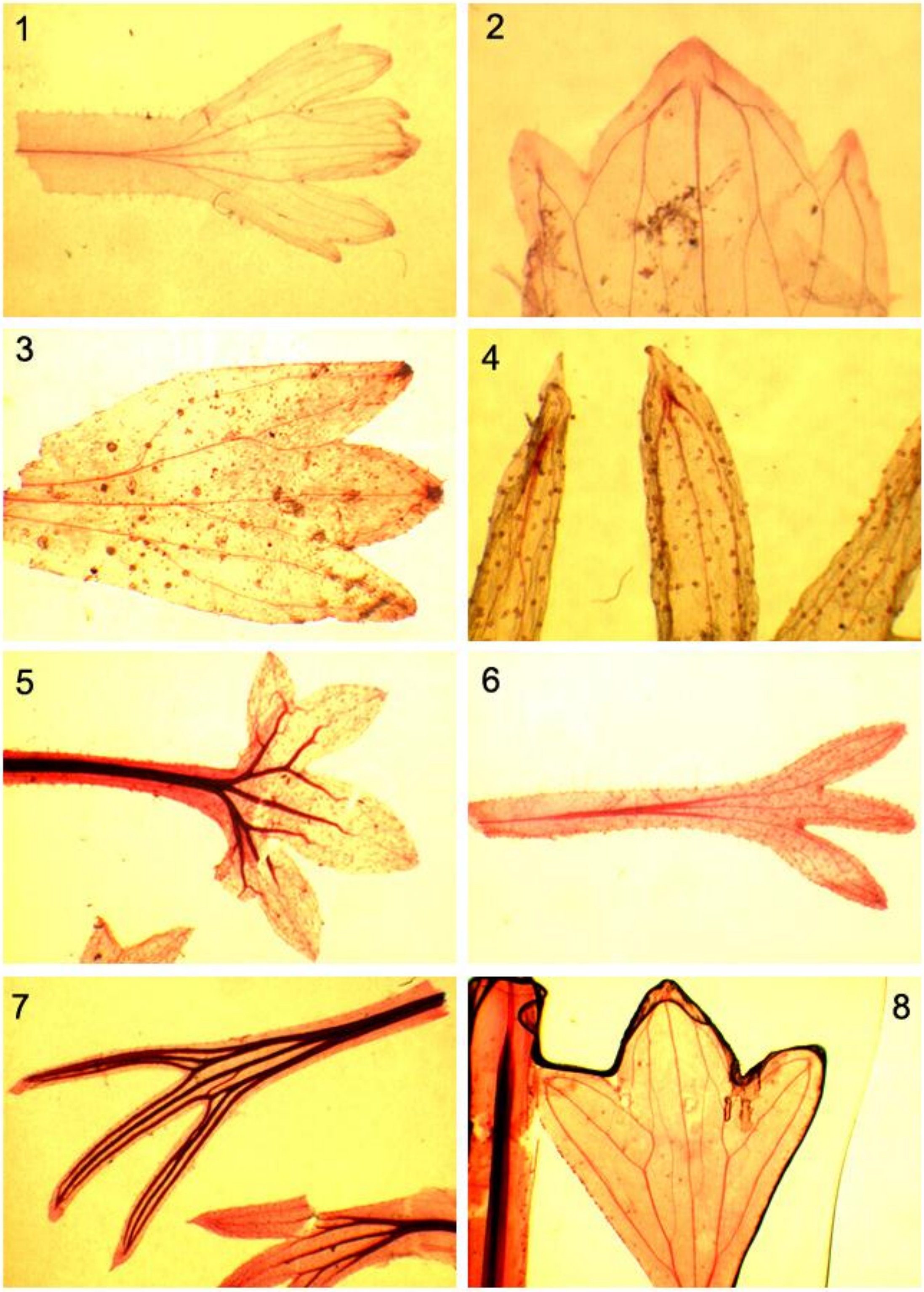


Figure 4.7. Pictures of actinodromous venation. 1: S. cebennensis; 2: S. pubescens; 3-4: S. globulifera; 5: S. dichotoma; 6: S. pedemontana; 7-8: S. praetermissat.

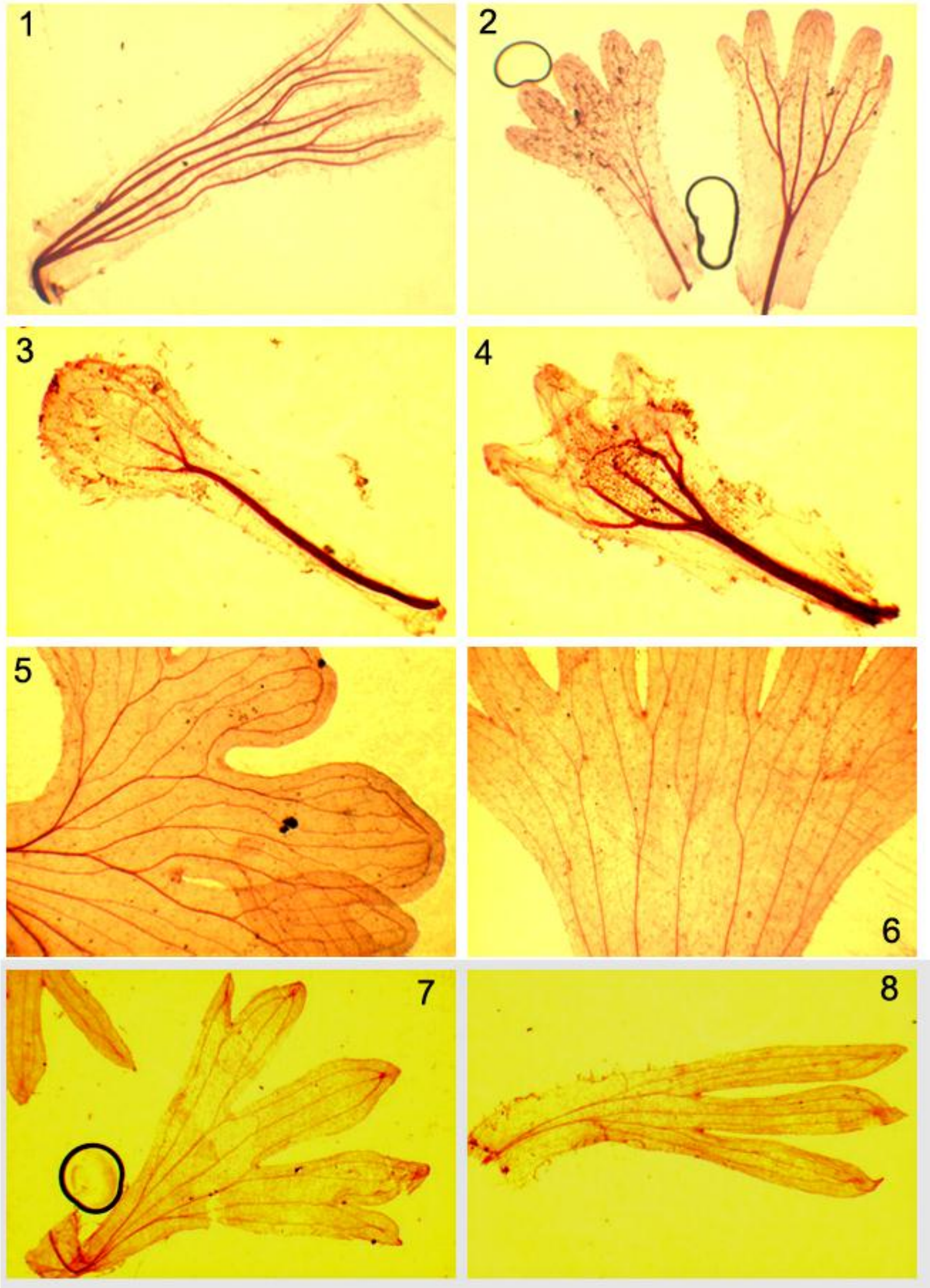


Figure 4.8. Pictures of camptodromous venation. 1: S. caesia; 2: S. squarrosa; 3: S. marginata; 4: S. scardica; 5: S. juniperifolia; 6: S. aretioides; 7-8: S. sempervivum.

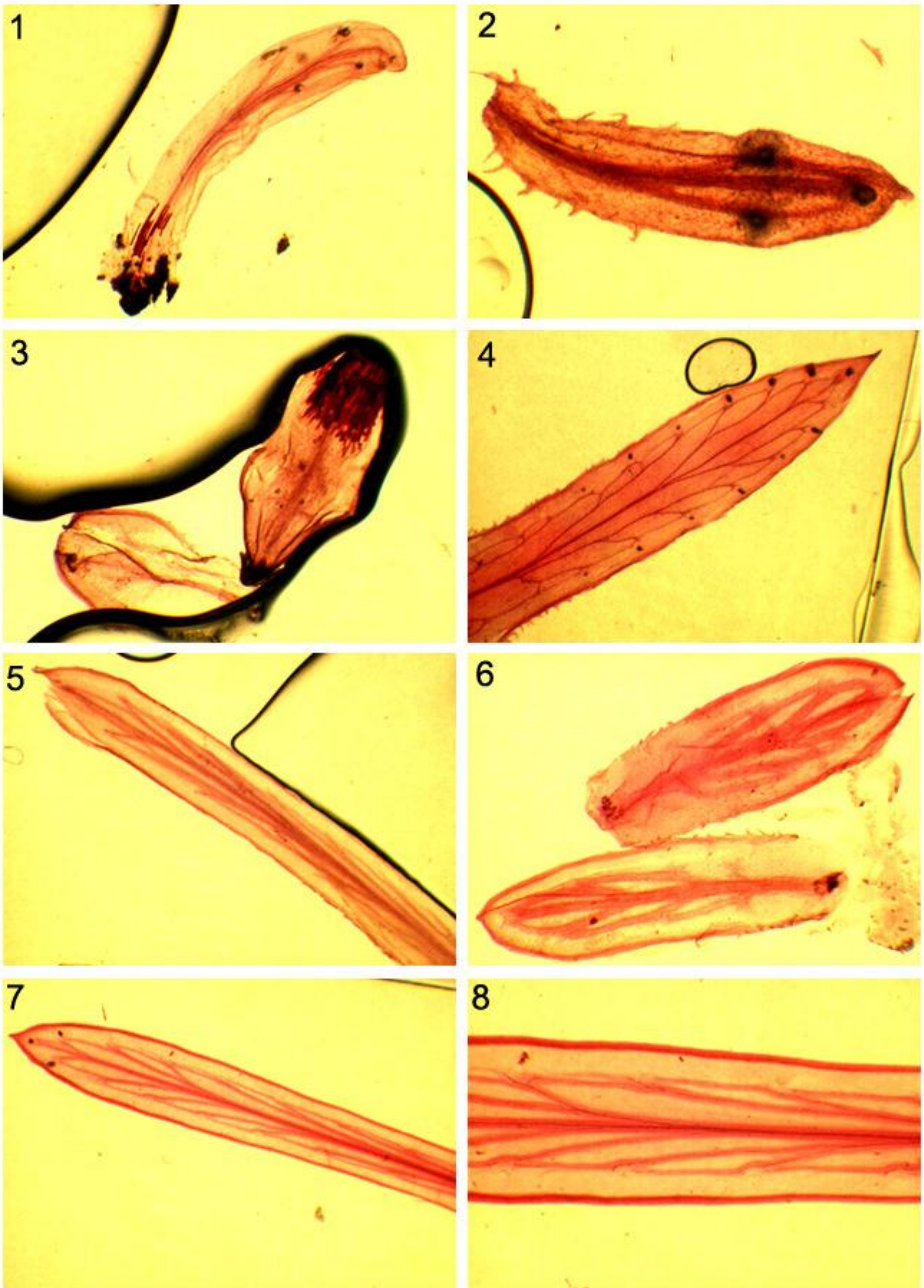


Figure 4.9. Pictures of camptodromous venation. 1-2: S. paniculata; 3-4: S. chionophila; 5: S. federici-augusti; 6-8: S. hostii.

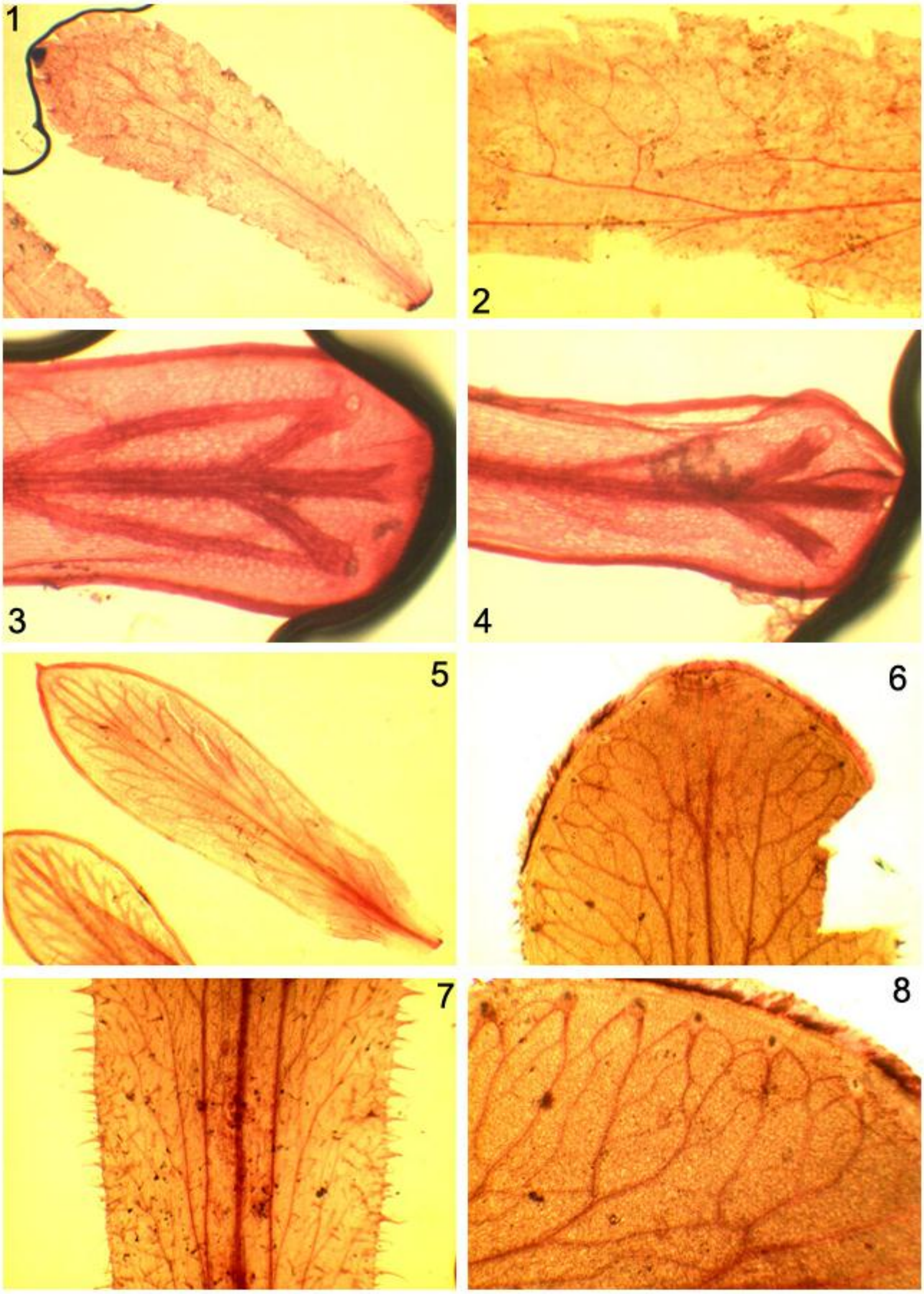


Figure 4.10. Pictures of camptodromous venation. 1: S. cochlearis 2: S. media; 3: S. porophylla; 4: S. florulenta; 5: S. aizoides; 6: S. biflora.

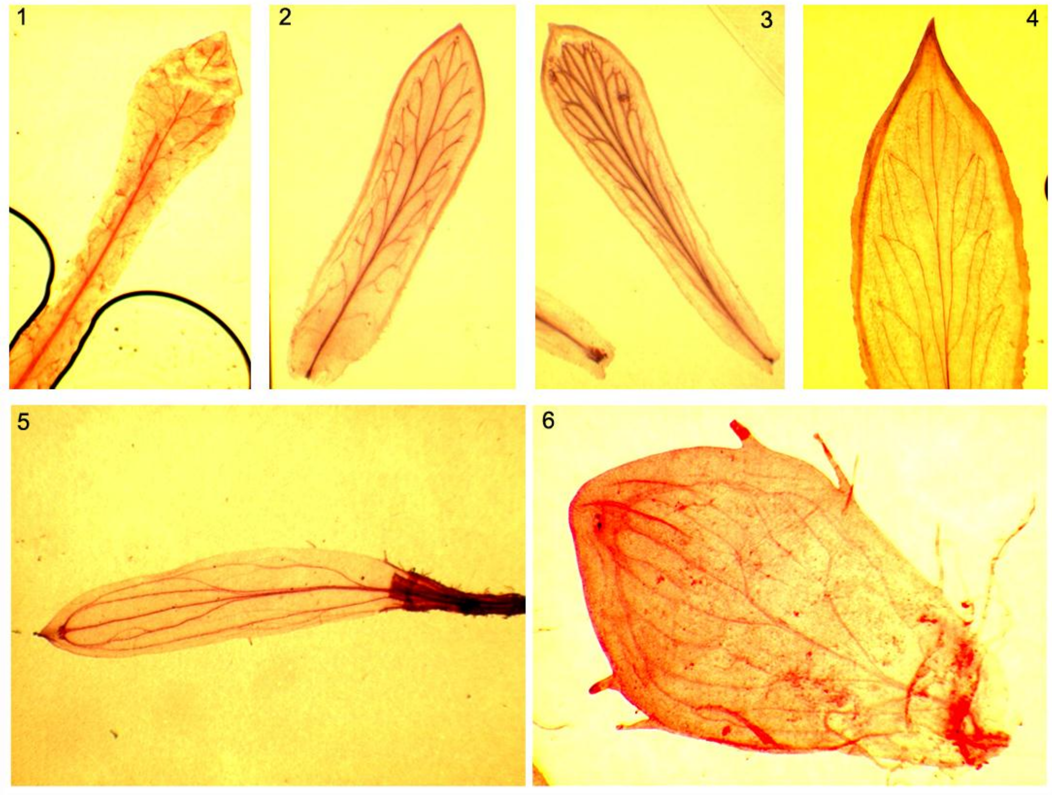


Figure 4.11. Pictures of sclaries (1-3) and dark stained vein (4). Under dissecting microscope, 1: S. marginata; 2: S. porophylla. 3: under compound microscope. 4: dark stained vein under compound microscope.

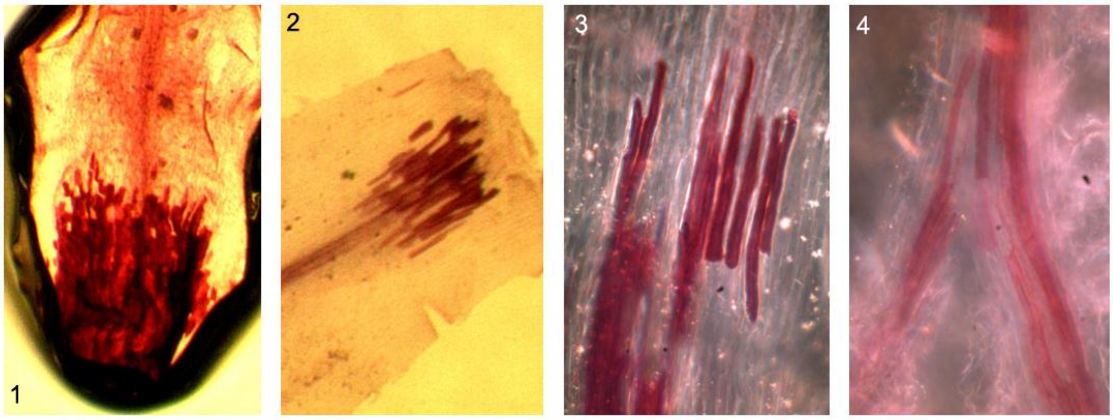


Figure 4.12. Acrodromous venation drawing.

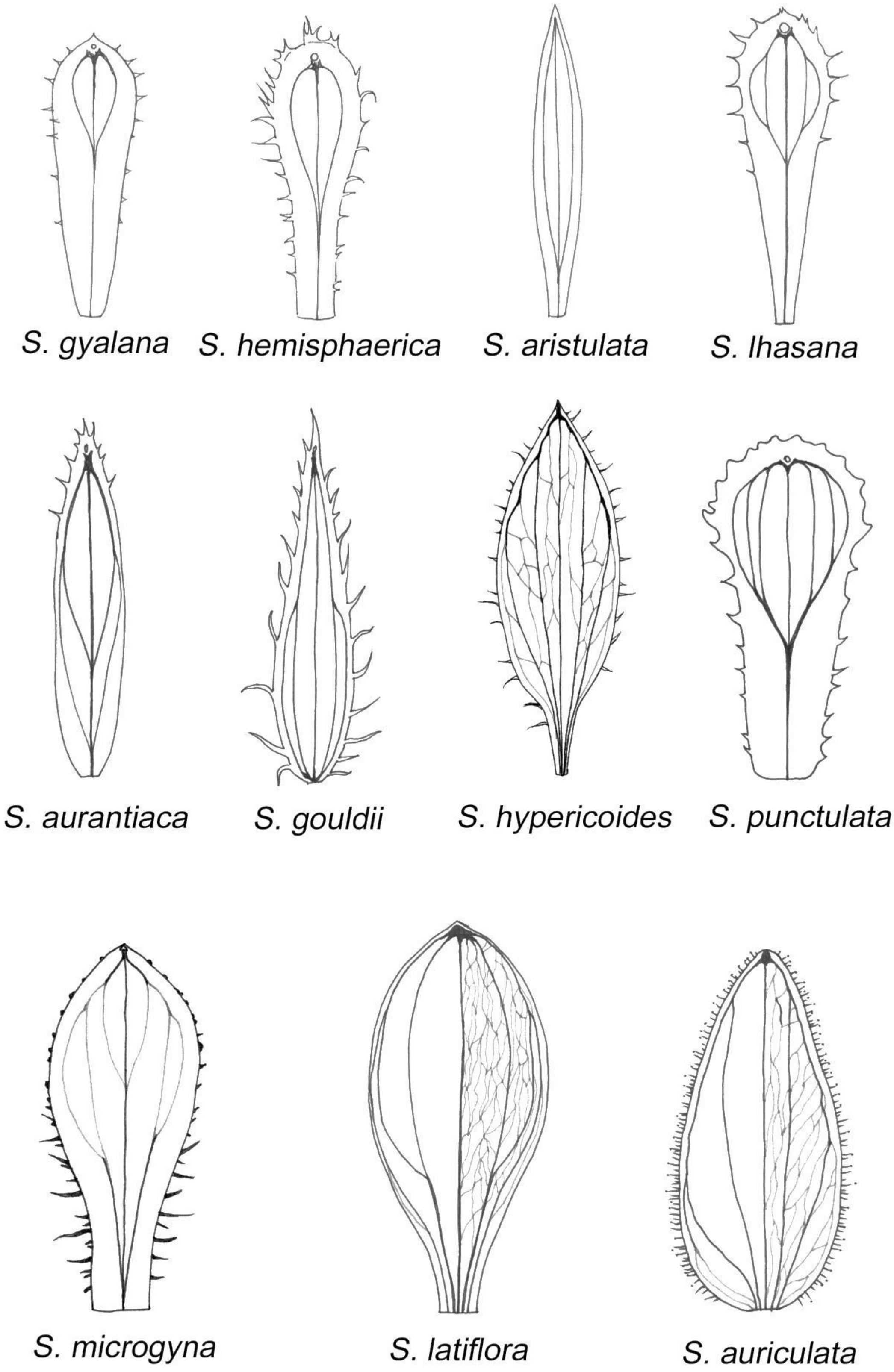


Figure 4.13. Acrodromous subtype Campylodromous venation, and actinodromous venation.

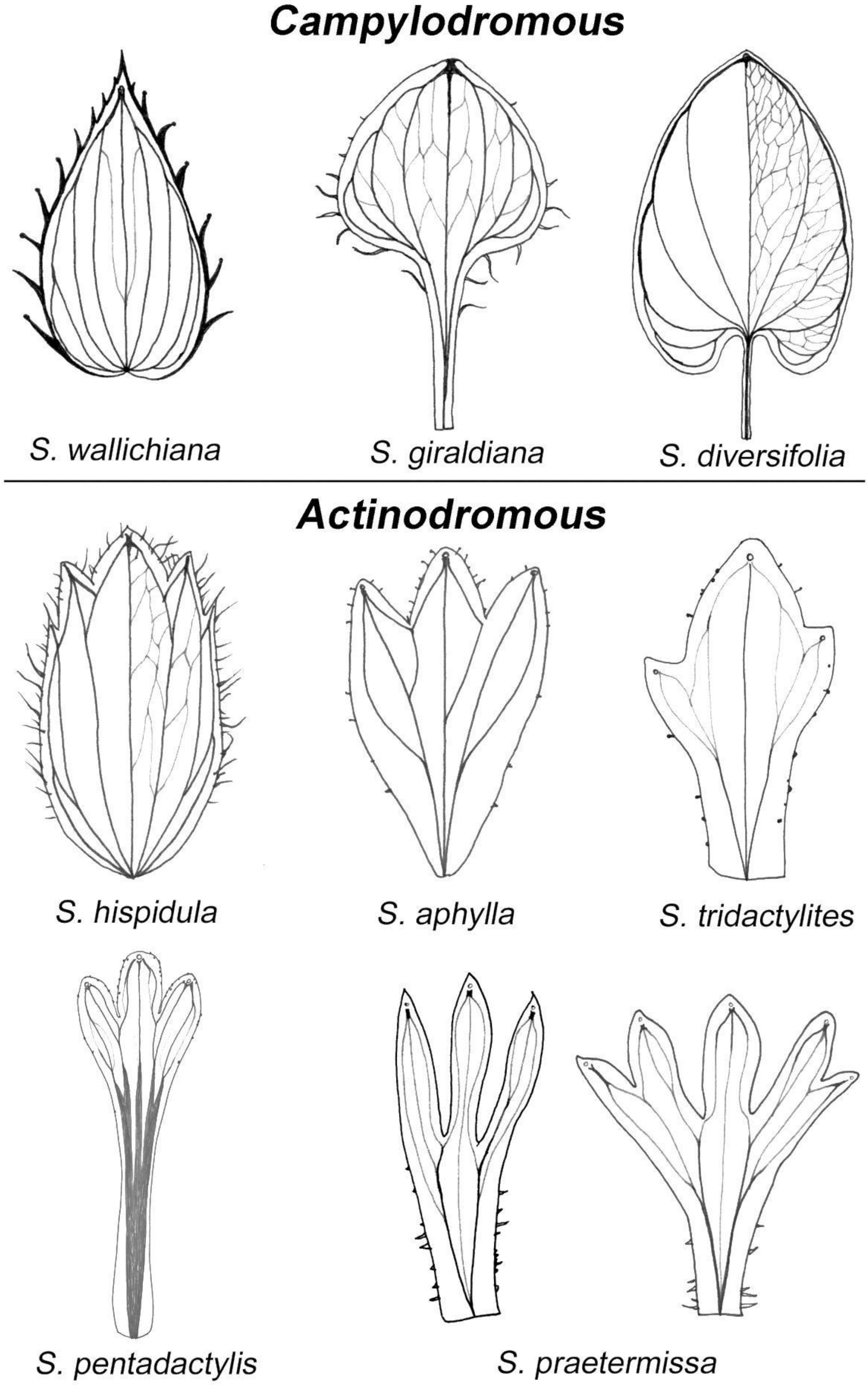
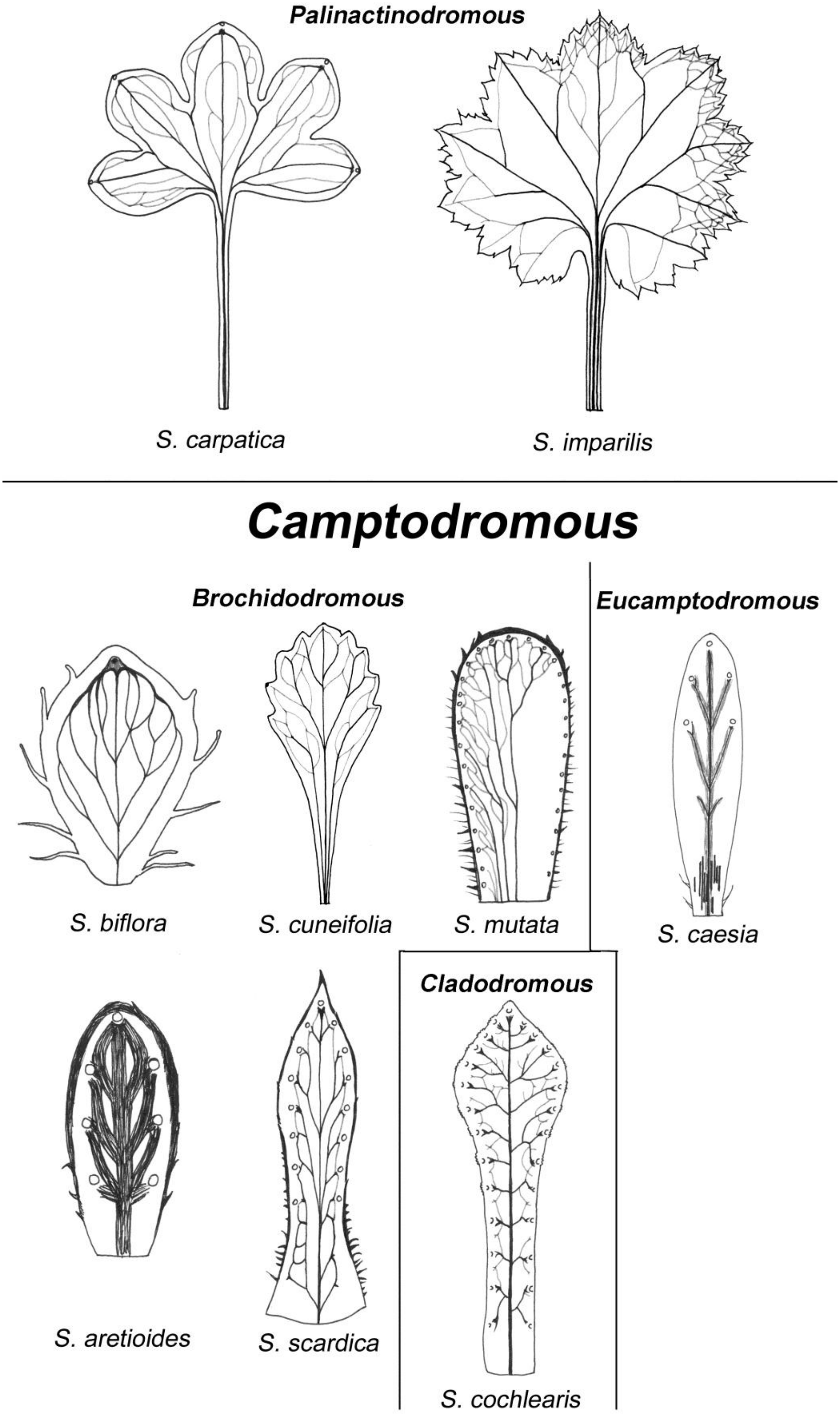


Figure 4.14. Actinodromous subtype palinactinodromous, camptodromous and its three subtypes—brochidodromous, eucamptodromous venation.



4.4 Discussion

4.4.1 Phylogenetic relationships

The distribution of venation types is constant in groups and therefore of systematic importance (Appendix 3, character 12-15). A single venation-type characterises subsections, sections and even groups of sections. The main groups are described here:

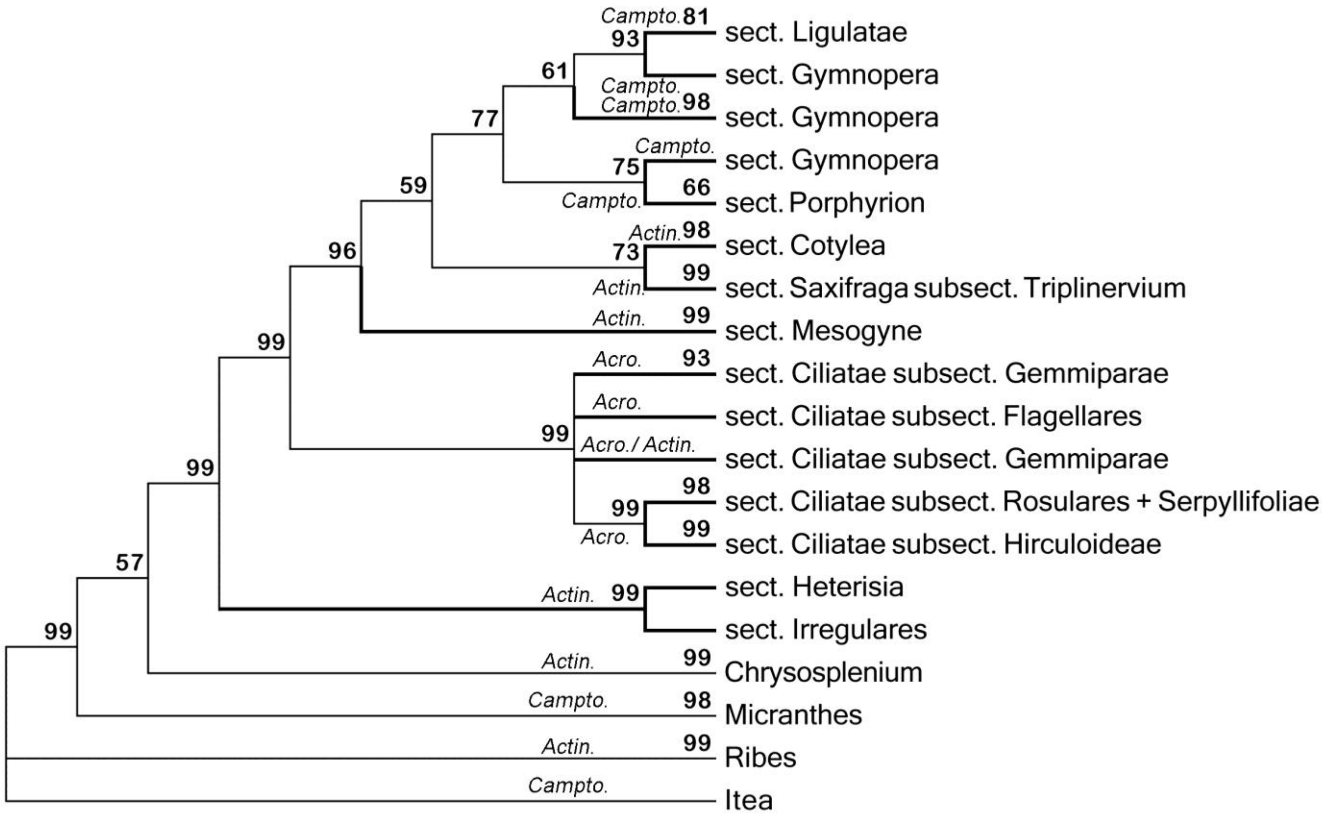
1. Species from sects. *Cotylea*, *Mesogyne*, *Irregulares* and *Heterisia* share actinodromous venation, corresponding to their similar leaf shapes (reniform-lobed). The latter two sections are closely related (Fig. 2.9).
2. Sect. *Ciliatae* species are uniformly of the acrodromous type, which occurs otherwise only in species of sect. *Trachyphyllum* and sect. *Saxifraga* subsect. *Holophyllae*. There is clearly as association with leaves that have only a single hydathode and, usually, an entire margin (Appendix 3, character 11, 16).
3. The most significant finding in this study is that all the lime-secreting species (sects. *Ligulatae*, *Xanthizoon* and *Porphyrion*) have a unique (in the genus), camptodromous (pinnate) leaf venation. Sect. *Gymopera*, which is water-secreting, also has camptodromous venation. This is a potential morphological synapomorphy for the clade Ligulatae + Gymopera + Xanthizoon + Porphyrion which was recovered in the matK MP tree (BS 77%, PP 98%, Fig. 4.15). It is noticeable that the open and intermediate marginal ultimate veins only occur in camptodromous species; they seem to have a different water transport strategy to those with closed vein networks.

4. Species in sect. *Saxifraga* are all actinodromous, except for the entire-leaved species in subsect. *Holophyllae*, which are acrodromous as noted above.

4.4.2 Evolution of leaf venation patterns

Venation types of the species sampled was plotted on the taxonomically compressed matK MP tree (Fig. 4.15).

Figure 4.15. The tree is the compressed view of matK MP tree with the venation types of species examined in each group. Numbers above branches indicate bootstrap values.



The ancestral state in *Saxifraga* is suggested to be actinodromous as it is shared by two of the outgroups (*Ribes* and *Chrysosplenium*) and the basal sections of *Saxifraga* (sect. *Irregulares* and *Heterisia*). Acrodromous venation evolved either once or up to three times: in sect. *Ciliatae*, sect. *Trachyphyllum* and sect. *Saxifraga* subsect. *Holophyllae*. *Trachyphyllum* is not in the combined MP tree but from the ITS MP tree (Fig. 2.9) and six gene tree (Soltis et al. 2001a). It is in a position that is clearly ancestral to sect. *Ciliatae* and a descendant of *Irregulares*. Furthermore, acrodromous venation evolved at sect. *Saxifraga* subsect. *Holophyllae*, where subsect. *Triplinervium* is.

There is a close relationship between acrodromous and actinodromous venation. Basically, the former is a simplified form of the latter. The two states are connected by an ontological relationship. Leaves with actinodromous venation look like those with more than three acrodromous patterns joined together. Figure 4.3 shows a young leaf (2) and developed ones (1, 3, 4) of *S. cernua*; the young one is acrodromous while the mature one is actinodromous. Another example is *S. globulifera* (Figure 4.7: 3-4). The cause of the difference is the development of lobes or teeth, which in turn involves an increase in the number of hydathodes. *S. hispidula* and S. substrigosa have three teeth and three hydathodes and actinodromous venation (Figure 4.2: 1), whereas its close relatives with entire leaves and only one hydathode, like *S. wallichiana* (Figure 4.1: 3), are acrodromous. For this reason, these two types sometimes co-occur in some groups. It is not surprising if acrodromous venation has arisen from the actinodromous state more than once.

Leaf shape is correlated with venation pattern (Beck 2010). The small, almost entire-leaved species of subsect. *Holophyllae* of sect. *Saxifraga* have acrodromous leaves, while the rest of the section has actinodromous leaves.

Palinactinodromous is a very complicated version of an actinodromous pattern. It is almost like a fractal pattern based on an acrodromous unit. The teeth form on the sides of lobes with same curving acrodromous pattern (Figure 4.2: 4). It also has more vein orders to form a reticulate network. This kind of venation may relate to a large leaf size and a humid habitat (Roth-Nebelsick et al. 2001).

Some of the species in sect. *Ciliatae* subsect. *Hirculoideae*, especially those allied to *S. diversifolia*, tend to have prominent intra-marginal veins (Figure 4.1: 4, *S. diversifolia*) but the degree is very variable. For instance, the thick intra-marginal veins of *S. giraldiana* and *S. hypericoides* occur only in the distal part of the leaf. This kind of venation helps water supply to the margin which is high in water stress (Roth-Nebelsick et al. 2001).

It is quite likely that camptodromous venation evolved just once at the common ancestor of sect. *Ligulatae* + *Gymopera* + *Xanthizoon* + *Porphyrion* clade (Fig. 4.15). Sect. *Ligulatae* is unresolved in the ITS+matK MP tree but clustered with *Porphyrion* and *Gymopera* in the matK tree. *Xanthizoon* is nested into the *Porphyrion* clade.

In two species (classified as camptodromous) the venation pattern is somewhat intermediate to either acrodromous (Figs. 4.10: 5, 6; *S. aizoides* and S. biflora, respectively) or actinodromous (S. cuneifolia, Fig. 4.14). They were assigned to the camptodromous state owing to their clearly pinnate secondary veins, but they may represent something like the ancestral groups of the camptodromous species.

An open vein network is less efficient at water transport than a closed one (Roth-Nebelsick et al. 2001). In *Saxifraga*, the open and intermediate marginal ultimate veins only occur in camptodromous species. These species are mostly lime-secreting plants (sects. *Ligulatae*, *Xanthizoon* and *Porphyrion*). Is there a physiological link between lime-secretion and an open network? No conclusion can be made without experiments but a hypothesis is that an open network might help remove calcium from the hydrothodes more effectively and stop it being transported back into the system. On the other hand, xeromorphic leaves tend to have more closed venation (Roth et al. 1995). However, some people believe that the closed and open veins have little to do with leaf water relations, but instead are more important for mechanical support (Walls 2011).

Another interesting phenomenon is the occurrence of dark-stained vascular cells and folia sclereids (Figure 4.5-9, 4.11). Since safranin O stains xylem cells with secondary wall thickenings, these dark-stained cells have very strong secondary wall thickenings. This is accentuated with age because the veins of mature leaves can be thickened completely (Figure 4.6: 7, S. canaliculata). Species from sect. *Porphyrion* subsect. *Engleria* and some of the species of subsect. *Kabschia* are completely thickened; this is presumably one important cause of the stiffness of their leaves. In sect. *Saxifraga* subsect. *Triplinervium,* some species, especially those in series *Ceratophyllae*, have leaves with pronounced thickening in their petiole and proximal region of the lamina.

As veins are important leaf components with functional roles in water transport, carbohydrate export, and mechanical support (Walls 2011), an evolutionary study will need an extensive survey set in the context of both leaf function and habitat preference of the species.

Chapter Five: Morphological and total data analysis

"Data! Data! Data!" he cried impatiently. "I can't make bricks without clay."

Sherlock Holmes -*The Adventure of the Copper Beeches*

Unlike many molecular characters, morphological characters are more likely to be directly under the pressure of natural selection and thus are telling the evolutionary story in a different way. Although morphological characters are of great systematic importance, they are more difficult to collect and analyses than the DNA ones. In this chapter, data on micro- and macro-morphological features are given and included in an overall analysis.

5.1 Introduction

Morphological characters formed the basis of systematics before molecular systems were produced. The previous systems for *Saxifraga* (Engler and Irmscher 1916/19; Gornall 1987a; Haworth 1803; Haworth 1812, 1821; Pan et al. 2001; Seringe 1830; Sternberg 1810, 1822, 1831; Tausch 1823; Webb and Gornall 1989) are all derived from morphological traits (see Chapter 1).

Microscopic characters are often of systematic importance. Gornall (1986) studied trichome anatomy on pedicels and leaf surfaces and found this to be a good systematic character. Foliar crystals were also studied and found only in sect. *Irregulares* in *Saxifraga* (Gornall 1987b). Seed epidermal ribs characterise the genus *Micranthes* while *Saxifraga* species have smooth, tuberculate or palilate seed surface (Kaplan 1981). Unfortunately, this character does not appear to be taxonomically informative above the species level within *Saxifraga*: indeed, there is often considerable infra-specific variation (Kaplan 1981), and thus after a brief initial study (see later) it was not considered further.

Morphological analysis does not require the alignment process as in the molecular studies but first it needs to compare measurements between taxa so that character states can be properly built or grouped (Stevens 2000). In this process, the delimitation of characters, not only the state, is also being considered. In the pollen morphology study of genus *Parkia*, the basic types of exine were found fail to determine an intermediate form between verrucae and reticulate type. Therefore the character was broke into more than one characters to distinguish the homology of these two exine types (Gustafsson and Bremer 1995). But study also shows that simply breaking down multiple state characters to individual nominal variable codes (present / absent) is not necessarily the solution (Forey and Kitching 2000).

The criteria for an appropriate character (Mishler 2005) are:

1. Homology and heritability;
2. Independent evolution from other characters;
3. Having at least two discrete states.

5.2 Methods

The morphological data matrix (Appendix 3) was made from a list of possible characters (Table 5.1). Characters were chosen from the vegetative organs to the reproductive organs plus life history, chromosome base number. Initially a larger number of characters were considered but the ones not been widely studied or not appropriate were deleted (Appendix 4). Character states were collected from literature (Ferguson and Webb 1970; Gornall 1986, 1987b; Hideux and Ferguson 1976; Kaplan 1981; Kubitzki 2007; Pan et al. 2001; Soltis 2007; Webb and Gornall 1989; Weigend 2007), as well as by examining herbarium specimens and plant pictures. Character 38-42 and 12-15 are taken from Chapters Three and Four, respectively.

Table 5.1. The list of characters with states. Terminology follows Hickey (1973, 1979), Hideux &Ferguson (1976), Webb & Gornall (1989) and Hickey & King (2000).

	Character	**States**
1	Life form	perennial, annual, biennial
2	Rhizomatous perennation	absent, bulbil, tuber
3	Axillary leafy buds	absent, present
4	Axillary runners	absent, present
5	Node trichomes	absent, gland, egland, crisp-rufous
6	Pedicel glandular trichomes	multiseriate, intermediate, uniseriate, sessile.
7	Leaf arrangement	alternate, opposite
8	Leaf texture	herbaceous, leathery, fleshy
9	Leaf margin differentiation	cartilaginous, absent
10	Leaf lamina outline	entire, toothed, lobed, lobed-toothed, compound lobed
11	Lamina shape	cordate, deltoid, (elliptic, ovate, orbiculate, lanceolate, oval, oblong), (ensiform, ligulate, subulate), (obovate, spathulate, oblanceolate), (palmatifid, palmatisect), reniform
12	Venation type	acrodromous, actinodromous, and camptodromous
13	Marginal ultimate venation	close, intermediate, open
14	Vein expansion	absent, complete, partial
15	Foliar sclereids	absent, present

16	Hydathodes number	>1, 1
17	Hydathodes insertion	surface, sunken
18	Hydathodes position	adacial, marginal, pseudo-marginal
19	Hydathodes secretion	$CaHCO_3$, H2O
20	Leaf epidermal tannin cells	absent, present
21	Leaf crystal	absent, druse
22	Inflorescence branching	solitary, simple cyme, racemose cyme, compond cyme, umbel, raceme
23	Flower symmetry	radial, bilateral
24	Sepal number	4, 5
25	Sepal orientation	erect, spread, reflexed
26	Petal number	0, 5
27	Petal spots	absent, present
28	Petal callosese	absent, present
29	Petal:sepals length ratio	petal length > sepals, <= sepals
30	Petal colour	white, cream, yellow, greenish yellow, pink, red, various
31	Petal shape	round, ensiform
32	Stamen number	5, 10, 4/5, 4/8
33	Filament shape	clavate, linear
34	Anther connective	obtuse, apiculate
35	Anther dehiscence	parallel, divergent, T-shaped
36	Pollen ecto-aperture	porus, colpus
37	Pollen endo-aperture	pore, diffuse
38	Pollen surface: lirae & striae	absent; lirae coarse and ribbon-like, striae ill-defined; lirae & striae fine; lirae coarse and marginal ridges present, striae slit-like; lirae coarse and marginal ridges absent, striae of variable width
39	Tectum structure	single, double
40	Tectum perforation	reticulate, perforate/complete, micro-capillaries, primary tectum perforate, perforate-reticulate/perforate-rugulate
41	Secondary columellae	absent, low, high
42	Supra-tectal processes	absent, verrucae, granual, granules mostly with expanded base, spinules
43	Pollen cell number	2, 3
44	Ovary position	inferior, semi-inferior, superior, semi-superior
45	Carpel number	2, 3
46	Carpel fusion	basal, >50% ovary
47	Placentation	parietal, axile
48	Fruit type	baccate, capsule
49	Chromosome base number	each treated as a separate state

The venation types and other venation characters were included in the morphological data matrix (Appendix 3, characters 12-15). Only the main types were recognized as character states in the morphological data matrix because the recognition of subtypes was sometimes blurred by intermediates.

The matrix (Appendix 3) was coded by treating all states as unordered. The total data matrix contains 131 species; there is also a condensed data matrix which only has 37 species as representatives of the sections from the total matrix. An MP tree was made by selecting parameters BS test = 1000 replications, Gap/Missing Data Treatment = Use all site, MP Search Method = Close-Neighbour-Interchange (CNI) on Random Trees, No. of Initial Trees (random addition) = 10 and MP Search Level = 2. The total data set was used to make a strict consensus tree; bootstrap support values from the 50% bootstrap consensus tree were added where topologies permitted. The smaller data set was used to make a 50% bootstrap consensus tree.

Bayesian trees were produced with MrBayes version 3.2.0 (Ronquist et al. 2012). The evolutionary model is Jukes Cantor (JC) model and Gamma. State and frequence = fix (equal). Each analysis ran two simultaneous independent analyses and computed diagnostics every 1000th generation with three heated and one cold chains, discarded the first 25 % samples from the cold chain, and sample the chain every 100th generation. The average standard deviation of split frequencies was ca. 0.012 after $1{\times}10^6$ generations; the smaller set was after generations. The Potential Scale Reduction Factor (PSRF) for all data set is larger than 1. The PP values for clades congruent with the MP trees were plotted onto the latter.

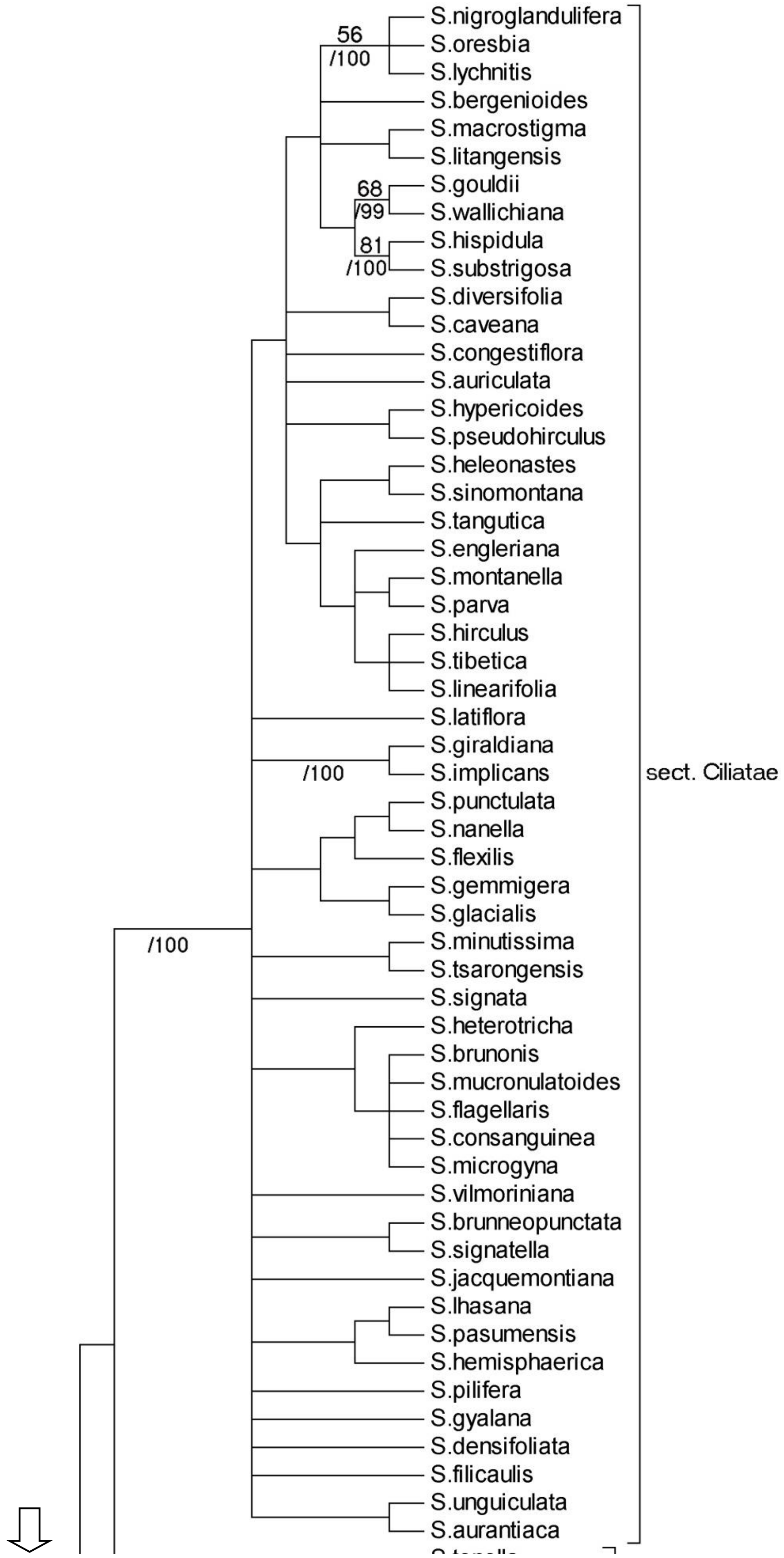
5.3 Results

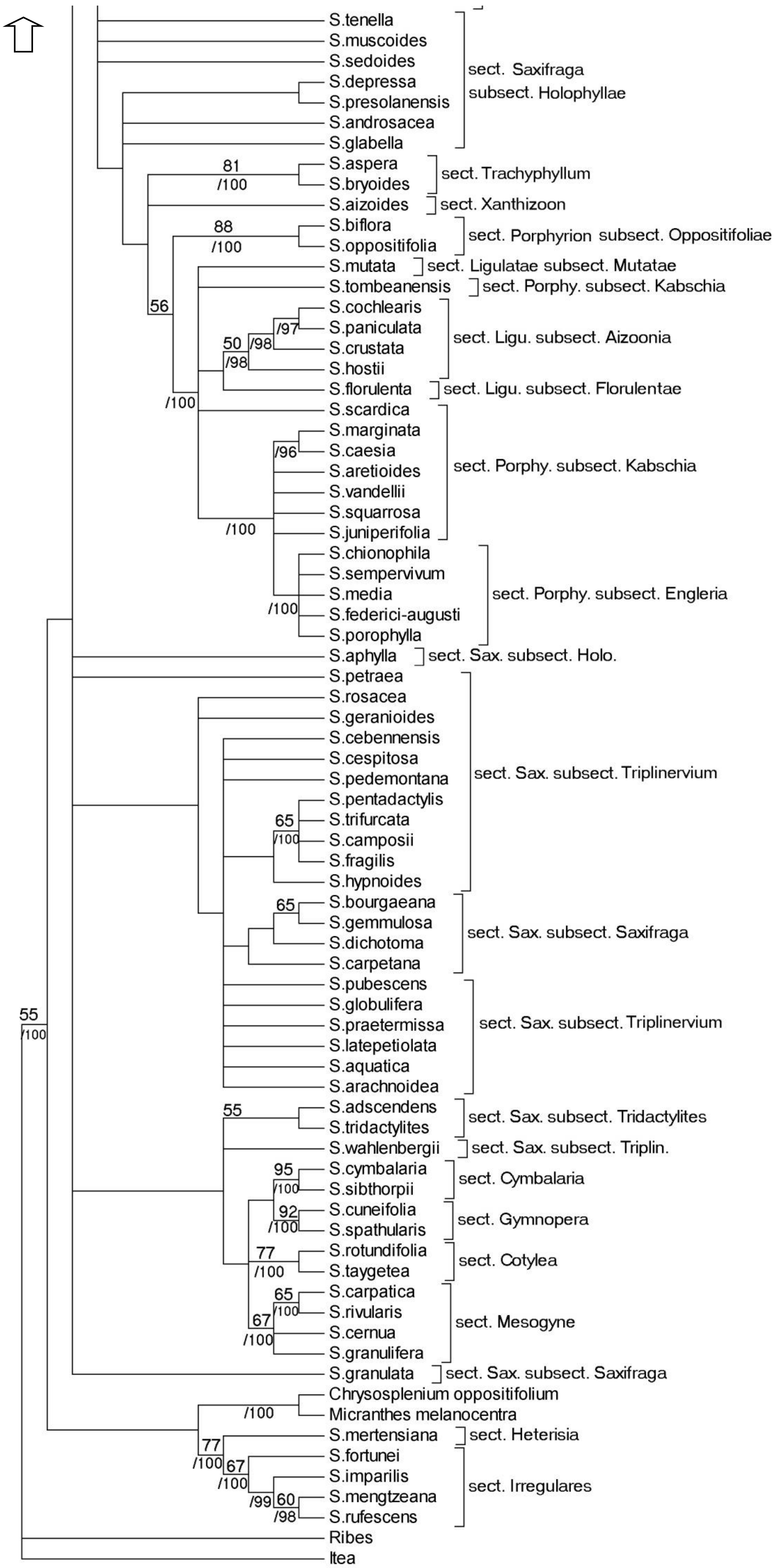
5.3.1 The total data matrix

The analysis involved 131 species and 49 characters; a total of 376 trees were found; CI = 0.28, RI = 0.78. The MP tree is shown in Fig. 5.1.

Most clades are less than 50%BS supported and very few are 100%PP supported. Most clades have very low (<10%) support in the bootstrap consensus tree (not shown) which explains why so few clades are higher than 50%BS supported.

Figure 5.1. The strict consensus MP tree of 131 species with BS support (above branches) and PP values (below branches).




S.tenella
S.muscoides
S.sedoides
S.depressa
S.presolanensis
S.androsacea
S.glabella
sect. Saxifraga subsect. Holophyllae
81 /100
S.aspera
S.bryoides
sect. Trachyphyllum
S.aizoides
sect. Xanthizoon
88 /100
S.biflora
S.oppositifolia
sect. Porphyrion subsect. Oppositifoliae
S.mutata
sect. Ligulatae subsect. Mutatae
S.tombeanensis
sect. Porphy. subsect. Kabschia
56
/97
/98
50 /98
S.cochlearis
S.paniculata
S.crustata
S.hostii
sect. Ligu. subsect. Aizoonia
S.florulenta
sect. Ligu. subsect. Florulentae
/100
S.scardica
/96
S.marginata
S.caesia
S.aretioides
S.vandellii
S.squarrosa
S.juniperifolia
sect. Porphy. subsect. Kabschia
/100
/100
S.chionophila
S.sempervivum
S.media
S.federici-augusti
S.porophylla
sect. Porphy. subsect. Engleria
S.aphylla
sect. Sax. subsect. Holo.
S.petraea
S.rosacea
S.geranioides
S.cebennensis
S.cespitosa
S.pedemontana
65 /100
S.pentadactylis
S.trifurcata
S.camposii
S.fragilis
S.hypnoides
sect. Sax. subsect. Triplinervium
65
S.bourgaeana
S.gemmulosa
S.dichotoma
S.carpetana
sect. Sax. subsect. Saxifraga
55 /100
S.pubescens
S.globulifera
S.praetermissa
S.latepetiolata
S.aquatica
S.arachnoidea
sect. Sax. subsect. Triplinervium
55
S.adscendens
S.tridactylites
sect. Sax. subsect. Tridactylites
S.wahlenbergii
sect. Sax. subsect. Triplin.
95 /100
S.cymbalaria
S.sibthorpii
sect. Cymbalaria
92 /100
S.cuneifolia
S.spathularis
sect. Gymnopera
77 /100
S.rotundifolia
S.taygetea
sect. Cotylea
65 /100
67 /100
S.carpatica
S.rivularis
S.cernua
S.granulifera
sect. Mesogyne
S.granulata
sect. Sax. subsect. Saxifraga
/100
Chrysosplenium oppositifolium
Micranthes melanocentra
S.mertensiana
sect. Heterisia
77 /100
67 /100
/99
60 /98
S.fortunei
S.imparilis
S.mengtzeana
S.rufescens
sect. Irregulares
Ribes
Itea

The outgroup *Itea* and *Ribes* clustered together but *Micranthes* and *Chrysosplenium* species are sister to the sect. *Irregulares* & *Heterisia* clade and then the other *Saxifraga* (55%BS/100%PP). The sect. *Irregulares* & *Heterisia* clade is well supported (77%BS/100%PP).

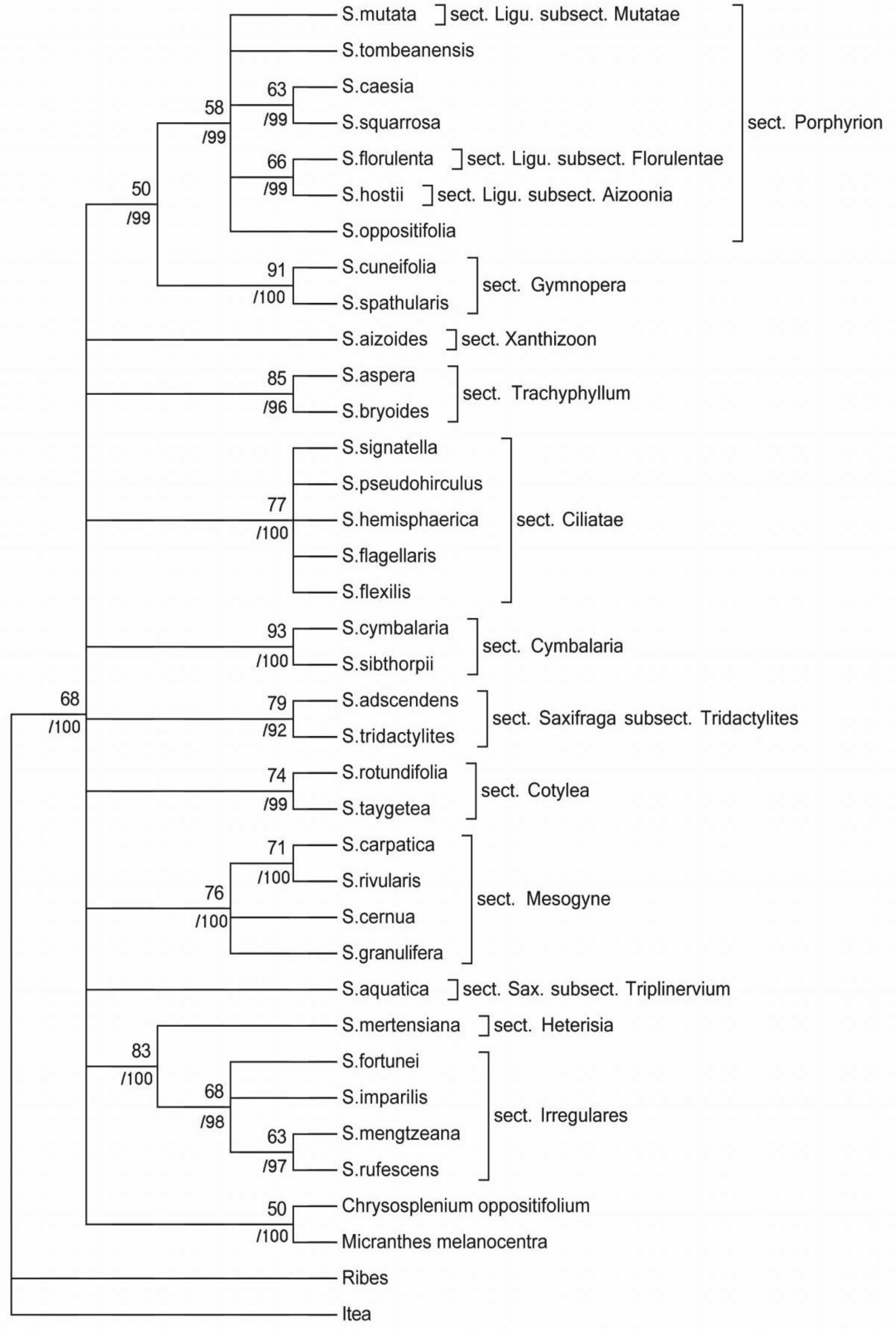
The rest of *Saxifraga* is largely unresolved. Sect. *Cymbalaria* (95%BS/100%PP), *Gymnopera* (92%BS/100%PP), *Cotylea* (77%BS/100%PP), and *Mesogyne* (67%BS/100%PP) clades are monophyletic and the topology suggests they having a close relationship with sect. *Saxifraga*. Species from sect. *Saxifraga* are separated and the group is polyphyletic. Species of sect. *Porphyrion* and *Ligulatae* clustered together with 56%BS value. Subsect. *Aizoonia* of *Ligulatae* is monophyletic with 50%BS/98%PP support. So also are subsect. *Oppositifoliae* of *Porphyrion* (88%BS/100%PP), sect. *Trachyphyllum* (81%BS/100%PP) and sect. *Ciliatae* (100%PP). Subsections in *Ciliatae* do not appear as monophyletic.

5.3.2 The smaller data matrix

The analysis contains 37 species and 49 characters with total 10 most parsimonious trees, CI = 0.50, RI = 0.71. The MP tree is shown in Fig. 5.2.

It is obvious that the CI and RCI improved in this data set but RI decreased slightly. Meanwhile, the topology is showing a clearer story and the BS supports were increased dramatically in most clades.

Figure 5.2. The bootstrap consensus (50%) MP tree with 37 species. The numbers above branches are BS values; the numbers with a slash and below branches are PP values >90.



5.4 Discussion

5.4.1 Tree analysis

The low statistical support of the total data set MP tree is possibly due to the paucity of characters. The consistency index (CI) measures the amount of homoplasy in a cladogram and is negatively correlated with the number of species sampled (Judd 2007). The low CI (0.28) for morphological traits suggests that the traits are too variable to differentiate homologies and homoplasies or the number of species (131) included in the analysis is too many for the number of characters (49). This is demonstrated by the 37 species MP tree which is much better supported; the CI is 0.5 while RI was slightly decreased.

The complete data set's high retention index (RI=0.78), a measure of the amount of homoplasy and how well synapomorphies explain the tree (Judd 2007), indicates that the characters fits the tree perfectly and which means that the characters I used are good ones.

The 37 species tree is well supported at the section level but not at the inter-sectional level. All sectional clades except sect. *Saxifraga* have more than 50% BS value and are monophyletic but the relationships between them are largely unresolved. This suggests that the characters can specify sections do not provide the synapomorphies necessary to group them.

Considering the number of species in the genus (ca. 460), it is necessary to have more species to cover the morphological diversity in the genus. Therefore, the morphological data matrix should include more traits which can specify not only inter sectional but also infra-sectional taxa.

5.4.2 Phylogenetic consideration

Due to the poor resolution of the morphological trees, only a few phylogenetic conclusions can be made. The strongly supported sect. *Irregulares* & *Heterisia* clade (77%BS in the complete tree and 83%BS in the 37-species tree) again showed the very close relationship of these two sections, a relationship also demonstrated in the matK MP tree in this study and in the six gene tree of Soltis, et al. (2001a).

Similar to the matK MP tree, sect. *Gymnopera* has a close relationship with sect. *Porphyrion* (61%BS in matK, 50%BS in 37-species morphological tree). They share the same venation type. Similarly, *S. florulenta* and *S. mutata* from sect. *Ligulatae* join into sect. Porphyrion (BS 58% in 37-species tree), just as in the molecular trees, indicating the close relationship of these two species with sect. *Porphyrion*. However, species from sect. *Ligulatae* subsect. *Aizoonia* (S. *hostii*) was not separated from sect. *Porphyrion* as in the DNA trees.

The monophyletic status of sects. *Cymbalaria*, *Gymnopera*, *Cotylea*, *Mesogyne*, *Trachyphyllum*, *Xanthizoon* and *Ciliatae* indicate that these sections are well founded in the Gornall (1987) system. Overall, morphological characters seem to be good for defining sections but are much less useful at identifying inter-sectional relationships.

5.5 Combining molecular and morphological data

Characters from molecular and morphological (structural) sources have different and complementary strengths and weakness. DNA markers are better for covering all branches of the evolutionary tree due to the numerous sites (Mishler 2005). There is no argument about the determination of character states for DNA markers (Mishler 2005; Stevens 2000). Highly conserved genes can be homologized in a group of species divergent in structure (Mishler 2005). The problems of DNA characters are several. First of all, the false alignment of sequences can cause severe failures in the evolutionary tree (Stevens 2000). Secondly, the congruence of homology (=synapomorphy) from the DNA data is merely based on the similarity between two sequences—a statistical problem (homoplasy) rather than ancestral (Mooi and Gill 2010; Stevens 2000). In the end, the lack of understanding about individual molecular characters, their quality and distribution, raises questions about the phylogeny they make (Mooi and Gill 2010).

Morphological characters are much more complex. The characters are in different levels of the tree and may exhibit sequences or relations of states from ontological studies (Mishler 2005). They sometimes have more character states than the molecular markers so that there is less possibility of the problem of long branch attraction (Mishler 2005). Moreover, morphological characters can be collected from more species, like rare species and fossils, which otherwise cannot provide sufficient DNA (Mishler 2005). The disadvantages of morphological characters are their much smaller number and the possible subjectivity of data. Informative structural characters are only a few compared to the DNA ones; the reduced possibility of finding new characters is another problem (Olmstead and Scotland 2005). The definitions of not only character states but also characters are seriously subject to personal decisions (especially for quantitative characters) and may have been misinterpreted by earlier systematic studies (Stevens 2000).

Morphological characters are more likely to experience natural selection and may be subject therefore to adaptive convergence (Mishler 2005). The results in the end affect the DNA sequences, especially in deeper levels (Mishler 2005). However, each change of morphological character state may include many changes in genetic markers (Stevens 2000). In contrast, most DNA markers are invisible to selection and behave in a neutral fashion (Kimura 1985), more like clocks or finger- prints for species. It may be that some morphological characters behave similarly, e.g. the exine surface of *Saxifraga* (Chapter 3) and seed testa pattern (see later); the relationship between structure and function is far from clear, although future study might reveal more.

In summary, molecular and morphological characters can complement one another to product a better estimate of evolutionary history. So, the results for DNA sequences and morphological features were combined and analysed as follows.

5.5.1 Method

Morphological data was added to the matK + ITS sequence data for the same species except that *S. stolonifera* sequence was connected to the morphological states of *S. imparilis* to represent section *Irregulares*.

The Bayesian inferences for the tree were conducted by MrBayes 3.2 (Ronquist et al. 2012). The model for the DNA portion is GTR + G (nst=6, rates= Dirichlet) while the morphological portion is JC + G (nst=1, rates=fixed). The average standard deviation of split frequencies were lower than 0.01. The clade credibility tree preserved the PP values >90.

The MP tree with 1000 bootstrap replicates was conducted by PAUP 4.0 (Swofford 2002). The 50% majority-rule consensus tree was shown with BS supports. The BS values were plotted to the Bayesian tree where topologies are the same. The reason of not plotting the PP value to the MP tree like Chapter 2 is that Mega 5 software does not accept combined data and MrBayes 3.2 is better than PAUP 4.0 in tree output.

To understand the influences of morphological characters, the same data set was used for Bayesian and MP analyses but with the morphological data removed. BS values were plotted to the Bayesian tree as before.

5.5.2 Results

The combined DNA and morphology tree has 39 species and a total of 1718 characters; number of trees retained = 5 (Fig. 5.3). The tree without morphology data has 39 species and 1669 characters; number of trees retained = 117 (Fig. 5.4).

Figure 5.3. The Bayesian tree from a data set combined ITS, matK, and morphological data. The PP values >90% are above branches; BS supports from MP analysis were plotted under branches which were shared by two analysis. Triangles are to highlight *S. mutata* and *S. florulenta*.

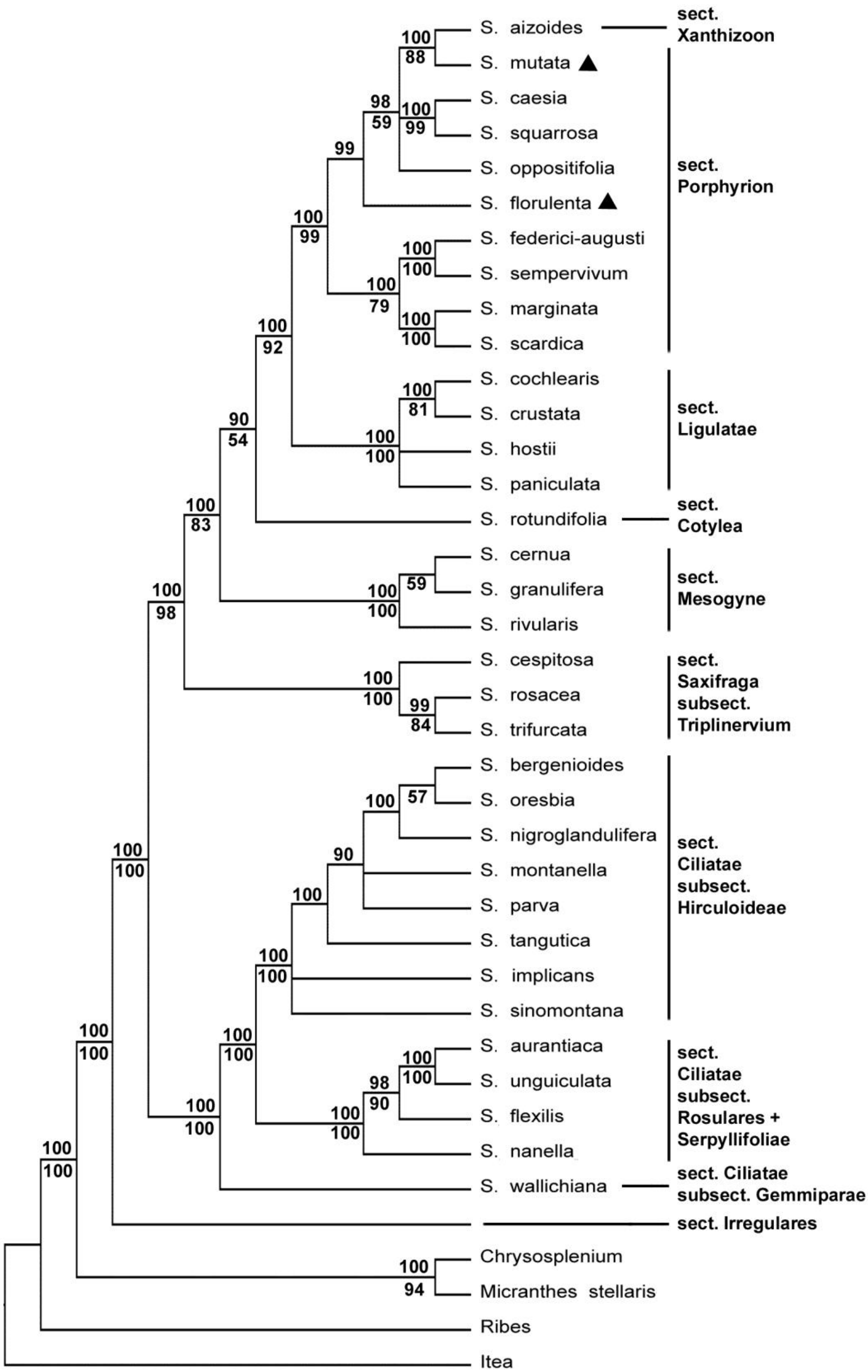
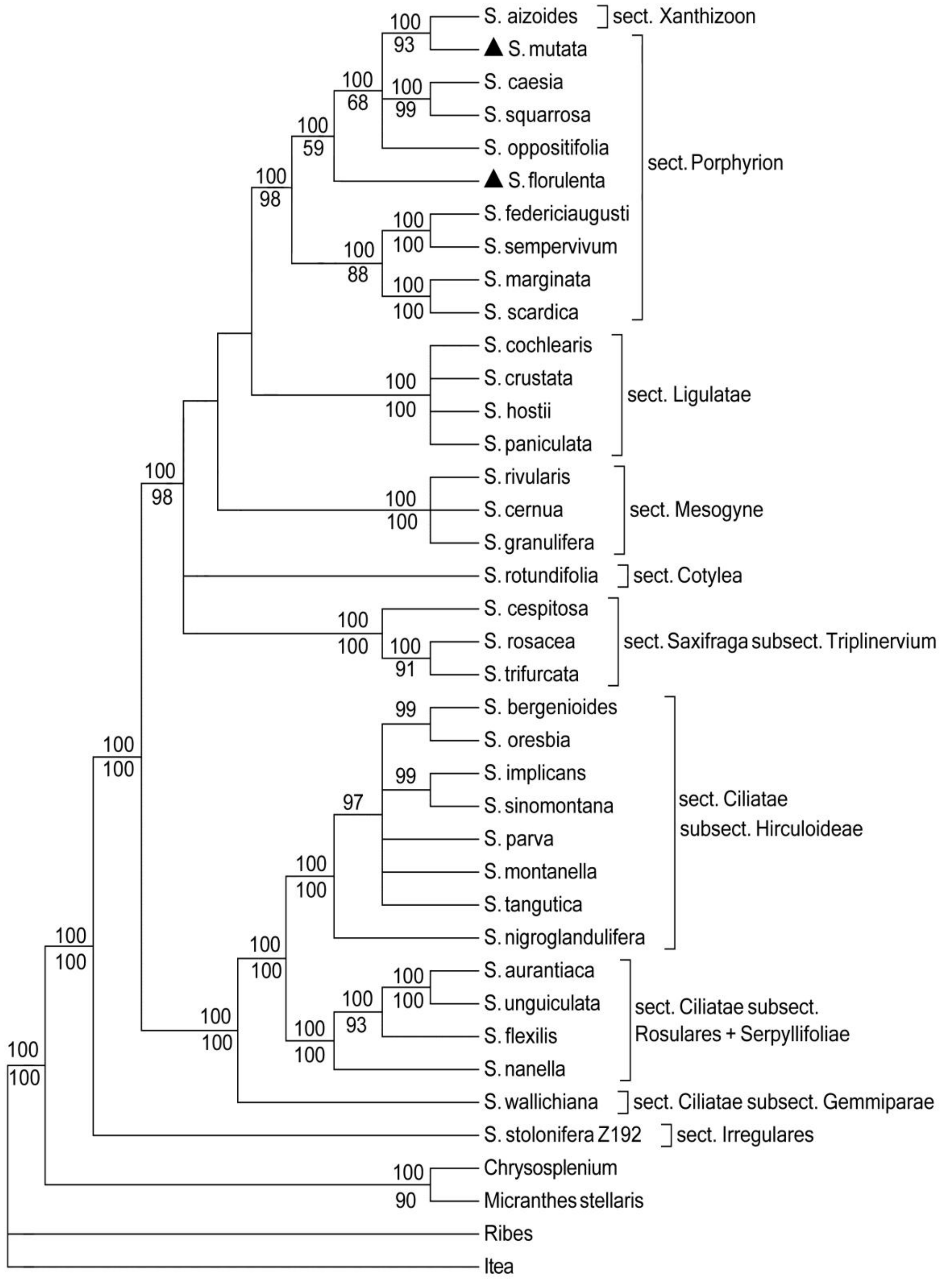


Figure 5.4. Bayesian tree from ITS + matK sequences (no morphological characters). The PP values >90% are above branches; the BS supports for the same topologies in the MP analysis are below the branches. Triangles are to highlight *S. mutata* and *S. florulenta*.



The topologies of the two trees are similar. The statistic support differences in the main clades between combined DNA + morphology tree and the DNA-only tree are as follows:

1. The BS for the *Xanthizoon* and *S. mutata* clade is higher on the DNA-only tree (93%) than the other one (88%);
2. The supports for the *S. oppositifolia* to *Xanthizoon* clade is higher on the DNA-only (PP 100%, BS 68%) than the total evidence tree (PP 98%, BS 59%);
3. The supports for the *S. florulenta* to *Xanthizoon* clade is higher on the DNA-only (PP 100%, BS 59%) than the total evidence tree (PP 99%, BS n/a);
4. The BS support for the *S. scardica* to *S. federici-augusti* clade is higher on the DNA-only (88%) than total evidence tree (79%);
5. The BS for the sect *Porphyrion* clade is higher on the total evidence tree (99%) than the DNA-only one (98%);
6. The sect. *Ligulatae* & *Porphyrion* clade is not supported on the DNA-only tree but strongly supported on the total evidence tree (PP 100%, BS 92%);
7. S. rotundifolia (sect. *Cotylea*) is unresolved and sister to sect. *Saxifraga* clade on the DNA-only tree but on the total evidence tree it is sister to the *Porphyrion* + *Ligulatae* clade (PP 90%, BS 54%);
8. The position of sect. *Mesogyne* clade is unsupported on the DNA-only tree but well supported (PP 100%, BS 83%) on the total evidence tree and sister to the *Cotylea* clade.

5.5.3 Discussion

Since the total evidence data analysis has only 10 most parsimonious trees compared to more than a hundred for the DNA-only data set, it seems that morphological characters can improve the phylogeny. Adding morphological data decreased the support values in some places but increased it in others, particularly at the intra-sectional level (result 5-8).

Moreover, the results show that there is no significant incongruence between DNA and morphological data. Most sections on the morphological MP tree are monophyletic and show agreement with the ITS + matK MP tree (Figs. 2.14).

The total evidence tree is better resolved than the ITS + matK tree (Fig. 2.14) in the positions of sects. *Ligulatae*, *Porphyrion*, and *Cotylea*. The main weakness for the total evidence tree is that the number of species studied in the matK analysis limited the final number of species in the tree.

Chapter Six: Taxonomic conclusions and discussion

"Having gathered these facts, Watson, I smoked several pipes over them, trying to separate those which were crucial from others which were merely incidental."

Sherlock Holmes -*The Crooked Man*

6.1 General conclusions and major results

The study considered both molecular and morphological evidences to estimate a putative evolutionary relationship of the genus *Saxifraga*. Phylogenetic trees were produced from both evidences and the combined data set. The major findings are summarised here.

Molecular data comprised DNA sequences from the chloroplast gene (*matK*) and nuclear regions (*ITS* and *DUO1*). The ITS maximum parsimony tree shows that subsections *Gemmiparae* and *Flagellares* in section *Ciliatae* have a close relationship and the former is paraphyletic to the latter (Fig. 2.8). However, the matK analysis does not confirm this combination. In the ITS (Fig. 2.8) and matK (Fig. 2.10) MP trees of sect. *Ciliatae*, species from subsection *Rosulares* and *Serpyllifoliae* cluster together and cannot be assigned to separate groups. Moreover, they have similar genetic distances in the ITS neighbour joining tree (Fig. 2.4). The sect. *Porphyrion* clade (in MP trees of ITS, matK and the combined data) includes *S. florulenta* (formerly as sect. *Ligulatae* subsect.

Florulentae), *S. mutata* (formerly as sect. *Ligulatae* subsect. *Mutatae*) and *S. aizoides* (formerly as sect. *Xanthizoon*).

The sequences were also used in plant identification. However, the molecular limits to species in *Saxifraga* are impossible to define. In many cases genetic distances between species in terms of DNA sequences were very low, indicating rapid evolution of morphology or DNA coding regions. On the other hand, the polyphyly of some species raises questions about whether the species are artificial and whether the limited collections of populations can give a true picture of the species.

Morphological evidence, including microscopic characters from pollen nuclear number, exine and leaf venation, was also collected. Trinucleate pollen is confined to section *Ciliatae* subsection *Hirculoideae*, where it is a putative synapomorphy, and to two species of subsection *Serpyllifoliae*. Types of exine morphology and anatomy were observed and compared with that of Ferguson & Webb (1970). A novel Type 2.3, in which the single tectum is punctured with numerous micro-capillaries, is a putative synapomorphy for sects. *Heterisia* and *Irregulares*. Three species from sect. *Ciliatae* have a novel Type 5 while the rest of the section is uniformly Type 3. In leaf venation study, the pinnate type (camptodromous venation) was firstly observed in the genus *Saxifraga* in sect. *Ligulatae*, *Gymopera* and *Porphyrion*.

Morphological matrix has only 49 characters thus the dataset with small amount of species was better performed. The 37 species tree is well supported at the sectional level but not at the inter-sectional level. This suggests that the characters can specify sections do not provide the synapomorphies necessary to group them.

Molecular and morphological characters have different and complementary strengths and weakness. In this study, morphological features proved informative and improved the resolution of the molecular tree, particularly at the intra-sectional level.

The description of a new species, *S. minutissima* from N. India (Gornall, Rawat and Zhang 2012; Appendix 5), is part of the systematic study. It is a good example of how morphology characters correspond to molecular data. *S. minutissima* had pollen exine with fine parallel lirae and granular supratectal processes or verrucae, a pattern that is a synapomorphy for section *Ciliatae*. Within section *Ciliatae*, the presence in the new species of somewhat fleshy leaves, prostrate axillary leafy shoots, and the absence of rosette leaves and rufous, crisped hairs, indicates a position in subsection *Serpyllifoliae* (Gornall 1987a). The assumption was proved by ITS sequence data which clustered *S. minutissima* and other sect. Ciliatae subsect. *Serpyllifoliae* species (Fig. 2.9).

6.2 Taxonomic treatment of Saxifraga

A number of realignments, new combinations and new taxa are needed, to make the sections and subsections monophyletic, as a consequence of the research presented in this thesis. A revised classification of *Saxifraga* primarily to subsection level is presented below, with description and comments on each of the groups where appropriate.

The main changes from previous classification (Gornall 1987a) are:

(1) genus *Saxifragella* is included as a monotypic section in *Saxifraga*;

(2) sect. *Ciliatae* subsect. *Serpyllifoliae* is merged into sect. *Ciliatae* subsect. *Rosulares*;

(3) sect. *Ciliatae* subsect. *Cinerascentes* is merged with subsection *Gemmiparae*;

(4) section *Porphyrion* is monophyletic only if *S.mutata* and *S. florulenta* (formerly in sect. *Ligulatae*) are included; this transfer also makes sect. *Ligulatae* monophyletic;

(5) *S.mutata* and *S.aizoides* (the later formerly in sect. *Xanthizoon*) are closely related and are now placed together in ser. *Xanthizoon* of sect. *Porphyrion*.

A key to each group is given.

Key to *Saxifraga* groups

1	Flowering stem leafless; basal rosette leaf palmate with long petiole; pollen exine punctured with radially orientated micro-capillaries or fissures	—2
	Flowering stem leafy; rosette leaf sometimes absent; pollen exine without micro-capillaries or fissures	—3
2	Flower zygomorphic; leaf with crystal druse	Sect. ***Irregulares***
	Flower actinomorphic; leaf without crystal druse	Sect. ***Heterisia***
3	Leaf apex deeply bifid, with an acute sinus; stamens 5; petals 0	Sect. ***Saxifragella***
	Leaf apex acute, obtuse or rounded; stamens usually 10, occasionally 8, in one species 5; petals usually 5, rarely 0	—4
4	Leaf hydathodes secreting water, superficial	—5
	Leaf hydathodes secreting calcium bicarbonate (chalk-glands), usuallysunken	—16
5	Petal usually yellow, rarely red or white, usually with proximal calloses	—6
	Petal usually white, rarely yellow, green or cream; calloses absent	—11
6	Annual or biennial; rosette leaves usually palmately lobed or toothed; leaf epidermis with tannin cells; pollen exine coarsely striate	Sect. ***Cymbalaria***
	Usually perennial; rosette leaves usually entire; leaf epidermal cells not tanniniferous; pollen exine finely striate	—7
7	At least proximal stem nodes and petiole bases with brown, crisped, villous hairs; pollen trinucleate	Sect. ***Ciliatae*** subsect. ***Hirculoideae***
	Proximal stem nodes glabrous or with colourless/white glandular hairs; pollen nearly always binucleate	—8
8	Leaves often shiny or hairy; leafy buds produced in axils of cauline leaves	Sect. ***Ciliatae*** subsect. ***Gemmiparae***
	Not as above.	—9
9	Rosette leaves producing axillary, filiform stolons.	Sect. ***Ciliatae*** subsect. ***Flagellares***

	Stolons absent	—10
10	Basal leaves with a dense distal fringe of eglandular hairs which often united to form a scarious margin	Sect. ***Ciliatae*** subsect. ***Hemisphaericae***
	Basal leaves setose-ciliate or glandular-ciliate, but the hairs not united into a fringe	Sect. ***Ciliatae*** subsect. ***Rosulares***
11	Leaf entire; with axillary leafy buds	Sect. ***Trachyphyllum***
	Leaf toothed or lobed, usually without axillary leafy buds	—12
12	Leaf leathery and thick; leaf venation pinnate	Sect. ***Gymnopera***
	Leaf herbaceous, venation palmate	—13
13	Rhizomes with tubers; sometimes tubers in leaf axils	Sect. ***Mesogyne***
	Rhizomes without tubers	—14
14	Leaves rounded or reniform, with teeth or slightly lobed; petals white with many spots; ovary superior	Sect. ***Cotylea***
	Leaf lobed; usually with leafy shoots; petals usually white without spots; ovary inferior	—15
15	Annual or biennial; bulbils or leafy shoots absent; leaves entire to shortly 3-5 lobed.	Sect. ***Saxifraga*** subsect. ***Tridactylites***
	Perennial; bulbils or axillary leafy shoots often present; leaves lobed	Sect. ***Saxifraga*** subsect. ***Saxifraga***
16	Rosettes die after flowering; cyme compound; petals white, often spotted	Sect. ***Ligulatae***
	Rosettes persist after flowering; cyme usually simple; petals white, pink or yellow, usually without spots	—17
17	Leaves opposite, rarely alternate (*S. oppositifolia* ssp. *paradoxa*)	—18
	Leaves alternate	—19
18	Petals four; leaf-pairs confluent at the base; nodes glabrous	Sect. ***Porphyrion*** subsect. ***Oppositifoliae*** ser. ***Tetrameridium***

	Petals five; leaf-pairs not confluent; nodal hairs present	Sect. ***Porphyrion*** subsect. ***Oppositifoliae*** ser. ***Oppositifoliae***
19	Chalk-glands superficial; petals ensiform	Sect. ***Porphyrion*** subsect. ***Oppositifoliae*** ser. ***Xanthizoon***
	Chalk-glands sunken; petal apex rounded	—20
20	Rosette leaves strongly recurved to give a dome-like shape to the rosette	Sect. ***Porphyrion*** subsect. ***Oppositifoliae*** ser. ***Squarrosae***
	Rosette leaves patent or ascending, rosettes not dome-like	—21
21	Carpels three	Sect. ***Porphyrion*** subsect. ***Florulentae***
	Carpels two	—22
22	Rosettes few; cyme raceme-like; petals concealed by sepals	Sect. ***Porphyrion*** subsect. ***Engleria***
	Rosettes forming dense cushions; cyme simple, few-flowered; petals exceeding sepals	Sect. ***Porphyrion*** subsect. ***Kabschia***

Saxifraga L., Sp. Pl. 398 (1753). TYPE *S. granulata* L., designated by Small (1905)

1) Sect. ***Heterisia*** (Raf. ex Small) A.M. Johnson, Minnesota Stud. Pl. Sci. 4: 4, 65 (1923). TYPE *S. mertensiana* Bong.

Basionym: *Heterisia* Raf. ex Small, N. Amer. Fl. 22: 155 (1905).

This section contains a single species of woodland herbs from western N.America. It has usually leafless flowering stems, a superior ovary, and pollen whose smooth exine has micro-capillaries. It is strongly supported as sister to sect. *Irregulares* (matK 99%BS, 100%PP; ITS no reliable data; morphology 83%BS, 100%PP).

2) Sect. ***Irregulares*** Haw., Misc. Nat. 155, 158 (1803). TYPE *S. stolonifera* W. Curtis

Synonyms: *Diptera* Borkhausen, Roem. Neues Mag. Bot., I : 29 (1794); TYPE: S. stolonifera W. Curtis

section *Diptera* (Borkhausen) Sternberg, Revis. Saxzfrag. Suppl. 11: 12 (1831).

Eleven species of woodland herbs distributed from Japan, through north-eastern to southern China. The group is distinguished primarily by its distinctive zygomorphic flowers. Ovary is superior. Pollen exine is smooth with micro-capillaries. Monophyletic (ITS 99%BS, 100%BS; matK insufficient taxonomic sample; morphology 68%BS, 98%PP).

3) *Saxifraga* section ***Saxifragella*** (Engler) Gornall & Zhang, comb. nov.

Basionym: *Saxifragella* Engler, in Engler & Prantl., Nat. Pflanzenfam. 3(2a): 61 (1891). TYPE: *Saxifraga bicuspidata* Hook. f., Bot. Antarct. Voy. I. (Fl. Antarct.). 2: 281, t.97 (1846 [ante 2 Mar 1846]).

The section contains a single, mat-forming species from Tierra del Fuego. It is recognised mainly by its uniquely bifid leaves and calyx. The ovary is superior and the pollen exine is coarsely striate, lacking supra-tectal processes. Sister to sect. *Trachphyllum* (ITS 58%BS, 98%PP; matK no data; morphology no data) or to the rest of the genus in the six-region tree of Soltis et al. (2001) (100%BS). The latter evidence indicates a separate status is warranted.

4) Sect. ***Trachyphyllum*** (Gaud.) W.D.J. Koch, Syn. Fl. Germ. Helv. 270 (1837). 10 spp. TYPE *S. aspera* L.

Synonyms: *Ciliaria* Haworth, *Saxlfrag. Enum.:* **4,** 41 (1821), *non* Stackhouse (1809) *nom. rej.;* TYPE: C. *aspera* (L.) Haworth *(S. aspera* L.), designated by Gornall (1987a).

group *Trachyphyllum* Gaudin, *Fl. Helv.,* 3: 85, *108* (1828). TYPE: C. *aspera* (L.) Haworth *(S. aspera* L.), designated by Gornall (1987a).

A section of about ten species of cushion- or mat-forming herbs, distributed chiefly in north-eastern Asia, north-western North America and the European Alps. They are characterised by largely entire leaves, with acrodromous venation; ovary is usually superior; pollen exine is coarsely striate with supra-tectal processes. Monophyletic (ITS 99%BS, 100%PP; matK no data; morphology 85%BS, 96%PP).

5) Sect. ***Ciliatae*** Haw., Misc. Nat. 155, 160 (1803). TYPE S. hirculus L., designated by Siplivinsky (1983).

Synonym: section *Hirculus* (Haworth) Sternberg, *Revis. Saxifrag. Suppl. II*: 17 (1831). TYPE *S. hirculus* L.

This section contains ca 215 species, distributed mainly in the Sino-Himalayan region, but with a few species with a circum-boreal distribution. They are characterised by usually yellow flowers that have proximal calloses. The leaves are more or less entire with acrodromous venation. Ovary position varies from superior to inferior. The pollen nearly always has a finely-striate exine. Monophyletic (ITS 57%BS, 100%PP [excl.

S.hemisphaerica]; matK 99%BS, 100%PP; ITS+matK 99%BS, 100%PP; morphology 77%BS 100%PP).

a) subsect. ***Gemmiparae*** Engl. & Irmsch., Notes R. Bot. Gard. Edinb. 5(24): 140 (1912). TYPE *S. gemmipara* Franch., designated by Gornall (1987a).

Synonym: subsect. *Cinerascentes* Engl. & Irmsch., Notes R. Bot. Gard. Edinb. 5(24): 142 (1912). TYPE *S. cinerascens* Engl. & Irmsch.

The subsection contains 19 species of herbs usually found on cliffs and rock-faces. They are characterised by usually shiny abaxial leaf surfaces and by the presence of axillary leafy buds. Apparently paraphyletic. Monophyletic only if sect. *Flagellares* is included (ITS 66%, 100%PP; matk unresolved; morphology unresolved). Given the ambiguity and the incomplete sampling of sect. *Flagellares*, it is best to retain section *Gemmiparae* as circumscribed for now. The subsection name *Cinerascentes* was published at the same time as subsect. *Gemmiparae*. Here I choose the latter name to represent the combined subsections because it has been more widely used in the literature (Pan 1982).

b) subsect. ***Flagellares*** (C.B. Clarke) Engl. & Irmsch., Notes R. Bot. Gard. Edinb. 5(24): 146 (1912). TYPE *S. flagellaris* Willd., designated by Gornall (1987a).

Synonym: section *Flagellares* C.B. Clarke in J.D. Hooker, *Fl. Br. India, 2:* 397 (1878).

A subsection of 19 species of herbs usually growing on bare ground and talus. They are characterised by the unique presence of axillary thread-like stolons. Monophyletic (ITS 99%BS, 100%PP; matK and morphology taxonomic sampling insufficient).

c) subsect. ***Hirculoideae*** Engl. & Irmsch., Notes R. Bot. Gard. Edinb. 5(24): 135 (1912). TYPE *S. nigroglandulosa* Engl. & Irmsch., designated by Gornall (1987a).

Synonyms: subsection *Densae* Engler & Irmscher, Notes R. bot. Gard. Edinb., 5(24): 129 (1912). TYPE *S. macrostigma* Franchet, designated by Gornall (1987a).

subsection *Turfosae* Engler & Irmscher, Notes R. bot. Gard. Edinb., 5(24): 133 (1912); TYPE *S. turfosa* Engler & Irmscher, designated by Gornall (1987a).

subsection *Stellariifoliae* Engler & Irmscher, Notes R. Bot. Gard. Edinb., 5(24): 135 (1912). TYPE *S. cardiophylla* Franchet

The subsection contains about 110 species. It is characterised by crisped-rufous hairs at least at the proximal nodes, and by trinucleate pollen. Monophyletic (ITS 99%BS, 100%PP; matK 99%BS, 100%PP; ITS+matK 99%BS, 100%PP; morphology unretaxonomic sampling insufficient).

d) subsect. ***Rosulares*** Gornall, Bot. J. Linn. Soc. 95: 277 (1987a). TYPE *S. sediformis* Engl. & Irmsch., designated by Gornall (1987a).

Synonyms: subsection *Sediformes* Engler & Irmscher, *Notes R. Bot. Gard. Edinb., 5(24):* 143 (1912), *nom. illeg., non* group *Sedformes* H.G.L. Reichenbach (1832).

subsect. *Serpyllifoliae* Gornall, Bot. J. Linn. Soc. 95: 277 (1987a). TYPE *S. serpyllifolia* Pursh

A subsection of 62 species, characterised by the presence of well-defined basal leaf rosettes or by well-developed, proximal, axillary leafy shoots. Most species have binucleate pollen but two (*S. engleriana* and *S. jacquemontiana*) have the trinucleate condition. Monophyletic (ITS 58%BS, <90%PP; matK 98%BS, 100%PP; ITS+matK 99%BS, 100%PP; morphology unresolved). By combining subsects. *Rosulares* and *Serpyllifoliae*, which were published simultaneously, I am reverting to Engler & Irmscher's original, broad concept of this group. Their name for this subsection is illegitimate (later homonym) so I have chosen the subsection name that has the same type.

e) subsect. ***Hemisphaericae*** (Engl. & Irmsch.) Gornall, Bot. J. Linn. Soc. 95: 277 (1987a). TYPE *S. hemisphaerica* J.D. Hook. & Thoms., designated by Gornall (1987a).

This subsection contains six species of cushion-froming herbs. They are characterised by rosette leaves that have a distal fringe of eglandular hairs. Taxonomic sampling is insufficient to draw conclusions about evolutionary status. The ITS tree suggests the type species does not belong to sect. *Cilatae* (it is unresolved). Morphological evidence, especially pollen exine morphology (Chapter 3), strongly suggests, however, that it belongs in this section. Further study is needed to resolve the exact position of these species.

6) Sect. ***Cymbalaria*** Grisebach, Spicil. Fl. Rumel. 1: 336 (1843). TYPE *S. cymbalaria* L.

A small section of four, annual or biennial herbs native to Europe, the Middle-East and north-eastern Africa. Characterised by yellow petals with proximal yellow calloses, a superior ovary and by leaves with tanniniferous epidermal cells. Monophyletic (ITS 99%BS, 100%PP; matK no data; morphology 93%BS, 100%PP).

7) Sect. ***Saxifraga***

Synonyms: group *Nephrophyllum* Gaudin, *F1. Helv.,* 3: 85, 103 (1828); TYPE S. *granulata* L, designated by Gornall (1987a).

section *Nephrophyllum* (Gaudin) W. D. J. Koch, *Syn. Fl. Germ. Helv.:* 276 (1837).

section *Dactylites* Tausch, *Hart. Canal.:* 1 (1823); TYPE S. *granulata* L., designated by Gornall (1987a).

section *Dactyloides* Tausch; Seringe in DC., *Prodr.,* ***4:*** 23 (1830), later name for section *Dactylites* Tausch, orth. var.

Tridactylites Haworth, Saxlfrag. Enum.: 3, 2 1 (1821). TYPE *S. tridactylites* L.

section *Tridactylites* (Haworth) Grisebach, *Spicil. Fl. Rumel., 1:* 335 (1843).

This section contains some 85 species, distributed chiefly across the mountains of Europe. They are charatcterised by usually deeply lobed leaves and an inferior ovary. The pollen exine is often smooth, but without micro-capillaries. Monophyletic (ITS 99%BS, 100%PP; matK 99%BS, 100%PP (but limited sampling); ITS+matK 99%BS, 100%PP; morphology unresolved).

a) subsect. ***Tridactylites*** (Haw.) Gornall, Bot. J. Linn. Soc. 95: 291 (1987a). TYPE *S. tridactylites* L., designated by Gornall (1987a).

Synonyms: *Tridactylites* Haworth, Saxlfrag. Enum.: 3, 2 1 (1821). TYPE *S. tridactylites* L.

section *Tridactylites* (Haworth) Grisebach, *Spicil. Fl. Rumel., 1:* 335 (1843).

A small subsection of four species of annual or biennial herbs. The pollen differs from most other members of the section in having a coarsely striate exine. Monophyletic (ITS 92%BS, 100%PP; matK no data; morphology 79%BS, 92%PP).

b) subsect. ***Saxifraga***

Provisional synonyms: group *Holophyllae* Engler in Engler & Prantl, *Nat. Pflanzenfam., 3, 2a:* 55 (1891). TYPE S. *muscoides* All., designated by Gornall (1987a).

subsection *Holophyllae* (Engler) Engler & Irmscher, *Pflanzenr.,* 67: 284 (1916), basionym not cited directly.

subsection *Triplinervium* (Gaudin) R.J. Gornall, group *Triplinervium* Gaudin, *Fl. Helv.,* 3: 85, 116 (1828). TYPE S. *cespitosa* L., designated by Gornall (1987a).

The subsection contains about 80 species of perennial herb. They are characterised by mostly deeply lobed leaves (entire in subsect. *Holophyllae*). It is unclear from the analyses whether or not subsections *Holophyllae* and *Triplinervium* are monophyletic or whether they are distinct from subsect. *Saxifraga*. As defined here, the subsection s.l. is monophyletic (ITS 97%BS, 100%PP; matK 99%BS, 100%PP but taxonomic scope limited; ITS+matK 99%BS, 100%PP but taxonomic scope limited; morphology taxonomic sampling insufficient).

8) Sect. ***Cotylea*** Tausch, Hort. Canal. 1 (1823). TYPE *S. rotundifolia* L.

Synonyms: *Miscopetalum* Haworth, Enum. Pl. Succ.: 323 (1812); TYPE M. rotundifolium (L.) Haw. (*S. rotundifolia* L.)

section *Miscopetalum* (Haworth) Sternberg, Revis. Saxzfrag. Suppl. 11: 15 (1831).

A section of three morphologically very similar perennial herbs, native to European montane woodlands. They are characterised by a superior ovary and a coarsely striate pollen exine. Putatively monophyletic (ITS, matK taxonomic sampling insufficient; morphology 74%BS, 99%PP).

9) Sect. ***Mesogyne*** Sternb., Revis. Saxifrag. Suppl. II, 29 (1831). TYPE S. sibirica L., designated by Gornall (1987a).

Section *Nephrophyllum* grex *Sibiricae* Engler & Irmscher, *Pflanzenr.,* 67: 233, 262 (1916); TYPE *S. sibirica* L., designated by Gornall (1987a).

sect. *Odontophyllae* Gornall, Bot. J. Linn. Soc. 95: 286 (1987a). TYPE *S. odontophylla* Wall.

The section contains 16 species, distributed chiefly in the Sino-Himalaya but with circum-boreal extensions. The group is characterised by a distinctive multi-lobed leaf-shape, bulbiliferous rhizomes, a superior ovary, pollen with a coarsely striate exine, and large seeds. Monophyletic (ITS 99%BS, 100%PP; matK 99%BS, 100%PP; ITS+matK 99%BS, 100%PP; morphology 76%BS, 100%PP).

Study of herbarium material of *S. asarifolia* <authty> (=*S. odontophylla* Wall.) strongly suggests that this species at least belongs in sect. *Mesogyne*. It resembles *S. sibirica* in particular, and its distinctive seed characters also match those of this section (Kaplan 1981).

10) Sect. ***Gymnopera*** D. Don, Trans. Linn. Soc. 13: 343, 344 (1822). TYPE *S. hirsuta* L., designated by Gornall (1987a).

Synonyms: *Robertsonia* Haworth, Syn. PI. Succ.: 321 (1812); TYPE R. crenata Haw. (= S. hirsuta L.), designated by Gornall (1987a).

section *Robertsonia* (Haworth) Sternberg, Revis. Saxifrag. Suppl. II: 3 (1831).

This morphologically well-marked section contains four species of woodland herb, distributed in the mountains of Europe. They are characterised by nearly leafless stems, pinnately-veined leaves, flowers with a superior ovary and coarsely-striate pollen exines. The group is putatively monophyletic (ITS 91%BS, 100%PP; matK polyphyletic; morphology 91%BS, 100%PP), but there is a conflict between the ITS and matK trees for this section. The reason is likely to be due to hybridisation. There are documented hybrids with species of sect. *Ligulatae* (*S.spathularis x paniculata*) and sect. *Porphyrion* (*S. umbrosa x aizoides*) (Webb and Gornall 1989). Under these circumstances, chloroplast capture, a phenomenon which the cpDNA from one parent has been retained in the hybrids but not from the other parent (Judd 2007), could be involved. Therefore, the ITS topology given the monophyletic clade is more likely to reflect the relationship between *Gymnopera* species. More study is needed.

11) Sect. ***Ligulatae*** Haw., Misc. Nat. 155, 159 (1803). TYPE *S. cotyledon* L., designated by Siplivinsky (1983).

Synonym: subsection *Aizoonia* (Tausch) Schott in Schott, Nyman & Kotschy, *Analecta Bot.:* 20 (1854) *"Euaizoonia"*; TYPE *S. aizoon* Jacq. (= S. *paniculata* Miller), designated by Gornall (1987a).

This is a small section of eight species, known as the 'Silver Saxifrages' by gardeners. The species are characterised by monocarpic leaf rosettes, whose leaves have chalk-glandsand pinnate venation where the marginal veins end freely (= 'open'); the inflorescence is paniculate and the flowers have white petals, often with spots, and an inferior ovary. The group is monophyletic (ITS 98%BS, 100%PP; matK 81%BS, 100%PP; ITS+matK 99%BS, 100%PP; morphology taxonomic sampling insufficient).

The section now includes only subsection *Aizoonia* (Tausch) Schott, because *S. mutata* and *S. florulenta* have been moved to sect. *Porphyrion*.

12) Sect. ***Porphyrion*** Tausch, Hort. Canal. 1 (1823). TYPE *S. oppositifolia* L.

Synonyms: group *Porophyllum* Gaudin, *Fl. Helv.,* ***3:*** 84, 91 (1828); TYPE *S. caesia* L., designated by Gornall (1987a).

section *Porophyllum* (Gaudin) Engler, *Linnaea,* ***35:*** 14, 16 (1867).

This section contains 106 species of mostly cushion- or mat-forming herbs, distributed in a wide arc in the mountains of Europe and Asia. The species are characterised by chalk-glands in their leaves, with pinnate venation, and coarsely-striate pollen exines.

As defined here the group is monophyletic (ITS 87%BS, 100%PP; matK 66%BS, 97%PP; ITS+matK 95%BS, 100%PP; morphology polyphyletic).

Sect. *Porphyrion* formerly included only *S. oppositifolia* and its close relatives. It was later expanded by Gornall (1987a) to include all the *Kabschia* and *Engleria* saxifrages, with each group recognised as a subsection. It is now clear that three more species belong to the section, viz. *S. florulenta* (formerly sole member of sect. *Ligulatae* subsect. *Florulentae*), *S. mutata* (formerly sole member of sect. *Ligulatae* subsect. *Mutatae*), and *S. aizoides* (formerly sole member of sect. *Xanthizoon*).

a) subsect. ***Oppositifoliae*** Hayek, Denkschr. Akad. Wiss. Wien 77: 637 (1905). TYPE *S. oppositifolia* L., designated by Gornall (1987a).

The subsection as defined here contains 16 species of rosette-, mat- or cushion-forming herbs distributed in the mountains of Europe and the

Sino-Himalaya. The group is recognised on the basis of DNA sequence data and it is not yet possible of offer any morphological synapomorphies. The following series are tentatively regarded as forming a monophyletic group constituting subsect. *Oppositifoliae* (ITS unresolved; matK 67%BS, 93%PP; ITS+matK 58%BS, 99%PP; morphology unresolved).

i) ser. ***Oppositifoliae*** (Hayek) Gornall, Bot. J. Linn. Soc. 95: 282 (1987a). TYPE *S. oppositifolia* L.

Basionym: subsection *Oppositfoliae* Hayek, Denkschr. Akad. Wiss. Wien, 77: 637 (1905); TYPE *S. oppositifolia* L.

A group of nine species native to Europe and the Sino-Himalaya and with Arctic representatives. They are characterised by the opposite leaves meeting at an acute angle where the proximal margins are toothed-ciliate. Monophyletic (ITS 99%BS, 100%PP; matK taxonomic sampling insufficient; morphology insufficient data).

ii) ser. ***Tetrameridium*** (Engl.) Gornall, Bot. J. Linn. Soc. 95: 283 (1987a). TYPE *S. nana* Engl.

Basionym: section *Tetrameridium* Engler, Bull. Acad. St. Petersb., 29: 118 (1883). TYPE *S. nana* Engl.

A group of seven species native to the Sino-Himalaya and diagnosed by the confluent nature of the pairs of opposite leaves. No species from this series were included in this study. Its evolutionary status remains untested.

iii) ser. ***Xanthizoon*** (Griseb.) Gornall & Zhang, stat. nov.

Basionym: *Saxifraga* section *Xanthizoon* Griseb. Spic. fl. rumel. 1: 333 (1843). TYPE: *S. aizoides* L. Sp. Pl: 403. (1753), designated by Small (1905).

Synonyms: subsection *Mutatae* (Engl. & Irmsch.) Gornall, Bot. J. Linn. Soc. 95: 283 (1987a). TYPE *S. mutata* L.

This series contains two species, one with an Arctic/circum-boreal distribution and one native to the European Alps. The petals are ensiform and coloured yellow or orange; and the pollen exine is coarsely striate with supra-tectal processes. The group is probably monophyletic (ITS 89%BS, 99%PP; matK 81%BS, 100%PP; ITS+matk 97%BS, 100%PP; morphology paraphyletic).

Subsection *Mutatae* was formerly assigned to section *Ligulatae*, but the DNA evidence is clear that it is sister to *S. aizoides*, with which it hybridises (Webb and Gornall 1989).

iv) ser. ***Squarrosae*** (Engl. & Irmsch.) Gornall, Bot. J. Linn. Soc. 95: 282 (1987a).

Basionym: grex *Squarrosae* Engler & Irmscher, *Pflanzenr.,* 69: 535, 577 (1919). TYPE *S. squarrosa* Sieb., designated by Gornall (1987a).

A group of two species native to the European Alps, and characterised by dome-like basal leaf-rosettes; pollen exine is coarsely striate with supratectal processes. Monophyletic (ITS 96%BS, 100%PP; matK 89%BS, 100%PP; ITS+matK 99%BS, 100%PP; morphology 63%BS, 99%PP). This series was allocated to subsect. *Kabschia* by Gornall (1987a); it is fairly clear, however, that it belongs here. There is a natural hybrid between *S. caesia* and *S. aizoides* (Webb and Gornall 1989).

b) subsect. ***Florulentae*** (Engl. & Irmsch.) Gornall, Bot. J. Linn. Soc. 95: 283 (1987a). TYPE *S. florulenta* Moretti

Basionym: grex *Florulentae* Engler & Irmscher, *Pflanzenr.,* 69: 476, 526 (1919); TYPE *S. florulenta* Moretti

The single species occurs in the maritime Alps of Europe, where it is regarded as endangered. It is characterised by a racemiform inflorescence with flesh-pink petals and a tricarpellate gynoecium. Its pollen exine is coarsely straite but lacks supra-tectal processes. This subsection was allocated to sect. *Ligulatae* by Gornall (1987a). My analyses indicate, however, that it does not belong there but is instead nearer either subsect. *Engleria* or subsect. *Oppositifoliae*.

c) subsect. ***Engleria*** (Sundermann) Gornall, Bot. J. Linn. Soc. 95: 282 (1987a).

Basionym: section *Engleria* Sundermann, *Allg. Bot. Zeitschr., 21:* 23 (1915). TYPE *S. media* Gouan

Synonym: grex *Mediae* Engler & Irmscher, *Pflanzenr.,* 69: 535 (1919); TYPE S. *media* Gouan, designated by Gornall (1987a).

This exclusively European group of six species is diagnosed by a racemiform inflorescence, with petals included within the calyx, and by a coarsely-striate pollen exine that lacks supra-tectal processes. Monophyletic (ITS 88%BS, 100%PP; matK 99%, 100%PP; ITS+matK 99%BS, 100%PP; morphology insufficient data).

d) subsect. ***Kabschia*** (Engl.) Rouy & Camus, Fl. France 7: 72 (1901).

Basionym: section *Kabschia* Engler, *Linnaea, **35:*** 14, 16 (1867). TYPE *S. marginata* Sternb., designated by Gornall (1987a).

This is a large group of about 85 species of cushion- or mat-forming herbs, distributed in the mountains of Europe, the Caucasus and into the Sino-Himalaya. Morphological variation is extensive, but the inflorescence is a simple cyme with unspotted flowers; pollen exine is coarsely straite with supra-tectal processes. Notwithstanding the limited sampling, the group appears to be monophyletic (ITS 91%BS, 100%PP; matK 73%BS, 91%PP; ITS+matK 91%BS, 100%PP; morphology taxonomic sampling insufficient).

6.3 The origin of Saxifraga

The origin of *Saxifraga* was proposed to be in Europe due to the large number of sections that grow there (Cain 1944; Symkiewicz 1937). The northern shore of the Pacific Ocean (Beringia) was also considered as the original place because the primitive sections clustered in this place (Kaplan 1981). However, we now know that the primitive sections that were the focus of Kaplan's discussion (sects. *Micranthes* and *Merkianae*) have been shown to belong elsewhere, not in *Saxifraga* (Soltis 2001a). Webb and Gornall (1989) discussed the question of the place of origin but came to no firm conclusion, other than to suggest that the earliest *Saxifraga* stock comprised woodland plants.

Some of the species locality information from Table 2.1 was plotted on the global map (Fig. 6.1). These locality data are from the accessions used in the molecular phylogenetic analyses thus the map can link to the phylogenetic trees in this study. The species were chosen from each section (modified system) to show the range of species distribution within a section.

From my studies, it is clear that the basal clade in *Saxifraga* phylogenetic trees (mainly the ITS maximum parsimony tree in Fig. 2.9) is distributed around the Pacific Rim: thus sect. *Heterisia* grows in western North America and its sister, sect. *Irregulares*, is distributed through eastern China to Japan on woodland edge. The next section up the tree is sect. *Saxifragella* which is endemic to Tierra del Fuego in the southern Pacific. Following is sect. *Trachyphyllum*, which has a centre of diversity in the north Pacific Rim (Beringia) but with a secondary centre in alpine Europe. The next section is sect. *Ciliatae* which is almost entirely restricted to the Sino-Himalaya area. The European sections do not appear in the phylogeny until after this point. For this reason, it is highly possible that the origin of the genus lies in the north Pacific Rim (Fig. 6.1).

Since fossil plant material is missing for *Saxifraga*, it is difficult to date the migration and speciation events. Moreover, the potential migration routes and distribution patterns require future phylogeographic studies which are above the scale of this Ph.D. project.

Figure 6.1. The global map indicates where the sections of the genus *Saxifraga* (Saxifragaceae) grow, based on locality data of the species used in my molecular study. The species from the primitive sections *Irregulares* and *Heterisia*, showed with red and orange dotted bubbles respectively, are distributed around the north Pacific Rim.

sect. Irregulares
sect. Heterisia
sect. Saxifragella
sect. Trachyphyllum
sect. Ciliatae
sect. Mesogyne
sect. Porphyrion
sect. Cymbalaria
sect. Saxifraga
sect. Cotylea
sect. Gymnopera
sect. Ligulates
North Pacific Rim
Greenland
Iceland
Finland
Sweden
Norway
Russia
Poland
Ukraine
Spain
Turkey
Kazakhstan
Canada
United States
Mexico
North Atlantic Ocean
North Pacific Ocean
Afghanistan
Pakistan
Iraq
Iran
Saudi Arabia
India
Thailand
Algeria
Libya
Egypt
Mali
Niger
Chad
Sudan
Nigeria
Ethiopia
DR Congo
Kenya
Tanzania
Angola
Namibia
Botswana
Madagascar
South Africa
Indonesia
Papua New Guinea
Australia
New Zealand
Indian Ocean
South Atlantic Ocean
South Pacific Ocean
Southern Ocean
Venezuela
Colombia
Brazil
Peru
Bolivia
Chile
Argentina
2000 mi
2000 km

Appendices

Appendix 1: Species list of genus *Saxifraga* and data types collected.

	SPECIES	DNA sequence			Pollen cell no.	exine type no.	venation type	Other morphological features
		ITS	matK	DUO 1				
Outgroup								
	Itea ilicifolia Oliv.	√				√	√	√
	Itea virginica L.		√					
	Itea yunnanensis Franch	√						
	Ribes alpestre Wall. ex Decne.	√				√	√	√
	Ribes maximowiczianum Kom.		√					
	Ribes nigrum L.	√						
	Ribes soulieanum Jancz.		√					
	Chrysosplenium album Maxim.		√					
	Chrysosplenium hydrocotylifolium H. Léveillé & Vaniot	√						
	Chrysosplenium lanuginosum var. gracile (Franchet) H. Hara	√						
	Chrysosplenium oppositifolium L.					√	√	√
	Chrysosplenium tetrandrum Th.Fr.		√					
	Micranthes merkii (Fischer) comb. nov.							
	Micranthes tolmiei (Torr. & A. Gray) Brouillet & Gornall		√					
	Micranthes bryophora (A.Gray) comb. nov.		√					
	Micranthes clusii (Gouan) comb.nov.							√
	Micranthes ferruginea (Graham) comb.nov.		√					
	Micranthes foliolosa (R.Br.) comb.nov.		√					
	Micranthes laciniata (Nakai & Takeda) comb.nov.							√
	Micranthes redofskyi (Adams) comb. nov.							

	Micranthes stellaris (L.) comb.nov.	√	√					√
	Micranthes atrata (Engl.) Losinsk.	√						
	Micranthes clavistaminea (Engl.& Irmsch.) Losinsk.							
	Micranthes davidii (Franch.) Losinsk.							
	Micranthes davurica (Willd.) Small							
	Micranthes divaricata (Engl.& Irmsch.) Losinsk.	√	√		√			
	Micranthes dungbooi (Engl.& Irmsch.) comb.nov.							
	Micranthes gageana (W.W.Sm.) comb.nov.							
	Micranthes kermodei (H. Sm. ex Wadhwa) comb. nov.							
	Micranthes lumpuensis (Engl.) Losinsk.				√			
	Micranthes melaleuca (Fisch.) Losinsk.							
	Micranthes melanocentra (Franch.) Losinsk.	√	√	√	√	√	√	√
	Micranthes nudicaulis (D.Don) comb.nov.							
	Micranthes pallida (Wall.ex Sr.) Losinsk.	√						
	Micranthes paludosa (Anthony) comb.nov.							
	Micranthes parvula (Engl.& Irmsch.) Losinsk.							
	Micranthes pluviarum (W.W.Sm.) comb.nov.							
	Micranthes pseudopallida (Engl. & Irmsch.) Losinsk.							
	Micranthes rubriflora (H.Sm.) Gornall & Ohba							
	Micranthes tilingiana (Regel & Tiling) Kom. ex Gornall & Ohba							
	Micranthes zekoensis (J.T.Pan) comb.nov.							
	Micranthes astilbeoides (Losinsk.) comb. nov.							
	Micranthes calycina (Sternb.) comb. nov.		√					

	Micranthes fusca (Maximovicz) H.Hara ex Gornall & Ohba		√					
	Micranthes japonica (Boissieu) Hara ex Gornall & Ohba							
	Micranthes lyallii (Engl.) Small		√					
	Micranthes manchuriensis (Engl.) Gornall & Ohba							
	Micranthes nelsoniana (D.Don) Small							
	Micranthes odontoloma (Piper) A.A.Heller							
	Micranthes spicata (D.Don) Small		√					
	Micranthes unalaschensis (Sternb.) comb. nov.							
	Micranthes micranthidifolia (Haw.) Small							
	Micranthes careyana (A.Gray) Small							
	Micranthes caroliniana (A.Gray) Small							
	Micranthes eriophora (S.Wats.) Small							
	Micranthes howellii (Greene) Small		√					
	Micranthes marshallii (Greene) Small							
	Micranthes mexicana (Engl.& Irmsch.) comb.nov.							
	Micranthes nivalis (L.) Small		√					√
	Micranthes oblongifolia (Nakai) comb.nov.							
	Micranthes occidentalis (S.Wats.) Small		√					
	Micranthes palmeri Bush							
	Micranthes reflexa (W.J.Hook.) Small		√					
	Micranthes rufidula Small		√					
	Micranthes sachalinensis (F.Schmidt) Hara ex Gornall & Ohba							
	Micranthes tempestiva (Elvander & Denton) comb. nov.							
	Micranthes tenuis (Wahlenb.) Small							√

	Micranthes texana (Buckl.) Small		√					
	Micranthes virginiensis (Michx) Small		√					
	Micranthes apetala (Piper) Small							
	Micranthes aprica (Greene) Small		√					
	Micranthes brachypetala (Malyschev) comb.nov.							
	Micranthes californica (Greene) Small		√		√			
	Micranthes fragosa (Suksd. ex Small) Small							
	Micranthes gaspensis (Fern.) Small							
	Micranthes gormanii (Suksdorf) Brouillet & Gornall							
	Micranthes hieraciifolia (Waldst. & Kit. ex Willd.) Haw.		√					√
	Micranthes hitchcockiana (P.E. Elvander) comb. nov.							
	Micranthes idahoensis (Piper) Brouillet & Gornall							
	Micranthes integrifolia (W.J.Hook.) Small		√					
	Micranthes nidifica (Greene) Small		√					
	Micranthes oregana (Howell) Small		√					
	Micranthes pensylvanica (L.) Haw.		√					
	Micranthes petiolaris Bush							
	Micranthes punctata (L.) Losinsk.		√					
	Micranthes rhomboidea (Greene) Small		√					
	Micranthes subapetala (E.Nelson) Small							
Ingroup								
1	Saxifraga abchasica Oettingen							
2	Saxifraga adenodes Poepp. ex Sternb.							
3	Saxifraga adscendens L.	√			√	√	√	√
4	Saxifraga afghanica Aitch. & Hemsl.	√				√		
5	Saxifraga aizoides L.	√	√		√	√	√	√

6	Saxifraga albertii Regel & Schmalh.							
7	Saxifraga aleutica Hulten							
8	Saxifraga alpigena H. Sm.					√		
9	Saxifraga amabilis H. Ohba & M. Wakabayashi							√
10	Saxifraga andersonii Engl.					√		
11	Saxifraga androsacea L.	√			√	√	√	√
12	Saxifraga angustata H. Sm.							√
13	Saxifraga anisophylla H. Sm.							
14	Saxifraga aphylla Sternb.	√			√	√	√	√
15	Saxifraga aquatica Lapeyr.	√			√	√	√	√
16	Saxifraga arachnoidea Sternb.	√			√	√	√	
17	Saxifraga arctolitoralis B.A. Yurtsev & Petrovsky							
18	Saxifraga aretioides Lapeyr. = felineri				√	√	√	√
19	Saxifraga arinae P.Yu. Zhmylev							
20	Saxifraga aristulata J.D.Hook. & Thoms.				√		√	√
21	Saxifraga artvinensis Matthews							
22	Saxifraga asarifolia (syn. odontophylla)					√		√
23	Saxifraga asarifolia Sternb.							
24	Saxifraga aspera L.			√	√	√	√	√
25	Saxifraga atuntsiensis W.W.Sm.							√
26	Saxifraga aurantiaca Franch. (=confertifolia)	√			√	√	√	√
27	Saxifraga auriculata Engl.& Irmsch.		√		√	√	√	√
28	Saxifraga babiana T.E. Diaz Gonzalez & J.A.Fernandez Prieto	√						
29	Saxifraga baimashanensis C.Y. Wu							
30	Saxifraga balfourii Engl.& Irmsch.	√			√	√		√
31	Saxifraga bergenioides Marquand	√	√	√	√	√	√	√
32	Saxifraga berica (Beguinot) D.A. Webb					√		
33	Saxifraga bicuspidata	√				√		√

34	Saxifraga biebersteinii Sipliv.							
35	Saxifraga biflora All.	√			√	√	√	√
36	Saxifraga biternata Boiss.	√			√	√		√
37	Saxifraga blavii (Engl.) G.Beck von Managetta							
38	Saxifraga bourgaeana Boiss. & Reut.	√			√	√	√	√
39	Saxifraga boussingaultii Brongn.							
40	Saxifraga brachyphylla Franch.							√
41	Saxifraga brachypoda D. Don	√			√	√		√
42	Saxifraga brachypodoidea J-T. Pan							
43	Saxifraga bracteata D. Don							
44	Saxifraga brevicaulis H. Sm.					√		√
45	Saxifraga bronchialis L.	√						
46	Saxifraga brunneopunctata H. Sm.				√	√	√	√
47	Saxifraga brunonis Wall. ex Ser.				√	√	√	√
48	Saxifraga bryoides L.			√	√	√	√	√
49	Saxifraga buceras					√		√
50	Saxifraga bulbifera L.	√			√	√		√
51	Saxifraga bulleyana Engl.& Irmsch.							
52	Saxifraga burmensis H. Sm. ex B.M. Wadhwa							
53	Saxifraga burseriana L.				√	√		
54	Saxifraga cacuminum H. Sm.							
55	Saxifraga caesia L.	√	√	√	√	√	√	√
56	Saxifraga callosa Sm.	√	√		√	√		√
57	Saxifraga calopetala H. Sm.							
58	Saxifraga camposii Boiss. & Reut.	√				√	√	√
59	Saxifraga canaliculata Boiss. & Reut. ex Engl.	√	√				√	√
60	Saxifraga candelabrum Franch.				√	√		√
61	Saxifraga cardiophylla Franch.							
62	Saxifraga carinata Oettingen							
63	Saxifraga carpatica Sternb.				√	√	√	√

64	Saxifraga carpetana Boiss. & Reut.	√				√	√	√
65	Saxifraga caspica Sipliv.							
66	Saxifraga caucasica Somm. & Levier							
67	Saxifraga caveana W.W.Sm.				√	√	√	√
68	Saxifraga cebennensis Rouy & Camus	√			√	√	√	√
69	Saxifraga cernua L.	√	√	√	√	√	√	√
70	Saxifraga cespitosa L.	√	√	√	√	√	√	√
71	Saxifraga charadzeae Otsch.							
72	Saxifraga cherlerioides D. Don							
73	Saxifraga chionophila Franch.					√	√	√
74	Saxifraga chrysantha A. Gray							
75	Saxifraga chrysanthoides Engl.& Irmsch.							√
76	Saxifraga chrysospleniifolia Boiss.					√		√
77	Saxifraga chumbiensis Engl.& Irmsch.							
78	Saxifraga ciliatopetala (Engl. & Irmsch.) J-T. Pan							
79	Saxifraga cinerascens Engl.& Irmsch.	√	√		√	√		√
80	Saxifraga cinerea H. Sm.					√		
81	Saxifraga cintrana Willk.	√				√		
82	Saxifraga clivorum H. Sm.							
83	Saxifraga coarctata W.W.Sm.							
84	Saxifraga cochlearis Rchb.	√	√	√		√	√	√
85	Saxifraga colchica Alboff							
86	Saxifraga columnaris Schmalh.							
87	Saxifraga columnaris Schmalh. ex Akinfew							
88	Saxifraga congestiflora Engl.& Irmsch.				√	√	√	√
89	Saxifraga conifera Coss. & Dur.	√						√
90	Saxifraga consanguinea W.W.Sm.	√		√	√	√	√	√
91	Saxifraga continentalis (Engl. & Irmscher) D.A.Webb	√				√		√

92	Saxifraga contraria H. Sm.					√		√
93	Saxifraga cordigera J.D.Hook. & Thoms.				√		√	√
94	Saxifraga corsica (Ser.) Gren.& Godr.							√
95	Saxifraga cortusifolia Sieb. & Zucc.							
96	Saxifraga corymbosa Boiss.					√		√
97	Saxifraga cotyledon L.	√	√		√	√		√
98	Saxifraga crustata Vest	√	√			√	√	√
99	Saxifraga culcitosa Mattf.						√	√
100	Saxifraga cuneata Willd.	√					√	√
101	Saxifraga cuneifolia L.	√	√		√	√	√	√
102	Saxifraga cymbalaria L.	√		√	√	√	√	√
103	Saxifraga daochengensis J-T. Pan							
104	Saxifraga decora H. Sm.					√		√
105	Saxifraga decussata Anthony							
106	Saxifraga deminuta H. Sm.							
107	Saxifraga densifoliata Engl.& Irmsch.				√	√	√	√
108	Saxifraga depressa Sternb.	√			√	√	√	√
109	Saxifraga deqenensis C.Y. Wu							
110	Saxifraga derbekii Sipliv.							
111	Saxifraga desoulavyi Oettingen							
112	Saxifraga dianxibeiensis J-T. Pan							√
113	Saxifraga diapensia H. Sm.							√
114	Saxifraga diapensioides Bell.					√		
115	Saxifraga dichotoma Willd. in Sternb.	√				√	√	√
116	Saxifraga dielsiana Engl.& Irmsch.				√			√
117	Saxifraga diffusicallosa C.Y. Wu					√		√
118	Saxifraga dinnikii Schmalh.							
119	Saxifraga diversifolia Wall. ex Ser.					√	√	√
120	Saxifraga doyalana H. Sm.							
121	Saxifraga drabiformis Franch.							√
122	Saxifraga draboides C.Y. Wu							

123	Saxifraga dshagalensis Engl.							
124	Saxifraga eglandulosa Engl.							
125	Saxifraga egregia Engl.	√		√	√		√	√
126	Saxifraga egregioides J-T. Pan							
127	Saxifraga elatinoides Hand.-Mazz.							
128	Saxifraga elliotii H. Sm.							√
129	Saxifraga elliptica Engl.& Irmsch.							
130	Saxifraga embergeri Maire							
131	Saxifraga engleriana H. Sm.				√	√	√	√
132	Saxifraga epiphylla Gornall & H.Ohba	√					√	√
133	Saxifraga erectisepala J-T. Pan							
134	Saxifraga erinacea H. Sm.				√	√		√
135	Saxifraga erioblasta Boiss. & Reut.	√					√	√
136	Saxifraga eschholzii Sternb. =eschscholtzii Cham. ex DC							
137	Saxifraga exarata Vill.	√					√	√
138	Saxifraga excellens H. Sm.					√		
139	Saxifraga facchinii W.Koch	√						
140	Saxifraga federici-augusti Biasol.	√	√	√		√	√	√
141	Saxifraga felineri P. Vargas =aretioides							
142	Saxifraga ferdinandi-coburgi J.Kellerer & Sund.				√	√		√
143	Saxifraga filicaulis Wall. ex Ser.	√			√	√	√	√
144	Saxifraga filifolia Anthony							
145	Saxifraga finitima W.W.Sm.				√			√
146	Saxifraga flaccida J-T. Pan							
147	Saxifraga flagellaris Willd. in Sternb.	√			√	√	√	√
148	Saxifraga flavida H. Sm.							
149	Saxifraga flexilis W.W.Sm.	√	√	√	√	√	√	√
150	Saxifraga flexuosa Sternb.							
151	Saxifraga florulenta Moretti	√	√			√	√	√
152	Saxifraga forrestii Engl.& Irmsch.							√
153	Saxifraga fortunei	√			√	√	√	√

	J.D.Hook.							
154	Saxifraga fragilis Schrank (=corbariensis)	√				√	√	√
155	Saxifraga ganeshii Ohba & Akiyama							
156	Saxifraga gedangensis J-T. Pan							
157	Saxifraga gemmigera Engl.	√	√		√	√	√	√
158	Saxifraga gemmipara Franch.	√	√		√	√		√
159	Saxifraga gemmulosa Boiss.	√			√	√	√	√
160	Saxifraga genesiana P. Vargas	√						
161	Saxifraga georgei Anthony					√		
162	Saxifraga geranioides L.	√				√	√	√
163	Saxifraga giraldiana Engl.				√	√	√	√
164	Saxifraga glabella Bertol.			√	√	√	√	√
165	Saxifraga glabricaulis H. Sm.							
166	Saxifraga glacialis H. Sm.				√	√	√	√
167	Saxifraga glaucophylla Franch.							
168	Saxifraga globulifera Desf.	√			√	√	√	√
169	Saxifraga gonggashanensis J-T. Pan							
170	Saxifraga gongshanensis T.C. Ku							
171	Saxifraga gouldii C.E.C. Fischer				√	√	√	√
172	Saxifraga granulata L.	√		√	√	√	√	√
173	Saxifraga granulifera H. Sm.	√	√	√	√	√	√	√
174	Saxifraga grisea Sipliv.							
175	Saxifraga grisebachii							
176	Saxifraga gyalana Marquand & Airy-Shaw				√	√	√	√
177	Saxifraga haenseleri Boiss. & Reut.	√					√	√
178	Saxifraga haplophylloides Franch.						√	√
179	Saxifraga harai H. Ohba & M. Wakabayashi							
180	Saxifraga hariotii Luizet & Soulie	√						√
181	Saxifraga harry-smithii B.M. Wadhwa							
182	Saxifraga hederacea L.	√		√			√	√

183	Saxifraga hederifolia Hochst. ex A. Rich.							
184	Saxifraga heleonastes H. Sm.				√	√	√	√
185	Saxifraga hemisphaerica J.D.Hook. & Thoms.	√			√	√	√	√
186	Saxifraga heteroclada H. Sm.							
187	Saxifraga heterocladoides J-T. Pan							
188	Saxifraga heterotricha Marquand & Airy-Shaw				√	√	√	√
189	Saxifraga hirculoides Decne.							
190	Saxifraga hirculus L.	√			√	√	√	√
191	Saxifraga hirsuta L.	√	√		√	√		√
192	Saxifraga hispidula D. Don			√	√	√	√	√
193	Saxifraga hohenwartii Vest ex Sternb.							
194	Saxifraga hookeri Engl.& Irmsch.	√				√		
195	Saxifraga hostii Tausch	√	√		√	√	√	√
196	Saxifraga hyperborea R. Br.							
197	Saxifraga hypericoides Franch.	√		√	√	√	√	√
198	Saxifraga hypnoides L.	√			√	√	√	√
199	Saxifraga hypostoma H. Sm.					√		
200	Saxifraga imparilis I.B. Balf.				√	√	√	√
201	Saxifraga implicans H. Sm.				√	√	√	√
202	Saxifraga inconspicua W.W.Sm.							
203	Saxifraga insolens Irmsch.	√	√	√				
204	Saxifraga intricata Lapeyr. (=nervosa)	√				√		√
205	Saxifraga iranica Bornm.							
206	Saxifraga irrigua M.-Bieb.						√	√
207	Saxifraga isophylla H. Sm.	√	√		√	√		√
208	Saxifraga italica D.A. Webb							
209	Saxifraga jacquemontiana Decne.				√	√	√	√
210	Saxifraga jainzhuglaensis J-T. Pan							
211	Saxifraga jaljalensis Ohba & Akiyama							
212	Saxifraga josephi Engl.							

213	Saxifraga juniperifolia Adams				√	√	√	√
214	Saxifraga kashmeriana U. Dhar & P. Kachroo							
215	Saxifraga kinchingingae Engl.							
216	Saxifraga kingdonii Marquand						√	√
217	Saxifraga kingiana Engl.& Irmsch.	√						
218	Saxifraga koelzii Schonbeck-Temesy							
219	Saxifraga kongboensis H. Sm.					√		√
220	Saxifraga korshinskii Kom.							
221	Saxifraga kotschyi Boiss.							
222	Saxifraga kumaunensis Engl.							
223	Saxifraga kusnezowiana Oettingen							
224	Saxifraga kwangsiensis Chun & How ex C.Z. Gao & G.Z.Li							
225	Saxifraga lamarum H. Sm.					√		√
226	Saxifraga lamninamensis H. Ohba							
227	Saxifraga latepetiolata Willk.	√			√	√	√	√
228	Saxifraga latiflora J.D.Hook. & Thoms.				√	√	√	√
229	Saxifraga lepida H. Sm.							
230	Saxifraga lepidostolonosa H. Sm.							
231	Saxifraga lhasana H. Sm.				√	√	√	√
232	Saxifraga likiangensis Franch.					√		
233	Saxifraga lilacina Duthie							
234	Saxifraga limprichtii Engl.& Irmsch.							
235	Saxifraga linearifolia Engl.& Irmsch.				√	√	√	√
236	Saxifraga litangensis Engl.				√	√	√	√
237	Saxifraga lixianensis T.C. Ku							
238	Saxifraga llonakhensis W.W.Sm.					√		√
239	Saxifraga lolaensis H. Sm.					√		√
240	Saxifraga longifolia Lapeyr.	√	√		√	√		√
241	Saxifraga loripes Anthony				√			√
242	Saxifraga losae Sennen	√						

243	Saxifraga lowndesii H. Sm.					√		
244	Saxifraga ludlowii H. Sm.					√		√
245	Saxifraga luizetiana Emberger & Maire							
246	Saxifraga lungpanensis J-T.Pan & Gornall							
247	Saxifraga lychnitis J.D.Hook. & Thoms.				√	√	√	√
248	Saxifraga macrostigma Franch.	√		√	√	√	√	√
249	Saxifraga macrostigmatoides Engl.				√	√		√
250	Saxifraga maderensis D. Don	√	√					
251	Saxifraga magellanica Poir.					√		
252	Saxifraga maireana Luizet							
253	Saxifraga mallae H. Ohba & M. Wakabayashi							
254	Saxifraga mandenovae J. Sojak							
255	Saxifraga marginata Sternb.	√	√	√	√	√	√	√
256	Saxifraga matta-florida H. Sm.					√		√
257	Saxifraga matta-viridis H. Sm.							
258	Saxifraga maweana Baker							
259	Saxifraga maxionggouensis J-T. Pan							
260	Saxifraga mazanderanica K.H. Rechinger							
261	Saxifraga media Gouan					√	√	√
262	Saxifraga medogensis J-T. Pan							
263	Saxifraga meeboldii Engl.& Irmsch.							
264	Saxifraga mengtzeana Engl.& Irmsch.	√			√	√	√	√
265	Saxifraga mertensiana Bongard		√			√	√	√
266	Saxifraga micans H. Sm.					√		
267	Saxifraga microgyna Engl.& Irmsch.		√	√	√	√	√	√
268	Saxifraga microphylla Royle ex J.D.Hook. & Thoms.							
269	Saxifraga microviridis H. Hara							
270	Saxifraga minutissima	√				√	√	√

271	Saxifraga mira H. Sm.					√		
272	Saxifraga miralana H. Sm.							√
273	Saxifraga monantha H. Sm.					√		√
274	Saxifraga moncayensis D.A. Webb	√					√	√
275	Saxifraga montanella H. Sm.	√	√		√	√	√	√
276	Saxifraga montanelloides J-T. Pan							
277	Saxifraga moorcroftiana (Wall.ex Ser.) Sternb.							
278	Saxifraga moschata Wulf.	√				√		√
279	Saxifraga mucronulata Royle				√			√
280	Saxifraga mucronulatoides J-T. Pan				√	√	√	√
281	Saxifraga mundula H. Smith							√
282	Saxifraga muricola Marquand & Airy-Shaw				√	√		√
283	Saxifraga muscoides All.			√	√	√	√	√
284	Saxifraga mutata L.	√	√		√	√	√	√
285	Saxifraga nakaoi Kitamura							
286	Saxifraga nakaoides J-T. Pan							
287	Saxifraga nambulana H. Sm.					√		√
288	Saxifraga namdoensis H. Sm.							
289	Saxifraga nana Engl.					√		
290	Saxifraga nanella Engl.& Irmsch.	√	√		√	√	√	√
291	Saxifraga nanelloides C.Y. Wu							
292	Saxifraga nangqenica J-T. Pan							
293	Saxifraga nangxianensis J-T. Pan	√						
294	Saxifraga nathorstii (Dusen) Hayek					√		
295	Saxifraga neopropagulifera H. Hara							
296	Saxifraga nevadensis Boiss.	√					√	√
297	Saxifraga nigroglandulifera Balakr. (=nutans)	√	√	√	√	√	√	√
298	Saxifraga nigroglandulosa Engl.& Irmsch.							
299	Saxifraga nipponica Makino							√

300	Saxifraga numidica Maire							
301	Saxifraga obtusa (Sprague) R. Horny & K. Mirko Webr							
302	Saxifraga octandra							√
303	Saxifraga omphalodifolia Hand.-Mazz.						√	√
304	Saxifraga oppositifolia L.	√	√	√	√	√	√	√
305	Saxifraga oreophila Franch.							
306	Saxifraga oresbia Anthony	√	√	√	√	√	√	√
307	Saxifraga osloensis Knaben	√				√		√
308	Saxifraga paiquensis J-T. Pan							
309	Saxifraga pallasiana Sternb.							
310	Saxifraga palpebrata J.D.Hook. & Thoms.							
311	Saxifraga paniculata Mill.	√	√		√	√	√	√
312	Saxifraga paradoxa Sternb.					√		√
313	Saxifraga pardanthina Hand.-Mazz.						√	√
314	Saxifraga parkaensis J-T. Pan							
315	Saxifraga parnassiifolia D. Don							
316	Saxifraga parnassioides Regel & Schmalh. ex Regel							
317	Saxifraga parva Hemsl.	√	√			√	√	√
318	Saxifraga pasumensis Marquand & Airy-Shaw	√			√	√	√	√
319	Saxifraga pavonii D. Don							
320	Saxifraga pedemontana All.	√				√	√	√
321	Saxifraga pellucida C.Y. Wu							√
322	Saxifraga pentadactylis Lapeyr.	√			√	√	√	√
323	Saxifraga peplidifolia Franch.							
324	Saxifraga perpusilla J.D.Hook. & Thoms.						√	√
325	Saxifraga peruviana Sternb.							
326	Saxifraga petraea L.				√	√	√	√
327	Saxifraga petrophila Franch.							√
328	Saxifraga pilifera J.D.Hook. & Thoms.				√	√	√	√

329	Saxifraga pilopetala J-T. Pan							
330	Saxifraga platysepala (Trautv.) Tolmatch.							
331	Saxifraga poluniniana H. Sm.							
332	Saxifraga polytrichoides Sipliv.							
333	Saxifraga porophylla Bertol.					√	√	√
334	Saxifraga portosanctana Boiss.	√						
335	Saxifraga praetermissa D.A. Webb	√			√	√	√	√
336	Saxifraga pratensis Engl.& Irmsch.							
337	Saxifraga prattii Engl.& Irmsch.				√			√
338	Saxifraga prenja G.Beck							
339	Saxifraga presolanensis Engl.					√	√	√
340	Saxifraga przewalskii Engl.							√
341	Saxifraga pseudohirculus Engl.		√	√	√	√	√	√
342	Saxifraga pseudolaevis Oettingen							
343	Saxifraga pubescens Pourr.	√			√	√	√	√
344	Saxifraga pulchra Engl.& Irmsch.							
345	Saxifraga pulvinaria H. Sm.	√				√		
346	Saxifraga punctulata Engl.	√			√	√	√	√
347	Saxifraga punctulatoides J-T. Pan							
348	Saxifraga qinghaiensis J-T. Pan							
349	Saxifraga quadrifaria Engl.& Irmsch.							
350	Saxifraga radiata Small							
351	Saxifraga ramsarica Jamzad							
352	Saxifraga ramulosa Wall. ex Ser.					√		
353	Saxifraga razshivinii P.Yu. Zhmylev							
354	Saxifraga retusa Gouan					√		√
355	Saxifraga reuteriana Boiss.	√					√	√
356	Saxifraga rhodopetala H. Sm.					√		
357	Saxifraga rigoi Porta	√						
358	Saxifraga rivularis L.	√	√		√	√	√	√

359	Saxifraga rizhaoshanensis J-T. Pan							
360	Saxifraga rolwalingensis H. Ohba							
361	Saxifraga rosacea Moench	√	√		√	√	√	√
362	Saxifraga rotundifolia L.	√	√	√	√	√	√	√
363	Saxifraga rotundipetala J-T. Pan							
364	Saxifraga roylei H. Sm.					√		
365	Saxifraga rufescens I.B. Balf.	√			√	√	√	√
366	Saxifraga rupicola Franch.					√		
367	Saxifraga ruprechtiana Mandenova							
368	Saxifraga saginoides J.D.Hook. & Thoms.				√	√		√
369	Saxifraga sancta Griseb.	√	√	√				
370	Saxifraga sanguinea Franch.	√				√		√
371	Saxifraga saxatilis H. Sm.							√
372	Saxifraga saxicola H. Sm.							√
373	Saxifraga saxorum H. Sm.					√		
374	Saxifraga scardica Griseb.	√	√			√	√	√
375	Saxifraga scleropoda Somm. & Levier							
376	Saxifraga sediformis Engl.& Irmsch.	√						√
377	Saxifraga sedoides L.	√		√	√	√	√	√
378	Saxifraga seguieri Spreng.							√
379	Saxifraga selemdzhensis Gorovoi & Vorosch.							
380	Saxifraga sempervivum C. Koch	√	√	√	√	√	√	√
381	Saxifraga sendaica Maxim.							√
382	Saxifraga serotina Sipliv.							
383	Saxifraga serpyllifolia Pursh							
384	Saxifraga serrula H. Sm.							
385	Saxifraga sessiliflora H. Sm.				√			√
386	Saxifraga setigera Pursh							
387	Saxifraga sheqilaensis J-T. Pan							
388	Saxifraga sherriffii H. Sm.					√		
389	Saxifraga sibirica L.							
390	Saxifraga sibthorpii Boiss.				√	√	√	√

391	Saxifraga sieversiana Sternb.					√		
392	Saxifraga signata Engl.& Irmsch.				√	√	√	√
393	Saxifraga signatella Marquand					√	√	√
394	Saxifraga sikkimensis Engl.							
395	Saxifraga sinomontana J-T.Pan & Gornall	√	√		√	√	√	√
396	Saxifraga smithiana Irmsch.							
397	Saxifraga sommieri (Engl. & Irmsch.) Sipliv.							
398	Saxifraga sosnowskyi Mandenova							
399	Saxifraga spathularis Brot.	√			√	√	√	√
400	Saxifraga spathulata							√
401	Saxifraga sphaeradena H. Sm.							
402	Saxifraga spruneri Boiss.				√	√		
403	Saxifraga squarrosa Sieber	√	√		√	√	√	√
404	Saxifraga staintonii H. Sm.					√		
405	Saxifraga stella-aurea J.D.Hook. & Thoms.				√	√		√
406	Saxifraga stellariifolia Franch.							
407	Saxifraga stelleriana Merk. ex Ser.							
408	Saxifraga stenophylla Royle							
409	Saxifraga stolitzkae Duthie ex Engl.& Irmsch.					√		
410	Saxifraga stolonifera W. Curtis	√	√	√	√	√		√
411	Saxifraga stribrnyi (Velen.) Podpera	√	√		√	√		√
412	Saxifraga strigosa Wall. ex Ser.	√	√		√			√
413	Saxifraga subaequifoliata Irmsch.						√	√
414	Saxifraga subamplexicaulis Engl.& Irmsch.						√	√
415	Saxifraga sublinearifolia J-T. Pan							
416	Saxifraga subomphalodifolia J-T. Pan							
417	Saxifraga subrhombifolia Irmsch.							
418	Saxifraga subsessiliflora Engl.& Irmsch.							

419	Saxifraga subspathulata Engl.& Irmsch.							
420	Saxifraga substrigosa J-T. Pan	√	√		√	√	√	√
421	Saxifraga subternata H. Sm.					√		√
422	Saxifraga subtsangchanensis J-T. Pan							
423	Saxifraga subverticillata Boiss.							
424	Saxifraga svalbardensis D.O. Ovstedal							
425	Saxifraga tangutica Engl.	√	√	√	√	√	√	√
426	Saxifraga taraktophylla Marquand & Airy-Shaw				√			√
427	Saxifraga tatsienluensis Engl.							
428	Saxifraga taygetea Boiss. & Heldr.				√	√	√	√
429	Saxifraga taylori J.A.Calder & D.B.O.Savile							
430	Saxifraga tenella Wulf.					√	√	√
431	Saxifraga tentaculata C.E.C. Fischer							
432	Saxifraga terektensis Bunge							
433	Saxifraga thiantha H. Sm.					√		
434	Saxifraga tibetica Losinsk.	√			√	√	√	√
435	Saxifraga tigrina H. Sm.							√
436	Saxifraga tombeanensis Boiss. ex Engl.					√	√	√
437	Saxifraga trabutiana Engl.& Irmsch.	√						
438	Saxifraga triaristulata Hand.-Mazz.							
439	Saxifraga tricrenata Pau & Font Quer							
440	Saxifraga tricuspidata Rottb.	√		√		√		
441	Saxifraga tridactylites L.	√			√	√	√	√
442	Saxifraga trifurcata Schrad.	√	√	√	√	√	√	√
443	Saxifraga trinervia Franch.							
444	Saxifraga tsangchanensis Franch.							√
445	Saxifraga tsarongensis Anthony				√	√	√	√
446	Saxifraga umbellulata J.D.Hook. & Thoms.	√			√	√		√

447	Saxifraga umbrosa L.		√		√	√		√
448	Saxifraga unguiculata Engl.	√	√	√	√	√	√	√
449	Saxifraga unguipetala Engl.& Irmsch.							
450	Saxifraga uninervia Anthony							
451	Saxifraga vacillans H. Sm.					√		
452	Saxifraga valdensis DC.	√				√		√
453	Saxifraga vandellii Sternb.					√	√	√
454	Saxifraga vayredana Luizet	√				√		√
455	Saxifraga verticillata Losinsk.							
456	Saxifraga vespertina (Small) Fedde							
457	Saxifraga vilmoriniana				√	√	√	√
458	Saxifraga virgularis H. Sm.							
459	Saxifraga viscidula J.D.Hook. & Thoms.							
460	Saxifraga wahlenbergii J.Ball				√	√	√	√
461	Saxifraga wallichiana Sternb.	√	√	√	√	√	√	√
462	Saxifraga wardii W.W.Sm.	√	√	√	√		√	√
463	Saxifraga wenchuanensis T.C. Ku							
464	Saxifraga wendelboi Schonbeck-Temesy				√	√		
465	Saxifraga werneri Font Quer & Pau							
466	Saxifraga williamsii H. Sm.					√		
467	Saxifraga yaluzangbuensis J-T. Pan							√
468	Saxifraga yezhiensis C.Y. Wu							√
469	Saxifraga yushuensis J-T. Pan							
470	Saxifraga zayuensis T.C. Ku							
471	Saxifraga zhidoensis J-T. Pan	√						
472	Saxifraga zimmermannii Baehni							

Appendix 2: Sample list of venation study. System follows Gornall (1987a).

SPECIES	Sample No.	Voucher specimen
Sect. Heterisia		
Saxifraga mertensiana Bongard	387	Bohm 1224 (UBC)
Sect. Irregulares		
Saxifraga epiphylla Gornall & H.Ohba	290	Boufford 33058 (A)
Saxifraga fortunei J.D.Hook.	310	Hort. LTR
Saxifraga imparilis I.B. Balf.	103	Boufford 32626 (A)
Saxifraga mengtzeana Engl.& Irmsch.	107	Boufford 35811 (A)
Saxifraga rufescens I.B. Balf.	104	Boufford 33395 (A)
Sect. Trachyphyllum		
Saxifraga aspera L.	194b	Stace July 1971 (LTR)
Saxifraga bryoides L.	195	Polunin 11396 (LTR)
Sect. Ciliatae, subsect. Hirculoideae		
Saxifraga diversifolia Wall. ex Ser. (diversifolia var. soulieana)	125	Boufford 35748 (A)
Saxifraga egregia Engl.	311	Aufschnaiter 1946-50 (LTR)
Saxifraga giraldiana Engl.	249	Boufford 38592 (A)
Saxifraga haplophylloides Franch.	312	Forrest 4206. 1906 (BM)
Saxifraga implicans H. Sm.	161	D4719 (MB)
Saxifraga kingdonii Marquand	293	D5092 (MB)
Saxifraga latiflora J.D.Hook. & Thoms.	168	00-421-04 (MB)
Saxifraga omphalodifolia Hand.-Mazz.	315	Wilson 3601 (BM)
Saxifraga pardanthina Hand.-Mazz.	314	Forrest 20576 (BM)
Saxifraga subaequifoliata Irmsch.	316	Forrest 30583 (BM)
Saxifraga subamplexicaulis Engl.& Irmsch.	313	Forrest 2965. 1906 (BM)
Saxifraga aristulata J.D.Hook. & Thoms.	294	99-134-20 (MB)
Saxifraga auriculata Engl.& Irmsch.	247	Boufford 40445 (A)
Saxifraga caveana W.W.Sm.	167	D5267 (MB)
Saxifraga cordigera J.D.Hook. & Thoms.	244b	00-303-13 (MB)
Saxifraga hypericoides Franch.	15b	Boufford 39514 (A)
Saxifraga macrostigma Franch.	250	Boufford 42024 (A)
Saxifraga litangensis Engl.	251	Boufford 38591 (A)
Saxifraga lychnitis J.D.Hook. & Thoms.	160	D3819 (MB)
Saxifraga nigroglandulifera Balakr. (=*nutans*)	70	Boufford 32299 (A)
Saxifraga oresbia Anthony	62	Boufford 37414 (A)
Saxifraga pseudohirculus Engl.	63	Boufford 34485 (A)
Sect. Ciliatae, subsect. Rufolanatae		
Saxifraga bergenioides Marquand	159	94-100-7 (MB)
Saxifraga congestiflora Engl.& Irmsch.	38	Boufford 36119 (A)
Saxifraga culcitosa Mattf.	288	Boufford 34802 (A)
Saxifraga heleonastes H. Sm.	252	Boufford 40083 (A)
Saxifraga hirculus L.	169b	Halliday 1052a (LTR)
Saxifraga montanella H. Sm.	388	Chensl 20110061 (HWNP)
Saxifraga parva Hemsl.	389	Chensl 20110067 (HWNP)

Saxifraga sinomontana J-T.Pan & Gornall	46	Boufford 34837 (A)
Saxifraga tangutica Engl.	41	Boufford 34068 (A)
Saxifraga tibetica Losinsk.	30	Boufford 29453 (A)
Sect. Ciliatae, subsect. Gemmiparae		
Saxifraga filicaulis Wall. ex Ser.	155	D10581 (MB)
Saxifraga gouldii C.E.C. Fischer	153	D10921 (MB)
Saxifraga hispidula D. Don	102	Boufford 35124 (A)
Saxifraga substrigosa J-T. Pan	154	D4944 (MB)
Saxifraga wallichiana Sternb.	100	Boufford 35849 (A)
Saxifraga wardii W.W.Sm.	27	Boufford 30129 (A)
Sect. Ciliatae, subsect. Flagellares		
Saxifraga brunonis Wall. ex Ser.	163	00-298-01 (MB)
Saxifraga consanguinea W.W.Sm.	110	Boufford 32197 (A)
Saxifraga flagellaris Willd. in Sternb.	322	Harper R.82 (LTR)
Saxifraga microgyna Engl.& Irmsch.	109	Boufford 35760 (A)
Saxifraga mucronulatoides J-T. Pan	162	D10225 (MB)
Saxifraga pilifera J.D.Hook. & Thoms.	166b	D10023 (MB)
Sect. Ciliatae, subsect. Rosulares		
Saxifraga brunneopunctata H. Sm.	158	D3430 (MB)
Saxifraga gyalana Marquand & Airy-Shaw	28	Boufford 29783 (A)
Saxifraga heterotricha Marquand & Airy-Shaw	157	D11867 (MB)
Saxifraga lhasana H. Sm.	24	Boufford 30955 (A)
Saxifraga pasumensis Marquand & Airy-Shaw	254	Boufford 39069 (A)
Saxifraga punctulata Engl.	23	Boufford 31089 (A)
Saxifraga signata Engl.& Irmsch.	92	Boufford 33667 (A)
Saxifraga signatella Marquand	156	D5115 (MB)
Saxifraga unguiculata Engl.	72	Boufford 31481 (A)
Saxifraga vilmoriniana	88	Boufford 36019 (A)
Sect. Ciliatae, subsect. Serpyllifoliae		
Saxifraga aurantiaca Franch. (=*confertifolia*)	87	Boufford 35831 (A)
Saxifraga densifoliata Engl.& Irmsch.	74	Boufford 33197 (A)
Saxifraga engleriana H. Sm.	150	00-248-15 (MB)
Saxifraga flexilis W.W.Sm.	90	Boufford 34162 (A)
Saxifraga gemmigera Engl.	248	Boufford 39212 (A)
Saxifraga glacialis H. Sm.	96	Boufford 31827 (A)
Saxifraga jacquemontiana Decne.	152	D5301 (MB)
Saxifraga linearifolia Engl.& Irmsch.	52	Boufford 36197 (A)
Saxifraga nanella Engl.& Irmsch.	98	Boufford 33550 (A)
Saxifraga tsarongensis Anthony	291	D5252 (MB)
Saxifraga minutissima	320	Rawat 11. IX. 2001 (BM)
Sect. Ciliatae, subsect. Hemisphaericae		
Saxifraga hemisphaerica J.D.Hook. & Thoms.	151	D8529 (MB)
Saxifraga perpusilla J.D.Hook. & Thoms.	292	D3962 (MB)
Sect. Cymbalaria		
Saxifraga cymbalaria L.	230	Gornall 688 (LTR)
Saxifraga hederacea L.	331	Stace & Cotton 417 (LTR)
Saxifraga sibthorpii Boiss.	332	Akeroyd. etc. 381 (BM)

Sect. Mesogyne		
Saxifraga carpatica Sternb.	333	Chater 5096 (LTR)
Saxifraga cernua L.	237b	Polunin 15610 (LTR)
Saxifraga granulifera H. Sm.	114	Boufford 32169 (A)
Saxifraga rivularis L.	198b	Gornall & Hollingsworth 670 (LTR)
Sect. Gymnopera		
Saxifraga cuneifolia L.	141b	Walz VI.1900 (LTR)
Saxifraga spathularis Brot.	180b	Halliday 45/66 (LTR)
Sect. Ligulatae, subsect. Aizoonia		
Saxifraga cochlearis Rchb.	324	Brown 20. IX. 1978 (LTR)
Saxifraga crustata Vest	296	Halliday 22/8/1963 (LTR)
Saxifraga hostii Tausch	317	Conolly 6, IX. 1969 (LTR)
Saxifraga paniculata Mill.	232b	Horwood 16. VIII. 1963 L.30 (LTR)
Sect. Ligulatae, subsect. Florulentae		
Saxifraga florulenta Moretti	335	Orr 15. VII. 1871 (LTR)
Sect. Ligulatae, subsect. Mutatae		
Saxifraga mutata L.	193b	Podlech 32814 (LTR)
Sect. Xanthizoon		
Saxifraga aizoides L.	196b	Halliday 36/59 (LTR)
Sect. Porphyrion, subsect. Oppositifoliae		
Saxifraga biflora All.	326	Jury, et., al. 6312 (LTR)
Saxifraga oppositifolia L.	189b	T.D. Pennington 11.7.58 (LTR)
Sect. Porphyrion, subsect. Engleria		
Saxifraga chionophila Franch.	289	Boufford 33417 (A)
Saxifraga federici-augusti Biasol.	299	Bierbach V. 1904 (LTR)
Saxifraga media Gouan	328	Webb 6. V. 1959 (LTR)
Saxifraga porophylla Bertol.	330	Rigo 23 19. VII. 1899 (LTR)
Saxifraga sempervivum C. Koch	187b	D. A. Web 1. VII. 1963 (LTR)
Sect. Porphyrion, subsect. Kabschia		
Saxifraga aretioides Lapeyr.	186	Polunin 9437 (LTR)
Saxifraga juniperifolia Adams	184b	Polunin 10674 (LTR)
Saxifraga marginata Sternb.	176b	Chater 567 (LTR)
Saxifraga scardica Griseb.	178b	Polunin 11126 (LTR)
Saxifraga tombeanensis Boiss. ex Engl.	295	Porta 13.5.1867 (LTR)
Saxifraga vandellii Sternb.	297	Halliday 79/59 (LTR)
Saxifraga caesia L.	174b	Patzak 30.8.1959 (LTR)
Saxifraga squarrosa Sieber	175b	Horwood 15/8/1964 L.28 (LTR)
Sect. Cotylea		
Saxifraga rotundifolia L.	183b	Tutin 57229 (LTR)
Saxifraga taygetea Boiss. & Heldr.	173b	Polunin 13485 (LTR)
Sect. Saxifraga, subsect. Holophyllae		
Saxifraga tenella Wulf.	309	Marchesetti V. 1890 (LTR)
Saxifraga sedoides L.	343	Jury, et., al. 23. VII. 1985 (LTR)
Saxifraga aphylla Sternb.	218b	Gadella? 16. VII. 1868 (LTR)

Saxifraga muscoides All.	345	Webb 27. VII. 1959 (LTR)
Saxifraga depressa Sternb.	215b	Halliday 102/59 (LTR)
Saxifraga presolanensis Engl.	308	Webb 12. VII. 1959 (LTR)
Saxifraga androsacea L.	214b	Horwood 26. VII. 1960 (LTR)
Saxifraga glabella Bertol.	219b	Polunin 13799 (LTR)
Sect. Saxifraga, subsect. Saxifraga		
Saxifraga carpetana Boiss. & Reut.	303	Smythies 1150 (LTR)
Saxifraga dichotoma Willd. in Sternb.	334	Brummit & Emst 5804 (LTR); Reverchon V. 1895 (LTR)
Saxifraga granulata L.	149	Hort. LTR
Saxifraga haenseleri Boiss. & Reut.	302	Smythies 1165 (LTR)
Saxifraga bourgaeana Boiss. & Reut. (=*boissieri*)	201b	Tutin 10. V. 1968 (LTR)
Saxifraga gemmulosa Boiss.	200b	S. Silvestre et B. Valdes 230/70 (LTR)
Sect. Saxifraga, subsect. Tridactylites		
Saxifraga adscendens L.	341	Polunin 13498 (LTR)
Saxifraga tridactylites L.	220b	Stace V. 1968 (LTR)
Sect. Saxifraga, subsect. Triplinervium		
Saxifraga aquatica Lapeyr.	211b	Vallee D Eynes? 27. VIII. 1836 (LTR)
Saxifraga irrigua M.-Bieb.	348	Chater 80 (LTR)
Saxifraga latepetiolata Willk.	344	Vetter V. 1902 (LTR)
Saxifraga arachnoidea Sternb.	340	Webb 13. VII. 1959 (LTR)
Saxifraga petraea L.	342	Webb 8. VII. 1959 (LTR)
Saxifraga praetermissa D.A. Webb	339	Weyraut VII. 1905 (LTR)
Saxifraga wahlenbergii J.Ball	337	Chater 5047 (LTR)
Saxifraga camposii Boiss. & Reut.	305	Smythies 1003 (LTR)
Saxifraga canaliculata Boiss. & Reut. ex Engl.	306	Stace 191 (LTR)
Saxifraga cuneata Willd.	307	Montserrat 3670/69 (LTR)
Saxifraga fragilis Schrank (=*corbariensis*)	346	Adshead & Scott 264 (LTR)
Saxifraga geranioides L.	347	Horwood 27. VII. 1969 (LTR)
Saxifraga moncayensis D.A. Webb	338	Webb 2. VI. 1967 (LTR)
Saxifraga pedemontana All.	336	Fraser-Jenkins 58 (LTR)
Saxifraga pentadactylis Lapeyr.	209b	Smythies 1214 (LTR)
Saxifraga trifurcata Schrad.	231	Gornall 689 (LTR)
Saxifraga cebennensis Rouy & Camus	325	Webb 8 VI. 1967 (LTR)
Saxifraga cespitosa L.	206b	Lambley 49 (LTR)
Saxifraga exarata Vill.	323	Horwood 16. VII. 1967 (LTR)
Saxifraga nevadensis Boiss.	304	Webb 257 30. V. 1967 (LTR)
Saxifraga pubescens Pourr.	327	Pennington 18. VII. 1958 (LTR)
Saxifraga rosacea Moench	205b	Raven 24. V. 1947 (LTR)
Saxifraga erioblasta Boiss. & Reut.	301	Smythies 977 (LTR)
Saxifraga globulifera Desf.	329	Dubuis 4273 (LTR)
Saxifraga hypnoides var. muscosa Don	204b	Abbott & Burrow 11. V. 2005 (LTR)

Saxifraga reuteriana Boiss.	300	Smythies 925 (LTR)
Genus Micranthes		
Micranthes melanocentra (Franch.) Losinsk.	147	Boufford 34819 (A)

Appendix 3. The morphological data matrix. The numbers under the character names are character sequence numbers. System follows Gornall (1987a).

SPECIES	life form	bulbil	Leafy buds	Axillary runners	Node-trichomes	Pedicel-gland-trichomes	Leaf arrangement	Leaf texture	Leaf margin cartilage	Leaf lamina outline
	1	2	3	4	5	6	7	8	9	10
OUT GROUP										
Itea	perennial	presnt	absent	absent	absent	?	alt	herb	absent	tooth/entire
Ribes	perennial	absent	absent	absent	absent	?	alt	herb	absent	lob-tooth
Chrysosplenium oppositifolium	perennial	absent	absent	absent	absent	?	oppo	herb	absent	lob
Micranthes melanocentra (Franch.) Losinsk.	perennial	absent	absent	absent	absent	M/U/I	alt	herb	absent	tooth
Sect. Heterisia										
1 Saxifraga mertensiana Bongard	perennial	absent	absent	absent	absent	M	alt	herb	absent	lob-tooth
Sect. Irregulares										
4 Saxifraga fortunei J.D.Hook.	perennial	absent	absent	absent	absent	M	alt	herb	absent	lob-tooth
5 Saxifraga imparilis I.B. Balf.	perennial	absent	absent	absent	absent	?	alt	herb	absent	lob-tooth
7 Saxifraga mengtzeana Engl.& Irmsch.	perennial	absent	absent	absent	egland	M	alt	herb	absent	lob-tooth
9 Saxifraga rufescens I.B. Balf.	perennial	absent	absent	absent	egland	M	alt	herb	absent	lob-tooth
Sect. Trachyphyllum										
14 Saxifraga aspera L.	perennial	absent	present	absent	gland	M/U	alt	herb	absent	entire
16 Saxifraga bryoides L.	perennial	absent	present	absent	gland	M	alt	herb	absent	entire
Sect. Ciliatae, subsect. Hirculoideae										
26 Saxifraga diversifolia Wall. ex Ser.	perennial	absent	absent	absent	crisp-rufous	M	alt	herb	cart	entire
33 Saxifraga giraldiana Engl.	perennial	absent	absent	absent	crisp-rufous	M	alt	herb	cart	entire
38 Saxifraga implicans H. Sm.	perennial	absent	absent	absent	crisp-rufous	?	alt	herb	cart	entire
43 Saxifraga latiflora J.D.Hook. & Thoms.	perennial	absent	absent	absent	crisp-rufous	?	alt	herb	absent	entire

62	Saxifraga auriculata Engl.& Irmsch.	perennial	absent	absent	absent	crisp-rufous	M	alt	herb	absent	entire
65	Saxifraga caveana W.W.Sm.	perennial	absent	absent	absent	crisp-rufous	?	alt	herb	cart	entire
79	Saxifraga hypericoides Franch.	perennial	absent	absent	absent	crisp-rufous	?	alt	herb	absent	entire
83	Saxifraga macrostigma Franch.	perennial	absent	absent	absent	crisp-rufous	M	alt	herb	absent	entire
103	Saxifraga litangensis Engl.	perennial	absent	absent	absent	crisp-rufous	?	alt	herb	absent	entire
104	Saxifraga lychnitis J.D.Hook. & Thoms.	perennial	absent	absent	absent	crisp-rufous	M	alt	herb	absent	entire
105	Saxifraga nigroglandulifera Balakr. (=*nutans*)	perennial	absent	absent	absent	crisp-rufous	M	alt	herb	cart	entire
106	Saxifraga oresbia Anthony	perennial	absent	absent	absent	crisp-rufous	?	alt	herb	absent	entire
107	Saxifraga pseudohirculus Engl.	perennial	absent	absent	absent	crisp-rufous	M	alt	herb	absent	entire
Sect. Ciliatae, subsect. Rufolanatae											
110	Saxifraga bergenioides Marquand	perennial	absent	absent	absent	crisp-rufous	absent	alt	herb	absent	entire
112	Saxifraga congestiflora Engl.& Irmsch.	perennial	absent	absent	absent	crisp-rufous	M	alt	herb	absent	entire
115	Saxifraga heleonastes H. Sm.	perennial	absent	absent	absent	crisp-rufous	absent	alt	herb	absent	entire
117	Saxifraga hirculus L.	perennial	absent	absent	absent	crisp-rufous	absent	alt	herb	absent	entire
120	Saxifraga montanella H. Sm.	perennial	absent	absent	absent	crisp-rufous	absent	alt	herb	absent	entire
124	Saxifraga parva Hemsl.	perennial	absent	absent	absent	crisp-rufous	?	alt	herb	absent	entire
127	Saxifraga sinomontana J-T.Pan & Gornall	perennial	absent	absent	absent	crisp-rufous	absent	alt	herb	absent	entire
128	Saxifraga tangutica Engl.	perennial	absent	absent	absent	crisp-rufous	absent	alt	herb	absent	entire
129	Saxifraga tibetica Losinsk.	perennial	absent	absent	absent	crisp-rufous	absent	alt	herb	absent	entire
Sect. Ciliatae, subsect. Gemmiparae											
137	Saxifraga filicaulis Wall. ex Ser.	perennial	absent	present	absent	egland	M	alt	herb	absent	entire
139	Saxifraga gouldii C.E.C. Fischer	perennial	absent	present	absent	absent	?	alt	herb	cart	entire
140	Saxifraga hispidula D. Don	perennial	absent	present	absent	gland	M	alt	herb	absent	entire/tooth
146	Saxifraga substrigosa J-T. Pan	perennial	absent	present	absent	egland	?	alt	herb	absent	entire/tooth
147	Saxifraga wallichiana Sternb.	perennial	absent	present	absent	absent	?	alt	herb	cart	entire
Sect. Ciliatae, subsect. Flagellares											
152	Saxifraga brunonis Wall. ex Ser.	perennial	absent	absent	runner	gland	M	alt	herb	cart	entire
153	Saxifraga consanguinea W.W.Sm.	perennial	absent	absent	runner	gland	?	alt	herb	absent	entire

156	Saxifraga flagellaris Willd. in Sternb.	perennial	absent	absent	runner	gland	M/U	alt	herb	absent	entire
159	Saxifraga microgyna Engl.& Irmsch.	perennial	absent	absent	runner	gland	M/U	alt	herb	absent	entire
161	Saxifraga mucronulatoides J-T. Pan	perennial	absent	absent	runner	gland	?	alt	herb	cart	entire
165	Saxifraga pilifera J.D.Hook. & Thoms.	perennial	absent	absent	runner	gland	?	alt	herb	absent	entire
Sect. Ciliatae, subsect. Rosulares											
170	Saxifraga brunneopunctata H. Sm.	perennial	absent	absent	absent	gland	?	alt	herb	cart	entire
175	Saxifraga gyalana Marquand & Airy-Shaw	perennial	absent	absent	absent	gland	?	alt	herb	absent	entire
176	Saxifraga heterotricha Marquand & Airy-Shaw	perennial	absent	absent	absent	egland	?	alt	herb	cart	entire
177	Saxifraga lhasana H. Sm.	perennial	absent	absent	absent	gland	?	alt	herb	cart	entire
181	Saxifraga pasumensis Marquand & Airy-Shaw	perennial	absent	absent	absent	gland	?	alt	herb	cart	entire
183	Saxifraga punctulata Engl.	perennial	absent	absent	absent	gland	M	alt	herb	cart	entire
187	Saxifraga signata Engl.& Irmsch.	perennial	absent	absent	absent	gland	M	alt	herb	absent	entire
188	Saxifraga signatella Marquand	perennial	absent	absent	absent	gland	?	alt	herb	cart	entire
191	Saxifraga unguiculata Engl.	perennial	absent	absent	absent	gland	?	alt	herb	absent	entire
192	Saxifraga vilmoriniana	perennial	absent	absent	absent	gland	?	alt	herb	absent	entire
Sect. Ciliatae, subsect. Serpyllifoliae											
196	Saxifraga aurantiaca Franch.	perennial	absent	absent	absent	gland	M	alt	herb	absent	entire
202	Saxifraga densifoliata Engl.& Irmsch.	perennial	absent	absent	absent	gland	?	alt	fleshy	absent	entire
206	Saxifraga engleriana H. Sm.	perennial	absent	absent	absent	egland	?	alt	fleshy	absent	entire
	Saxifraga minutissima	perennial	absent	absent	absent	gland	?	alt	fleshy	absent	entire
209	Saxifraga flexilis W.W.Sm.	perennial	absent	absent	absent	gland	absent	alt	fleshy	absent	entire
210	Saxifraga gemmigera Engl.	perennial	absent	present	absent	absent	M	alt	fleshy	absent	entire
211	Saxifraga glacialis H. Sm.	perennial	absent	absent	absent	absent	?	alt	fleshy	absent	entire
212	Saxifraga jacquemontiana Decne.	perennial	absent	absent	absent	gland	M	alt	herb	absent	entire
214	Saxifraga linearifolia Engl.& Irmsch.	perennial	absent	absent	absent	crisp-rufous	?	alt	herb	absent	entire
220	Saxifraga nanella Engl.& Irmsch.	perennial	absent	absent	absent	gland	?	alt	fleshy	absent	entire
230	Saxifraga tsarongensis Anthony	perennial	absent	absent	absent	gland	?	alt	herb	absent	entire
Sect. Ciliatae, subsect. Hemisphaericae											

234	Saxifraga hemisphaerica J.D.Hook. & Thoms.	perennial	absent	absent	absent	gland	?	alt	herb	absent	entire
Sect. Cymbalaria											
238	Saxifraga cymbalaria L.	annual	absent	absent	absent	absent	U	alt	herb	absent	lob
241	Saxifraga sibthorpii Boiss.	annual	absent	absent	absent	absent	U	alt	herb	absent	lob
Sect. Mesogyne											
245	Saxifraga carpatica Sternb.	perennial	tuber	absent	absent	absent	?	alt	herb	absent	lob
246	Saxifraga cernua L.	perennial	tuber	absent	absent	absent	U	alt	herb	absent	lob
248	Saxifraga granulifera H. Sm.	perennial	tuber	absent	absent	absent	?	alt	herb	absent	lob
251	Saxifraga rivularis L.	perennial	tuber	absent	absent	gland	U/I	alt	herb	absent	lob
Sect. Gymnopera											
257	Saxifraga cuneifolia L.	perennial	absent	absent	absent	absent	U	alt	leathery	cart	tooth
259	Saxifraga spathularis Brot.	perennial	absent	absent	absent	absent	M/U/I	alt	leathery	cart	tooth
Sect. Ligulatae, subsect. Aizoonia											
262	Saxifraga cochlearis Rchb.	perennial	absent	absent	absent	absent	M	alt	leathery	cart	entire
264	Saxifraga crustata Vest	perennial	absent	absent	absent	absent	M	alt	leathery	cart	entire
265	Saxifraga hostii Tausch	perennial	absent	absent	absent	absent	M	alt	leathery	cart	entire
267	Saxifraga paniculata Mill.	perennial	absent	absent	absent	absent	M	alt	leathery	cart	entire
Sect. Ligulatae, subsect. Florulentae											
269	Saxifraga florulenta Moretti	perennial	absent	absent	absent	absent	?	alt	leathery	cart	entire
Sect. Ligulatae, subsect. Mutatae											
270	Saxifraga mutata L.	perennial	absent	absent	absent	gland	M	alt	leathery	cart	entire
Sect. Xanthizoon											
271	Saxifraga aizoides L.	perennial	absent	absent	absent	absent	M	alt	fleshy	absent	entire
Sect. Porphyrion, subsect. Oppositifoliae											
274	Saxifraga biflora All.	perennial	absent	absent	absent	absent	M/I	opp	leathery	cart	entire
279	Saxifraga oppositifolia L.	perennial	absent	absent	absent	absent	M	alt/opp	leathery	cart	entire
Sect. Porphyrion, subsect. Engleria											
285	Saxifraga chionophila Franch.	perennial	absent	absent	absent	gland	M/I	alt	leathery	cart	entire

287 Saxifraga federici-augusti Biasol.	perennial	absent	absent	absent	gland	?	alt	leathery	cart	entire
288 Saxifraga media Gouan	perennial	absent	absent	absent	gland	M	alt	leathery	cart	entire
289 Saxifraga porophylla Bertol.	perennial	absent	absent	absent	gland	M	alt	leathery	cart	entire
291 Saxifraga sempervivum C. Koch	perennial	absent	absent	absent	gland	M	alt	leathery	cart	entire
Sect. Porphyrion, subsect. Kabschia										
293 Saxifraga aretioides Lapeyr.	perennial	absent	absent	absent	gland	M/I	alt	leathery	cart	entire
314 Saxifraga juniperifolia Adams	perennial	absent	absent	absent	gland	absent	alt	leathery	cart	entire
344 Saxifraga marginata Sternb.	perennial	absent	absent	absent	gland	M	alt	leathery	cart	entire
351 Saxifraga scardica Griseb.	perennial	absent	absent	absent	gland	M	alt	leathery	cart	entire
356 Saxifraga tombeanensis Boiss. ex Engl.	perennial	absent	absent	absent	gland	M	alt	leathery	cart	entire
357 Saxifraga vandellii Sternb.	perennial	absent	absent	absent	gland	M	alt	leathery	cart	entire
359 Saxifraga caesia L.	perennial	absent	absent	absent	gland	M/I	alt	leathery	cart	entire
360 Saxifraga squarrosa Sieber	perennial	absent	absent	absent	gland	M	alt	leathery	cart	entire
Sect. Cotylea										
377 Saxifraga rotundifolia L.	perennial	absent	absent	absent	absent	U	alt	herb	cart	lob-tooth
379 Saxifraga taygetea Boiss. & Heldr.	perennial	absent	absent	absent	gland	U	alt	leathery	absent	lob
Sect. Saxifraga, subsect. Holophyllae										
380 Saxifraga tenella Wulf.	perennial	absent	absent	absent	absent	U	alt	herb	absent	entire
381 Saxifraga sedoides L.	perennial	absent	absent	absent	gland	U	alt	herb	absent	entire
384 Saxifraga aphylla Sternb.	perennial	absent	absent	absent	?	U	alt	herb	absent	entire/lob
386 Saxifraga muscoides All.	perennial	absent	absent	absent	gland	U	alt	herb	absent	entire
388 Saxifraga depressa Sternb.	perennial	absent	absent	absent	gland	?	alt	herb	absent	lob
390 Saxifraga presolanensis Engl.	perennial	absent	absent	absent	gland	U	alt	herb	absent	entire
392 Saxifraga androsacea L.	perennial	absent	absent	absent	gland	U	alt	herb	absent	entire
394 Saxifraga glabella Bertol.	perennial	absent	absent	absent	gland	U	alt	herb	absent	entire
Sect. Saxifraga, subsect. Saxifraga										
396 Saxifraga carpetana Boiss. & Reut.	perennial	presnt	absent	absent	?	U	alt	herb	absent	lob
399 Saxifraga dichotoma Willd. in Sternb.	perennial	presnt	absent	absent	gland	U	alt	herb	absent	lob

400	Saxifraga granulata L.	perennial	presnt	absent	absent	absent	U/I	alt	herb	absent	tooth
404	Saxifraga bourgaeana Boiss. & Reut.	perennial	presnt	absent	absent	?	U	alt	herb	absent	cpd-lobed!
405	Saxifraga gemmulosa Boiss.	perennial	presnt	absent	absent	gland	U	alt	herb	absent	cpd-lobed!
Sect. Saxifraga, subsect. Tridactylites											
406	Saxifraga adscendens L.	biennial/annual	absent	absent	absent	absent	U	alt	herb	absent	lob
409	Saxifraga tridactylites L.	annual	absent	absent	absent	gland	U	alt	herb	absent	lob
Sect. Saxifraga, subsect. Triplinervium											
410	Saxifraga aquatica Lapeyr.	perennial	absent	absent	absent	?	U	alt	herb	absent	lob
412	Saxifraga latepetiolata Willk.	biennial	absent	absent	absent	gland	U	alt	herb	absent	lob
413	Saxifraga arachnoidea Sternb.	perennial	absent	absent	absent	gland	U	alt	herb	absent	lob
416	Saxifraga petraea L.	biennial	absent	absent	absent	gland	U	alt	herb	absent	lob
417	Saxifraga praetermissa D.A. Webb	perennial	absent	absent	absent	?	U	alt	herb	absent	lob
418	Saxifraga wahlenbergii J.Ball	perennial	absent	absent	absent	absent	absent	alt	herb	absent	lob
420	Saxifraga camposii Boiss. & Reut.	perennial	absent	absent	absent	absent	S	alt	herb	absent	lob
423	Saxifraga fragilis Schrank	perennial	absent	absent	absent	absent	S	alt	herb	absent	lob
425	Saxifraga geranioides L.	perennial	absent	absent	absent	gland	U	alt	herb	absent	lob
431	Saxifraga pedemontana All.	perennial	absent	absent	absent	gland	U	alt	herb	absent	lob
432	Saxifraga pentadactylis Lapeyr.	perennial	absent	absent	absent	absent	S	alt	herb	absent	lob
434	Saxifraga trifurcata Schrad.	perennial	absent	absent	absent	absent	S	alt	herb	absent	lob
438	Saxifraga cebennensis Rouy & Camus	perennial	absent	absent	absent	gland	U	alt	herb	absent	lob
439	Saxifraga cespitosa L.	perennial	absent	absent	absent	gland	U	alt	herb	absent	lob
449	Saxifraga pubescens Pourr.	perennial	absent	absent	absent	gland	U	alt	herb	absent	lob
450	Saxifraga rosacea Moench	perennial	absent	absent	absent	gland	U	alt	herb	absent	lob
457	Saxifraga globulifera Desf.	perennial	absent	present	absent	gland	U	alt	herb	absent	lob
458	Saxifraga hypnoides L.	perennial	absent	present	absent	absent	U	alt	herb	absent	lob

SPECIES		lamina shape	venation type	marginal ultimate venation	vein expand	Fol-sclereids	Hydathodes number	Hydathodes insertion	Hydathodes position	Hydathodes secretion	tannin
		11	12	13	14	15	16	17	18	19	20
OUT GROUP											
Itea		elliptic	Campto	closed	absent	absent	>1	surf	marginal	H2O	absent
Ribes		palmatifid/palmatisect	Actin	closed	absent	absent	>1	surf	marginal	H2O	absent
Chrysosplenium oppositifolium		reniform	Actin	closed	absent	absent	>1	surf	marginal	H2O	absent
Micranthes melanocentra (Franch.) Losinsk.		ovate	Campto	intermedia	absent	absent	>1	surf	marginal	H2O	absent
Sect. Heterisia											
1	Saxifraga mertensiana Bongard	reniform	Actin	closed	absent	absent	>1	surf	ada	H2O	absent
Sect. Irregulares											
4	Saxifraga fortunei J.D.Hook.	reniform	Actin	closed	absent	absent	>1	surf	ada	H2O	absent
5	Saxifraga imparilis I.B. Balf.	reniform	Actin	closed	absent	absent	>1	surf	ada	H2O	absent
7	Saxifraga mengtzeana Engl.& Irmsch.	reniform	Actin	closed	absent	absent	>1	surf	ada	H2O	absent
9	Saxifraga rufescens I.B. Balf.	reniform	Actin	closed	absent	absent	>1	surf	ada	H2O	absent
Sect. Trachyphyllum											
14	Saxifraga aspera L.	ensiform	Acro	closed	absent	absent	1	surf	ada	H2O	absent
16	Saxifraga bryoides L.	ovate	Acro	closed	absent	absent	1	surf	ada	H2O	absent
Sect. Ciliatae, subsect. Hirculoideae											
26	Saxifraga diversifolia Wall. ex Ser.	cordate	Acro	closed	absent	absent	1	surf	ada	H2O	absent
33	Saxifraga giraldiana Engl.	deltoid	Acro	closed	absent	absent	1	surf	ada	H2O	absent
38	Saxifraga implicans H. Sm.	cordate	Acro	closed	absent	absent	1	surf	ada	H2O	absent
43	Saxifraga latiflora J.D.Hook. & Thoms.	obovate	Acro	closed	absent	absent	1	surf	ada	H2O	absent
62	Saxifraga auriculata Engl.& Irmsch.	ovate	Acro	closed	absent	absent	1	surf	ada	H2O	absent
65	Saxifraga caveana W.W.Sm.	obovate	Acro	closed	absent	absent	1	surf	ada	H2O	absent
79	Saxifraga hypericoides Franch.	elliptic	Acro	closed	absent	absent	1	surf	ada	H2O	absent
83	Saxifraga macrostigma Franch.	ensiform	Acro	closed	absent	absent	1	surf	ada	H2O	absent

103	Saxifraga litangensis Engl.	ensiform	Acro	closed	absent	absent	1	surf	ada	H2O	absent
104	Saxifraga lychnitis J.D.Hook. & Thoms.	elliptic	Acro	closed	absent	absent	1	surf	ada	H2O	absent
105	Saxifraga nigroglandulifera Balakr.	elliptic	Acro	closed	absent	absent	1	surf	ada	H2O	absent
106	Saxifraga oresbia Anthony	orbiculate	Acro	closed	absent	absent	1	surf	ada	H2O	absent
107	Saxifraga pseudohirculus Engl.	elliptic	Acro	closed	absent	absent	1	surf	ada	H2O	absent
Sect. Ciliatae, subsect. Rufolanatae											
110	Saxifraga bergenioides Marquand	ovate	Acro	closed	absent	absent	1	surf	ada	H2O	absent
112	Saxifraga congestiflora Engl.& Irmsch.	elliptic	Acro	closed	absent	absent	1	surf	ada	H2O	absent
115	Saxifraga heleonastes H. Sm.	ensiform	Acro	closed	absent	absent	1	surf	ada	H2O	absent
117	Saxifraga hirculus L.	elliptic	Acro	closed	absent	absent	1	surf	ada	H2O	absent
120	Saxifraga montanella H. Sm.	elliptic	Acro	closed	absent	absent	1	surf	ada	H2O	absent
124	Saxifraga parva Hemsl.	elliptic	Acro	closed	absent	absent	1	surf	ada	H2O	absent
127	Saxifraga sinomontana J-T.Pan & Gornall	ensiform	Acro	closed	absent	absent	1	surf	ada	H2O	absent
128	Saxifraga tangutica Engl.	elliptic	Acro	closed	absent	absent	1	surf	ada	H2O	absent
129	Saxifraga tibetica Losinsk.	elliptic	Acro	closed	absent	absent	1	surf	ada	H2O	absent
Sect. Ciliatae, subsect. Gemmiparae											
137	Saxifraga filicaulis Wall. ex Ser.	ligulate	Acro	closed	absent	absent	1	surf	ada	H2O	absent
139	Saxifraga gouldii C.E.C. Fischer	lanceolate	Acro	closed	absent	absent	1	surf	ada	H2O	absent
140	Saxifraga hispidula D. Don	oval	Actin	closed	absent	absent	1/>1	surf	ada	H2O	absent
146	Saxifraga substrigosa J-T. Pan	elliptic	Actin	closed	absent	absent	1	surf	ada	H2O	absent
147	Saxifraga wallichiana Sternb.	ovate	Acro	closed	absent	absent	1	surf	ada	H2O	absent
Sect. Ciliatae, subsect. Flagellares											
152	Saxifraga brunonis Wall. ex Ser.	elliptic	Acro	closed	absent	absent	1	surf	ada	H2O	absent
153	Saxifraga consanguinea W.W.Sm.	elliptic	Acro	closed	absent	absent	1	surf	ada	H2O	absent
156	Saxifraga flagellaris Willd. in Sternb.	obovate	Acro	closed	absent	absent	1	surf	ada	H2O	absent
159	Saxifraga microgyna Engl.& Irmsch.	obovate	Acro	closed	absent	absent	1	surf	ada	H2O	absent
161	Saxifraga mucronulatoides J-T. Pan	ensiform	Acro	closed	absent	absent	1	surf	ada	H2O	absent
165	Saxifraga pilifera J.D.Hook. & Thoms.	obovate	Acro	closed	absent	absent	1	surf	ada	H2O	absent

Sect. Ciliatae, subsect. Rosulares											
170	Saxifraga brunneopunctata H. Sm.	spathulate	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
175	Saxifraga gyalana Marquand & Airy-Shaw	ligulate	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
176	Saxifraga heterotricha Marquand & Airy-Shaw	spathulate	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
177	Saxifraga lhasana H. Sm.	spathulate	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
181	Saxifraga pasumensis Marquand & Airy-Shaw	spathulate	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
183	Saxifraga punctulata Engl.	spathulate	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
187	Saxifraga signata Engl.& Irmsch.	spathulate	Acro	closed	absent/partly	absent	1	surf	ada	H_2O	absent
188	Saxifraga signatella Marquand	spathulate	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
191	Saxifraga unguiculata Engl.	ensiform	Acro	closed	partly	absent	1	surf	ada	H_2O	absent
192	Saxifraga vilmoriniana	spathulate	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
Sect. Ciliatae, subsect. Serpyllifoliae											
196	Saxifraga aurantiaca Franch.	ensiform	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
202	Saxifraga densifoliata Engl.& Irmsch.	elliptic	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
206	Saxifraga engleriana H. Sm.	orbicular	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
	Saxifraga minutissima	oblanceolate	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
209	Saxifraga flexilis W.W.Sm.	ensiform	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
210	Saxifraga gemmigera Engl.	oblanceolate	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
211	Saxifraga glacialis H. Sm.	oblanceolate	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
212	Saxifraga jacquemontiana Decne.	obovate	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
214	Saxifraga linearifolia Engl.& Irmsch.	ensiform	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
220	Saxifraga nanella Engl.& Irmsch.	oblanceolate	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
230	Saxifraga tsarongensis Anthony	oblong	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
Sect. Ciliatae, subsect. Hemisphaericae											
234	Saxifraga hemisphaerica J.D.Hook. & Thoms.	oblanceolate	Acro	closed	absent	absent	1	surf	ada	H_2O	absent
Sect. Cymbalaria											
238	Saxifraga cymbalaria L.	reniform	Actin	closed	absent	absent	1	surf	ada	H_2O	present

241	Saxifraga sibthorpii Boiss.	reniform	Actin	closed	absent	absent	1	surf	ada	H2O	present
Sect. Mesogyne											
245	Saxifraga carpatica Sternb.	palmatifid	Actin	closed	absent	absent	>1	surf	marginal	H2O	absent
246	Saxifraga cernua L.	palmatifid	Actin	closed	absent	absent	>1	surf	marginal	H2O	absent
248	Saxifraga granulifera H. Sm.	palmatifid	Actin	closed	absent	absent	>1	surf	marginal	H2O	absent
251	Saxifraga rivularis L.	palmatifid	Actin	closed	absent	absent	>1	surf	marginal	H2O	absent
Sect. Gymnopera											
257	Saxifraga cuneifolia L.	spathulate	Campto	closed	absent	Absent	>1	surf	ada	H2O	absent
259	Saxifraga spathularis Brot.	spathulate	Campto	closed	absent	absent	>1	surf	ada	H2O	absent
Sect. Ligulatae, subsect. Aizoonia											
262	Saxifraga cochlearis Rchb.	spathulate	Campto	open	absent	absent	>1	sunk	pseudomarg	Ca	absent
264	Saxifraga crustata Vest	ensiform	Campto	open	absent	absent	>1	sunk	pseudomarg	Ca	absent
265	Saxifraga hostii Tausch	ensiform	Campto	intermedia	absent	absent	>1	sunk	pseudomarg	Ca	absent
267	Saxifraga paniculata Mill.	spathulate	Campto	open	absent	absent	>1	sunk	pseudomarg	Ca	absent
Sect. Ligulatae, subsect. Florulentae											
269	Saxifraga florulenta Moretti	ensiform	Campto	intermedia	absent	absent	>1	sunk	ada	Ca	absent
Sect. Ligulatae, subsect. Mutatae											
270	Saxifraga mutata L.	oblanceolate	Campto	intermedia	absent	absent	>1	surf	ada	Ca	absent
Sect. Xanthizoon											
271	Saxifraga aizoides L.	ensiform	Campto	closed	absent	absent	1/>1	surf	ada	Ca	absent
Sect. Porphyrion, subsect. Oppositifoliae											
274	Saxifraga biflora All.	obovate	Campto	closed	absent	absent	1	sunk	ada	Ca	absent
279	Saxifraga oppositifolia L.	obovate	Campto	closed	absent	present	1	sunk	ada	Ca	absent
Sect. Porphyrion, subsect. Engleria											
285	Saxifraga chionophila Franch.	oblong	Campto	open/intermedia	completely	absent	>1	sunk	ada	Ca	absent
287	Saxifraga federici-augusti Biasol.	oblanceolate	Campto	intermedia	completely	absent	>1	sunk	ada	Ca	absent
288	Saxifraga media Gouan	oblanceolate	Campto	open/intermedia	completely	absent	>1	sunk	ada	Ca	absent

289	Saxifraga porophylla Bertol.	oblanceolate	Campto	intermedia	complet ely	present	>1	sunk	ada	Ca	absent
291	Saxifraga sempervivum C. Koch	ensiform	Campto	intermedia	complet ely	present	>1	sunk	ada	Ca	absent
Sect. Porphyrion, subsect. Kabschia											
293	Saxifraga aretioides Lapeyr.	oblong	Campto	intermedia	complet ely	absent	>1	sunk	ada	Ca	absent
314	Saxifraga juniperifolia Adams	ligulate	Campto	intermedia	complet ely	absent	>1	sunk	ada	Ca	absent
344	Saxifraga marginata Sternb.	oval	Campto	open	complet ely	present	>1	sunk	ada	Ca	absent
351	Saxifraga scardica Griseb.	ensiform	Campto	intermedia	absent	absent	>1	sunk	ada	Ca	absent
356	Saxifraga tombeanensis Boiss. ex Engl.	lanceolate	Campto	intermedia	absent	absent	>1	sunk	ada	Ca	absent
357	Saxifraga vandellii Sternb.	lanceolate	Campto	intermedia	complet ely	absent	>1	sunk	ada	Ca	absent
359	Saxifraga caesia L.	oblanceolate	Campto	open	complet ely	present	>1	sunk	ada	Ca	absent
360	Saxifraga squarrosa Sieber	ensiform	Campto	intermedia	complet ely	absent	>1	sunk	ada	Ca	absent
Sect. Cotylea											
377	Saxifraga rotundifolia L.	reniform	Actin	closed	absent	absent	>1	surf	ada	H2O	absent
379	Saxifraga taygetea Boiss. & Heldr.	reniform	Actin	closed	absent	absent	>1	surf	ada	H2O	absent
Sect. Saxifraga, subsect. Holophyllae											
380	Saxifraga tenella Wulf.	subulate	Acro	closed	absent	absent	1	surf	ada	H2O	absent
381	Saxifraga sedoides L.	oblanceolate	Acro	closed	absent	absent	1	surf	ada	H2O	absent
384	Saxifraga aphylla Sternb.	palmatifid	Actin	closed	absent	absent	>1	surf	ada	H2O	absent
386	Saxifraga muscoides All.	oblanceolate	Acro	closed	absent	absent	1	surf	marginal	H2O	absent
388	Saxifraga depressa Sternb.	palmatifid	Actin	closed	absent	absent	>1	surf	marginal	H2O	absent
390	Saxifraga presolanensis Engl.	ensiform	Acro	closed	absent	absent	1	surf	marginal	H2O	absent
392	Saxifraga androsacea L.	spathulate	Acro	closed	absent	absent	1	surf	ada	H2O	absent
394	Saxifraga glabella Bertol.	spathulate	Acro	closed	absent	absent	1	surf	ada	H2O	absent
Sect. Saxifraga, subsect. Saxifraga											
396	Saxifraga carpetana Boiss. & Reut.	palmatifid	Actin	closed	absent	absent	>1	surf	marginal	H2O	absent
399	Saxifraga dichotoma Willd. in Sternb.	palmatisect	Actin	closed	absent	absent	>1	surf	marginal	H2O	absent

400	Saxifraga granulata L.	palmatifid	Actin	closed	absent	absent	>1	surf	both	H2O	absent
404	Saxifraga bourgaeana Boiss. & Reut.	palmatisect	Actin	closed	partly	absent	>1	surf	both	H2O	absent
405	Saxifraga gemmulosa Boiss.	palmatisect	Actin	closed	absent	absent	>1	surf	both	H2O	absent
Sect. Saxifraga, subsect. Tridactylites											
406	Saxifraga adscendens L.	palmatifid	Actin	closed	absent	absent	1/>1	surf	ada	H2O	absent
409	Saxifraga tridactylites L.	palmatisect	Actin	closed	absent	absent	1/>1	surf	marginal	H2O	absent
Sect. Saxifraga, subsect. Triplinervium											
410	Saxifraga aquatica Lapeyr.	palmatifid	Actin	closed	absent	absent	>1	surf	ada	H2O	absent
412	Saxifraga latepetiolata Willk.	palmatisect	Actin	closed	absent	absent	>1	surf	ada	H2O	absent
413	Saxifraga arachnoidea Sternb.	palmatifid	Actin	closed	absent	absent	>1	surf	ada	H2O	absent
416	Saxifraga petraea L.	palmatisect	Actin	closed	absent	absent	>1	surf	ada	H2O	absent
417	Saxifraga praetermissa D.A. Webb	palmatisect	Actin	closed	absent	absent	>1	surf	ada	H2O	absent
418	Saxifraga wahlenbergii J.Ball	palmatisect	Actin	closed	absent	absent	>1	surf	ada	H2O	absent
420	Saxifraga camposii Boiss. & Reut.	palmatisect	Actin	closed	partly	absent	>1	surf	ada	H2O	absent
423	Saxifraga fragilis Schrank	palmatisect	Actin	closed	partly	absent	>1	surf	ada	H2O	absent
425	Saxifraga geranioides L.	palmatisect	Actin	closed	partly	absent	>1	surf	ada	H2O	absent
431	Saxifraga pedemontana All.	palmatifid	Actin	closed	partly	absent	>1	surf	ada	H2O	absent
432	Saxifraga pentadactylis Lapeyr.	palmatifid	Actin	closed	partly	absent	>1	surf	ada	H2O	absent
434	Saxifraga trifurcata Schrad.	palmatisect	Actin	closed	partly	absent	>1	surf	ada	H2O	absent
438	Saxifraga cebennensis Rouy & Camus	palmatisect	Actin	closed	partly	absent	>1	surf	ada	H2O	absent
439	Saxifraga cespitosa L.	palmatifid	Actin	closed	partly	absent	>1	surf	ada	H2O	absent
449	Saxifraga pubescens Pourr.	palmatifid	Actin	closed	partly	absent	>1	surf	ada	H2O	absent
450	Saxifraga rosacea Moench	palmatisect	Actin	closed	partly	absent	>1	surf	ada	H2O	absent
457	Saxifraga globulifera Desf.	palmatifid	Actin	closed	partly	absent	>1	surf	ada	H2O	absent
458	Saxifraga hypnoides L.	palmatisect	Actin	closed	partly	absent	>1	surf	ada	H2O	absent

SPECIES	Leaf crystal	Inflorescence	Flower symmetry	sepal no.	Sepals orientation	petal number	Petal spots	petal callosese	petal:sepals	petal Colour
	21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
OUT GROUP										
Itea	druse	raceme	radial	5	erect	5	absent	absent	?	white
Ribes	druse	raceme	radial	5	?	5	absent	absent	<	?
Chrysosplenium oppositifolium	?	simple cyme	radial	4	spread	0	n/a	n/a	n/a	n/a
Micranthes melanocentra (Franch.) Losinsk.	druse	cpd cyme	radial	5	spread	5	present	absent	>	white
Sect. Heterisia										
1 Saxifraga mertensiana Bongard	absent	cpd cyme	radial	5	reflex	5	absent	absent	>	white
Sect. Irregulares										
4 Saxifraga fortunei J.D.Hook.	druse	cpd cyme	bi	5	spread	5	absent	absent	>	white
5 Saxifraga imparilis I.B. Balf.	druse	cpd cyme	bi	5	reflex	5	absent	absent	>	white
7 Saxifraga mengtzeana Engl.& Irmsch.	druse	cpd cyme	bi	5	erect	5	absent	absent	>	white
9 Saxifraga rufescens I.B. Balf.	druse	cpd cyme	bi	5	reflex	5	absent	absent	>	white
Sect. Trachyphyllum										
14 Saxifraga aspera L.	absent	cpd cyme	radial	5	erect	5	present	absent	>	cream
16 Saxifraga bryoides L.	absent	simple cyme	radial	5	erect	5	present	absent	>	cream
Sect. Ciliatae, subsect. Hirculoideae										
26 Saxifraga diversifolia Wall. ex Ser.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	yellow
33 Saxifraga giraldiana Engl.	absent	cpd cyme	radial	5	reflex	5	absent	present	>	yellow
38 Saxifraga implicans H. Sm.	absent	cpd cyme	radial	5	reflex	5	present	absent/present	>	yellow
43 Saxifraga latiflora J.D.Hook. & Thoms.	absent	?	radial	5	?	5			>	yellow
62 Saxifraga auriculata Engl.& Irmsch.	absent	cpd cyme	radial	5	erect	5	absent	present	>	yellow
65 Saxifraga caveana W.W.Sm.	absent	solitary	radial	5	spread	5		absent	>	yellow
79 Saxifraga hypericoides Franch.	absent	cpd cyme	radial	5	erect	5	absent	present	>	yellow
83 Saxifraga macrostigma Franch.	absent	solitary	radial	5	absent	5	absent	present	>	yellow
103 Saxifraga litangensis Engl.	absent	simple cyme	radial	5	erect	5	absent	present	>	yellow

104	Saxifraga lychnitis J.D.Hook. & Thoms.	absent	simple cyme	radial	5	erect	5	absent	absent	>	yellow
105	Saxifraga nigroglandulifera Balakr. (=*nutans*)	absent	racemose cyme	radial	5	erect	5	present	absent	>	yellow
106	Saxifraga oresbia Anthony	absent	simple cyme	radial	5	erect	5	absent	absent	>	yellow
107	Saxifraga pseudohirculus Engl.	absent	cpd cyme	radial	5	erect	5	absent	present	>	yellow
Sect. Ciliatae, subsect. Rufolanatae											
110	Saxifraga bergenioides Marquand	absent	simple cyme	radial	5	erect	5	absent	absent	>	pink
112	Saxifraga congestiflora Engl.& Irmsch.	absent	cpd cyme	radial	5	erect	5	absent	present	>	yellow
115	Saxifraga heleonastes H. Sm.	absent	simple cyme	radial	5	erect	5	present	present	>	yellow
117	Saxifraga hirculus L.	absent	simple cyme	radial	5	reflex	5	present	present	>	yellow
120	Saxifraga montanella H. Sm.	absent	solitary	radial	5	erect	5	absent	present	>	yellow
124	Saxifraga parva Hemsl.	absent	solitary	radial	5	erect	5	absent	present	=	yellow
127	Saxifraga sinomontana J-T.Pan & Gornall	absent	cpd cyme	radial	5	erect	5	present	present	>	yellow
128	Saxifraga tangutica Engl.	absent	cpd cyme	radial	5	erect	5	present	present	=	yellow
129	Saxifraga tibetica Losinsk.	absent	solitary	radial	5	reflex	5	present	present	>	yellow
Sect. Ciliatae, subsect. Gemmiparae											
137	Saxifraga filicaulis Wall. ex Ser.	absent	simple cyme	radial	5	erect	5	present	present	>	yellow
139	Saxifraga gouldii C.E.C. Fischer	absent	simple cyme	radial	5	erect	5	absent	absent	>	yellow
140	Saxifraga hispidula D. Don	absent	simple cyme	radial	5	erect	5	absent	present	>	yellow
146	Saxifraga substrigosa J-T. Pan	absent	cpd cyme	radial	5	erect	5	absent	present	>	yellow
147	Saxifraga wallichiana Sternb.	absent	simple cyme	radial	5	erect	5	absent	present	>	yellow
Sect. Ciliatae, subsect. Flagellares											
152	Saxifraga brunonis Wall. ex Ser.	absent	cpd cyme	radial	5	erect	5	absent	present	>	yellow
153	Saxifraga consanguinea W.W.Sm.	absent	cpd cyme	radial	5	erect	5	absent	present	< =	yellow
156	Saxifraga flagellaris Willd. in Sternb.	absent	simple cyme	radial	5	erect	5	absent	present	>	yellow
159	Saxifraga microgyna Engl.& Irmsch.	absent	cpd cyme	radial	5	erect	5	absent	present	< =	yellow/pink
161	Saxifraga mucronulatoides J-T. Pan	absent	cpd cyme	radial	5	erect	5	absent	present	>	yellow
165	Saxifraga pilifera J.D.Hook. & Thoms.	absent	cpd cyme	radial	5	erect	5	present	present	<	yellow

Sect. Ciliatae, subsect. Rosulares											
170	Saxifraga brunneopunctata H. Sm.	absent	cpd cyme	radial	5	erect	5	present	present	>	yellow
175	Saxifraga gyalana Marquand & Airy-Shaw	absent	cpd cyme	radial	5	erect	5	present	present	>	yellow
176	Saxifraga heterotricha Marquand & Airy-Shaw	absent	cpd cyme	radial	5	reflex	5	absent	present	>	yellow
177	Saxifraga lhasana H. Sm.	absent	umbel	radial	5	erect	5	absent	present	>	white
181	Saxifraga pasumensis Marquand & Airy-Shaw	absent	umbel	radial	5	erect	5	absent	present	>	yellow
183	Saxifraga punctulata Engl.	absent	simple cyme	radial	5	erect	5	present	absent/pre sent	>	yellow
187	Saxifraga signata Engl.& Irmsch.	absent	cpd cyme	radial	5	reflex	5	present	present	>	yellow
188	Saxifraga signatella Marquand	absent	cpd cyme	radial	5	erect	5	present	present	>	white
191	Saxifraga unguiculata Engl.	absent	cpd cyme	radial	5	reflex	5	present	present	>	yellow
192	Saxifraga vilmoriniana	absent	cpd cyme	radial	5	reflex	5	present	present	>	yellow
Sect. Ciliatae, subsect. Serpyllifoliae											
196	Saxifraga aurantiaca Franch.	absent	cpd cyme	radial	5	reflex	5	present	present	>	yellow
202	Saxifraga densifoliata Engl.& Irmsch.	absent	cpd cyme	radial	5	erect	5	present	absent/pre sent	>	yellow
206	Saxifraga engleriana H. Sm.	absent	solitary	radial	5	erect	5	present	present		yellow
	Saxifraga minutissima	absent	solitary	radial	5	erect	0	n/a	n/a	n/a	n/a
209	Saxifraga flexilis W.W.Sm.	absent	simple cyme	radial	5	reflex	5	present	present		yellow
210	Saxifraga gemmigera Engl.	absent	cpd cyme	radial	5	reflex	5	present	absent/pre sent	>	yellow
211	Saxifraga glacialis H. Sm.	absent	cpd cyme	radial	5	reflex	5	present	present		yellow
212	Saxifraga jacquemontiana Decne.	absent	solitary	radial	5	erect	5	present	present	>	yellow
214	Saxifraga linearifolia Engl.& Irmsch.	absent	solitary	radial	5	reflex	5	present	absent	>	yellow
220	Saxifraga nanella Engl.& Irmsch.	absent	simple cyme	radial	5	reflex	5	present	absent/pre sent	>	yellow
230	Saxifraga tsarongensis Anthony	absent	solitary	radial	5	reflex	5	present	present	>	yellow
Sect. Ciliatae, subsect. Hemisphaericae											
234	Saxifraga hemisphaerica J.D.Hook. & Thoms.	absent	solitary	radial	5	erect	5	absent	present	>	yellow
Sect. Cymbalaria											
238	Saxifraga cymbalaria L.	absent	cpd cyme	radial	5	erect/spr	5	present	present	>	yellow

						ead					
241	Saxifraga sibthorpii Boiss.	absent	cpd cyme	radial	5	reflex	5	present	present	>	yellow
Sect. Mesogyne											
245	Saxifraga carpatica Sternb.	absent	simple cyme	radial	5	erect	5	absent	absent	>	white
246	Saxifraga cernua L.	absent	solitary	radial	5	erect	5	absent	absent	>	white
248	Saxifraga granulifera H. Sm.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
251	Saxifraga rivularis L.	absent	simple cyme	radial	5	erect	5	absent	absent	>	white
Sect. Gymnopera											
257	Saxifraga cuneifolia L.	absent	cpd cyme	radial	5	reflex	5	absent	absent	>	white
259	Saxifraga spathularis Brot.	absent	cpd cyme	radial	5	reflex	5	present	absent	>	white
Sect. Ligulatae, subsect. Aizoonia											
262	Saxifraga cochlearis Rchb.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
264	Saxifraga crustata Vest	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
265	Saxifraga hostii Tausch	absent	cpd cyme	radial	5	erect	5	present	absent	>	white
267	Saxifraga paniculata Mill.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
Sect. Ligulatae, subsect. Florulentae											
269	Saxifraga florulenta Moretti	absent	racemose cyme	radial	5	erect	5	absent	absent	>	pink
Sect. Ligulatae, subsect. Mutatae											
270	Saxifraga mutata L.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	yellow
Sect. Xanthizoon											
271	Saxifraga aizoides L.	absent	cpd cyme	radial	5	erect	5	present	absent	>	yellow
Sect. Porphyrion, subsect. Oppositifoliae											
274	Saxifraga biflora All.	absent	simple cyme	radial	5	erect	5	absent	absent	>	variable
279	Saxifraga oppositifolia L.	absent	solitary	radial	5	erect	5	absent	absent	>	variable
Sect. Porphyrion, subsect. Engleria											
285	Saxifraga chionophila Franch.	absent	racemose cyme	radial	5	erect	5	absent	absent	>=	pink
287	Saxifraga federici-augusti Biasol.	absent	racemose cyme	radial	5	erect	5	absent	absent	=	red

288	Saxifraga media Gouan	absent	racemose cyme	radial	5	erect	5	absent	absent	=	red
289	Saxifraga porophylla Bertol.	absent	racemose cyme	radial	5	erect	5	absent	absent	=	yellow
291	Saxifraga sempervivum C. Koch	absent	racemose cyme	radial	5	erect	5	absent	absent	=	red
Sect. Porphyrion, subsect. Kabschia											
293	Saxifraga aretioides Lapeyr.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	yellow
314	Saxifraga juniperifolia Adams	absent	cpd cyme	radial	5	erect	5	absent	absent	>	yellow
344	Saxifraga marginata Sternb.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
351	Saxifraga scardica Griseb.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
356	Saxifraga tombeanensis Boiss. ex Engl.	absent	simple cyme	radial	5	erect	5	absent	absent	>	white
357	Saxifraga vandellii Sternb.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
359	Saxifraga caesia L.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
360	Saxifraga squarrosa Sieber	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
Sect. Cotylea											
377	Saxifraga rotundifolia L.	absent	cpd cyme	radial	5	erect	5	present	absent	>	white
379	Saxifraga taygetea Boiss. & Heldr.	absent	cpd cyme	radial	5	erect	5	present	absent	>	white
Sect. Saxifraga, subsect. Holophyllae											
380	Saxifraga tenella Wulf.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	yellow
381	Saxifraga sedoides L.	absent	simple cyme	radial	5	erect	5	absent	absent	>=	cream
384	Saxifraga aphylla Sternb.	absent	solitary	radial	5	erect	5	absent	absent	>	greenish yellow
386	Saxifraga muscoides All.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	yellow
388	Saxifraga depressa Sternb.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
390	Saxifraga presolanensis Engl.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
392	Saxifraga androsacea L.	absent	simple cyme	radial	5	erect	5	absent	absent	>	white
394	Saxifraga glabella Bertol.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
Sect. Saxifraga, subsect. Saxifraga											
396	Saxifraga carpetana Boiss. & Reut.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
399	Saxifraga dichotoma Willd. in Sternb.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white

400	Saxifraga granulata L.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
404	Saxifraga bourgaeana Boiss. & Reut.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
405	Saxifraga gemmulosa Boiss.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
Sect. Saxifraga, subsect. Tridactylites											
406	Saxifraga adscendens L.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
409	Saxifraga tridactylites L.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
Sect. Saxifraga, subsect. Triplinervium											
410	Saxifraga aquatica Lapeyr.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
412	Saxifraga latepetiolata Willk.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
413	Saxifraga arachnoidea Sternb.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
416	Saxifraga petraea L.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
417	Saxifraga praetermissa D.A. Webb	absent	simple cyme	radial	5	erect	5	absent	absent	>	white
418	Saxifraga wahlenbergii J.Ball	absent	simple cyme	radial	5	erect	5	absent	absent	>	?
420	Saxifraga camposii Boiss. & Reut.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
423	Saxifraga fragilis Schrank	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
425	Saxifraga geranioides L.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
431	Saxifraga pedemontana All.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
432	Saxifraga pentadactylis Lapeyr.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
434	Saxifraga trifurcata Schrad.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
438	Saxifraga cebennensis Rouy & Camus	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
439	Saxifraga cespitosa L.	absent	cpd cyme	radial	5	erect	0/5	absent	absent	>	white
449	Saxifraga pubescens Pourr.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
450	Saxifraga rosacea Moench	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
457	Saxifraga globulifera Desf.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white
458	Saxifraga hypnoides L.	absent	cpd cyme	radial	5	erect	5	absent	absent	>	white

SPECIES		Petal Shape	Stamen number	Filament	Anther-connective	Anther dehiscence	Pollen-ECA	Pollen-ENA	Lirae & striae	Tectum structure	Tectum perforation
		31	32	33	34	35	36	37	38	39	40
OUT GROUP											
Itea		round	5	linear	apiculate	parallel	porus	pore	absent	single	perforate/complete
Ribes		round	4/5	linear	obtuse	?	porus	pore	absent	single	perforate/complete
Chrysosplenium oppositifolium		n/a	4/8	linear	obtuse	flip	colpus	diffuse	absent	single	reticulate
Micranthes melanocentra (Franch.) Losinsk.		round	10	linear	obtuse	diverg	colpus	diffuse	n/a	single	reticulate
Sect. Heterisia											
1	Saxifraga mertensiana Bongard	round	10	clavate	obtuse	diverg	colpus	diffuse	absent	single	micro-capillaries
Sect. Irregulares											
4	Saxifraga fortunei J.D.Hook.	round	10	clavate	obtuse	diverg	colpus	diffuse	absent	single	micro-capillaries
5	Saxifraga imparilis I.B. Balf.	round	10	clavate	obtuse	diverg	colpus	diffuse	absent	single	micro-capillaries
7	Saxifraga mengtzeana Engl.& Irmsch.	round	10	clavate	obtuse	diverg	colpus	diffuse	absent	single	?
9	Saxifraga rufescens I.B. Balf.	round	10	clavate	obtuse	diverg	colpus	diffuse	absent	single	micro-capillaries
Sect. Trachyphyllum											
14	Saxifraga aspera L.	round	10	linear	obtuse	diverg	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary tectum perforate
16	Saxifraga bryoides L.	round	10	linear	obtuse	diverg	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary tectum perforate
Sect. Ciliatae, subsect. Hirculoideae											
26	Saxifraga diversifolia Wall. ex Ser.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
33	Saxifraga giraldiana Engl.	round	10	linear	obtuse	flip	colpus	diffuse	lirae & striae fine	single	perforate/complete
38	Saxifraga implicans H. Sm.	round	10	linear	obtuse	diverg	colpus	diffuse	lirae & striae fine	single	perforate/complete
43	Saxifraga latiflora J.D.Hook. & Thoms.	round	10		obtuse		colpus	diffuse	lirae & striae fine	single	perforate/complete
62	Saxifraga auriculata Engl.& Irmsch.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
65	Saxifraga caveana W.W.Sm.	round	10		obtuse		colpus	diffuse	lirae & striae fine	single	perforate/complete
79	Saxifraga hypericoides Franch.	round	10	linear	obtuse	diverg	colpus	diffuse	lirae & striae fine	single	perforate/complete

83	Saxifraga macrostigma Franch.	round	10	linear	obtuse	diverg	colpus	diffuse	lirae & striae fine	single	perforate/complete
103	Saxifraga litangensis Engl.	round	10	linear	obtuse	diverg	colpus	diffuse	lirae & striae fine	single	perforate/complete
104	Saxifraga lychnitis J.D.Hook. & Thoms.	round	10	linear	obtuse	parallel	colpus	diffuse	n/a	single	perforate-reticulate/perforate-rugulate
105	Saxifraga nigroglandulifera Balakr.	round	10	linear	obtuse	parallel	colpus	diffuse	n/a	single	perforate-reticulate/perforate-rugulate
106	Saxifraga oresbia Anthony	round	10	linear	obtuse	parallel	colpus	diffuse	n/a	single	perforate-reticulate/perforate-rugulate
107	Saxifraga pseudohirculus Engl.	round	10	linear	obtuse	diverg	colpus	diffuse	lirae & striae fine	single	perforate/complete
Sect. Ciliatae, subsect. Rufolanatae											
110	Saxifraga bergenioides Marquand	round	10	linear	obtuse	diverg	colpus	diffuse	lirae & striae fine	single	perforate/complete
112	Saxifraga congestiflora Engl.& Irmsch.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
115	Saxifraga heleonastes H. Sm.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
117	Saxifraga hirculus L.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
120	Saxifraga montanella H. Sm.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
124	Saxifraga parva Hemsl.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
127	Saxifraga sinomontana J-T.Pan & Gornall	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
128	Saxifraga tangutica Engl.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
129	Saxifraga tibetica Losinsk.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
Sect. Ciliatae, subsect. Gemmiparae											
137	Saxifraga filicaulis Wall. ex Ser.	round	10	linear	obtuse	diverg	colpus	diffuse	lirae & striae fine	single	perforate/complete
139	Saxifraga gouldii C.E.C. Fischer	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
140	Saxifraga hispidula D. Don	round	10	linear	obtuse	flip	colpus	diffuse	lirae & striae fine	single	perforate/complete
146	Saxifraga substrigosa J-T. Pan	round	10	linear	obtuse	flip	colpus	diffuse	lirae & striae fine	single	perforate/complete
147	Saxifraga wallichiana Sternb.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
Sect. Ciliatae, subsect. Flagellares											
152	Saxifraga brunonis Wall. ex Ser.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
153	Saxifraga consanguinea W.W.Sm.	round	10	linear	obtuse	flip	colpus	diffuse	lirae & striae fine	single	perforate/complete

156 Saxifraga flagellaris Willd. in Sternb.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
159 Saxifraga microgyna Engl.& Irmsch.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
161 Saxifraga mucronulatoides J-T. Pan	round	10	linear	obtuse	diverg	colpus	diffuse	lirae & striae fine	single	perforate/complete
165 Saxifraga pilifera J.D.Hook. & Thoms.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
Sect. Ciliatae, subsect. Rosulares										
170 Saxifraga brunneopunctata H. Sm.	round	10	linear	obtuse	diverg	colpus	diffuse	lirae & striae fine	single	perforate/complete
175 Saxifraga gyalana Marquand & Airy-Shaw	round	10	linear	obtuse	diverg	colpus	diffuse	lirae & striae fine	single	perforate/complete
176 Saxifraga heterotricha Marquand & Airy-Shaw	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
177 Saxifraga lhasana H. Sm.	round	10	linear	obtuse	flip	colpus	diffuse	lirae & striae fine	single	perforate/complete
181 Saxifraga pasumensis Marquand & Airy-Shaw	round	10	linear	obtuse	flip	colpus	diffuse	lirae & striae fine	single	perforate/complete
183 Saxifraga punctulata Engl.	round	10	linear	obtuse	flip	colpus	diffuse	lirae & striae fine	single	perforate/complete
187 Saxifraga signata Engl.& Irmsch.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
188 Saxifraga signatella Marquand	round	10	linear	obtuse	diverg	colpus	diffuse	lirae & striae fine	single	perforate/complete
191 Saxifraga unguiculata Engl.	round	10	linear	obtuse	diverg	colpus	diffuse	lirae & striae fine	single	perforate/complete
192 Saxifraga vilmoriniana	round	10	linear	obtuse	diverg	colpus	diffuse	lirae & striae fine	single	perforate/complete
Sect. Ciliatae, subsect. Serpyllifoliae										
196 Saxifraga aurantiaca Franch.	round	10	linear	obtuse	diverg	colpus	diffuse	lirae & striae fine	single	perforate/complete
202 Saxifraga densifoliata Engl.& Irmsch.	round	10	linear	obtuse	diverg	colpus	diffuse	lirae & striae fine	single	perforate/complete
206 Saxifraga engleriana H. Sm.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
Saxifraga minutissima	n/a	5	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
209 Saxifraga flexilis W.W.Sm.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
210 Saxifraga gemmigera Engl.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
211 Saxifraga glacialis H. Sm.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
212 Saxifraga jacquemontiana Decne.	round	10	linear	obtuse	diverg	colpus	diffuse	lirae & striae fine	single	perforate/complete
214 Saxifraga linearifolia Engl.& Irmsch.	round	10	linear	obtuse	diverg/parallel	colpus	diffuse	lirae & striae fine	single	perforate/complete
220 Saxifraga nanella Engl.& Irmsch.	round	10	linear	obtuse	flip	colpus	diffuse	lirae & striae fine	single	perforate/complete
230 Saxifraga tsarongensis Anthony	round	10	linear	obtuse	flip	colpus	diffuse	lirae & striae fine	single	perforate/complete

Sect. Ciliatae, subsect. Hemisphaericae												
234	Saxifraga hemisphaerica J.D.Hook. & Thoms.	round	10	linear	obtuse	diverg	colpus	diffuse	lirae & striae fine	single	perforate/complete	
Sect. Cymbalaria												
238	Saxifraga cymbalaria L.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
241	Saxifraga sibthorpii Boiss.	round	10	linear	obtuse	diverg	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
Sect. Mesogyne												
245	Saxifraga carpatica Sternb.	round	10	linear	obtuse	?	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
246	Saxifraga cernua L.	round	10	linear	obtuse	flip	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
248	Saxifraga granulifera H. Sm.	round	10	linear	obtuse	?	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
251	Saxifraga rivularis L.	round	10	linear	obtuse	flip	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
Sect. Gymnopera												
257	Saxifraga cuneifolia L.	round	10	clavate	obtuse	diverg	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
259	Saxifraga spathularis Brot.	round	10	clavate	obtuse	diverg/parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
Sect. Ligulatae, subsect. Aizoonia												
262	Saxifraga cochlearis Rchb.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
264	Saxifraga crustata Vest	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
265	Saxifraga hostii Tausch	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
267	Saxifraga paniculata Mill.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent,	double	primary perforate	tectum

								striae of variable width			
Sect. Ligulatae, subsect. Florulentae											
269 Saxifraga florulenta Moretti	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	?	
Sect. Ligulatae, subsect. Mutatae											
270 Saxifraga mutata L.	ensiform	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
Sect. Xanthizoon											
271 Saxifraga aizoides L.	ensiform	10	linear	obtuse	diverg/parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
Sect. Porphyrion, subsect. Oppositifoliae											
274 Saxifraga biflora All.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	?	
279 Saxifraga oppositifolia L.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	?	
Sect. Porphyrion, subsect. Engleria											
285 Saxifraga chionophila Franch.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
287 Saxifraga federici-augusti Biasol.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	?	
288 Saxifraga media Gouan	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	?	
289 Saxifraga porophylla Bertol.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	?	
291 Saxifraga sempervivum C. Koch	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	?	
Sect. Porphyrion, subsect. Kabschia											
293 Saxifraga aretioides Lapeyr.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum

314 Saxifraga juniperifolia Adams	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
344 Saxifraga marginata Sternb.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
351 Saxifraga scardica Griseb.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
356 Saxifraga tombeanensis Boiss. ex Engl.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
357 Saxifraga vandellii Sternb.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
359 Saxifraga caesia L.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
360 Saxifraga squarrosa Sieber	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
Sect. Cotylea											
377 Saxifraga rotundifolia L.	round	10	linear	obtuse	flip	colpus	diffuse	lirae coarse and marginal ridges present, striae slit-like	double	primary perforate	tectum
379 Saxifraga taygetea Boiss. & Heldr.	round	10	linear	obtuse	flip	colpus	diffuse	lirae coarse and marginal ridges present, striae slit-like	double	primary perforate	tectum
Sect. Saxifraga, subsect. Holophyllae											
380 Saxifraga tenella Wulf.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and ribbon-like, striae ill-defined	single	perforate/complete	
381 Saxifraga sedoides L.	ensiform	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and ribbon-like, striae ill-defined	single	perforate/complete	
384 Saxifraga aphylla Sternb.	ensiform	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and ribbon-like, striae ill-defined	single	perforate/complete	
386 Saxifraga muscoides All.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and ribbon-like, striae ill-defined	single	perforate/complete	
388 Saxifraga depressa Sternb.	round	10	linear	obtuse	diverg/parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum
390 Saxifraga presolanensis Engl.	ensiform	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary perforate	tectum

392	Saxifraga androsacea L.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary tectum perforate
394	Saxifraga glabella Bertol.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary tectum perforate
Sect. Saxifraga, subsect. Saxifraga											
396	Saxifraga carpetana Boiss. & Reut.	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
399	Saxifraga dichotoma Willd. in Sternb.	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
400	Saxifraga granulata L.	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
404	Saxifraga bourgaeana Boiss. & Reut.	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
405	Saxifraga gemmulosa Boiss.	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
Sect. Saxifraga, subsect. Tridactylites											
406	Saxifraga adscendens L.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary tectum perforate
409	Saxifraga tridactylites L.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary tectum perforate
Sect. Saxifraga, subsect. Triplinervium											
410	Saxifraga aquatica Lapeyr.	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
412	Saxifraga latepetiolata Willk.	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
413	Saxifraga arachnoidea Sternb.	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
416	Saxifraga petraea L.	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and ribbon-like, striae ill-defined	single	perforate/complete
417	Saxifraga praetermissa D.A. Webb	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
418	Saxifraga wahlenbergii J.Ball	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and marginal ridges absent, striae of variable width	double	primary tectum perforate
420	Saxifraga camposii Boiss. & Reut.	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
423	Saxifraga fragilis Schrank	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
425	Saxifraga geranioides L.	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
431	Saxifraga pedemontana All.	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
432	Saxifraga pentadactylis Lapeyr.	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete

434	Saxifraga trifurcata Schrad.	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
438	Saxifraga cebennensis Rouy & Camus	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
439	Saxifraga cespitosa L.	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
449	Saxifraga pubescens Pourr.	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
450	Saxifraga rosacea Moench	round	10	linear	obtuse	parallel	colpus	diffuse	lirae coarse and ribbon-like, striae ill-defined	single	perforate/complete
457	Saxifraga globulifera Desf.	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete
458	Saxifraga hypnoides L.	round	10	linear	obtuse	parallel	colpus	diffuse	absent	single	perforate/complete

SPECIES	Secondary columellae	Supra-tectal processes	Pollen cell number	Ovary position	G-no	Carpel fusion	Placentation	fruit	Ch'some X#
	41	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**
OUT GROUP									
Itea	absent	absent	?	semi-inf	2	>50% ovary	axile	capsure	11
Ribes	absent	absent	?	inferior	2	>50% ovary	parietal	baccate	8
Chrysosplenium oppositifolium	absent	absent	?	semi-inf	2	basal	parietal	capsure	11, 12
Micranthes melanocentra (Franch.) Losinsk.	absent	absent	2!	superior	2	basal	axile	capsure	11
Sect. Heterisia									
1 Saxifraga mertensiana Bongard	absent	spinules		superior	2	>50% ovary	axile	capsure	18
Sect. Irregulares									
4 Saxifraga fortunei J.D.Hook.	absent	spinules	2!	superior	2	>50% ovary	axile	capsure	11
5 Saxifraga imparilis I.B. Balf.	absent	spinules	2!	superior	2	>50% ovary	axile	capsure	
7 Saxifraga mengtzeana Engl.& Irmsch.	absent	spinules	2!	superior	2	>50% ovary	axile	capsure	
9 Saxifraga rufescens I.B. Balf.	absent	spinules	2!	superior	2	>50% ovary	axile	capsure	
Sect. Trachyphyllum									
14 Saxifraga aspera L.	high	?	2!	superior	2	>50% ovary	axile	capsure	13
16 Saxifraga bryoides L.	high	?	2!	superior	2	>50% ovary	axile	capsure	13
Sect. Ciliatae, subsect. Hirculoideae									
26 Saxifraga diversifolia Wall. ex Ser.	absent	?		superior	2	>50% ovary	axile	capsure	8
33 Saxifraga giraldiana Engl.	absent	granules	3	superior	2	>50% ovary	axile	capsure	
38 Saxifraga implicans H. Sm.	absent	granules	3	superior	2	>50% ovary	axile	capsure	8
43 Saxifraga latiflora J.D.Hook. & Thoms.	absent	?	3		2	>50% ovary	axile	capsure	
62 Saxifraga auriculata Engl.& Irmsch.	absent	granules, most with base expanded	3	superior	2	>50% ovary	axile	capsure	
65 Saxifraga caveana W.W.Sm.	absent	granules, most with base expanded	3		2	>50% ovary	axile	capsure	
79 Saxifraga hypericoides Franch.	absent	granules, most with base expanded	3	superior	2	>50% ovary	axile	capsure	
83 Saxifraga macrostigma Franch.	absent	granules, most with base expanded	3	superior	2	>50% ovary	axile	capsure	

103	Saxifraga litangensis Engl.	absent	granules, most with base expanded	3	superior	2	>50% ovary	axile	capsure	
104	Saxifraga lychnitis J.D.Hook. & Thoms.	absent	verrucae	3	superior	2	>50% ovary	axile	capsure	8
105	Saxifraga nigroglandulifera Balakr.	absent	verrucae	3	superior	2	>50% ovary	axile	capsure	
106	Saxifraga oresbia Anthony	absent	verrucae	3	superior	2	>50% ovary	axile	capsure	
107	Saxifraga pseudohirculus Engl.	absent	granules, most with base expanded	3	superior	2	>50% ovary	axile	capsure	
Sect. Ciliatae, subsect. Rufolanatae										
110	Saxifraga bergenioides Marquand	absent	?	3	superior	2	>50% ovary	axile	capsure	
112	Saxifraga congestiflora Engl.& Irmsch.	absent	granules, most with base expanded	3	superior	2	>50% ovary	axile	capsure	
115	Saxifraga heleonastes H. Sm.	absent	granules, most with base expanded	3	superior	2	>50% ovary	axile	capsure	
117	Saxifraga hirculus L.	absent	?	3	superior	2	>50% ovary	axile	capsure	8
120	Saxifraga montanella H. Sm.	absent	granules, most with base expanded	3	superior	2	>50% ovary	axile	capsure	8
124	Saxifraga parva Hemsl.	absent	granules, most with base expanded			2	>50% ovary	axile	capsure	
127	Saxifraga sinomontana J-T.Pan & Gornall	absent	granules, most with base expanded	3	semi-inf	2	>50% ovary	axile	capsure	
128	Saxifraga tangutica Engl.	absent	?	3	superior	2	>50% ovary	axile	capsure	
129	Saxifraga tibetica Losinsk.	absent	granules, most with base expanded	3	superior	2	>50% ovary	axile	capsure	8
Sect. Ciliatae, subsect. Gemmiparae										
137	Saxifraga filicaulis Wall. ex Ser.	absent	granules, most with base expanded	2!	superior		>50% ovary	axile	capsure	8
139	Saxifraga gouldii C.E.C. Fischer	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	
140	Saxifraga hispidula D. Don	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	8
146	Saxifraga substrigosa J-T. Pan	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	
147	Saxifraga wallichiana Sternb.	absent	granules, most with base expanded	2!	semi-inf	2	>50% ovary	axile	capsure	8
Sect. Ciliatae, subsect. Flagellares										
152	Saxifraga brunonis Wall. ex Ser.	absent	?	2!	superior	2	>50% ovary	axile	capsure	8
153	Saxifraga consanguinea W.W.Sm.	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	

156	Saxifraga flagellaris Willd. in Sternb.	absent	?	2!	superior		>50% ovary	axile	capsure	8
159	Saxifraga microgyna Engl.& Irmsch.	absent	?	2!	inferior	2	>50% ovary	axile	capsure	
161	Saxifraga mucronulatoides J-T. Pan	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	
165	Saxifraga pilifera J.D.Hook. & Thoms.	absent	?	2!	inferior	2	>50% ovary	axile	capsure	
Sect. Ciliatae, subsect. Rosulares										
170	Saxifraga brunneopunctata H. Sm.	absent	?	2!	superior	2	>50% ovary	axile	capsure	
175	Saxifraga gyalana Marquand & Airy-Shaw	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	
176	Saxifraga heterotricha Marquand & Airy-Shaw	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	
177	Saxifraga lhasana H. Sm.	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	
181	Saxifraga pasumensis Marquand & Airy-Shaw	absent	granules, most with base expanded	2!	inferior	2	>50% ovary	axile	capsure	
183	Saxifraga punctulata Engl.	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	
187	Saxifraga signata Engl.& Irmsch.	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	
188	Saxifraga signatella Marquand	absent	?		superior	2	>50% ovary	axile	capsure	
191	Saxifraga unguiculata Engl.	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	
192	Saxifraga vilmoriniana	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	
Sect. Ciliatae, subsect. Serpyllifoliae										
196	Saxifraga aurantiaca Franch.	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	
202	Saxifraga densifoliata Engl.& Irmsch.	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	
206	Saxifraga engleriana H. Sm.	absent	granules, most with base expanded	3	superior	2	>50% ovary	axile	capsure	8
	Saxifraga minutissima	absent	granules, most with base expanded		semi-superior	2	>50% ovary	axile	capsure	
209	Saxifraga flexilis W.W.Sm.	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	
210	Saxifraga gemmigera Engl.	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	
211	Saxifraga glacialis H. Sm.	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	
212	Saxifraga jacquemontiana Decne.	absent	granules, most with base	3	superior	2	>50% ovary	axile	capsure	8

			expanded							
214	Saxifraga linearifolia Engl.& Irmsch.	absent	granules, most with base expanded	3	superior	2	>50% ovary	axile	capsure	
220	Saxifraga nanella Engl.& Irmsch.	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	
230	Saxifraga tsarongensis Anthony	absent	granules, most with base expanded	2!	superior	2	>50% ovary	axile	capsure	
Sect. Ciliatae, subsect. Hemisphaericae										
234	Saxifraga hemisphaerica J.D.Hook. & Thoms.	absent	granules, most with base expanded	2!		2	>50% ovary	axile	capsure	
Sect. Cymbalaria										
238	Saxifraga cymbalaria L.	high	granules	2!	superior	2	>50% ovary	axile	capsure	9
241	Saxifraga sibthorpii Boiss.	high	granules	2!	superior	2	>50% ovary	axile	capsure	9
Sect. Mesogyne										
245	Saxifraga carpatica Sternb.	low	granules	2!	semi-inf	2	>50% ovary	axile	capsure	12
246	Saxifraga cernua L.	low	?	2!	superior	2	>50% ovary	axile	capsure	various
248	Saxifraga granulifera H. Sm.	low	granules	2!	superior	2	>50% ovary	axile	capsure	
251	Saxifraga rivularis L.	low	?	2!	semi-inf	2	>50% ovary	axile	capsure	13
Sect. Gymnopera										
257	Saxifraga cuneifolia L.	high	?	2!	superior	2	>50% ovary	axile	capsure	14
259	Saxifraga spathularis Brot.	high	?	2!	superior	2	>50% ovary	axile	capsure	14
Sect. Ligulatae, subsect. Aizoonia										
262	Saxifraga cochlearis Rchb.	high	granules		semi-inf	2	>50% ovary	axile	capsure	14
264	Saxifraga crustata Vest	high	granules		semi-inf	2	>50% ovary	axile	capsure	14
265	Saxifraga hostii Tausch	high	?	2!	semi-inf	2	>50% ovary	axile	capsure	14
267	Saxifraga paniculata Mill.	high	?	2!	inferior	2	>50% ovary	axile	capsure	14
Sect. Ligulatae, subsect. Florulentae										
269	Saxifraga florulenta Moretti	high	absent		?	3	>50% ovary	axile	capsure	14
Sect. Ligulatae, subsect. Mutatae										
270	Saxifraga mutata L.	high	?	2!	semi-inf	2	>50% ovary	axile	capsure	13, 14
Sect. Xanthizoon										

271	Saxifraga aizoides L.	high	?	2!	superior/semi-inferior	2	>50% ovary	axile	capsure	13
Sect. Porphyrion, subsect. Oppositifoliae										
274	Saxifraga biflora All.	high	absent	2!	semi-inf	2	>50% ovary	axile	capsure	13
279	Saxifraga oppositifolia L.	high	absent	2!	semi-superior	2	>50% ovary	axile	capsure	13
Sect. Porphyrion, subsect. Engleria										
285	Saxifraga chionophila Franch.	high	granules		inferior	2	>50% ovary	axile	capsure	
287	Saxifraga federici-augusti Biasol.	high	absent		inferior	2	>50% ovary	axile	capsure	13
288	Saxifraga media Gouan	high	absent or sparse verrucae		inferior	2	>50% ovary	axile	capsure	13
289	Saxifraga porophylla Bertol.	high	absent		inferior	2	>50% ovary	axile	capsure	13
291	Saxifraga sempervivum C. Koch	high	absent	2!	inferior	2	>50% ovary	axile	capsure	12
Sect. Porphyrion, subsect. Kabschia										
293	Saxifraga aretioides Lapeyr.	high	?	2!	semi-inf	2	>50% ovary	axile	capsure	13
314	Saxifraga juniperifolia Adams	high	?	2!	inferior	2	>50% ovary	axile	capsure	13
344	Saxifraga marginata Sternb.	high	?	2!	semi-inf	2	>50% ovary	axile	capsure	
351	Saxifraga scardica Griseb.	high	granules		semi-inf	2	>50% ovary	axile	capsure	13
356	Saxifraga tombeanensis Boiss. ex Engl.	high	granules		semi-inf	2	>50% ovary	axile	capsure	13
357	Saxifraga vandellii Sternb.	high	?		inferior	2	>50% ovary	axile	capsure	13
359	Saxifraga caesia L.	high	?	2!	inferior	2	>50% ovary	axile	capsure	13
360	Saxifraga squarrosa Sieber	high	?	2!	inferior	2	>50% ovary	axile	capsure	13
Sect. Cotylea										
377	Saxifraga rotundifolia L.	low	?	2!	superior	2	>50% ovary	axile	capsure	11
379	Saxifraga taygetea Boiss. & Heldr.	low	granules	2!	superior	2	>50% ovary	axile	capsure	11
Sect. Saxifraga, subsect. Holophyllae										
380	Saxifraga tenella Wulf.	absent	?		inferior	2	>50% ovary	axile	capsure	11
381	Saxifraga sedoides L.	absent	?	2!	semi-inf to inferior	2	>50% ovary	axile	capsure	16
384	Saxifraga aphylla Sternb.	absent	spinules	2!	inferior	2	>50% ovary	axile	capsure	15, 16

386 Saxifraga muscoides All.	absent	spinules	2!	inferior	2	>50% ovary	axile	capsure	19
388 Saxifraga depressa Sternb.	high	granules	2!	inferior	2	>50% ovary	axile	capsure	
390 Saxifraga presolanensis Engl.	high	granules		inferior	2	>50% ovary	axile	capsure	16
392 Saxifraga androsacea L.	high	granules	2!	inferior	2	>50% ovary	axile	capsure	11, various
394 Saxifraga glabella Bertol.	high	?	2!	inferior	2	>50% ovary	axile	capsure	
Sect. Saxifraga, subsect. Saxifraga									
396 Saxifraga carpetana Boiss. & Reut.	absent	spinules		inferior	2	>50% ovary	axile	capsure	10, 16
399 Saxifraga dichotoma Willd. in Sternb.	absent	?		inferior	2	>50% ovary	axile	capsure	16
400 Saxifraga granulata L.	absent	?	2!	semi-inf	2	>50% ovary	axile	capsure	various
404 Saxifraga bourgaeana Boiss. & Reut.	absent	?	2!	inferior	2	>50% ovary	axile	capsure	16
405 Saxifraga gemmulosa Boiss.	absent	spinules	2!	inferior	2	>50% ovary	axile	capsure	16
Sect. Saxifraga, subsect. Tridactylites									
406 Saxifraga adscendens L.	high	?	2!	inferior	2	>50% ovary	axile	capsure	11
409 Saxifraga tridactylites L.	high	?	2!	inferior	2	>50% ovary	axile	capsure	11
Sect. Saxifraga, subsect. Triplinervium									
410 Saxifraga aquatica Lapeyr.	absent	?	2!	inferior	2	>50% ovary	axile	capsure	11
412 Saxifraga latepetiolata Willk.	absent	?	2!	inferior	2	>50% ovary	axile	capsure	11
413 Saxifraga arachnoidea Sternb.	absent	?	2!	inferior	2	>50% ovary	axile	capsure	11
416 Saxifraga petraea L.	absent	?	2!	inferior	2	>50% ovary	axile	capsure	16
417 Saxifraga praetermissa D.A. Webb	absent	spinules	2!	inferior	2	>50% ovary	axile	capsure	11
418 Saxifraga wahlenbergii J.Ball	high	granules	2!	inferior	2	>50% ovary	axile	capsure	11
420 Saxifraga camposii Boiss. & Reut.	absent	?		inferior	2	>50% ovary	axile	capsure	16
423 Saxifraga fragilis Schrank	absent	?		inferior	2	>50% ovary	axile	capsure	16
425 Saxifraga geranioides L.	absent	?		inferior	2	>50% ovary	axile	capsure	13
431 Saxifraga pedemontana All.	absent	?		inferior	2	>50% ovary	axile	capsure	various
432 Saxifraga pentadactylis Lapeyr.	absent	spinules	2!	inferior	2	>50% ovary	axile	capsure	16
434 Saxifraga trifurcata Schrad.	absent	?	2!	inferior	2	>50% ovary	axile	capsure	14

438	Saxifraga cebennensis Rouy & Camus	absent	spinules	2!	inferior	2	>50% ovary	axile	capsure	13, 8
439	Saxifraga cespitosa L.	absent	spinules	2!	inferior	2	>50% ovary	axile	capsure	16, 39
449	Saxifraga pubescens Pourr.	absent	spinules	2!	inferior	2	>50% ovary	axile	capsure	13, 14
450	Saxifraga rosacea Moench	absent	spinules	2!	inferior	2	>50% ovary	axile	capsure	13
457	Saxifraga globulifera Desf.	absent	spinules	2!	inferior	2	>50% ovary	axile	capsure	11
458	Saxifraga hypnoides L.	absent	spinules	2!	inferior	2	>50% ovary	axile	capsure	13, 12

Appendix 4. Other morphological characters

There are some morphological characters that were not used in the analysis, mainly owing to incomplete studies or lack of material. Some of these characters are discussed here.

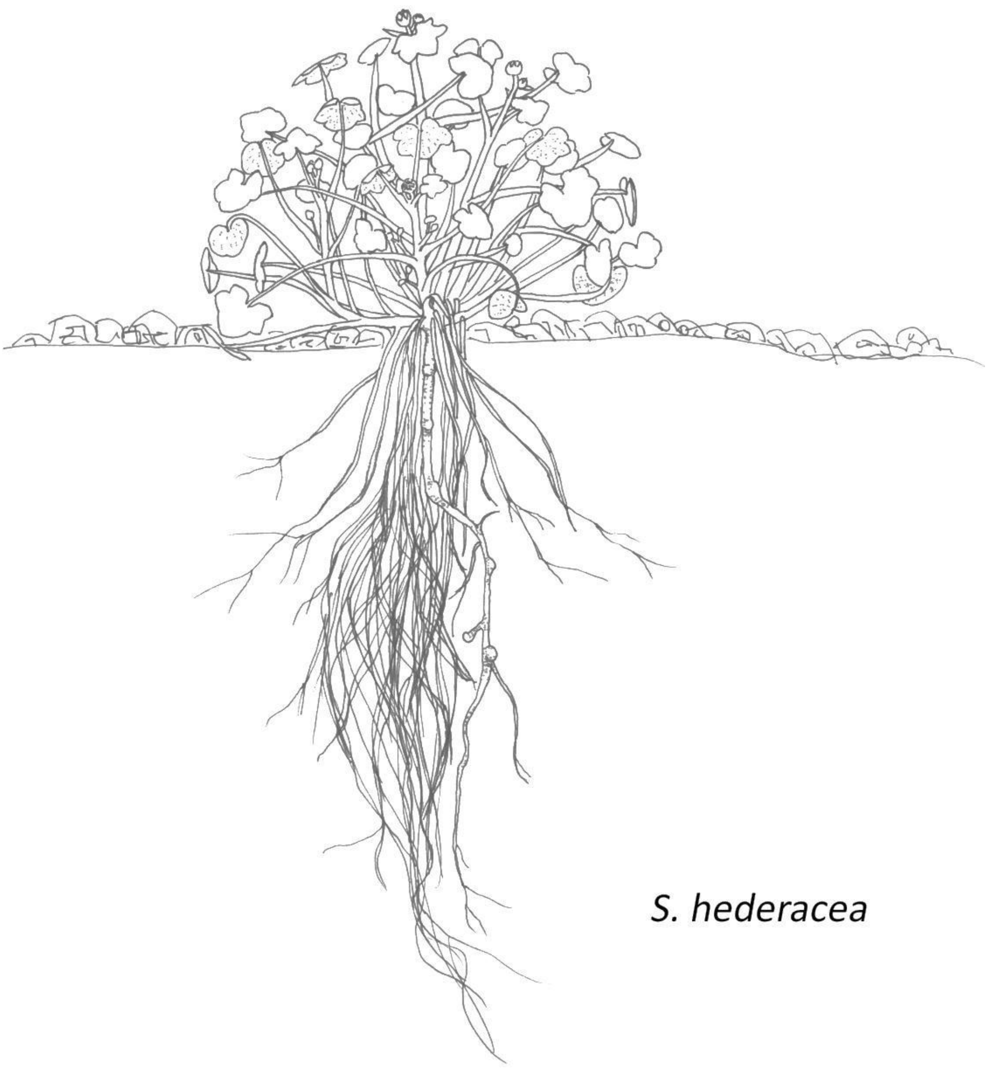
1 Vegetative reproduction

Six states were recognised in a preliminary analysis:

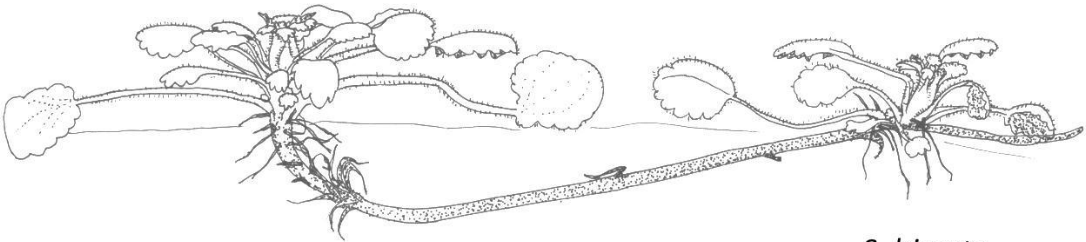
1. n/a (simple plant)
2. cespitose
3. main root branch
4. rosette shoot
5. axillary bulbil
6. runner

After going through the specimens, however, I was confused with states 1--4. It is difficult to define these states without a study of the developing living plant. I managed to complete some work on this but was unable to finish owing to time constraints. My observations on three species recorded below (Fig. App.1).

Figure App.1. The habits of *S. hederacea*, *S. hirsuta*, and *S. paniculata*.

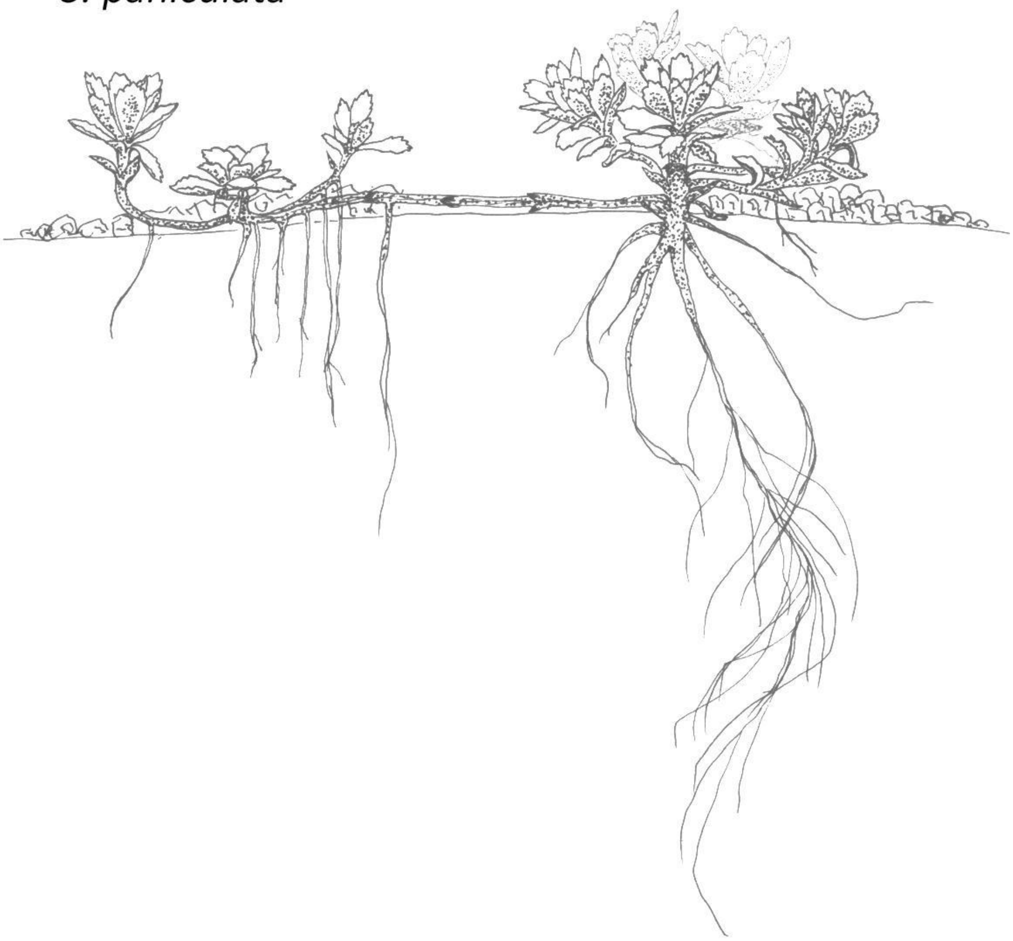


S. hederacea



S. hirsuta

S. paniculata



2 Nodal anatomy

This character was studied by Watari (1939). The various states involve the number of nodal traces vs. the number of gaps. It seems that *Saxifraga* is mostly 1trace/1gap, except in sect. *Heterisia* and *Irregulares* (Table App.4.1). The character is worth further study.

Table App.4.1. Nodal anatomy data from Watari (1939).

	Species	**Nodal anatomy**
1	Saxifraga mertensiana Bongard	3t/?g
2	Saxifraga cortusifolia Sieb. & Zucc.	3t/3g
3	Saxifraga epiphylla Gornall & H.Ohba	1t/1g
4	Saxifraga fortunei J.D.Hook.	3t/3g
5	Saxifraga imparilis I.B. Balf.	3t/?g
7	Saxifraga mengtzeana Engl.& Irmsch.	1t/?g
8	Saxifraga nipponica Makino	3t/3g
9	Saxifraga rufescens I.B. Balf.	3t/?g
10	Saxifraga sendaica Maxim.	3t/3g
12	Saxifraga stolonifera W. Curtis	3t/3g
14	Saxifraga aspera L.	1t/1g
16	Saxifraga bryoides L.	1t/1g
17	Saxifraga cherlerioides D. Don	1t/1g
26	Saxifraga diversifolia Wall. ex Ser.	1t/1?
28	Saxifraga egregia Engl.	1t/1?
33	Saxifraga giraldiana Engl.	1t/1?
38	Saxifraga implicans H. Sm.	1t/1?
40	Saxifraga kingdonii Marquand	1t/1?
46	Saxifraga omphalodifolia Hand.-Mazz.	1t/1?
56	Saxifraga subamplexicaulis Engl.& Irmsch.	1t/1?
61	Saxifraga aristulata J.D.Hook. & Thoms.	1t/1g
65	Saxifraga caveana W.W.Sm.	1t/1g
67	Saxifraga cordigera J.D.Hook. & Thoms.	1t/1g
79	Saxifraga hypericoides Franch.	3t/?g
83	Saxifraga macrostigma Franch.	1t/1g
103	Saxifraga litangensis Engl.	1t/1g
104	Saxifraga lychnitis J.D.Hook. & Thoms.	1t/1g
105	Saxifraga nigroglandulifera Balakr. (=*nutans*)	1t/1g
107	Saxifraga pseudohirculus Engl.	1t/1g
110	Saxifraga bergenioides Marquand	1t/1g
112	Saxifraga congestiflora Engl.& Irmsch.	1t/1g
113	Saxifraga culcitosa Mattf.	1t/1g
115	Saxifraga heleonastes H. Sm.	1t/1g
117	Saxifraga hirculus L.	3t/?g
128	Saxifraga tangutica Engl.	3t/?g

129 Saxifraga tibetica Losinsk.	1t/1g
137 Saxifraga filicaulis Wall. ex Ser.	1t/1g
139 Saxifraga gouldii C.E.C. Fischer	1t/1g
140 Saxifraga hispidula D. Don	1t/1g
146 Saxifraga substrigosa J-T. Pan	1t/1g
147 Saxifraga wallichiana Sternb.	1t/1g
152 Saxifraga brunonis Wall. ex Ser.	1t/1g
156 Saxifraga flagellaris Willd. in Sternb.	1t/1g
159 Saxifraga microgyna Engl.& Irmsch.	1t/1g
161 Saxifraga mucronulatoides J-T. Pan	1t/1g
165 Saxifraga pilifera J.D.Hook. & Thoms.	1t/1g
170 Saxifraga brunneopunctata H. Sm	1t/1g
175 Saxifraga gyalana Marquand & Airy-Shaw	1t/1g
176 Saxifraga heterotricha Marquand & Airy-Shaw	1t/1g
177 Saxifraga lhasana H. Sm.	1t/1g
181 Saxifraga pasumensis Marquand & Airy-Shaw	1t/1g
183 Saxifraga punctulata Engl.	1t/1g
187 Saxifraga signata Engl.& Irmsch.	1t/1g
188 Saxifraga signatella Marquand	1t/1g
191 Saxifraga unguiculata Engl.	1t/1g
192 Saxifraga vilmoriniana	1t/1g
196 Saxifraga aurantiaca Franch. (=*confertifolia*)	1t/1g
202 Saxifraga densifoliata Engl.& Irmsch.	1t/1g
206 Saxifraga engleriana H. Sm.	1t/1g
209 Saxifraga flexilis W.W.Sm.	1t/1g
210 Saxifraga gemmigera Engl.	1t/1g
211 Saxifraga glacialis H. Sm.	1t/1g
212 Saxifraga jacquemontiana Decne.	1t/1g
214 Saxifraga linearifolia Engl.& Irmsch.	1t/1g
220 Saxifraga nanella Engl.& Irmsch.	1t/1g
230 Saxifraga tsarongensis Anthony	1t/1g
234 Saxifraga hemisphaerica J.D.Hook. & Thoms.	1t/1g
235 Saxifraga perpusilla J.D.Hook. & Thoms.	1t/1g
244 Saxifraga bracteata D. Don	1t/1g
246 Saxifraga cernua L.	1t/1g
267 Saxifraga paniculata Mill.	1t/1g
450 Saxifraga rosacea Moench	1t/1g
1. Micranthes merkii (Fischer) comb. nov.	1t/1g
7. Micranthes laciniata (Nakai & Takeda) comb.nov.	1t/1g
32. Micranthes fusca (Maximovicz) H.Hara ex Gornall & Ohba	4t/1g
33. Micranthes japonica (Boissieu) Hara ex Gornall & Ohba	3t/1g
36. Micranthes nelsoniana (D.Don) Small	2t/1g
53. Micranthes sachalinensis (F.Schmidt) Hara ex Gornall & Ohba	5t/1g
68. Micranthes integrifolia (W.J.Hook.) Small	5t/1g

3 Seed ribs

Because the genus *Micranthes* has seed testa ribs as a synapomorphy, I examined several species of *Saxifraga* under the scanning electron microscope (SEM) (Figs. App.2 & 3). As suggested by Kaplan (1981), the main sections or groups of *Saxifraga* cannot be characterised: variation is below series level.

Figure App.2. The seed testa cells of one sect. *Ciliatae* and several sect. *Porphyrion* species. 1: *S. minutissima* (sect. *Ciliatae*), 2: *S. chionophila*, 3: *S. nambulana*, 4: *S. subsessilifolia*, 5: *S. georgeii*, 6: *S. calcicola*, 7: *S. subternata*, 8: *S. saxatilis*.

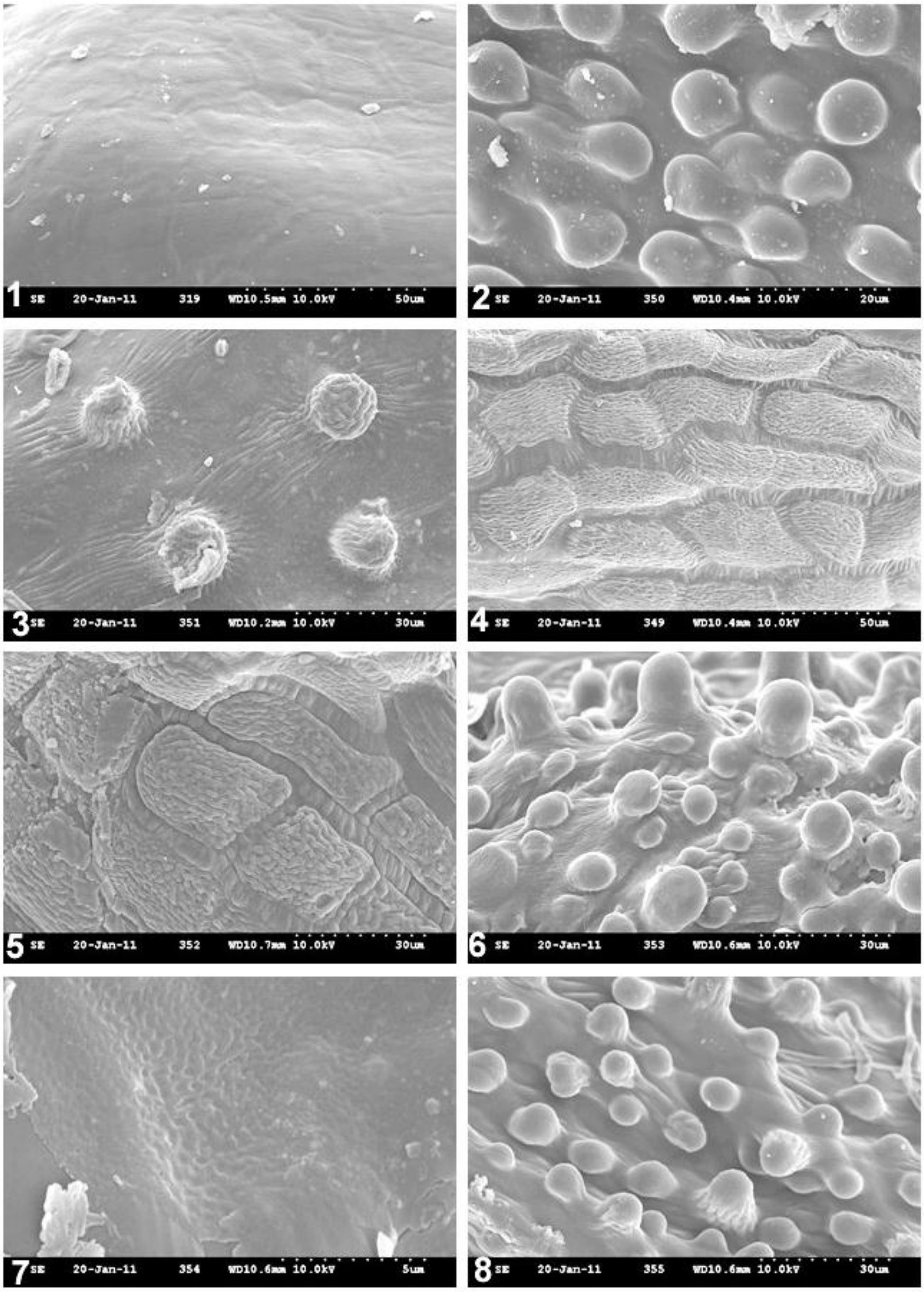
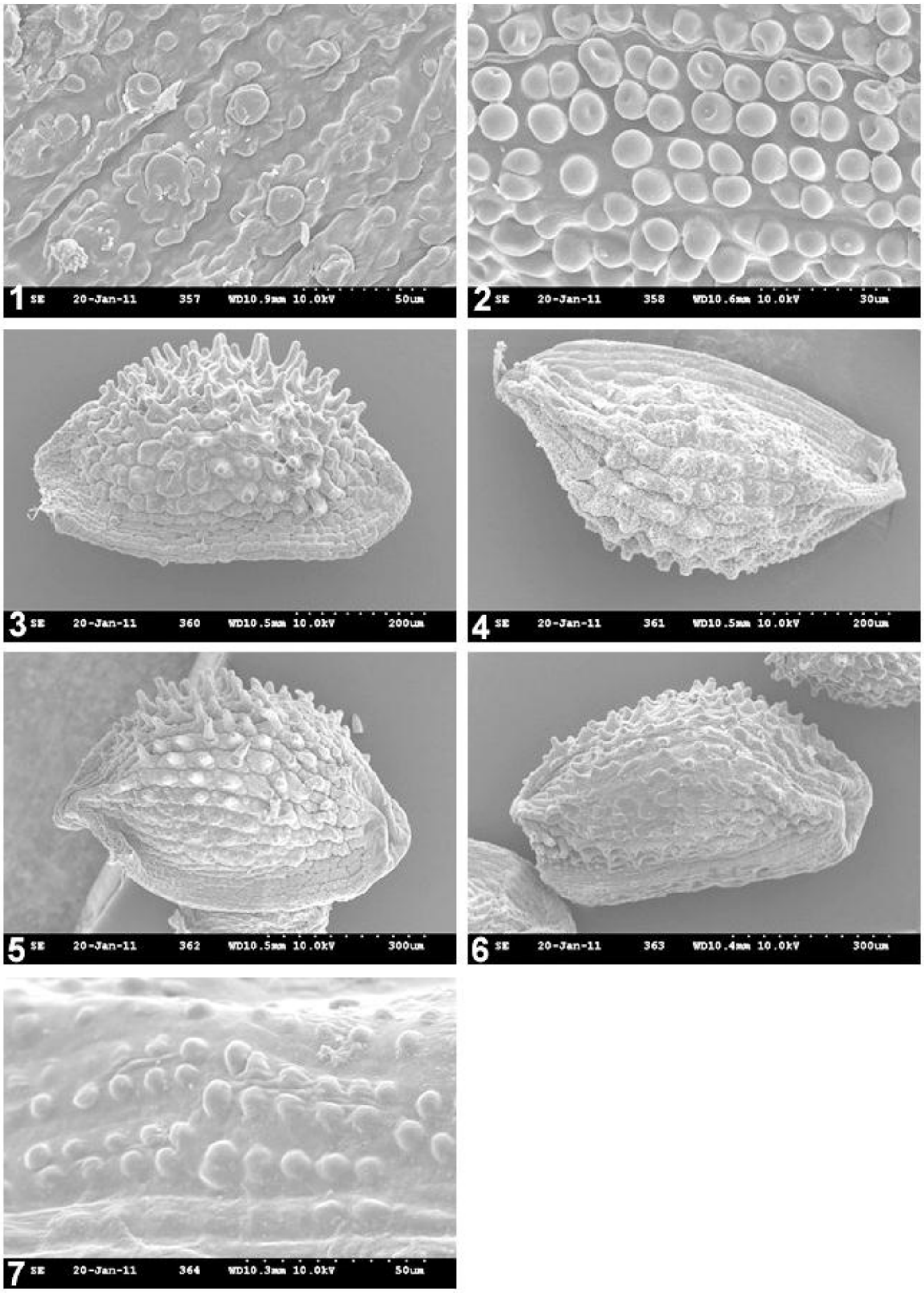


Figure App.3. The seed testa cells of sect. *Porphyrion* species. 1: *S. kongboensis*, 2: *S. octandra*, 3: *S. corymbosa*, 4: *S. federici-augusti*, 5: *S. sempervivum*, 6: *S. aretioides*, 7: *S. rupicola*.



4 Sex expression

Sex expression in *Saxifraga* is variable. There are three types: hermaphrodite, gynodioecious and dioecious.

Gynodioecious species recorded are *S. aizoides* and *S. granulata* (Fig. App.4). Male-sterile plants have also been found in *S. oppositifolia*, *S. hirculus*, *S. tricuspidata*, *S. cernua* and *S. cespitosa*, although they appear not be consistently so (Webb and Gornall 1989).

Figure App.4. *S. granulata*. Hermaphrodite flower on the left; female flower on the right.

The one of dioecious species recorded in the literature is *S. eschscholtzii* Sternberg (Chambers 1964), a species of sect. *Ciliatae* subsect. *Hemisphaericae*. But there are many records of dioecious plants in the herbarium specimens of sect. *Ciliatae*. For instance, specimens of *S. microgyna*, *S. pilifera* and *S. consanguinea* were recorded as dioecious by Harry Smith {British Museum (Natural History)}. However, it is not possible from herbarium material to pronounce on the population or species level whether dioecy occurs. Instead field studies are needed. Table App.4.2 lists the sex expression observed in nine species based on population studies in Qinghai province, China.

Table App.4.2. Sex expression in species of *Saxifraga* sect*. Ciliatae* from Qinghai Province, China.

Species	Section/Genus	Sex expression
S. unguiculata	Ciliatae	hermaphrodite
S. consanguinea	Ciliatae	dioecious
S. pseudohirculus	Ciliatae	hermaphrodite
S. tangutica	Ciliatae	dioecious
S. sinomontana	Ciliatae	hermaphrodite
S. montanella	Ciliatae	dioecious
S. gemmigera var. *gemmuligera*	Ciliatae	Hermaphrodite?
S. parva	Ciliatae	dioecious
S. tibetica	Ciliatae	dioecious

Appendix 5 New species

Contribution: the new species was found by D.S. Rawat; the new species were identified and named by R.J. Gornall; this paper is modified by R.J. Gornall; pollen SEM picture is taken and the manuscript is written by Zhuoxin Zhang.

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Saxifraga minutissima, a new species from the Garhwal Himalaya, India, and its implications for the taxonomy of the genus *Saxifraga* (Saxifragaceae)

R.J. GORNALL[1]*, D.S. RAWAT[2] & ZHUOXIN ZHANG[1]

[1] *Dept. of Biology, University of Leicester, Leicester, Leicestershire, LE1 7RH, UK*

[2] *Dept. of Biological Sciences, College of Basic Sciences and Humanities, G.B.Pant University of Agriculture and Technology, Pantnagar-263 145, Uttarakhand, INDIA*

Saxifraga minutissima, a new and extremely small species of *Saxifraga* (Saxifragaceae), is described from the Garhwal Himalaya, India. It differs from all other species of *Saxifraga*, except *Saxifraga bicuspidata*, in having five stamens and lacking petals. It can be distinguished from *S. bicuspidata* in having leaves and sepals entire. The finely striate pollen exine pattern of *S. minutissima* indicates that the species belongs to *Saxifraga* section *Ciliatae*. Its prostrate, axillary leafy shoots and lack of crisped, rufous hairs strongly suggests a place in subsection *Serpyllifoliae*, where one of its closest relatives may be *Saxifraga stella-aurea*.

Introduction

Routine floristic surveys have been conducted by one of the authors (DSR) in alpine zones of the Garhwal Himalaya over the last two decades. During these surveys, a remarkable new species belonging to *Saxifraga* section *Ciliatae* Haw. was discovered and is described and illustrated here with notes on its habitat and discussion of its taxonomic relationships. The species is discussed in the context of the classification by Gornall (Gornall 1987a).

Species description

Saxifraga minutissima D.S.Rawat, **sp. nov. Fig 1.**

A congeneribus omnibus differt, praeter *Saxifraga bicuspidata* Hook., floribus apetalis et staminibus 5 (–6). A *Saxifraga bicuspidata* foliis et sepalis integris differt. Species habitu cum *Saxifraga stella-aurea* Hook. f. & Thomson optime congruens, sed differt statura perpusilla. — TYPE: India, Garhwal Himalaya, Rudraprayag District, Vasuki Tal area near Kedarnath, in alpine zones with mosses, 4600-4800m asl, 30°44’27”N 79°01’25”E, 10 ix 2001, D.S. Rawat. (holo GBPUH Acc. No. 442 (G.B. Pant University Herbarium, Pantnagar, Uttarakhand, India); iso BM).

Perennial herb, 8—13 mm tall, branched at base to form loose cushions up to 5cm across. Proximal axillary shoots leafy, prostrate, with small, distal leaf rosettes. Rosette leaves sessile, linear to oblong-lanceolate, margin entire, glabrous or rarely with eglandular hairs, apex acute or obtuse, adaxial surface sometimes with eglandular hairs, 1.5—2.5 × 0.5—1 mm. Horizontal stem leaves sessile, linear to oblong-lanceolate, margin entire, glabrous or rarely with eglandular hairs, apex acute or obtuse, surface glabrous, 1.5—2 × 0.5 mm, somewhat fleshy. Flowering stem terminal, leafless, without bracts. Flowers solitary; pedicel (1-)3—6 mm, brown-glandular hairs. Sepals 5, erect, 1—1.2 × 0.6—1 mm, ovate to oblong, apex reddish, acute or subacute, adaxial surface and margins glabrous, or rarely with a few brown-glandular hairs; veins 3, confluent. Petals 0. Stamens 5, rarely 6 (seen in only one out of 26 flowers dissected), antisepalous, equalling sepals, filaments linear, ca. 0.7 mm, the dehisced anthers ca 0.3 mm, yellow, thecae parallel on dehiscence. Ovary semi-inferior at anthesis, ovoid to oblong, to 2.5 mm long in fruit; carpels tapered to short conical styles, styles 0.2—0.3 mm long, exceeding sepals, stigmas capitate. Seeds spherical to ovoid, shining brown, smooth, 0.3—0.4mm (Fig. App.5 & 6).

Phenology. Flowering: July-September; fruiting: September-October.

Conservation status. Data Deficient (apparently rare).

Distribution. *Saxifraga minutissima* is restricted to a small area of the Garhwal Himalaya. Only two populations are currently known: one from the Vasuki Tal area, where the type was collected, and the other from 4—5 km away in Madhuganga valley, also near Kedarnath. Specimens from the second locality are suitable paratypes: India, Garhwal Himalaya, Rudraprayag district, Madhuganga Valley near Kedarnath, 4200-4500m, 24 viii 2005, *D.S.Rawat* (BM); ibid, 4 x 2007, *D.S.Rawat* (BM).

Ecology. The plant is very small, scarcely more than 1 cm tall, forming loose cushions reaching up to 5 cm in diameter. It grows in moist sandy places on scree slopes or along streams. Its immediate associates are mosses, from which to the casual observer it is scarcely distinguishable in its vegetative state.

At both of its localities, the species inhabits the higher alpine zone, being found at altitudes of 4600—4800m in Vasuki Tal and slightly lower, 4200-4500m, in the Madhuganga valley (Fig. App.7).

Figure App.5. *Saxifraga minutissima* sp. Nov. A: whole plant; B: flower; C-D: dissected flowers show carpel and stamens; E: stamen; F: sepals, one showing antisepalous stamen; G: seeds; H: mature carpel; I: leaves from basal rosette; J: leaves from vegetative shoots. Drawn by Dharmendra S. Rawat.

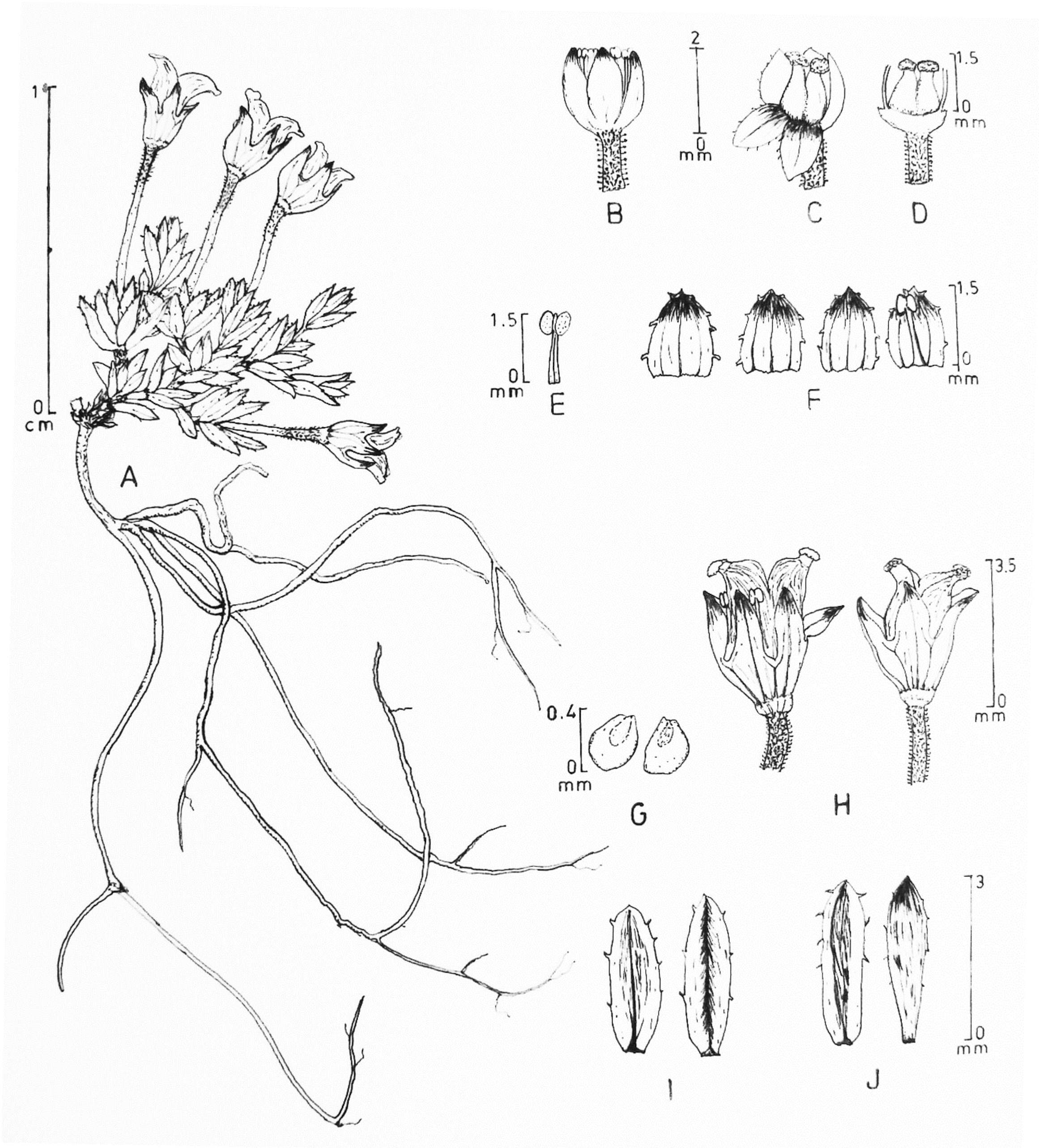


Figure App.6. A) *S. minutissima* growing with mosses; B) seed of *S. minutissima* showing its smooth surface; C) pollen exine of *S. minutissima* showing the finely striate pattern characteristic of section *Ciliatae*; D) pollen exine of *S. stella-aurea*, a putatively related species (Table App.5.1).

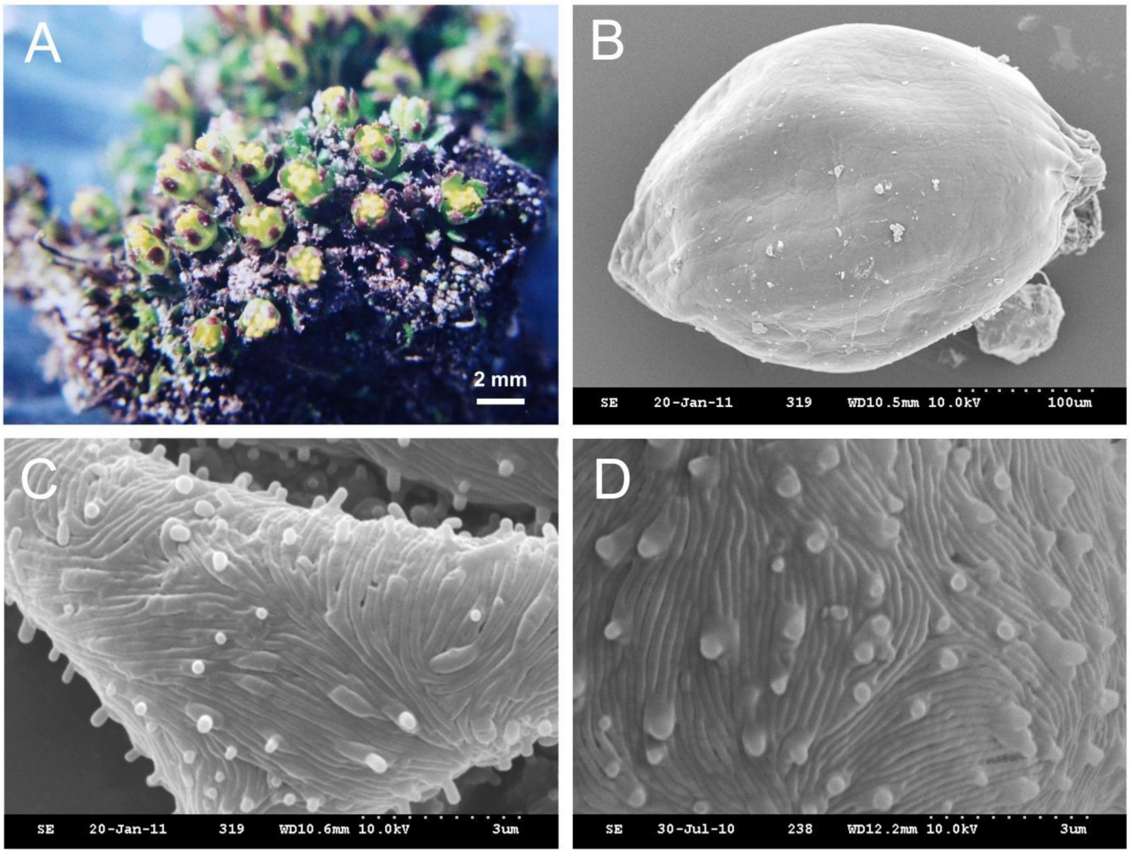
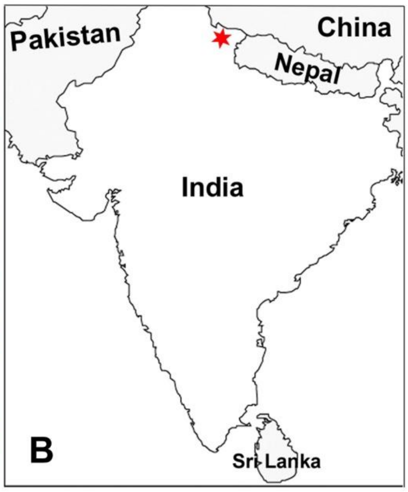


Figure App.7. Habitat (A) of *Saxifraga minutissima* (marked by a ring) in the Madhuganga valley, Garwhal Himalaya (B, marked by a star), India.



Etymology. The specific epithet refers to the impressively small size of the plants; the species is among the smallest known saxifrages.

Taxonomic relationships. The new species is assigned to *Saxifraga* on account of its alternate, exstipulate leaves, and flowers with five sepals, absence of a free hypanthium, presence of two carpels, and axile placentation. Its pollen exine is characterised by fine parallel lirae with granular supratectal processes or verrucae (Fig. App.6 C), a pattern that is a synapomorphy for section *Ciliatae*, e.g. *S. stella-aurea* (Fig. App.6 D; Ferguson and Webb 1970; Kaplan 1981). The monophyletic status of the section also has statistically significant support from ITS sequence data (Zhang et al. 2008). Within section *Ciliatae*, the presence in the new species of somewhat fleshy leaves, prostrate axillary leafy shoots, and the absence of rosette leaves and rufous, crisped hairs, indicates a position in subsection *Serpyllifoliae* (Gornall 1987a). Within that subsection, the only other very tiny species, which may be related, are *S. stella-aurea* and *S. inconspicua* W.W.Sm. These differ from our new species as outlined in Table App.5.1.

Table App.5.1. Main morphological differences between *S. minutissima* and its closest putative relatives in section *Ciliatae* subsection *Serpyllifoliae* and *S. bicuspidata*.

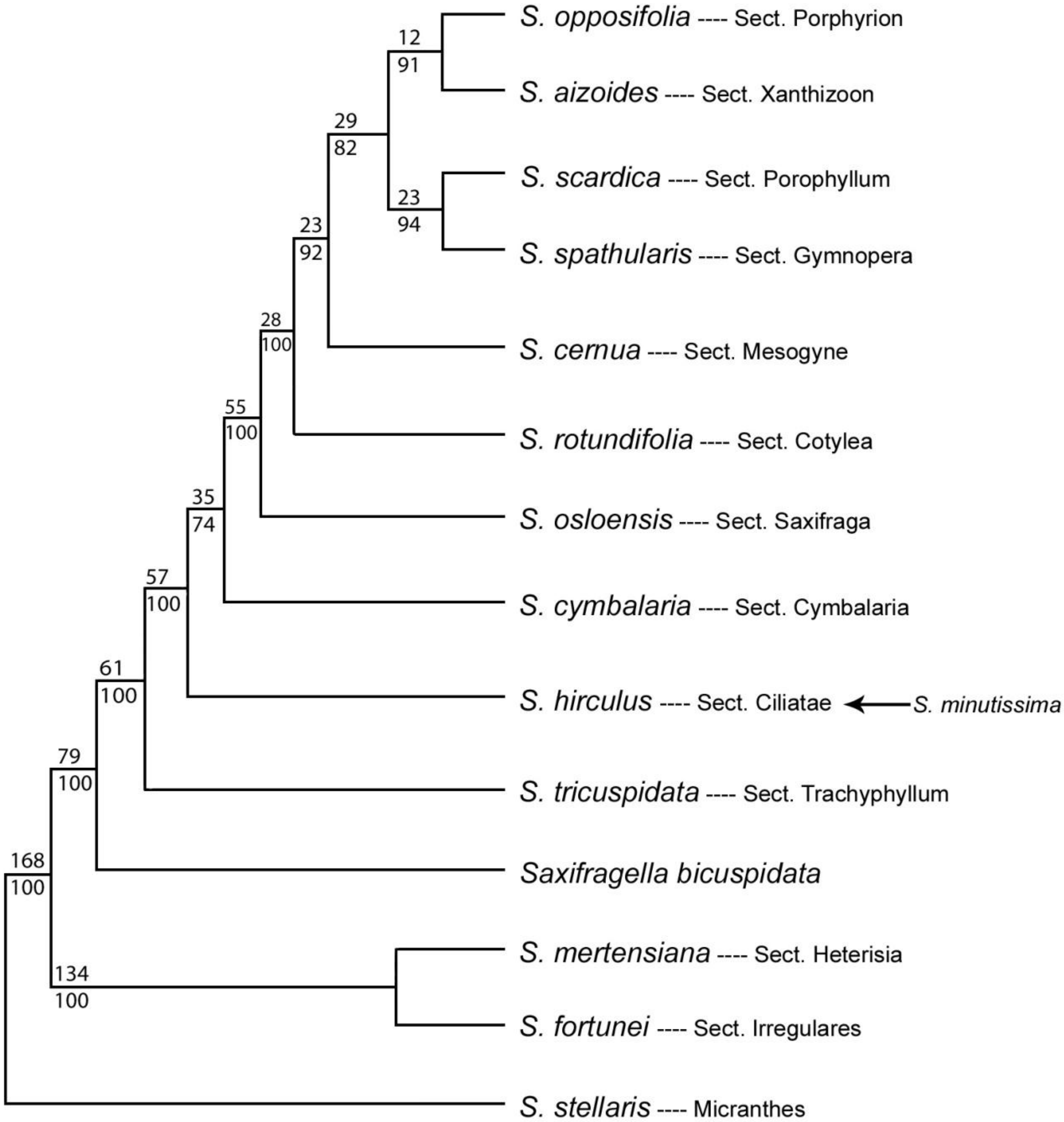
Species	*Basal leaves*	*Pedicel hairs*	*Sepals*	*Petals*	*Stamen number*
S. minutissima	linear to oblanceolate, 1.5—2.5 × 0.5—1 mm, apex acute to obtuse, adaxial surface and margin glabrous or rarely with eglandular hairs	brown-glandular pubescent	erect, apex acute to subacute	absent	5
S. inconspicua	elliptic, 1.5-3 × 0.3-1 mm, apex obtuse, glabrous	glabrous	erect, apex obtuse	present, 1.5 × 0.6-0.7 mm	10
S. stella-aurea	ovate to elliptic, 2—5 × 1—2 mm, apex obtuse, margin brown-glandular hairy, adaxial surface glabrous	brown-glandular pubescent	reflexed, apex acute to obtuse	present, 4–7 × 1.5–3.4 mm	10
S. bicuspidata	linear-spathulate, (2.5-)3-5(-7.5) × 0.7-1.4 mm, apex deeply emarginate with acute sinus, glabrous	glabrous	erect, apex deeply emarginate	absent	5

Discussion

The striking features of the plant are the lack of petals (instead of five) and the presence of only five stamens (instead of eight or the more usual ten), with the antipetalous whorl missing. The genus *Saxifraga* contains some 450 species, which display a remarkably constant floral morphology, viz. pentamerous, diplostemonous, bicarpellate flowers with axile placentation and lacking a free hypanthium. Deviations from this combination of characters are remarkably few but include a small group of species belonging to subsection *Kabschia* which possess tetramerous flowers with eight stamens. The occurrence of only five stamens is found otherwise only in *Saxifraga bicuspidata* (Table App.5.1), in which they are also antisepalous and which, interestingly, is similarly apetalous (Moore 1983). Apetaly is found in two other species, i.e. *S. eschscholtzii* Cham. ex DC. (section *Ciliatae* subsection *Hemisphaericae*), and *S. cespitosa* f. *apetala* Engl. (section *Saxifraga* series *Caespitosae*). *Saxifraga bicuspidata* comes from the southern tip of South America, in an area around Tierra del Fuego. Although it was originally assigned to *Saxifraga* (Hooker 1846), it was soon segregated as the monotypic genus *Saxifragella* (Engler and Irmscher 1916/19), on account of the reduced stamen number. This arrangement has been accepted by botanists ever since (Moore 1969), that is until Soltis et al. (2001a) demonstrated by means of a phylogenetic analysis of six DNA regions (four chloroplast and two nuclear sequences) that the species was nested within *Saxifraga*, albeit near the base alongside species of section *Irregulares* (which have uniquely zygomorphic corollas) and *S. mertensiana* Bong. (Fig. 4).

Any notion of increasing the homogeneity of *Saxifraga* by redrawing its boundaries to exclude these basal groups clearly now has much less appeal. If our sectional assigment of the haplostemonous, apetalous *Saxifraga minutissima* is phylogenetically correct, then it is nested so deeply in the genus (Soltis et al. 2001a; Zhang et al. 2008) that we must accept that *Saxifraga*, on occasion, may lack petals and may even have as few as five stamens. It seems probable that the very rare (for *Saxifraga*) mutations causing apetaly and five stamens in both the Himalayan *S. minutissima* and the Fuegian *S. bicuspidata* are the result of parallel evolution.

Figure 4. Phylogenetic tree of *Saxifraga*, redrawn from the 6-gene analysis by Soltis, et. al. (2001). Percentages below branches are bootstrap values; numbers above branches are the number of base substitutions. It shows the position of *Saxifraga bicuspidata* and the putative position of *S. minutissima* (indicated by an arrow) inferred from the pollen synapomorphy it shares with sect. Ciliatae [confirmed in Chapter 2].



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