

Leaf venation patterns in *Saxifraga* L. species (Saxifragaceae) of Indian Western Himalaya and its taxonomic implications

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Abstract

Leaf venation is an important trait used for specifying and grouping plants. In the present study leaf venation types of 30 *Saxifraga* L. species, found in Indian Western Himalaya, were studied. Out of these, leaf venation patterns of 20 species were not previously studied. In total, five major types of leaf venations *viz.* acrodromous (perfect-basal; perfect-suprabasal; imperfect-suprabasal), camptodromous (eucamptodromous), campylodromous, hyphodromous, and palinactinodromous – were recorded. Section *Micranthes* showed palinactinodromous; section *Ciliatae* showed mainly acrodromous; section *Mesogyne* showed palinactinodromous; and sect. *Porphyron* showed acrodromous, eucamptodromous and hyphodromous types of venations. Palinactinodromous venation type was found to be the ancestral type. Further molecular study is needed to be done to solve the taxonomic complexity of the genus.

Keywords: leaf venation; *Micranthes*; *Saxifraga*; Saxifragaceae; Western Himalaya; Uttarakhand

Introduction

Leaf venation refers to the pattern of veins and vein branching in a leaf (Simpson, 2019). Foliar veins and veinlets help to conduct water, minerals, hormones and food between the leaf and the stem, and within the leaf. They also provide mechanical support to the leaf. Different variations in leaf vein patterns in different plant species have been used in plant identification in palaeobotany. In the flowering plants two traditional categories (dicotyledons and monocotyledons) can simply be differentiated on the basis of leaf venation bearing few exceptions. It is thus an important trait of plants used in specifying and grouping plants (Walls, 2011). Patterns of veins can be used as fingerprints to characterize plant taxa (Corner, 1964). The most widely used and comprehensive classification of leaf venation was given by Hickey (1973, 1979). The Hickey's classification was developed further in the manual of leaf architecture (Ellis *et al.*, 2009).

Saxifraga L. (*sensu lato*), the largest genus in the family Saxifragaceae, is found mostly in the cold rocky terrains of Europe, North America and Sino-Himalayan region. The genus has around 495-543 species in the

world (Akiyama and Gornall, 2012; The Plant List, 2013; POWO, 2017). The Sino-Himalayan region is one of the diversity centres for this genus and the Indian Himalayan region adjacent to China sustains 64 species, 4 subspecies and 5 varieties of *Saxifraga* (Agnihotri and Husain, 2013) while Nepal Himalaya has 91 species (Akiyama and Gornall, 2012), and Bhutan has about 75 species (Grierson, 1987). In India, about 61 species of *Saxifraga* are known in Western Himalaya (Uniyal, 2016, unpublished) and the members of this genus are found growing in altitudinal range of 1500-6000 m above sea level.

The earliest reference of leaf venation study is of Galloë (1910) who provided an account of 10 arctic *Saxifraga* species. Engler and Irmscher (1916, 1919) in their monograph on *Saxifraga* have also drawn leaf venation in some species. Fuller and Hickey (2005) studied venation of 2 species of *Saxifraga* as out group while making their studies in Gunneraceae. Zhang *et al.* (2015) have reported leaf venation patterns in 150 species of *Saxifraga* including many Sino-Himalayan species and representing 13 sections of the genus. Three main types of venation patterns in the genus were reported as - acrodromous, camptodromous and palinactinodromous.

In the present study, 30 species of *Saxifraga* L., collected from subalpine and alpine regions of Uttarakhand and available in G.B. Pant University herbarium, Pantnagar, India, were studied for the type of leaf venation pattern present in them.

Materials and Methods

The study area of the present work is the land area covered by the Western Indian Himalaya comprising of three states - Jammu & Kashmir, Himachal Pradesh and Uttarakhand, extending at latitudinal range of 28°43' N - 37°05' N and at longitudinal range of 72°02' E - 81°02' E (Sidhu and Surya, 2014). Samples of species were collected by D.S. Rawat over the years from different localities of Western Himalaya. The present research was carried out for the duration of one year. Leaves from the 30 species (including 5 unidentified species) of genus *Saxifraga* available in G.B. Pant University Herbarium were collected. Leaf venation of the specimens were drawn and studied by modifying the method given by Payne (1969). Leaves from different species were submerged in 10% KOH solution in separate petri-dishes at room temperature for 3-10 days (depending upon the thickness/ texture of the leaves) till they became translucent. Then the translucent leaves are washed with tap water 2-3 times and placed in 50% commercial bleach solution for 10-20 minutes. The leaves are again washed with tap water 2-3 times and mounted in glycerine on separate glass slides and covered with cover slips. The leaves are then observed under a dissecting microscope (MSW-108DM, 10x). The venation pattern is identified using the classification of leaf architecture described by Hickey (1973, 1979).

Results and Discussion

Patterns of veins can be used as fingerprints to characterize plant taxa (Corner, 1964). The most widely used and comprehensive classification of leaf venation was produced by Hickey (1973, 1979). He based his classification on the pattern of distribution of primary veins in a leaf. He gave 6 major types of leaf venation subdividing further into different sub-types. In this study five major types and four sub types of leaf patterns were found. These leaf venation types are:

1. Acrodromous (perfect-basal, perfect-suprabasal, imperfect-basal)
2. Palinactinodromous
3. Campylodromous
4. Camptodromous (Eucamptodromous)
5. Hyphodromous

The types of venations in studied 30 species are listed in Table 1 and compared with the earlier report by Zhang *et al.* (2015). The illustrations of the types of leaf venation patterns are drawn in Figure 1.

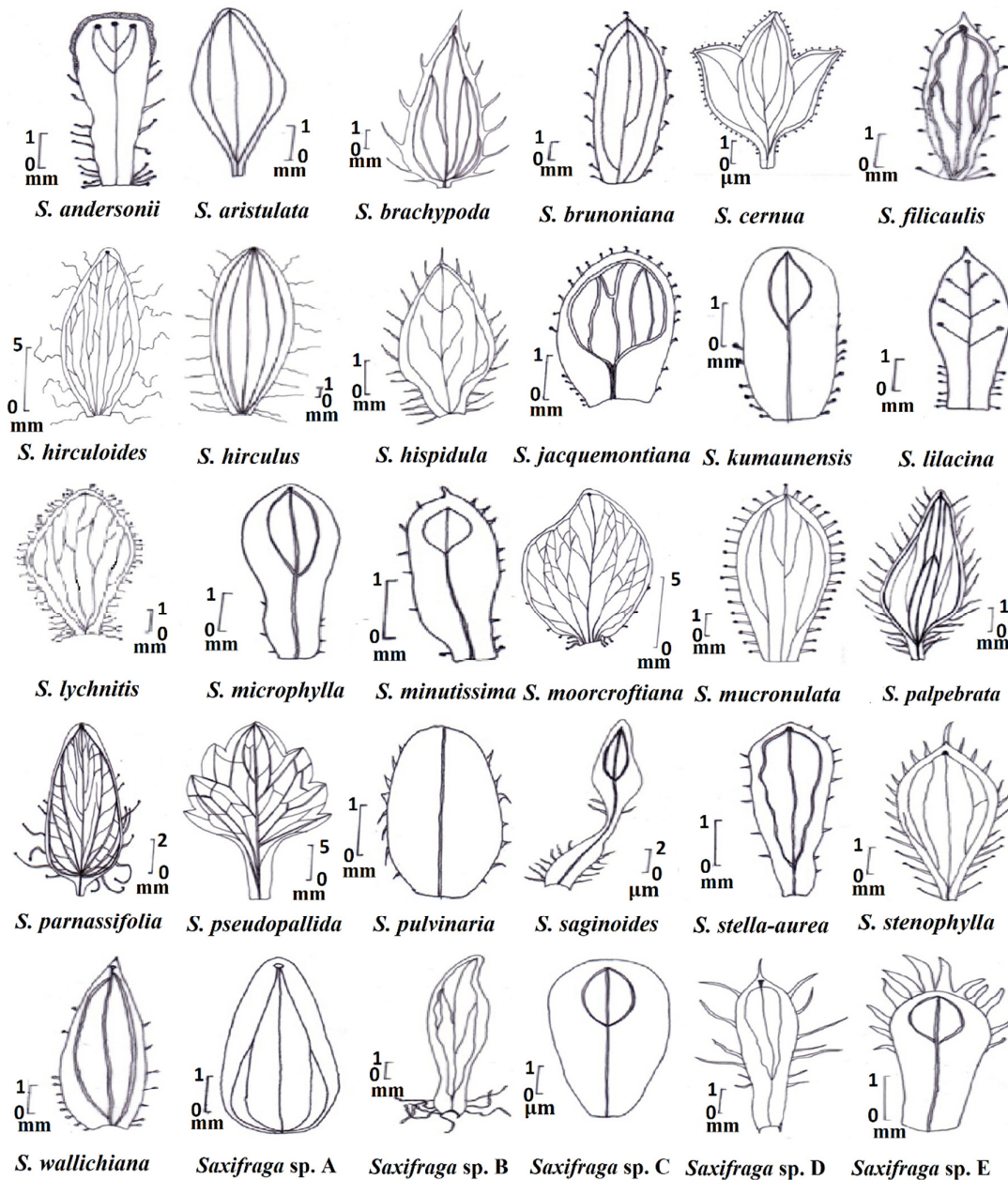


Figure 1. Venation patterns in studies species of *Saxifraga*

Section wise listing of the species shows that four sections (*Porphyron*, *Mesogyne*, *Ciliatae*, *Micranthes*) are represented by studied species. *Micranthes* section of *Saxifraga* (*sensu lato*) is considered as a separate genus currently which is basal to *Saxifraga* (*sensu stricto*) in the phylogenetic tree (Tkach *et al.*, 2015; Gao *et al.*, 2015) as shown in Figure 2. One species of this section *S. pseudopallida* (now *Micranthes melanocentra*) was studied in the present work and palinactinodromous venation was recorded in it. This section is known to have palinactinodromous venation as reported by Zhang *et al.* (2015).

Another section *Mesogyne* is represented by *S. cernua* L. in present study. In this species young leaves showed acrodromous (perfect-basal) type of venation but the mature leaves showed palinactinodromous venation. This species and other species of this section are reported to have palinactinodromous venation (Zhang *et al.*, 2015).

In this study, section *Porphyron* is represented by five species - *S. andersonii*, *S. kumaunensis*, *S. lilacina*, *S. pulvinaria* and an unidentified species mentioned as *Saxifraga* species 'C'. This unidentified species have characteristic lime secreting glands based on which it is placed here. This section has three different types of venation. *S. andersonii* showed acrodromous (imperfect-suprabasal) venation while *Saxifraga* species 'C' and *S. kumaunensis* showed acrodromous (perfect-suprabasal) venation. Differing from these species, *S. lilacina* showed eucamptodromous venation and *S. pulvinaria* showed hyphodromous venation. Zhang *et al.* (2015) studied 15 species in this section and reported only acrodromous and camptodromous venation in this section as they have not studied *S. pulvinaria* which show hyphodromous venation.

Table 1. Leaf venation in species of *Saxifraga* L.

Species and sections of <i>Saxifraga</i> (<i>s.l.</i>) genus studied		Venation (present study)	Venation (Zhang <i>et al.</i> , 2015)
<i>Sect. Porphyron</i>			
1.	<i>S. andersonii</i> Engl.	Acrodromous (Imperfect-suprabasal)	-
2.	<i>S. kumaunensis</i> Engl.	Acrodromous (Perfect-suprabasal)	-
3.	<i>S. lilacina</i> Duthie	Eucamptodromous	-
4.	<i>S. pulvinaria</i> Harry Sm.	Hyphodromous	-
5.	<i>Saxifraga</i> species C	Acrodromous (Perfect-suprabasal)	-
<i>Sect. Mesogyne</i>			
6.	<i>S. cernua</i> L.	Acrodromous (Perfect-basal), Palinactinodromous	Palinactinodromous
<i>Sect. Ciliatae</i>			
7.	<i>S. aristulata</i> Hook.f. & Thomson	Acrodromous (Perfect-basal)	Acrodromous
8.	<i>S. hirculoides</i> Decne.	Acrodromous (Perfect-basal)	-
9.	<i>S. hirculus</i> L.	Acrodromous (Perfect-basal)	Acrodromous
10.	<i>S. lychnitis</i> Hook.f. & Thomson	Acrodromous (Perfect-basal)	Acrodromous
11.	<i>S. moorcroftiana</i> (Ser.) Wall. ex Sternb.	Acrodromous (Perfect-basal)	-
12.	<i>S. palpebrata</i> Hook.f. & Thomson	Acrodromous (Perfect-basal)	-
13.	<i>S. parnassifolia</i> D.Don	Campylodromous	-
14.	<i>S. saginoides</i> Hook.f. & Thomson	Acrodromous (Perfect-suprabasal)	-
15.	<i>S. brachypoda</i> D.Don	Campylodromous	-
16.	<i>S. filiculis</i> Wall. ex Ser.	Acrodromous (Perfect-basal)	Acrodromous
17.	<i>S. hispidula</i> D.Don	Acrodromous (Perfect-basal)	Palinactinodromous
18.	<i>S. wallichiana</i> Sternb.	Acrodromous (Perfect-basal)	Acrodromous
19.	<i>S. Jacquemontiana</i> Decne.	Acrodromous (Perfect-suprabasal)	Acrodromous
20.	<i>S. microphylla</i> Royle ex Hook.f. & Thomson	Acrodromous (Perfect-suprabasal)	-
21.	<i>S. stella-aurea</i> Hook.f. & Thomson	Acrodromous (Perfect-suprabasal)	-
22.	<i>S. minutissima</i> D.S.Rawat	Acrodromous (Perfect-suprabasal)	Acrodromous
23.	<i>S. brunoniana</i> Wall. ex Sternb.	Acrodromous (Perfect-suprabasal)	Acrodromous
24.	<i>S. mucronulata</i> Royle	Acrodromous (Perfect-basal)	-
25.	<i>S. stenophylla</i> Royle	Acrodromous (Perfect-basal)	-
26.	<i>Saxifraga</i> species A	Campylodromous	-
27.	<i>Saxifraga</i> species B	Acrodromous (Perfect-basal)	-
28.	<i>Saxifraga</i> species D	Acrodromous (Perfect-suprabasal)	-
29.	<i>Saxifraga</i> species E	Acrodromous (Perfect-suprabasal)	-
<i>Sect. Micranthes</i>			
30.	<i>S. pseudopallida</i> Engl. & Irmsch.	Palinactinodromous	-

The section with largest number of species studied here is *Ciliatae* which is represented by 23 species. It is the largest section of *Saxifraga* and highly diversified in the Himalaya. Unidentified *Saxifraga* species 'A', 'B', 'D' and 'E' showed morphological characters resembling to *Ciliatae* hence placed here. Despite large number of species only two types- acrodromous (perfect-basal/-suprabasal) and campylodromous venation were recorded. In this study, *S. parnassifolia* and *Saxifraga* species 'A' showed campylodromous venation. Within acrodromous venation group 12 species (*S. aristulata*, *S. hirculoides*, *S. hirculus*, *S. lychnitis*, *S. moorcroftiana*, *S. palpebrata*, *S. filicaulis*, *S. hispidula*, *S. wallichiana*, *S. mucronulata*, *S. stenophylla*, and unidentified *Saxifraga* species 'B') showed perfect-basal acrodromous venation, while eight species (*S. saginoides*, *S. Jacquemontiana*, *S. microphylla*, *S. stella-aurea*, *S. minutissima*, *S. brunoniana*, and unidentified *Saxifraga* species 'D' and 'E' showed perfect-suprabasal acrodromous venation. Zhang *et al.* (2015) also reported acrodromous types of venation in this section, bearing *S. substrigosa* and *S. hispidula* which showed palinactinodromous venation. For the species common (*S. aristulata*, *S. hirculus*, *S. lychnitis*, *S. filicaulis*, *S. wallichiana*, *S. Jacquemontiana*, *S. minutissima*, *S. brunoniana*) same type of venations are recorded in present study and Zhang *et al.* (2015), except *S. hispidula* in which palinactinodromous venation was reported earlier but acrodromous (perfect-basal) venation was recorded here. This species (*S. hispidula*) is known to occur in China, Tibet, Eastern Himalaya, Bhutan, Nepal, Uttarakhand Himalaya, and morphologically variable. Specimens of Western Himalaya (Uttarakhand) have entire leaves (typical var. *hispidula*) while in Nepal and eastwards var. *doniana* with toothed leaves is more common (Grierson, 1987; Pan *et al.*, 2001; Akiyama and Gornall, 2012). It is possible that Western Himalayan populations (*S. hispidula* var. *hispidula*, with entire leaves) may have evolved acrodromous venation considering high diversification of *Ciliatae* section in the Himalaya.

Zhang *et al.* (2015) studied evolution of venation types in *Saxifraga* sections by mapping this character in the phylogenetic tree proposed by Gao *et al.* (2015). Their study concludes that palinactinodromous venation is the putative ancestral condition which is shared by outgroups and basal sections like *Irregulares* and *Heterisia*. Our results for studied four sections (*Porphyron*, *Mesogyne*, *Ciliatae* and *Micranthes*) and the results of Zhang *et al.* (2015) for rest of the sections are depicted in the current phylogenetic tree of the genus (Tkach *et al.*, 2015) in Figure 2.

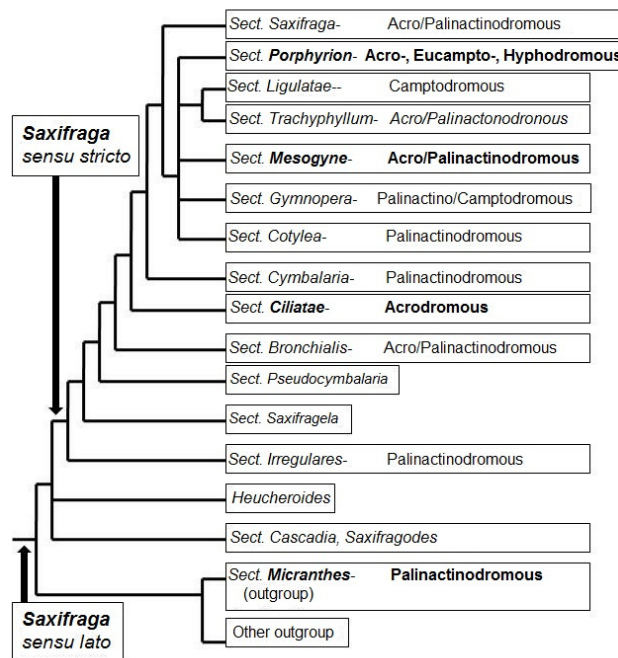


Figure 2. Phylogenetic tree of *Saxifraga* sections and venation patterns reported in present study (in bold face) and by Zhang *et al.* (2015)

Figure 2 shows that the basal groups (*Micranthes*, *Irregulares*) and relatively advance groups (*Cymbalaria*, *Cotylea*, *Mesogyne*) have retained this venation. But some members of relatively advanced groups (*Bronchialis*, *Trachyphyllum*, *Saxifraga*) have also evolved acrodromous venation in addition to ancestral venation. This character has evolved few times and the section *Ciliatae* exclusively (or mainly) has evolved acrodromous venation. Section *Ligulatae* has evolved camptodromous venation. However, section *Porphyron* has shown relatively more diversification in venation type evolving acrodromous, eucamptodromous and hyphodromous venations in the members found in the Himalaya.

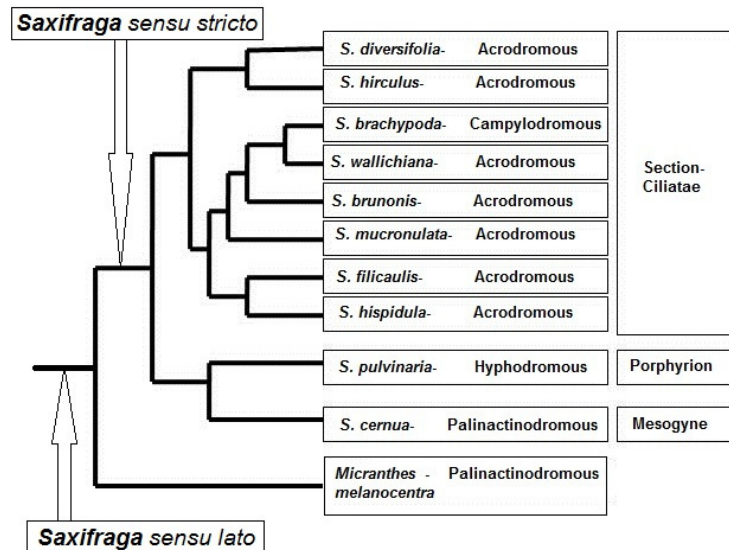


Figure 3. Venation pattern in the species common in present study and phylogenetic tree of Gao *et al.* (2015)

Venations of the taxa common in phylogenetic tree of Gao *et al.* (2015) and present study are depicted in the Figure 3. It shows palinactinodromous venation as putative ancestral character shown by *Micranthes* and section *Mesogyne*. However, *S. pulvinaria* of section *Porphyron*, a closer group of *Mesogyne*, has evolved hyphodromous venation. In the *Ciliatae* group where majority of the species show acrodromous venation, the most advanced member *S. brachypoda* has evolved campylodromous venation.

Results of venation patterns in present study resonate with the results of Zhang *et al.* (2015) for the species common to both studies (*S. cernua*, *S. aristulata*, *S. hirculus*, *S. lychnitis*, *S. filicaulis*, *S. hispidula*, *S. wallichiana*, *S. Jacquemontiana*, *S. minutissima*, *S. brunoniana*) except *S. hispidula*. The reason for this disagreement may be variations in leaf morphology. Our results further stabilize that *Saxifraga* species have similar venation in their entire range of distribution. The venation types in 20 species are being reported for the first time and, therefore, are additions to the pool of 150 species in which venation is known. However, present results also suggest more variability to only three venation types (acrodromous, camptodromous and palinactinodromous) reported earlier (Zhang *et al.*, 2015) by adding campylodromous and hyphodromous venations.

Conclusions

Saxifraga is one of the larger genera in angiosperms and many species, particularly those occurring in the Sino-Himalayan region, are not very well known and studied. Present study adds information on venation pattern of 20 Himalayan *Saxifraga* species, hitherto, not known. The results resonate with earlier studies and show palinactinodromous venation as ancestral type being present in the basal group *Micranthes* and evolving

in to acrodromous, campytodromous, campylodromous, hyphodromous in more evolved groups. 23 studied species of Ciliatae, the most diversified section of *Saxifraga* in the Himalaya, show mainly acrodromous venation bearing three species (*S. brachypoda*, *S. parnassifolia*, *Saxifraga* species A) which show campylodromous venation. Combined with leaf shape, venation can be useful even in the identification of herbarium specimens lacking flowering and fruiting stages. Molecular phylogenetics of Sino-Himalayan species needs to be studied in greater details to decipher evolution and diversification of genus in this important center of diversity.

Authors' Contributions

DSR: conceptualized and supervised the study, refined the original draft and have drawn figure-2 and 3. PU: conducted the study, prepared original draft and have drawn figure-1. SC: helped in conducting the study and in writing the draft and later reviewed it. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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