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Taxonomic significance of Micromorphology in *Senecio* Linnaeus (Senecioneae: Asteraceae)

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Abstract

Micromorphological characters like stomata and epidermal cell variations of twelve species of the genus *Senecio* Linnaeus of Asteraceae from Nepal Himalaya were carried out for solving the taxonomic problems within the genus. Out of the 12 species studied, 6 species showed amphistomatic and 6 species showed hypostomatic leaf surface. Four types of stomata were recorded in the genus; anomocytic, tricytic, anisocytic and tetracytic stomata. The contiguous stomata were of common occurrence in all species. The epidermal cells of leaf were found to be irregular to polygonal to rectangular. The stomata were elliptical and circular. The distribution of stomata in the leaf surface and type of stomata and epidermal cell type were found to be taxonomically significant. It could be used as new parameter to delimit the species in the genus *Senecio* Linnaeus. An artificial dichotomous key is presented to delimit the taxa of the genus *Senecio* L. from Nepal Himalaya.

***Key words*:** New parameter, Micromorphological character, Stomata, *Senecio*, Taxonomic significance.

INTRODUCTION

Senecio Linnaeus is the largest and core genus of the tribe Senecioneae of Asteraceae Berchtold& J. Presl (Compositae Gisek*enom. cons*.). The species of the genus are characterized by the annual herbs or perennial shrubby herbs, often scandent, with alternate leaves; capitulum often subtended by calyculate bracts.

Leaf epidermal anatomy particularly of stomata was reported to provide valuable taxonomic and systematic evidence in both living and fossil plants (Florin 1951; Bailey & Nast 1948; Stebbins & Khush 1961; Stace 1965; Van Cotthem 1970; den Hartog & Baas 1978; Wilkinson 1979; Kong 2001). The stomatal types had been reported to be significant in resolving many taxonomic problems; in establishing the various ranks and clarifying the interrelationship among the different taxa (Baranova 1992). Stomatal types had been used by different authors in resolution of taxonomic problems and delimiting the species (Adedeji & Jewoola 2008).

The genus, *Senecio* Linnaeus traditionally with ca. 3000 spp. (Jeffrey *et al*. 1977) was perceived as highly artificial (Bremer 1994; Vincent 1996) because of the fact that many species assigned to the genus *Senecio* was found to be closely related to the species of other genus. A number of taxonomic works had been carried out to make the genus more homogenous. However, the application of leaf epidermal characters in the taxonomy of

Senecio is so far been neglected till now. Study of distribution of stomata on adaxial and abaxial surfaces of leaf, stomatal types and the epidermal cell types can be useful parameters in delimiting these taxa.

The genus *Senecio* Linnaeus with at least 1200 spp. is worldwide in distribution except Antartica (Chen *et al*. 2011). In Nepal Himalaya, it is represented by 14 species. The genus was first established by Linnaeus (1753) based on *Senecio vulgaris* Linnaeus, the type species of the genus.

MATERIALS AND METHODS

In the present study 12 species of *Senecio* Linnaeus *viz*. *S. albopurpureus* Kitamura, *S. biligulatus* W.W. Smith, *S. echaetus*Y.L.Chen & K.Y.Pan, *S. graciliflorus* A.P. de Candolle, *S. kumaonensi*s Duthie *ex* C.Jeffrey & Y.L.Chen, *S. laetus* Edgewarth, *S. nudicaulis* Buchanan-Hamilton *ex* D. Don, *S. ramosus* Wallich *ex* DC., *S. raphanifolius* Wallich *ex* DC., *S. royleanus* A.P. de Candolle, *S. scandens* Buchanan-Hamilton *ex* D. Don and *S. topkegolensis* Kitamura from Nepal Himalaya were studied. The study was primarily based on the voucher specimens and live collections (Examples: *S. albopurpureus*: Stainton 5157 BM, Dhwoj 296 BM; *S. biligulatus*: Dhwoj 543 BM, Shakya *et al*. 4957 KATH; *S. echaetus*: Polunin 880 BM, Joshi SJ1955 KATH & TUCH; *S. graciliflorus*: Shakya *et al*. 8657 KATH, Shakya 6779 KATH; *S. kumaonensis*: Shrestha & Ghimire 395 KATH, Ohba 60414 *et al.* KATH; *S. laetus*: Joshi SJ10514 KATH, Joshi SJ2131 KATH & TUCH, Joshi SJ2116 KATH & TUCH; *S. nudicaulis*: Joshi SJ2130 KATH & TUCH, Joshi SJ10515 KATH & TUCH; *S. ramosus*: Kanai & Bista 11848 KATH, Williams 22 BM; *S. raphanifolius*: Joshi SJ2122 KATH & TUCH, Shakya 7311 KATH; *S. royleanus*: Joshi SJ1004 KATH & TUCH, Joshi SJ1960 KATH & TUCH, Joshi SJ2124 KATH & TUCH; *S. scandens*: Joshi SJ1910 KATH & TUCH, Joshi SJ2109 KATH & TUCH, Joshi SJ2120 KATH & TUCH; *S. topkegolensis*: Miyamito *et al*. 9440041 BM) deposited at different herbaria (Holmgren *et al.*1990). The specimens were identified and authenticated by consulting the protologue texts and lieratures (Don 1825; De Candollle 1838; M. Pakenham 1846; Clarke 1876; Hooker 1882; Kihara 1952; Kitamura 1981; Smith 1911; Liang Sung-yun & Kai-yu 1981; Jeffrey& Chen 1984) and by matching with type specimens housed at different herbaria.

For the study of stomata, the method described by Carpenter (2005), with some modifications, was followed. The mature leaf was selected from the plant specimen. The selected leaf was taken out and immersed in 5% potassium hydroxide solution overnight, rinsed with deionised water and again immersed in fresh solution of potassium hydroxide solution for few hours. The leaf was then washed with water and treated with glacial acetic acid for 2-5 minutes. The epidermal layers from adaxial and abaxial surfaces were peeled with fine forceps. The peeled epidermal layer was then treated with 4% Sodium Hypochlorite until the tissue got discolored. The time for the discoloration of the tissue varied from half an hour to 3 hours depending upon the peeled material. It was then washed with water for several times. The material was stained in 1 % aqueous safranin and excess stain was washed by water, mounted in glycerine and observed under light microscope.

The terminologies for stomatal types used were those of Metcalfe & Chalk (1950), Metcalfe (1961) and Pant & Kidwai (1966). The term contact cell was used to refer any specialized or not that is adjacent (i.e. in contact) to the stoma as adopted by Upchurch (1984) and Carpenter (2005).

The stomatal distribution on the both surfaces were observed and noted. The densities of stomata on the microscopic field (10 X, eye piece lens × 40 X, objective lens) in triplicate

were recorded and mean value was calculated. The types of stomata viz. anomocytic and anisocytic (Metcalfe & Chalk 1950); teracytic (Metcalfe, 1961); tricytic (Pant & Kidwai 1966) and contiguous stomata were carefully recorded. The size of stomatal pore, width and length were also measured by using the ocular micrometer. Besides, the type of epidermal cell and its wall and number of contact cells and cell wall nature were also recorded.

RESULT AND DISCUSSION

The species of *Senecio* were found to have amphistomatic as well as hypostomatic leaf surfaces. The stomata were largely of anomocytic type, but however close observation showed the presence of tricytic, anisocytic and tetracytic stomata also (Fig. 1). The contiguous stomata (Fig. 1) were of common occurrence in all the species (Fig. 1. A/B/C/D/E/F/H/I/J/L).

Stomatal distribution on the leaf surface, density, length and width of stoma, length of stomatal pore and shape and size of the stomata and type of epidermal cells on the adaxial and abaxial surface of leaves was found to be variable in different species of *Senecio* from Nepal Himalaya.

Species of *Senecio* viz. *S. albopurpureus*, *S. echaetus*, *S. laetus*, *S. nudicaulis*, *S. ramosus* and *S. raphanifolius* have amphistomatic leaf surface while all other species viz.

Table 1. Quilitative and quantitative characteristics of stomata and epidermal cell of leaf of 12 species of *Senecio* from Nepal
[*Abbreviations used*: LS = Leaf surface; Hypo = Hypostomatic; Amphi = Amphistomatic; Den = Density; L = Length of Ledge; W = Width of ledge; OL= Opening length; Ano =Anomocytic stomata; Tri = Tricytic Stomata; Ani = Anisocytic stomata; Tet = Tetracytic Stomata; Con. = Contiguous stomata; Cont. = Contact; Irr = Irregular; Poly = Polygonal; Rect = rectangular]

Taxon	LS	Den (10 x 40)	Measurement of Stomata				Type of Stomata					Epidermal Cells	
			L. (µm)	W (µm)	OL (µm)	Area (µm^2)	Ano	Tri	Ani	Tet	Con	Cont. Cells	Shape
S. albopurpureus	Amphi	11	52.0-72.0	36.0-40.0	32.0-60.0	1664-2400.0	+	-	-	+	+	4-6	Irr-Poly
S. biligulatus	Hypo	5	40.0	20.0	28.0	800.0	+	-	-	-	+	5-6	Irr-poly
S. echaetus	Amphi	18	28.0-44.0	24.0-28.0	16.0-24.0	672.0-1232.0	+	-	-	+	+	4-6	Irr-Poly
S. kumaonensis	Hypo	13	32.0-40.0	32.0-44.0	28.0-36.0	1024.0-1760.0	+	-	-	-	+	5-7	Irr
S, graciliflorus	Hypo	12	42.0-52.0	34.0-42.0	20.0-28.0	1428.0-2184.0	+	+	-	+	+	3-7	Irr
S. laetus	Amphi	10	48.0-	32.0	16.0	1536.0	+	-	-	+	+	4-6	Irr-Poly
S. nudicaulis	Amphi	7.0	44.0	32.0-36.0	28.0-32.0	1408.0-1584.0	+	+	-	+	+	3-5	Poly-Irr
S. ramosus	Amphi	23	44.0-52.0	32.0-44.0	24.0-40.0	1408.0-2288.0	+	+	+	+	+	3-5	Irr-poly-ract
S. raphanifolius	Amphi	7	44.0	28.0	24.0-28.0	1232.0	+	-	-	+	+	4-5	Poly-rect
S. royleanus	Hypo	13	44.0-52.0	36.0-44.0	20.0-28.0	1584.0-2288.0	+	+	-	+	+	3-7	Irr
S. scandens	Hypo	25	28.0-32.0	16.0-18.0	12.0-18.0	448.0-576.0	+	+	-	+	+	3-5	Irr
S. topkegolensis	Hypo	15	32.0-52.0	32.0	20.0-28.0	1024.0-1664.0	+	-	-	-	+	5-6	Irr

S. biligulatus, S. graciliflorus, S. kumaonensis, S. royleanus, S. scandens and *S. topkegolensis* have the hypostomatic leaf surface (Table 1). However in case of species with amphistomatic leaf surfaces, density of stomata was more or multistomatic on abaxial surface and less or paucistomaticon adaxial surface. Amphistomatic leaf surface with multistomatic on lower surface and paucistomatic on upper surface was reported as the condition found in herbaceous dicots (Pandey & Chand 1996). The number of contact cells varied from 3 – 7 in the different species of *Senecio*. The contact cells were indistinguishable from the other epidermal cells i.e. anomocytic type of stomata.

In *S. graciliflorus, S. nudicaulis, S. ramosus, S. royleanus* and *S. scandens*, the stomata were largely anomocytic, occasionally tricytic and tetracytic. Moreover, within those, *S. ramosus* could be delimited from others by the presence of anisocytic type of stomata. Tetracytic stomata were also observed more frequently in the species *viz. S. albopurpureus, S. echaetus, S. graciliflorus, S. laetus, S. nudicaulis, S. ramosus, S. raphanifolius, S. royleanus* and *S. scandens*. Tricytic, anisocytic and tetracytic stomata were lacking in *S. biligulatus, S. kumaonensis* and *S. topkegolensis* and were characterized by anomocytic type of stomata surrounded by more than four contact cells. Tetracytic type of stomata had been reported in many monocotyledons (Metcalfe 1961) and some dicotyledons, for e.g.in the families like Piperaceae (Pant &Banerji 1965); Malvaceae, Bombaceae, Sterculiacea and Elaeocarpaceae (Shanmukha Rao & Ramayya 1977, 1983); Portulacaceae (Nyananyo 1986); few members of Euphorbiaceae (Raju & Rao 1977). However, there were no previous reports of the presence of anisocytic, tricytic and tetracytic stomata in the members of the genus *Senecio* as well as in Asteraceae. Present study thus revealed the taxonomic significance of micromorphological characters like stomata in delimiting the species or group of species from the allied species.

The epidermal cells on the adaxial surface of the leaves were polygonal to rectangular to irregular. The species like *S. albopurpureus, S. biligulatus, S. echaetus, S. laetus*, S. *nudicaulis, S. ramosus* and *S. raphanifolius* had more or less polygonal epidermal cells with more or less straight wall or slightly wavy margin on the adaxial surface. However, the epidermal cells at the abaxial surface were found to be slightly irregular to distinctly irregular and rarely polygonal to rectangular. The cell wall also varied from more or less straight to wavy, sinuous and undulating to deeply undulating. All the epidermal cells were parenchymatous in nature.

Shape of stomata in *Senecio* varied from elliptical to broadly elliptical to nearly circular and circular. Most of the species had elliptical as well as circular stomata on surface view. The species like *S. albopurpureus, S. biligulatus, S. echaetus, S. nudicaulis, S. raphanifoilus* and *S. scandens* were characterized largely by the presence of elliptical type of stomata while the species, *S. kumaonensis* had the circular type of stomata usually.

The high density of stomata was found in *S. scandens* followed by *S. ramosus* and *S. echaetus* and the lowest in *S. biligulatus* and *S. raphanifolius*. The largest stoma was found in *S. albopurpureus* with the area of 2400.0μm^2 and the smallest in *S. scandens* with the area of 448.0 μm^2. However, stomatal distribution, number and size of stomata are correlated with the soil moisture and habit of plant and did not have the taxonomic significance.

The contiguous stomata were present in all species of *Senecio*. The occurrence of contiguous was reported as a feature associated with polyploidy (Ikechukwu & Bosa 2006). Many species of *Senecio* were also reported as polyploids (Mabberley 1997). Presence of contiguous stoma thus reflected the polyploid nature of some species of *Senecio* Linnaeus.

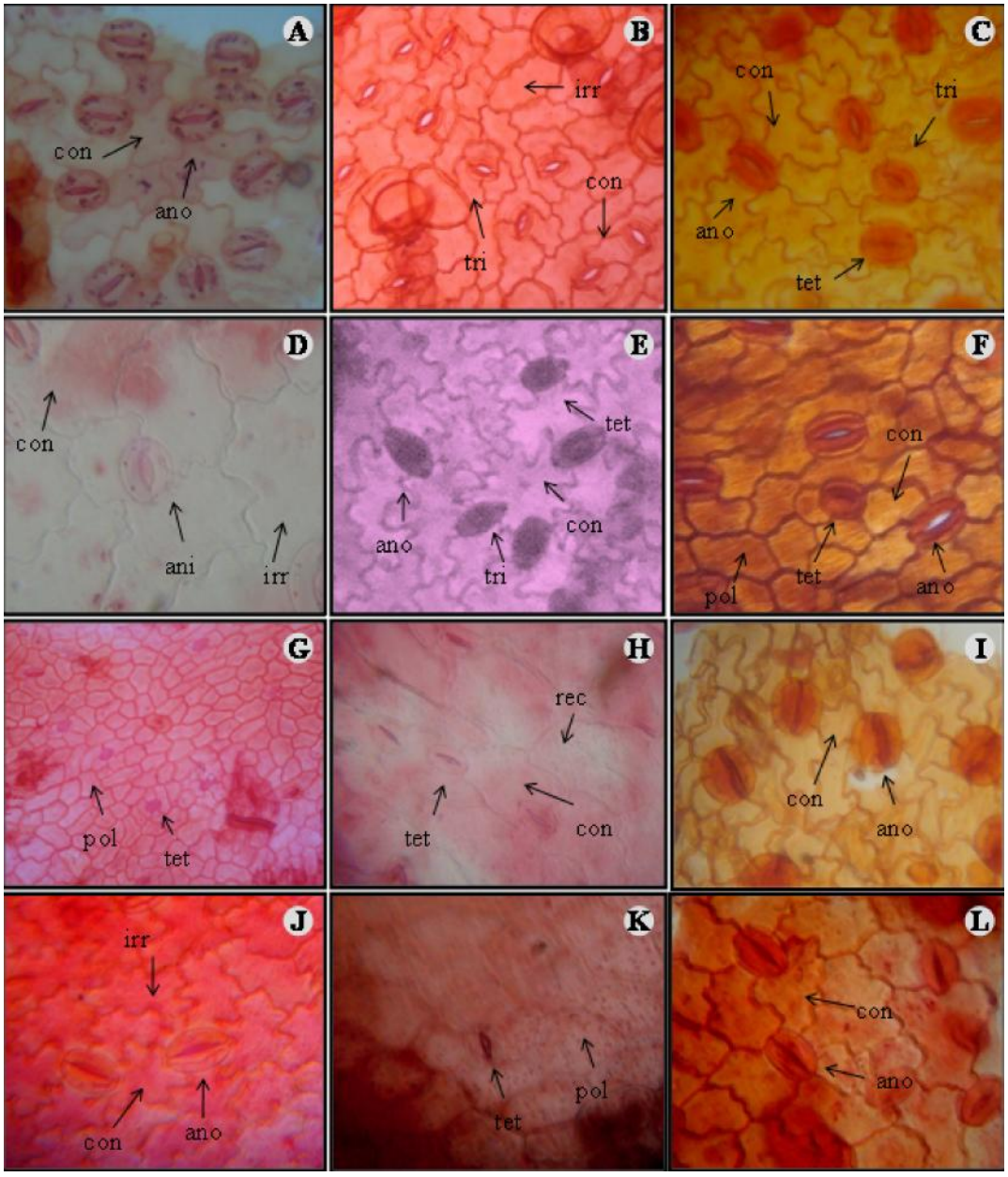

PLATE - I. Stomatal variations in different species of *Senecio* Linnaeus: A. *S. topkegolensis* (abaxial surface); **B.** *S. nudicaulis* (abaxial surface); **C.** *S. royleanus* (abaxial surface); **D.** *S. ramosus* (abaxial surfacea); **E.** *S. scandens* (abaxial surface); **F.** *S. raphanifolius* (abaxial surface); **G.** *S. albopurpureus* (adaxial surface); **H.** *S. ramosus* (adaxial surface); **I.** *S. kumaonensis* (abaxial surface); **J.** *S. laetus* (abaxial surface); **K.** *S. echaetus* (adaxial surface); **L.** *S. echaetus* (abaxial surface). *[Abbreviations used on photos*: ano = anisocytic stomata; pol = polygonal epidermal cell; irr = irregular epidermal cell; rec = rectangular epidermal cell.]

The micromorphological characters; distribution of stomata on leaf surface, type of stomata and shape of epidermal cells, in *Senecio* species of Nepal Himalaya thus revealed as taxonomically significant. This new parameter can be extrapolated in resolving the existing

problems in the *Senecio* species of the world as well as in other taxa. Application of this new parameter will probably be helpful in removing the anomalous species from the genus *Senecio* Linnaeus and in making it more homogenous.

Based on the distribution and type of stomata and type of epidermal cells, an artificial dichotomous key has been prepared and presented below to delimit the species in the genus *Senecio* Linnaeus in Nepal:

1a.	Leaf surface amphistomatic ……...…….……	2
1b.	Leaf surface hypostomatic ……...…….…….	4
2a	Tricytic stomata absent ……...……..… ……	***S. albopurpureus, S. echaetus, S. laetus, S. raphanifolius***
2b.	Tricytic stomata present ……...…….. ……..	3
3a.	Presence of anisocytic stomata ……...…….……	***S. ramosus***
3b.	Absence of anisocyticstomata ……...…….……	***S. nudicaulis***
4a.	Tricytic stomata present ……...……..… ……	***S. graciliflorus, S. royleanus, S. scandens***
4b.	Tricytic stomata absent ……...……..… …….	5
5a.	Presence of poloygonal epidermal cells ……...…..	***S. biligulatus***
5b.	Absence of polygonal epidermal cells ……...…….	***S. kumaonensis, S. topkegolensis***

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