RESEARCH ARTICLE

The state of *Lopholejeunea sikkimensis* (Lejeuneaceae; Marchantiophyta) in Western Himalaya, India

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ABSTRACT

A new variety of *Lopholejeunea sikkimensis* Steph., namely, *kumaunii var. nov.*, collected from Kumaun region in the state of Uttarakhand in Western Himalaya, is being described. The new taxon can be distinguished from the type variety, *sikkimensis*, in having inflated perianth with highly reduced to rudimentary teeth on perianth keels, a comparatively thinner stem with fewer medullary cells and the presence of some weak dentitions at the margin of female bracts. A comparison amongst the three varieties of *L. sikkimensis*, namely, *sikkimensis*, *kumaunii* and *tenuicostata*, occurring in Western Himalaya has been made.

Keywords: *Lopholejeunea,* New veriety, Leafy liveworts, Kumaun region, Western Himalaya

INTRODUCTION

The leafy liverwort, *Lopholejeunea sikkimensis* Steph., belonging to family Lejeuneaceae subfamily Ptychanthoidae, is an Asiatic taxon. The species is characterized by the leaf-lobes with rounded apices, slightly wider than long orbicular underleaves, dentate bracts, entire bracteoles and 5-keeled dentate perianth. In India, the species is widespread and has been recorded from various regions across the country including Eastern and Western Himalaya, Central India, South Indian hills and Andaman & Nicobar Islands (Mizutani, 1976; Lal & Parihar, 1979; Parihar *et al.* 1994; Awashthi *et al.*, 2000; Langer & Tanwir, 2002; Singh & Singh, 2005; Singh & Nath, 2004 & 2006; Dey & Singh, 2011; Barbhuiya & Singh, 2012; Singh & Barbhuiya, 2012; Alam, 2012).

So far as its distribution in Western Himalaya is concerned, *L. sikkimensis*, besides its type variety, is represented by another variety, var. *tenuicostata*, described by Singh & Singh (2005). While the typical *L. sikkimensis* Steph. is well recorded from Almora (Mizutani, 1976), Nainital (Tiwari & Pant, 1994), some unknown place in Uttar Pradesh (Awasthi *et al.* 2000) – all these localities presently lying in the state of Uttarakhand; and the Jammu region in the state of Jammu & Kashmir (Langer & Tanwir, 2002), the latter taxon is known only from its 'type' locality, Sanj Valley in Kullu in the state of Himachal Pradesh (Singh & Singh, 2005).

In course of survey of the liverwort flora of Kumaun region in the state of Uttarakhand we encountered some fascinating blackish populations of Lopholejeunea growing on old tree trunks of the oak, Quercus leucotrichophora A. Camus, in an oak-dominated forest at Munsyari (30 ° 3' 50" N; 80° 14' 12.3" E; 2195 m) in Pithoragarh district (Figure 1). A closer examination of the population revealed that it was a mixture of two distinct kinds of populations growing together. Though there were common features between the two kinds of forms including the leaf lobe with rounded apex, orbicular underleaves which are slightly wider than long, entire margined bract-lobule and 5-keeled perianth; yet, they differed considerably in the larger vs. highly reduced size of dentitions on the keels in perianth, the flattened vs. inflated symmetry of the perianth, the massive vs. delicate stem anatomy and the presence vs. absence of the dentitions in female bracts. The first form is identified as *L. sikkimensis* var. sikkimensis and is briefly described. The other form is treated as L. sikkimensis var. kumaunii, var. nov., named after Kumaun, the region from where it has been collected and is being described (Figures 1 & 2) in detail. Besides these the isotype (99594-b in BSD, Dehra Dun) specimen of Lopholejeunea sikkimensis var. tenuicostata Singh & Singh was also studied for comparison with the present specimens.

As the situation stands today, the West Himalayan region is represented by three varieties of *L. sikkimensis*, namely, var. *sikkimensis*, var. *kumaunii* var. nov. and var. *tenuicostata*. A comparison amongst all the three forms has been discussed and illustrated in Fig. 3.

MATERIAL AND METHODS

The morphological studies of collected specimens were made using Olympus SZ61Stereo-microscope and Leica DM 2500 Digital microscope. The slides of various plant parts including hand sections were mounted in glycerine jelly. The field photographs were taken by Olympus camera.

Description

Lopholejeunea sikkimensis var. **kumaunii** B. K. **Kushwaha, S. N. Srivast., M. Rai et Prateek Srivast.** (Figure 1 & 2)

Plants blackish green and closely appressed on bark of the tree. Shoots 0.6 - 2.5 cm long, 0.4 - 1.0 mm wide, well branched, branching of *Lejeunea*-type. Stem 110×94 µm in diameter, in transverse section consisting of

13 - 14 cortical cells ($14-32 \times 13 - 20 \mu m$) and 14 - 16medullary cells (10-16x 14-22µm), ventral merophytes 3-4 cell wide. Leaves imbricate, spreading from stem at an angle of $50 - 80^{\circ}$. Lobes ovate, 0.6 - 0.7 mm long and 0.45 - 0.60 mm wide, somewhat concave, apex rounded to rounded obtuse, generally incurved, entire, lobe cells thin-walled, trigones small, intermediate thickenings common, marginal cells subquadrate, 10-17x7-13 μm, median cells somewhat isodiametric, 14 - 25 x 12-18 μm, basal cells 18-35 x 13-25 μm; oil bodies 6 - 10 per cell, homogeneous, spherical to slightly ellipsoidal, 2–4 x 1–2 μm. Lobules ovate, nearly 1/3 as long as the lobe, inflated, apex attached to lobe by one cell, hyaline papilla present, keel arched, free margin with a notch. Under leaves distant, suborbicular, 0.30-0.45 long and 0.32-0.50 mm wide, 3-4 times wider than the stem, entire, almost transversely inserted. Androecia not seen. Gynoecia terminal on branches, without subfloral innovations, bracts in one pair; bract-lobe broadly ovate, 1.15 -1.25 mm long and 0.56 - 0.65 mm wide, margin usually entire to weakly denticulate, apex nearly entire; bract-lobule narrowly oblong, 1/2 or more longer as the bract-lobe, occasionally deeply notched, margin entire; bracteole suborbicular, 0.80 - 0.82 mm long and 0.65 - 0.68 mm wide, longer than wide, almost covering the entire perianth, margin entire, partly or entirely recurved. Perianth obovate, inflated with radial symmetry, 1.1 - 1.3 mm long and 0.65 -0.70 mm wide, with 5 keels (2 lateral, 1 dorsal and 2 ventral), keels with rudimentary or small teeth or protuberances up to 2/3 in upper part, one of the lateral keels.

Specimens examined: WHKP 0262 L/15 (Holotype) and WHKP 0263 L/15 (Isotype). Munsyari (30° 3′ 50″ N; 80° 14′ 12.3″ E; 2195 m; 06 October 2015) in Pithoragarh district, Kumaun Region, Uttarakhand state, Western Himalaya, leg. S. N. Srivastava, Meena Rai & B. K. Kushwaha, det. S. N. Srivastava, Meena Rai & B. K. Kushwaha (Deposited in Duthie Herbarium, Botany Department, University of Allahabad, Allahabad).

Habitat: Growing on the bark of the old tree trunks of *Quercus leucotrichophora* along with *Lopholejeunea* sikkimensis Steph. var. sikkimensis, *Plagiochila* parvifolia Lindenb. and some pleurocarpous mosses in an oak-dominated forest.

Etymology: The variety has been named after Kumaun, the region of the collection of the specimens.



Figure 1: Lopholejeunea sikkimensis var. kumaunii var. nov.**1.** Map of India showing the collection site Munsyari (\bullet) in the state of Uttarakhand in Western Himalaya, India; **2.** Plant, habit; **3.** A plant (holotype); **4.** Plant with perianth, ventral view; **5.** Perianth with bracts. (Bars: Figure 2 = 0.5 cm; Figure 3 = 2 mm; Figure 4 = 0.5 mm; Figure 5 = 30 μ m)

Lopholejeunea sikkimensis Steph. var. sikkimensis

Spec. Hepat. 5: 87 (1912); Mizutani, Journ. Hatt. bot. Lab. 40: 444 (1976); Awasthi *et al.*, Geophytology 29: 53 (2000); Singh & Nath, Phytotaxonomy 6: 30 (2006); Zhu & Gradstein, As *Lopholejeunea nigricans* (Lindenb.) Schiffn., Syst. Bot. Monograph 74: 44 (2005)

Plants blackish green. Shoots 0.6 - 1.5 cm long, 0.5 - 1.0 mm wide, well branched, branching of *Lejeunea*-type. Stem 160 x 105 μ m in diameter, in transverse section consisting of 13 – 16 cortical cells (23 – 35 x 15 – 30 μ m) and 23 –30 smaller medullary cells (16 – 31 x 12 –

17 µm), ventral merophytes 3 – 4 celled wide. Leaves imbricate, spreading from stem at an angle of 60–85°. Lobes ovate, 0.65 – 0.79 mm long and 0.54 – 0.60 mm wide, apex rounded to rounded obtuse, incurved, margin entire, lobe cells thin-walled, trigones small, marginal cells subquadrate, 10 – 18 x 10 – 14 µm, median cells 15 – 30 x 11 – 18 µm, basal cells 26 – 34 x 15 – 23 µm; oil bodies 7 – 11 per cell, homogeneous, spherical, 2 – 3 x 1 – 3 µm. Lobules ovate, nearly 2/5 as long as the lobe, inflated, apex attached to lobe by one cell, somewhat truncate, entire or with occasionally indistinct teeth, keel arched, free margin with a notch.

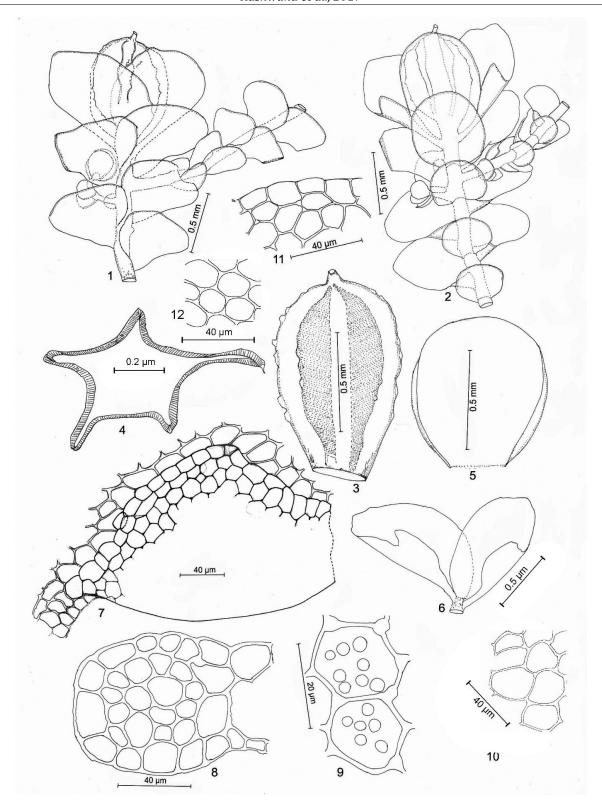


Figure 2: *Lopholejeunea sikkimensis* var. *kumaunii*.**1.** Part of plant with perianth, dorsal view; **2.**Plant with perianth, ventral view; **3.**Perianth; **4.**Perianth, t.s.; **5.**Bracteole; **6.**Bracts; **7.**Leaf lobule; **8.** Stem, t.s.; **9.**Cells having oil bodies; **10.**Basal cells of the leaf; **11.**Marginal cells of the leaf; **12.** Median cells of the leaf. forming a wing, beak 2 -3 celled long. Capsule spherical, bistratose, the outer layer with sinuate – nodular thickenings on radial walls, inner layer with fenestrate thickenings. Elaters $170 - 200 \, \mu m \log and 9 - 12 \, \mu m$ wide, with one spiral thickening. Spores oblong, $32 - 40 \times 26 - 32 \, \mu m$, with small papillae.

	L. sikkimensis Steph. var. sikkimensis	L. sikkimensis var. kumaunii B. K. Kushw., S. N. Sriv., M. Rai & P. Sriv.	L. sikkimensis var. tenuicostata S. K. Singh & D. K. Singh
Part of Plant, ventral view	0.5 mm	0.5 mm	0.5 mm
Perianth	0.5 mm	0.5 mm	0.5 mm
Perianth, t.s.	0.2 mm	0.2 mm	0.2 mm
Stem, t.s.	mud OE	30 mrl 08	30 km
Female bracts	0.6 mm	0.6 mm	0.6 mm

Figure 3: A comparative account of some salient features of the three West Himalayan varieties of *Lopholejeunea sikkimensis*

Underleaves distant, approximate or imbricate, 3 – 4 times as wide as the stem, rotund, 0.40 - 0.55 long and 0.35 - 0.58 mm wide, entire, slightly recurved, almost transversely inserted. Androecia not seen. Gynoecia terminal on branches, without subfloral innovations, bracts in one pair; bract-lobe broadly ovate, 0.8 - 1.1 mm long and 0.45 – 0.90 mm wide, apex acute, margin dentate; bract-lobule narrowly oblong to somewhat triangular, 2/3 or more longer as the bract-lobe, occasionally deeply notched, margin weakly toothed; bracteole very large, nearly rotund, 0.70 - 0.82 mm long and 0.65 - 0.70 mm wide, longer than wide, almost entirely covering the perianth, margin recurved throughout. Perianth pyriform, with flattened symmetry, 0.9 - 1.3 mm long and 0.65 - 0.75 mm wide, with 5 keels (2 lateral, 1 dorsal and 2 ventral), keels with prominent lacineae in upper half, except dorsal other keels forming distinct wing, beak prominent.

The West Himalayan specimens are larger than their counterparts of East Himalayan and South India described by Awasthi *et al.* (2000) and Singh & Nath (2006).

Type locality: Sikkim (Eastern Himalaya)

Specimens examined: WHKP 0260 L/15 and WHKP 0261 L/15 Munsyari (30° 3' 50" N; 80° 14' 12.3" E; 2195 m; 06 October 2015) in Pithoragarh district, Kumaun Region, Uttarakhand state, Western Himalaya, leg. S. N. Srivastava, Meena Rai & B. K. Kushwaha, det. S. N. Srivastava, Meena Rai & B. K. Kushwaha (Deposited in Duthie Herbarium, Botany Department, University of Allahabad, Allahabad).

Habitat: Growing on the bark of the old tree trunks of *Quercus leucotrichophora* along with *Lopholejeunea* sikkimensis var. kumaunii, Plagiochila parvifolia and some pleurocarpous mosses in an oak-dominated forest.

Ecology

The altitudinal distribution of the genus, *Lopholejeunea*, is interesting. Awasthi *et al.* (2000), in Indian context, noted that "*Lopholejeunea* has the capacity to grow even under totally exposed, warm conditions where many liverworts fail to grow; the genus is found from sea level (near the sea coast, Trivandrum) to higher attitudes of ca. 2000m (Ootakmund, Nilgiri Hills)". When the altitudinal distribution of *L. sikkimensis* in the Western Himalaya

is observed, we find that Tiwari & Pant (1994) had collected the species growing as a corticolous liverwort on the trees of Shorea robusta Gaertn. and Mallotus philippensis (Lam.) Müll. Arg. in the sal forest of Chourgalia at a very low altitude (326 m) in the Nainital district. The present collection of the taxa, both var. sikkimensis and var. kumaunii, was made from the trees of Quercus leucotrichophora A. Camus at a much higher altitude in an oak-dominated forest at Munsyari (2195 m) in Pithoragarh district. As cited by Mizutani (1976), the collection of Parihar from Almora (7000 feet or 2133.6 m) is also from a higher altitude. Langer & Tanwir (2002) have also recorded the species growing as epiphyte on the oak trees from Banola in Jammu but at a lesser altitude of 1200 m.On the other hand, L. sikkimensis var. tenuicostata is terrestrial, and has been collected from rocks in Himachal Pradesh at an altitude of 1800 m (Singh & Singh 2005).

DISCUSSION

Described by Franz Stephani (1912) from Sikkim, Lopholejeunea sikkimensis is known to be largely confined to India and Nepal. Two varieties of the species, namely, var. dentata U.S.Awasthi, from Meghalaya in Eastern Himalaya and var. tenuicostata from Himachal Pradesh in Western Himalaya were later added by Awasthi et al. (2000) and Singh & Singh (2005) respectively. The first variety differed from L. sikkimensis var. sikkimensis in having 4-keeled against 5-keeled perianth, presence of teeth between the keels of perianth against the absence of such teeth; and, the dentate margin of female bracteole against the entire margin. The second variety, namely, var. tenuicostata differed from var. sikkimensis in having thinner stem (13-16 rows of the medullary cells) against the robust stem (comprising of 23-30 rows of medullary cells) and the dentate against entire to finely denticulate female bract-lobules. The present variety kumaunii var. nov., can be sharply distinguished from var. sikkimensis in having rudimentary dentitions on perianth keels, the inflated perianth, the female bractlobes and bract-lobules being finely denticulate and a thinner stem (14 – 16 rows of medullary cells). In stem anatomy it resembles var. tenuicostata. The common characters in all the 3 varieties, however, include: the leaf-lobes always with rounded apex, wider than long orbicular underleaves, dentate to denticulate female bracts, the obovate large female bracteole with

recurved margin, large-sized bract-lobule and 5-4 keeled perianth.

Zhu & Gradstein (2005), on the basis of their observation of overlapping gynoecial characters and the consistency of leaf-lobule morphology, however, have reduced L. sikkimensis and its variety dentata along with 31 other taxa to synonymy and made them conspecific with L. nigricans (Lindenb.) Schiffn. It is, however, important to note that these authors under *L*. nigricans acknowledge the identity of five 'phases' each phase presumably representing a set of environmentally-guided variant -- wherein L. sikkimensis represents 'Phase 2' and L. nigricans 'Phase 5'. Interestingly, plants of these phases can be clearly distinguished morphologically. Our present variety, kumaunii var. nov. is, however, closer to their 'Phase 3', an entirely different category. Singh et al. (2016) in their latest checklist of Indian liverworts and hornworts have observed that "----as these taxa are distinct enough to be categorized in different phases, we feel that they together represent 'L. nigricans-complex' rather than a single taxonomic unit." As such, these authors have retained *L. sikkimensis* as a species in their checklist.

The issue as to whether *L. sikkimensis* and *L. nigricans* are two separate species or they should be treated as two ecologically different variants of the latter species may further be resolved with the help of molecular studies. Meanwhile, the sharp distinctions between two sets of forms and also, following Awasthi *et al.* (2000), Singh & Nath (2006) and Singh et al. (2016), we are treating these as two distinct taxa. The salient points of distinction between the two species, namely, *L. sikkimensis* and *L. nigricans*, include the rounded vs. acute leaf apex, distinct vs. indistinct dorsal keel of perianth, entire vs. denticulate bracteole and recurved vs. plane dorsal margin of the leaf-lobe.

It is important to note that *Lopholejeunea* (Spruce) Schiffn. is known to be a highly plastic genus and therefore care must be taken in creating any new infraspecific or infrageneric taxon. We, however, have watchfully noted that populations of the two forms of *L. sikkimensis*, collected by us, were growing together in an intermixed state on the bark of same oak trees almost exposed to similar set of environmental conditions, yet showing a remarkably different degree of variations. Besides, we didn't observe any set of overlapping traits between these two distinctly varying populations which were growing together in

the same patches. Looking at the consistency of characters and a considerable degree of morphological variation despite under similar set of environmental conditions led us to believe that the basis of such a deviation is genetic and demands the new form the status of a new variety. As such, *L. sikkimensis* var. *kumaunii* is being proposed. Our observations are also suggestive of the fact that even in term of Zhu & Gradstein (2005) the 'Phase 2' and 'Phase 3' of *L. nigricans* might be having a genetic basis and not purely environmental

CONCLUSION

A comparison of the salient features of the three varieties of *L. sikkimensis*, namely, *sikkimensis*, *kumaunii* and *tenuicostata*, occurring in Western Himalaya has been presented in

Figure 3. While in stem anatomy, var. *kumaunii* and var. *tenuicostata* come closer, in the presence of lacinae on the perianth keels, var. *sikkimensis* and var. *tenuicostata* look similar.

The new variety *kumaunii*, in having rudimentary teeth on perianth keels and occasionally having somewhat entire looking female bracts, may look similar to *L. abortiva* (Mitt.) Steph., yet the former can be distinguished from the latter in having large bractlobule and finely denticulate bracts. We observed the female bract-lobule in var. *kumaunii* to be often bifurcated.

Key to varieties of *Lopholejeunea sikkimensis* Steph. in Western Himalaya

- 2. Stem massive, comprising of 26-30 rows of medullary cells, female bract-lobule margin entire to finely denticulate......var. sikkimensis

 Stem delicate, comprising of 13 16 rows of medullary cells female bract-lobule margin dentate......var. tenuicostata.

Conflict of Interest/ Author Contributions

Prof.S.Y.Anwar designed the work, Narendar.V performed studies, Sudhakar Chekuri and Dr.R. Chandrashekhar helped the data interpretations. All the authors reviewed the manuscript.

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