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Source: Lindbergia, 39(4) : 7-11

Published By: Dutch Bryological and Lichenological Society and Nordic Bryological Society

URL: https://doi.org/10.25227/linbg.01067

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Lindbergia 39: 7–11, 2016
ISSN 2001-5909
Accepted 11 March 2016

Cytological studies of some Indian liverworts

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Cytological studies are made in nine species of liverworts belonging to seven genera collected from different areas of Chakrata and Deoban in Uttarakhand, W. Himalaya. The chromosome numbers in four species (*Calypogeia renistipula* Steph., n = 9; *Cephalozia gollani* Steph., n = 9; *Chiloscyphus campanulatus* Steph., n = 9; *C. coadunatus* (Sw.) Engel & Schust., n = 9) are recorded for the first time. In *Porella platyphylla* (L.) Pfeiff. n = 9 is counted for the first time in India. Precocious disjunction of one bivalent in the chromosome complement is observed in *Frullania retusa* Mitt. Laggards are observed in *Calypogeia renistipula.*

Of the nearly 9000 liverworts species (Crandall-Stotler and Stotler 2000), so far only 697 species representing 156 genera are known cytologically (Anand et al. 1989, Kumar and Anand 1990, Fritsch 1991, Kumar and Kapila 2003).

In India, so far 850 species of liverworts belonging to 140 genera and 52 families are reported (Norkett, cf. Chopra 1975). Despite the rich diversity of bryophytes in the Indian subcontinent, cytological study of this group has not received deserved attention.

Mehra (1938) initiated some work on Indian liverworts. He for the first time reported the chromosome numbers for three members of family Codoniaceae. Most of the earlier chromosome number reports (Kashyap and Pande 1922, Chavan 1937, Mehra and Mehra 1939, Srinivasan 1940, 1944, Mahabale and Bhate 1945, Mahabale and Gorji 1947, Mahabale and Deshpande 1947, Kachroo 1953, 1955, Mehra and Handoo 1953, Mehra and Sokhi 1972, 1977, Sokhi and Mehra 1973) were incidental to morphological and developmental studies on liverworts.

Studies focused on cytology of Indian liverworts were pioneered by Mehra (1948, 1959, 1977), Srinivasan (1940, 1944), Mahable and Gorji (1941), Chopra and Udar (1957), Udar and Chopra (1957), Mehra and Pathania (1959), Kanwal (1974, 1975, 1976a, 1976b), Mehra and Kumar (1979, 1980). Later, Anand et al. (1989), Kumar and Anand (1990) and Kumar and Kapila (2003) made significant contributions to the cytology of West Himalayan liverworts.

The goal of present study is to gain cytological information about the liverworts of Deoban and Chakrata in Uttarakhand and further see how far this data could be useful in taxonomy and phylogeny of the bryophytes.

Material and methods

Collections were from Deoban and Chakrata in Uttarakhand. To study the meiotic chromosomes, plants with suitable capsules of green colour were fixed in Farmer's fluid 1:3 acetic alcohol for 24 h and then stored in 90% alcohol. To prepare slides of dividing spore mother cells, the capsule was slightly punctured with a needle in a drop of 2% acetocarmine. The debris was removed with a fine tipped iron needle. A cover slip was put on and the slide was heated to 50–60º C for a few seconds and pressed in the folds of blotting paper to squeeze out the sporogenous tissue. To make a slide permanent, its cover slip was separated by placing the slide in absolute alcohol or n-butyl alcohol in a petri dish. Then the cover slip and the slide were passed separately through 1:3, 1:6, 1:9 acetic alcohol and finally through absolute alcohol and mounted separately in euparal.

The families and genera of liverworts are arranged according to Schuster (1984). The voucher specimens of the studied taxa are deposited in the herbarium of Dept of Botany, Panjab University, Chandigarh and their reference numbers with further relevant data are given in Table 1.

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Table 1. Summary of chromosome numbers of the studied taxa.

Name of the taxon	Locality and altitude	Herbarium reference no. (PAN no.)	Chromosome number n =	Fig. no.
DIVISION: BRYOPHYTA				
SUB-DIVISION: HEPATOPHYTINA				
CLASS: HEPATICOPSIDA				
ORDER: JUNGERMANNIALES				
FAMILY: CALYPOGEIACEAE				
***Calypogeia renistipula* Steph.**	Deoban (Uttarakhand) ; 2360 m	391	9	1-2
FAMILY: CEPHALOZIACEAE				
***Cephalozia gollani* Steph.**	Deoban (Uttarakhand); 2360 m	392	9	3
***Cephalozia* Sp.**	Chakrata (Uttarakhand); 1814 m	393	9	4
FAMILY: GEOCALYCACEAE				
***Chiloscyphus campanulatus* Steph.**	Chakrata (Uttarakhand); 1814 m	394	9	5
***Chiloscyphus coadunatus* (Sw.) Engel & Schust.**	Deoban (Uttarakhand); 2360 m	395	9	6-7
FAMILY: RADULACEAE				
***Radula complanata* (L.) Dum.**	Deoban (Uttarakhand); 2360 m	396	9	8
FAMILY: PORELLACEAE				
***Porella platyphylla* (L.) Pfeiff.**	Deoban (Uttarakhand); 2360 m	397	9	9
***Porella plumosa* (Mitt.) Parihar**	Deoban (Uttarakhand); 2360 m	398	18	10
FAMILY: JUBULACEAE				
***Frullania retusa* Mitt.**	Deoban (Uttarakhand); 2360 m	399	9	11

Observations and results

FAMILY: CALYPOGEIACEAE

***Calypogeia renistipula* Steph. – n = 9 (Fig. 1–2)**

The plants with sporophytes in the right stage were collected in Deoban (altitude 2360 m), and found growing on wet soil. Shoots with more or less imbricate leaves, each leaf ovate entire and the reniform amphigastria distant helped in identification of the species.

The chromosome number of this species was not known previously.

Of the nine darkly stained and well spread bivalents observed at metaphase-I (Fig. 1), two were larger in size and the remaining ones showed gradation in size. One laggard was observed at telophase-I (Fig. 2).

Fourteen species of *Calypogeia* are known cytologically. The distribution of chromosome numbers: n = 9 (11 species) and n = 18 (6 species) indicates the base number to be x = 9 and doubling of chromosomes has played a significant role in the evolution within the genus.

FAMILY: CEPHALOZIACEAE

***Cephalozia gollani* Steph. – n = 9 (Fig. 3)**

This species, also collected in Deoban (altitude 2360 m), grew on moist soil. Leaves were imbricate, the lack of amphigastria and a capsule borne on a long seta helped in identification of the plant.

The chromosomes of this species are also studied for the first time.

The chromosome complement comprises nine darkly stained bivalents at metaphase-I (Fig. 3). One bivalent was relatively small, the remaining members of the set were of variable size. The course of meiosis was observed to be normal.

***Cephalozia* sp. Dumort. – n = 9 (Fig. 4)**

This taxon, collected at Chakrata (altitude 1814 m), was found growing on soil mixed with humus. Leaves were obliquely inserted, succubous, generally bilobed and with entire margin.

The chromosome complement comprised nine darkly stained bivalents at metaphase-I (Fig. 4). The course of meiosis was observed to be normal.

Of the 43 species included in the genus, only six are known cytologically. The recorded chromosome numbers are: n = 9 (2 species), n = 18 (3 species) and n = 27 (1 species). The frequent occurrence of multiplied genomes (n = 18 and n = 27) shows that polyploidy is fairly widespread in the genus.

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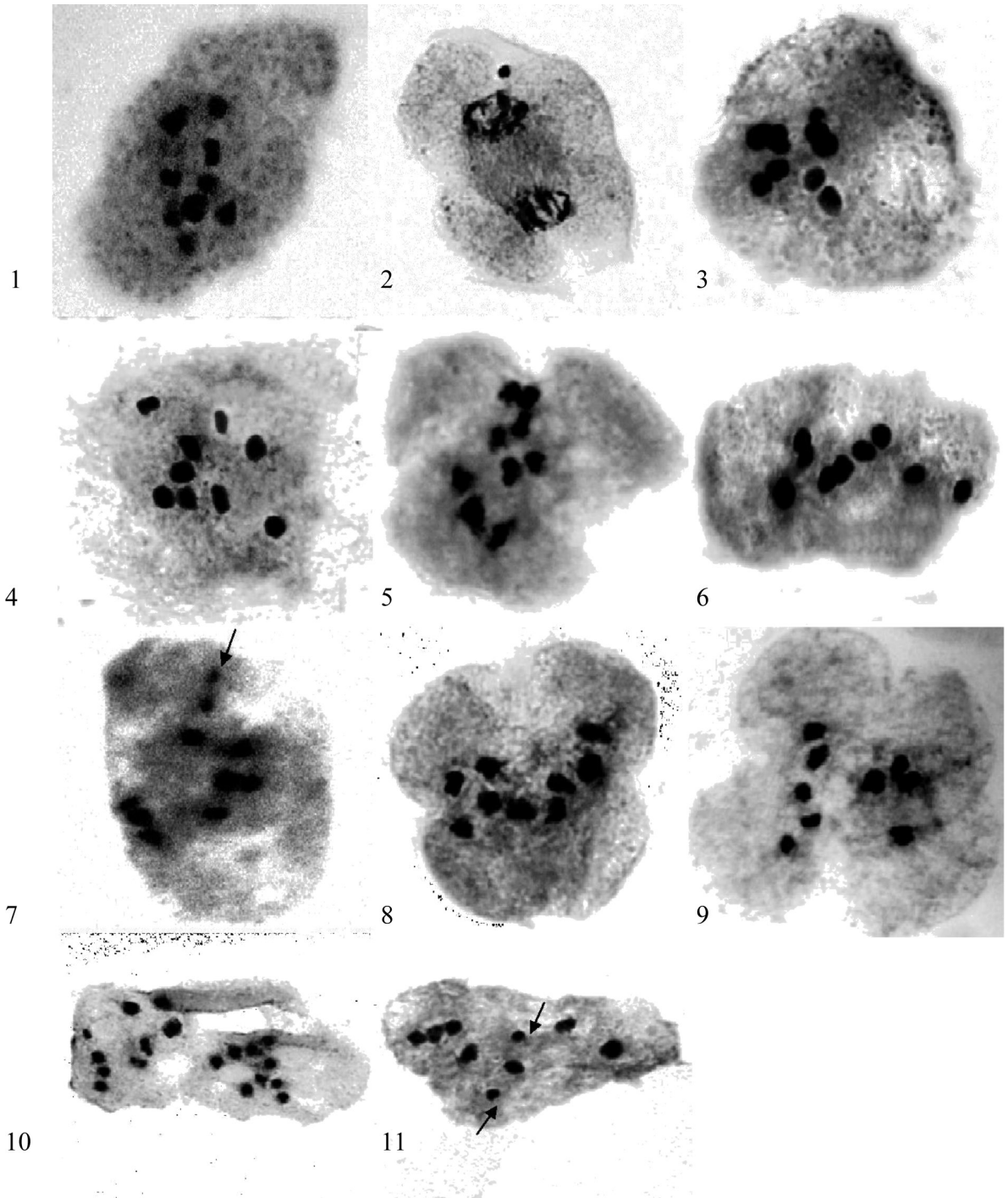


Figure 1–11. (1) *Calypogeia renistipula* – n = 9, metaphase - I, showing nine bivalents, (2) telophase - I, showing a laggard; (3) *Cephalozia gollani* – n = 9, metaphase - I, showing nine bivalents; (4) *Cephalozia* sp. – n = 9, metaphase - I, showing nine bivalents; (5) *Chiloscyphus campanulatus* – n = 9, metaphase - I, showing nine bivalents; (6) *C. coadunatus* – n = 9, metaphase - I, showing nine bivalents, (7) note the precociously disjoined one bivalent; (8) *Radula complanata* – n = 9, metaphase - I, showing nine bivalents; (9) *Porella platyphylla* – n = 9, metaphase - I, showing nine bivalents; (10) *P. plumosa* – n = 9, metaphase - I, showing eighteen bivalents; (11) *Frullania retusa* – n = 9, metaphase - I, showing nine bivalents. Note the precociously disjoined one bivalent.

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FAMILY: GEOCALYCACEAE

***Chiloscyphus campanulatus* Steph. – n = 9 (Fig. 5)**

The plants with sporophytes of the right stage were collected from Chakrata (altitude 1814 m) and were found growing on soil. This species is characterized by small amphigastria divided at apex, with a v-shaped notch and long ellipsoidal capsule.

This species is also studied cytologically for the first time.

The nine bivalents observed at metaphase-I were fastly stained and well spread (Fig. 5). The bivalents varied from one large to five of median and three of smaller size.

***C. coadunatus* (Sw.) Engel & Schust. –n = 9 (Fig. 6-7)**

This taxon is characterized by alternate leaves, small underleaves with a long, widely spreading, subulate tooth on both sides. The material from Deoban (altitude 2360 m) grew on stony soil.

The chromosome number of this species was not known previously.

Of the nine bivalents observed at metaphase-I, the smallest one showed precocious disjunction (Fig. 7). The bivalents of the chromosome complement showed clumping and stickiness.

Of the twelve cytologically known species of *Chiloscyphus*, eleven had n = 9 which is considered the base number in the genus. The occurrence of n = 18 in one species, *C. pallescens* (Enrh *ex* Hoffm.) Dumort. was shown to be the result of doubling (autodiploidy) (Newton 1973).

FAMILY: RADULACEAE

***Radula complanata* (L.) Dumort. – n = 9 (Fig. 8)**

This taxon, collected from Deoban (altitude 2360 m), was found growing on soil mixed with pebbles. Imbricate and strongly spreading leaves helped in easy identification of the species.

The present chromosome count, n = 9 differs from earlier reports from Japan (n = 6; Tatuno 1935), France (n = 8), Germany (n = 8; Müller 1951–1958, Fritsch 1984), India (n = 12; Mehra and Pathania 1959), Europe (n = 16; Heitz 1942) and Caucasus (n = 16; Fritsch 1987).

Nine darkly stained and well spread bivalents were observed at metaphase-I (Fig. 8). The course of meiosis was normal.

Sixteen species of *Radula* are known cytologically. Of these, eight species counted to n = 6 and ten species had n = 8. The base number of this genus could be x = 6 or x = 8. The present finding of n = 9 may be the result of an aneuploid gain of one chromosome from x = 8.

FAMILY: PORELLACEAE

***Porella platyphylla* (L.) Pfeiff. – n = 9 (Fig. 9)**

This taxon is characterized by large lobed leaves, dentate female bracts and a ciliate perianth mouth. The plants were found growing on soil at Deoban (altitude 2360 m).

This species is studied for the first time in India.

The present chromosome count n = 9 agrees with the previous findings in some European populations (Newton 1973, Fritsch 1984, Kuta et al. 1984). Nine darkly stained and well spread bivalents were observed at metaphase-I (Fig. 9). The course of meiosis was normal.

***Porella plumosa* (Mitt.) Parihar – n = 18 (Fig.10)**

This taxon is characterized by having leaves with small ovate lobule and by having amphigastria slightly larger than the lobule of the leaves. The fertile material, collected from Deoban (altitude 2360 m), was found growing on stony soil.

The present chromosome count n = 18, varies from an earlier finding (n = 7+m, Mehra and Pathania 1959) from an Indian population. Of the 18 bivalents observed at metaphase-I 6 (Fig. 10) two were relatively larger in size. The course of meiosis was normal.

The genus *Porella* includes 108 species, of which chromosomes have been counted in only 20. The chromosome numbers vary from n = 6 (1 species), n = 8 (17 species) and n = 9 (4 species). The prevalence of n = 8 suggests that to be the base number in this genus, which might in four species have given rise to n = 9 through aneuploid gain of one chromosome.

FAMILY: JUBULACEAE

***Frullania retusa* Mitt. – n = 9 (Fig. 11)**

This taxon, collected from Deoban (altitude 2360 m), was found growing on soil. The species is characterized by entire leaves and narrowly inserted amphigastria.

The chromosome number n = 9 agrees with the previous findings (Mehra and Pathania 1959). Of the nine darkly stained bivalents observed at metaphase-I (Fig. 11), one showed precocious disjunction. The course of meiosis was normal.

The genus *Frullania* includes 613 species, of which 62 are known cytologically. The chromosome numbers vary from n = 8 (23 species), n = 9 (57 species), n = 10 (4 species) and n = 17 (2 species). The cited cytological data suggest that x = 9 probably is the base number of the genus and n = 8 and n = 10 having arisen from it through aneuploidy. Polyploidy followed by aneuploid loss of one chromosome has given rise to n = 17 in two species.

The frequent occurrence of n = 9 accompanied by its multiples (n = 18, 27) also known from other taxa of Jungermanniales indicates it to be the basic chromosome number of the order.

Since cytological data are known for less than 8% of all liverworts, it is desirable to continue such studies to gain more of such information from this group of plants.

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