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Authors: Sharma, Meenakshi, and Kapila, Sunita

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Research article

Cytological studies of east Himalayan hornworts and liverworts species

Meenakshi Sharma and Sunita Kapila

Department of Botany, Panjab University, Chandigarh, India

Correspondence: Meenakshi Sharma (sharmameenakshi1312@gmail.com)

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Meiotic analysis were made in three hornwort, 12 leafy and six thalloid liverwort taxa collected from different areas of eastern Himalayas. The chromosome numbers in *Anthoceros alpinus n*=6, *A. subtilis* n=6, *Porella campylophylla* n=9, *Radula tjibodensis* n=8, *Cololejeunea jelinekii* n=8, *C. latilobula* n=9, *Solenostoma atrobrunneum* n=9, *S. purpuratum* n=9, one variety (*Pallavicinia lyellii* var. *lyellii.* n=8 and in *Asterella wallichiana* n=9, 18 are recorded for the first time. In *Phaeoceros laevis* n=6, *Frullania muscicola* n=18 (the first chromosome count for this species); *F. nepalensis* n=9, *Heteroscyphus argutus* n=9, *Fossombronia cristula* n=9 and *Cyathodium aureonitens* n=9 are counted for the first time in India. *Frullania ericoides* n=8, *Plagiochasma pterospermum* n=9, *Targionia hypophylla* n=9, *Dumortiera hirsuta* n=9 and *Calycularia crispula* n=9 are investigated for the first time from eastern Himalayas. An anaphasic bridge was also observed at anaphase-I in *F. ericoides*. An m-chromosome and an early disjunction of one bivalent is observed in the chromosome complements of *S. atrobrunneum*. Precocious disjunction of one bivalent is observed in the chromosome complements of *A. wallichiana* and *C. crispula*, of two bivalents in *P. pterospermum* and of three bivalents in *T. hypophylla*.

Keywords: cytology, chromosome number, hornworts, liverworts, polyploidy

Introduction

The eastern Himalayas are considered as one of the biodiversity hotspots in the world favouring the growth and proliferation of bryophytes. Despite the rich diversity of hornworts and liverworts in this area, their cytological studies remained unattended so far.

The aim of present study was to carry out studies of cytologically unattended liverworts of eastern Himalayas, India and to see how this data could be useful in taxonomy and phylogeny of this group.

Material and methods

The plant materials were collected from different areas of eastern Himalayas (Shillong, Mawsynram, Sikkim, Kalimpong and Darjeeling) in September 2016. The cytological observations were made on twelve species belonging to seven genera of liverworts including one variety. The young green sporophytes were fixed in acetic ethanol (1:3) and were observed by examining the meiotic cells using squashing technique (Kapila 2016). The families and genera of liverworts are arranged according to Söderström et al. (2016). The voucher specimens of the studied taxa are deposited in the herbarium of Department of Botany, Panjab University, Chandigarh, India (PAN) and their reference numbers with collection data are given in Table 1.

Results

Anthoceros alpinus Steph. n = 6 (Fig. 1.1)

This taxon was collected from three widely separated areas of eastern Himalayas – Shillong (Elephant Falls, 1525 m a.s.l.), East Sikkim (Lhasa Falls, 1650 m a.s.l.) and Darjeeling (Japanese Peace Pagoda, 2000 m a.s.l.), found growing on wet soils under shade and was rich in fruiting.

The chromosome number for this species was not known previously.

In all the three presently studied populations, six well spread and fastly stained bivalents were observed at first metaphase. One of the bivalents in the complement was conspicuously large. The other members showed gradation in size (Fig. 1.1).

Anthoceros subtilis Steph. n = 6 (Fig. 1.2)

The plants with sporophytes in the right stage collected at Shillong (Botanical Survey of India; 1525 m a.s.l.), were found growing on soil.

The number, n = 6 is the first chromosome count for this species.

Of the six darkly stained and well spread bivalents, four were of large size. The other members were relatively of smaller size as observed at metaphase-I (Fig. 2).

Only nine species of *Anthoceros* are known cytologically. The distribution of chromosome numbers: n = 5 (seven species), n = 6 (four species), n = 4, 9, 10 (one species each) suggest that this genus is based on x = 5 and the other numbers are derived through aneuploidy (gain or loss of chromosomes) and polyploidy (Fritsch 1991).

Phaeoceros laevis Prosk. n = 6 (Fig. 1.3)

This species was collected from Darjeeling (Rock garden, Chunnu Fall, 2134 m a.s.l.), found growing on shaded wet soil.

The chromosome count, n = 6 is the first report from India which is in agreement with earlier reports of French and Japanese populations of the same species from Japan, Great Britain and Taiwan (Fritsch 1991).

The six bivalents observed at metaphase-I were darkly stained and well spread. Three bivalents of the set were large sized and three small-sized (Fig. 1.3).

Only seven species are known cytologically, of which four species exclusively show n = 5, one species shows n = 6; one species shows n = 5, 6, 7 and one species shows n = 4, 5, 6, 8 (Fritsch 1991).

Solenostoma atrobrunneum (Amak.) Váňa & Long. n = 9 (Fig. 1.4)

The species was collected from two widely separated localities (Eastern Sikkim, Lhasa Falls, alt. 1650 m a.s.l. and Darjeeling, Rock Garden, Chunnu Falls, alt. 2134 m a.s.l.).

Previously, this species was not known cytologically. In both the populations, chromosome number n = 9 was observed at first metaphase. Out of the nine bivalents, one bivalent was exceptionally small and may be regarded as m-chromosome, another one showed tendency towards early disjunction. The remaining members of the complement showed gradation in size (Fig. 1.4).

Solenostoma purpuratum (Mitt.) Steph. n = 9 (Fig. 1.5)

The collection for this species was done from Darjeeling (Victoria Fall, alt. 2042 m a.s.l.).

The present count, n = 9 is the first cytological report for this species. The complement showed nine bivalents that were well spread and fastly stained. Of these nine bivalents, four were conspicuously large and the remaining ones small-sized (Fig. 1.5).

Cytologically, the genus is known from five species (four species show n = 9 and one species shows n = 9, 18) (Fritsch 1991), suggests x = 9 may be regarded as the base number of this genus.

Heteroscyphus argutus (Nees) Schiffn. n = 9 (Fig. 1.6)

The species was collected from Shillong (Botanical Survey of India, alt. 1525 m a.s.l.). It was found growing on trunk of *Rhododendron* plant.

The chromosome number n = 9 was also previously reported by Inoue (1977) in *Chiloscyphus argutus*. The bivalents were well spread and darkly stained. The complement consisted of one large bivalent, while the remaining members were nearly of the same size (Fig. 1.6). The course of meiosis was normal resulting in tetrad formation.

Cytologically the genus is known by five species and all of them show n = 9. This uniformity in chromosome number is indicative of the cytological conservativeness of the genus.

Frullania ericoides (Nees) Mont. n = 8 (Fig. 1.7–9)

The collection was made from Shillong (Elephant Falls, alt. 1525 m a.s.l.). The plants were growing on maple tree near a waterfall.

The observed chromosome number, n = 8 agrees with previous reports based on some other populations of this species

Table 1. Summary of chromosome numbers of studied taxa.

Name of the taxon	Locality and altitude (a.s.l.)	Herbarium reference no.	Chromosome number n =	Fig. no.
Division: Anthocerotophyta				
Family: Anthocerotaceae				
Anthoceros alpinus Steph.	Shillong, 1525 m	PAN 6279	6	1
	East Sikkim, 1650 m	PAN 6280		
Anthoceros subtilis Steph.		PAN 6281	6	2
	Darjeeling, 2000 m	PAN 6282		
	Shillong, 1525 m			
Family: Notothyladaceae				
Subfamily: Phaeocerotoideae				
Phaeoceros laevis Prosk.	Darjeeling, 2134 m	PAN 6283	6	3
Division: Marchantiophyta				
Family: Solenostomataceae				
Solenostoma atrobrunneum (Amak.)Váňa &Long.	East Sikkim, 1650 m	PAN 6304	9	4
		PAN 6305		
	Darjeeling, 2134 m			
Solenostoma purpuratum (Mitt.)Steph.		PAN 6306	9	5
	Darjeeling, 2042 m			
Family: Lophocoleaceae				
Heteroscyphus argutus (Nees) Schiffn.	Shillong, 1525 m	PAN 6303	9	6
Family: Frullaniaceae				
Frullania ericoides (Nees) Mont.	Shillong, 1525 m	PAN 6298	8	7–9
Frullania muscicola Steph.	Shillong, 1966 m	PAN 6299	18	10
Frullania nepalensis (Spreng.) Lehm.& Lindenb.	East Sikkim, 1780 m	PAN 6300	9	11
Family: Lejeunaeaceae				
Sub Family: Lejeuneoideae				
SubTribe: Cololejeuneinae				
Cololejeunea jelinekii Steph.	Shillong, 1525 m	PAN6301	8	12
Cololejeunea latilobula (Herzog) Tixier.	East Sikkim, 1650 m	PAN 6302	9	13
Family: Porellaceae				
Porella campylophylla (Lehm. & Lindenb.) Trevis.	Shillong, 1525 m	PAN 6296	9	14
Family: Radulaceae				
Radula tjibodensis Goebel	Shillong, 1525 m	PAN6297	8	15
Family: Fossombroniaceae				
Fossombronia cristula Austin	Shillong, 1496 m	PAN6294	9	16
Family: Calyculariaceae				
Calycularia crispula Mitt.	Darjeeling, 2134 m	PAN 6293	9	17
Family: Pallaviciniaceae				
Sub Family: Pallavicinioideae				
Pallavicinia lyellii (Hook.) Gray var. *lyellii*.	Shillong, 1525 m	PAN6295	8	18
Family: Aytoniaceae				
Asterella wallichiana (Lehm.) Grolle	East Sikkim, 1780 m	PAN 6284	9	19
	Darjeeling, 2134 m	PAN 6285	18	20
Plagiochasma pterospermum	Mawsynram, 1400 m	PAN 6286	9	21
C. Massal.		PAN 6287		
	Darjeeling, 2134 m			
Family: Cyathodiaceae				
Cyathodium aureonitens	Kalimpong, 2134 m	PAN 6288	9	22
(Griff.) Mitt.	Darjeeling, 2042 m	PAN 6289		
Family: Dumortieraceae				
Dumortiera hirsuta (Sw.) Nees.	East Sikkim, 1650 m	PAN 6291	9	23
		PAN 6292		
	Mawsynram, 1400 m			
Family: Targioniaceae				
Targionia hypophylla		PAN 6290	9	24
	Shillong, 1525 m			

(Fritsch 1991). The complement showed five large and three small-sized bivalents (Fig. 1.7). An anaphasic bridge was also observed at anaphase-I (Fig. 1.8–9).

Frullania muscicola Steph. n = 18 (Fig. 1.10)

The material of this species was collected from Shillong (Shillong Peak, alt. 1966 m a.s.l.). The plants were found growing on trunk of a maple tree.

The chromosome number n = 18 is the first chromosome count for this species. The bivalents were fastly stained and well spread. The members of the complement showed a regular gradation in the size (Fig. 1.10).

Frullania nepalensis (Spreng.) Lehm.& Lindenb. n = 9 (Fig. 1.11)

The material of this species was collected from Eastern Sikkim (Bulbulay Dara, alt. 1780 m a.s.l.). The plants were growing on an oak tree near a waterfall.

The present count, n = 9 is in conformity with the previous reports in some other population of same species (Fritsch 1991). The bivalents were well spread and darkly stained. The chromosomes in the complement showed a regular gradation in size (Fig. 1.11).

The cytological data available for 60 species so far (21 species with n = 8, 9; 34 species with n = 9; two species with n = 8, 9, 10; one species with n = 9, 10; one species with n = 10 and one species with n = 17) indicate that number, n = 9 is of most frequent occurrence in this genus and may be regarded as its base number. The other numbers are derived through aneuploidy or polyploidy.

Cololejeunea jelinekii Steph. n = 8 (Fig. 1.12)

The collection of this species was made from Shillong (Campus of Botanical Survey of India, alt. 1525 m a.s.l.). The plants were found growing on lower trunk of an oak tree.

Previously this species was not known cytologically. Presently eight bivalents were observed at first metaphase. The complement showed eight same-sized, well spread and fastly stained bivalents (Fig. 1.12). The course of meiosis was orderly resulting in normal tetrads.

Cololejeunea latilobula (Herzog) Tixier. n = 9 (Fig. 1.13)

The species was collected from Eastern Sikkim (Bhanjhakri Water Falls, alt. 1650 m a.s.l.). The plants were found growing on an oak tree trunk.

The number n = 9 is the first cytological report for this species. The bivalents were well spread and darkly stained. The complement showed three conspicuously large bivalents. The other members showed gradation in size (Fig. 1.13).

The genus is cytologically known by nine species (three species show n = 9 and six species show n = 8). More studies are required to ascertain the cytological status of this genus.

Porella campylophylla (Lehm.& Lindenb.) Trevis. n = 9 (Fig. 1.14)

This species is commonly found in the Himalayas. Presently, it was collected from Shillong (Campus of Botanical Survey of India, alt. 1525 m a.s.l.). It was found growing on the trunk of a sal (*Shorea robusta*) tree.

The number n = 9 is the first chromosome count for this species. The nine fastly stained bivalents were well spread as observed at first metaphase. The complement included two noticeably larger bivalents. Of the other bivalents, five were medium-sized and two relatively smaller sized (Fig. 1.14).

Cytologically, only 20 species are known (15 species show n = 8, three species show n = 8, 9, one species shows n = 10 and one species shows n = 8, 18). The available data suggest that x = 8 is the base number of the genus.

Radula tjibodensis Goebel n = 8 (Fig. 1.15)

The material was collected from Shillong (Elephant Falls, alt. 1525 m a.s.l.). The plants were found on an oak tree.

Previously, the chromosome number of this species was not known. Of the eight bivalents observed at first metaphase, two were conspicuously large. The remaining members of the set show gradation in size (Fig. 1.15).

The cytological data available for 15 species are available for this genus (Fritsch 1991). Six species show n = 6; n = 6, 8 in one species; n = 8 in eight species; n = 9 in one species and n = 6, 8, 12, 16 in one species showing n = 8 in 10 species suggest that x = 8 may be the base number of this genus. The other numbers may be derived through aneuploidy from the base chromosome number.

Fossombronia cristula Austin n = 9 (Fig. 1.16)

The studied material was collected from Shillong (Sweet Fall, alt. 1496 m a.s.l.) near waterfall, growing on wet soil.

The spore mother cells were large-sized. The complement included nine bivalents, as observed at metaphase-I (Fig. 1.16). One of the bivalents, was large and the remaining ones showed gradation in size. The course of meiosis looked orderly might resulting in normal tetrads.

Of the16 cytologically investigated species, 14 show n = 9, one shows n = 8 and another one shows n = 8, 9; seems x = 9 may be the base number of the genus. The other number, n = 8 is derived through aneuploid loss of one chromosome.

Calycularia crispula Mitt. n = 9 (Fig. 1.17)

The present collection was made from Darjeeling (Road to Zoological Park, alt. 2134 m a.s.l.). The plants were found growing on tree trunk.

The chromosome number n = 9 (Fig. 1.17) observed for the species is in line with the previous reports (Fritsch 1991). The complement showed nine well spread, faintly stained bivalents at metaphase-I. Two bivalents in the complement were large-sized. One of the bivalents was heteromorphic (presumably the sex-bivalent) and showed precocious

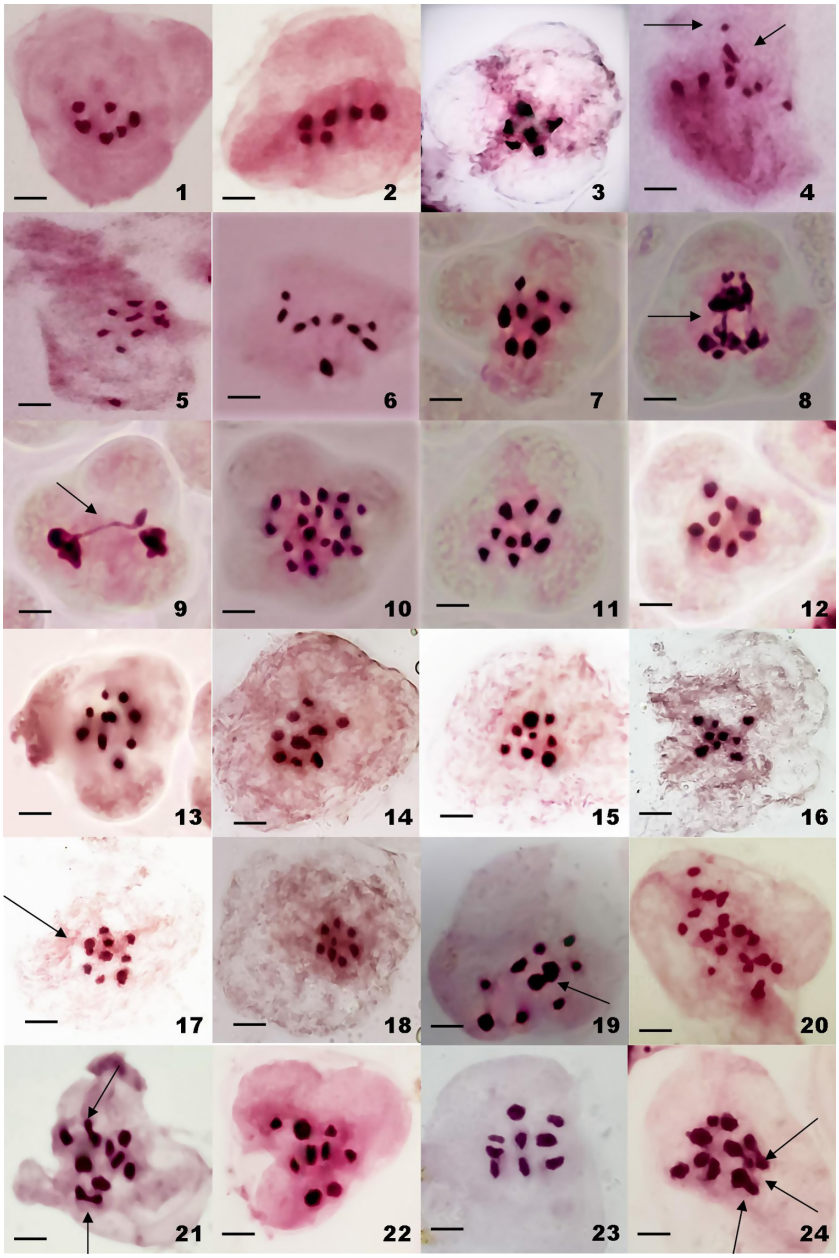

Figure 1. (1) *Anthoceros alpinus n*=6. Metaphase-I, showing six bivalents. (2) *Anthoceros subtilis* n=6. Metaphase-I, showing six bivalents. (3) *Phaeoceros laevis* n=6. Metaphase-I, showing six chromosomes. (4) *Solenostoma atrobrunneum* n=9 (8+1 m). Metaphase-I, showing eight bivalents, one m-chromosome and early disjunction in one bivalent (arrow marked). (5) *Solenostoma purpuratum* n=9. Metaphase-I, showing nine bivalents. (6) *Heteroscyphus argutus* n=9. Metaphase-I, showing nine chromosomes. (7–9) *Frullania ericoides* n=8. (7) Metaphase-I, showing eight bivalents. (8–9) Anaphasic bridge formation at anaphase- I. (10) *Frullania muscicola* n=18. Metaphase-I, showing 18 bivalents. (11) *Frullania nepalensis* n=9. Metaphase-I, showing nine chromosomes. (12) *Cololejeunea jelinekii* n=9. Metaphase-I, showing nine bivalents. (13) *Cololejeunea latilobula* n=9. Metaphase-I, showing nine bivalents. (14) *Porella campylophylla* n=9. Metaphase-I, showing nine bivalents. (15) *Radula tjibodensis* n=8. Metaphase-I, showing eight bivalents. (16) *Fossombronia cristula* n=9. Metaphase-I, showing nine bivalents. (17) *Calycularia crispula* n=9. Metaphase-I, showing eight bivalents and one precociously disjoined bivalent (arrow marked). (18) *Pallavicinia lyellii* var. *lyellii.* n=8. Metaphase-I, showing eight bivalents. (19–20) *Asterella wallichiana* n=9, 18. (19) Metaphase-I, showing eight bivalents and one precociously disjoined bivalent (arrow marked). (20) Metaphase-I, showing 18 bivalents. (21) *Plagiochasma pterospermum* n=9. Metaphase-I, showing nine bivalents including two bivalents showing precocious disjunction (arrow marked). (22) *Cyathodium aureonitens* n=9. Metaphase-I, showing 9 chromosomes. (23) *Dumortiera hirsuta* n=9. Metaphase-I, showing 9 bivalents. (24) *Targionia hypophylla* n=9. Metaphase-I, showing nine bivalents and precocious disjunction in three bivalents (arrow marked). Scale bar=20 μm.

disjunction. The other members of the set showed gradation in size.

This genus belongs to a monotypic family Calyculariaceae (Crandall-Stotler et al. 2009) and consists of only two accepted species, *C. crispula* Mitt and *C. laxa* Lindenb. & Arnell, out of which only the former is known cytologically.

Pallavicinia lyellii (Hook.) Carruth var. *lyellii*. n = 8 (Fig. 1.18)

The present collection was made from Shillong (Botanical Survey of India, alt. 1525 m a.s.l.) and was found growing near a waterfall.

This variety was not known cytologically. The present study showed eight bivalents at metaphase-I. One of the bivalents was large-sized while the remaining members of the set were same-sized (Fig. 18). The bivalents were closely packed and darkly stained. The meiotic divisions were normal resulting in regular tetrad formation.

Cytologically, the genus is known by five species. The occurrence of n = 8 in four species and n = 8, 9, 18 in one species suggests that x = 8 may be the base number of this genus.

Asterella wallichiana (Lehm.) Grolle. n = 9, 18 (Fig. 1.19–1.20)

The material of this species was collected from Eastern Sikkim (Bulbulay Dara, 1780 m a.s.l.) and Darjeeling (Road to Zoological Park, alt. 2134 m a.s.l.). Both the populations were found growing on walls and bricks.

This species was not cytologically investigated so far. It has been found to exist in two cytological forms, i.e. n = 9 (Darjeeling population) and n = 18 (Sikkim population).

Of the nine bivalents observed at metaphase-I, two bivalents were of large size. One of the bivalents showed precocious disjunction. The remaining members of the set showed gradation in size (Fig. 1.19).

In the East Sikkim population, 18 bivalents were observed out of which one was conspicuously large-sized, distinctly heteromorphic (presumably the sex-bivalent). The remaining members of the complement showed gradation in size (Fig. 20). In both the populations, the bivalents were well spread and darkly stained.

The chromosome counts n = 8, 9, 18, 26 are reported in 15 species, n = 8 is reported in one species; n = 9 in nine species exclusively; n = 18 in two species; n = 9, 18 in one species; n = 18, 26 in one species and n = 26 in one species. The genus seems to be based on x = 9.

Plagiochasma pterospermum C. Massal. n = 9 (Fig. 1.21)

The taxon was collected from two widely separated localities (Mawsynram, alt. 1400 m a.s.l. and Darjeeling, Rock Garden, Chunnu Falls, alt. 2134 m a.s.l.). Both populations were found growing on the wet cliff wall and bricks.

The East Himalayan population of this species is cytologically investigated for the first time. The present count agrees with an earlier report in some other population of this species (Mehra 1977). In the two presently studied populations, bivalents were well spread and darkly stained. Of the nine bivalents observed at first metaphase, two showed early disjunction (Fig. 1.21).

It is cytologically known by 13 species (n = 8, 9 in one species, n = 9 in nine species; n = 9, 16 in one species, n = 16, 18 in one species and n = 18 in three species). The genus seems to be based on n = 9 from which n = 18 is derived through euploidy and n = 8 through aneuploid loss of one chromosome.

Cyathodium aureonitens (Griff.) Mitt. n = 9 (Fig. 1.22)

This taxon collected from Kalimpong (Changey Falls, Lava; alt. 2134 m a.s.l.) and Darjeeling (Victoria Fall, alt. 2042 m a.s.l.).

Cytologically, this Indian species was not known previously. In both the populations, nine bivalents were counted at metaphase-I. The complement included three conspicuously large, well spread and fastly stained bivalents. Other bivalents showed a regular gradation in size (Fig. 1.22).

The family Cyathodiaceae includes a single genus *Cyathodium*. Only six are known cytologically of which one species showed erroneous chromosome number, n = 3 (Chavan 1937). Two species show n = 8, two species are recorded with n = 8, 9 and one species with n = 9). The chromosome number, n = 8 seems to be the base number of this genus.

Dumortiera hirsuta (Sw.) Nees n = 9 (Fig. 1.23)

The plants collected from two widely separated localities (Eastern Sikkim, Banjhakri Water Falls, alt. 1650 m a.s.l. and Mawsynram; alt. 1400 m a.s.l.), were found growing in abundance on shaded wet soil.

The family Dumortieraceae includes single genus *Dumortiera* represented by single species which is known to exist in four cytological forms (n = 9, 10, 18, 27) (Fritsch 1991).

The present count, n = 9 (Fig. 1.23) is in conformity with some of the previous reports based on some other populations of this species (Fritsch 1991). In both the populations, well spread, darkly stained nine bivalents were observed at metaphase-I. The bivalents showed gradation in size.

Targionia hypophylla n = 9 (Fig. 1.24)

Fertile material was collected from Shillong (Campus of Botanical Survey of India, alt. 1525 m a.s.l.) growing on soil.

This first study of the meiotic chromosomes in the East Himalayan population agrees with the earlier reports (Fritsch 1991).

The nine bivalents observed at first metaphase were well spread and darkly stained. Three members of the set showed tendency towards early disjunction (Fig. 1.24).

The family Targioniaceae is known by a single genus *Targionia*. The chromosome counts known in three species of the genus, n = 9 in two species; n = 18 in one species; n = 24 in two species; n = 26 in one species and n = 27 in two species indicate that n = 9 is of most frequent occurrence and may be the base number of the genus.

Discussion

In hornworts, only 21 species included in five genera are known cytologically. The predominant chromosome number is n = 5 considered as the base number in hornworts (Fritsch 1991).

In the present study, three species belonging to two genera (*Anthoceros* and *Phaeoceros*) showed n = 6 which can be an aneuploid number, showing intrageneric aneuploidy in *Anthoceros*, whereas *P. laevis* showed intraspecific aneuploidy.

In liverworts, 890 species belonging to 160 genera are known cytologically (Fritsch 1991, Yun-long et al. 1995, 1999, Chudzínska et al. 2001, Tong et al. 2002, Kumar and Kapila 2003, Wei et al. 2003, Min et al. 2004, Zheng et al. 2005, Zheng and Zhu 2008a, b, 2009, 2010, Bahti 2011, Kapila 2016).

Presently, in leafy liverworts, seven species and all the five thalloid liverworts species showed n = 9 (also reported by several other authors, e.g. Fritsch 1991). Thus, n = 9 is considered as the basic chromosome number for the liverworts.

Presently, the intrageneric polyploidy is observed in *Frullania* (n = 9, 18) where n = 18 is a polyploid chromosome number and in *A. wallichiana* (n = 9 and 18) showed intraspecific polyploidy indicating its role in the evolution and speciation of these genera.

In the present investigations, distinctly heteromorphic 'H' bivalent, presumably the sex bivalent was observed in two taxa (*A. wallichiana* and *C. crispula*).

Two species belonging to two genera (*Frullania* and *Cololejeunea*) showed n = 8 which is an aneuploid number, showing intrageneric aneuploidy in *Frullania* (n = 8, 9) and *Cololejeunea* (n = 8, 9).

Presently, in *F. ericoides*, a bridge formation can be observed at late anaphase-I.

No meiotic abnormality was observed in any of the taxa.

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Author contributions

The research work is carried by the author Dr Meenakshi Sharma under the guidance of Prof. Sunita Kapila, Department of Botany, Panjab University, Chandigarh, India.

Data availability statement

There are no additional data for this paper.

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