Environmental and Experimental Biology (2024) 22: 95–104
http://doi.org/10.22364/eeb.22.10

Original Paper

Influence of bark chemistry on distribution of epiphytic mosses on basal trunk of *Cryptomeria japonica*

ISSN 2255-9582

Kheyali Halder[1], Subhra Chakraborti[2], Projjwal Chandra Lama[3], Souvik Mitra[4]*

[1]Department of Botany, Acharya Prafulla Chandra Roy Government College, Siliguri-734010, Dist. Darjeeling, West Bengal, India
[2]Molecular Biology Laboratory, RKVY Uttar Banga Krishi Vishwavidyalaya, Pundibari-736165, West Bengal, India
[3]Post Graduate Department of Botany, Darjeeling Government College, Darjeeling-734101, West Bengal, India
[4]Department of Botany, Taki Government College, Taki-743429, North 24 Parganas, West Bengal, India

*Corresponding author, E-mail: ssouvikmitra1687@gmail.com

Abstract

Epiphytic mosses are integral parts of forest community structure in the Darjeeling Hills of the Eastern Himalayan region with remarkable contributions to the ecosystem functionality. The study was framed to assess the richness and spatial distribution of epiphytic mosses growing on the basal trunk of *Cryptomeria japonica* (Thunb. ex. L.) D.Don, and also to evaluate the explanatory host traits for shaping the moss assemblage. Field measurements and sampling were performed near Lamahatta village within Darjeeling district on 270 microplots placed on tree trunks. A total of twelve mosses represented by the members of Dicranales and Hypnales were recorded. Low species diversity was observed with dominance and maximum cover of *Syrrhopodon confertus*. Canonical correspondence analysis predicted a distinct combination of chemical requirements for local colonization of each moss. The results also demonstrated influence of bark acidic inputs on abundance and co-existence of bryophytes. The outcome can be potentially helpful in depicting the community structure of non-vascular epiphytes, which may further be considered while developing forest management strategies.

Key words: bark acidity, *Cryptomeria japonica*, epiphyte, moss, species richness.
Abbreviations: CCA, canonical correspondence analysis; DBH, diameter at breast height; DW, dry weight; IVI, Important Value Index; RC, relative coverage; RF, relative frequency.

Introduction

Non-vascular epiphytes are important components of forest ecosystems and contribute significantly to species diversity (Tatsumi et al. 2017). They represent a substantial proportion of photosynthetic biomass and play a vital role in maintaining biogeochemical cycles (Zechmeister et al. 2003). Epiphytic mosses are also efficient accumulators of minerals and contaminants from deposition due to their unique morphological and physiological features (Glime 2007). They can also transform nutrients to make them accessible to the surroundings (Coxson 1991). In spite of enormous ecological significance, they are under serious threat due to the destruction of habitat and changes in air quality (Rose 1992). Therefore, this plant group requires much attention in developing proper strategies for their conservation. To attain this goal, basic knowledge on aspects of phytosociology and their association with phorophytes, more precisely the factors modulating their phorophyte specificity, are necessary.

The complex canopy structures and trunks of large trees provide habitat for a diverse range of non-vascular epiphytes (Azuma et al. 2022). Distribution patterns and community structure of the epiphytic bryophytes are greatly influenced by the host physicochemical traits (Bates 1992; Callaway et al. 2002; Ezer 2017; Whitelaw 2015). Even bark properties have impact on their species and functional diversity (Putna, Mežaka 2014; Shao et al. 2023). Host preference of bryophytes can also be explained by pH and nutrient content of phorophyte bark (Gonzalez-Mancebo et al. 2003; Mitchell et al. 2021). Although neutral pH of bark is mostly favoured by bryophytes, occurrence of taxa on low bark pH has also been recorded (Strazdiņa 2010; Pereira et al. 2014). In addition, bark nitrogen, carbon, phosphorus, and potassium content determine the bryophyte host specificity (Studlar 1982). The effect of bark

calcium content on abundance of non-vascular epiphytes was also recorded (Gauslaa 1985). Bark calcium, being the dominant exchangeable cation in bark, has been predicted as the key factor in shaping the bryophyte composition (Gustafsson, Eriksson 1995). Bark phenolics and flavonoids may influence affinity of epiphytic lichens to bark, and may also influence metabolic shifts in the epiphytes (Paukov et al. 2022; Blatt-Janmaat et al. 2023). However, no such information on the effect of secondary metabolites on bryophyte cover is yet available on the epiphytic bryophytes.

The Darjeeling Hills of the Eastern Himalayan region are rich reservoirs of epiphytic bryoflora. Variations in temperature and humidity within a favourable range allow distribution of a diverse range of these epiphytes, but due to increasing deforestation and anthropogenic effects in this region, epiphytes are facing serious threat. Despite having a huge resource, very few works have been performed in India to understand the aspects of community structure and dynamics of their assemblages. The conducted studies were mostly confined to the distribution and diversity of bryophytes in other forest zones (Alam et al. 2011; Bansal et al. 2011; Nath et al. 2012). *Cryptomeria japonica* (Thunb. ex. L.) D.Don, one of the dominant conifers of Darjeeling hills, supports prolific growth of the epiphytic bryophytes on their trunks. Mukhia et al. (2019) recently demonstrated the influence of host traits on the association of epiphytic liverworts with *C. japonica* in Senchal Wildlife Sanctuary, Darjeeling. However, more comprehensive knowledge on epiphyte diversity and their assemblage patterns is necessary, which may further be employed for developing effective forest management strategy in this region. The taxa confined primarily to the basal zone of trunks require more attention as they are most vulnerable to forest management (McCune 1993). Thus, the present study was framed to assess the assemblages of epiphytic mosses on the basal trunk of *C. japonica* and to predict the underlying factors influencing their composition.

Materials and methods

Study area

The study was performed near Lamahatta Village within Takdah Forest Range, situated in Darjeeling District, West Bengal, India, extending between 27°03.091' to 27°03.132' N and 88°21.120' to 88°21.140' E. The terrain of the forest zone is hilly covering an approximate altitude range between 1750 to 1950 m above sea level. The mean annual precipitation in the area is 3110 mm and the mean annual temperature is about 17.9 °C. The montane evergreen forest of this area is characterized by prolific and homogeneous occurrence of old-growth *Cryptomeria japonica*, a predominant plantation tree belonging to the conifer family Cupressaceae. The tree favours luxuriant growth of epiphytes on their trunks, which are primarily bryophytes. The surrounding field vegetation mostly is represented by tall herbs and dwarf shrubs, indicating that the soil is nutrient-rich. The area has not been used for intensive forestry, and also without any visible anthropogenic activity.

Field sampling and identification

The field survey and sampling were performed during pre-monsoon of 2021 in between the first and third week of April. During the month of sampling, the average temperature was around 17.6 °C, and the total precipitation was approximately 101 mm. The occurrence of any epiphyte (presence or absence), their abundance (cover) and life forms were recorded in the field. In the study area, five plots of 50× 50 m were chosen having a minimum gap of 100 m between each plot, and three random trees from each plot were considered for the study. The occurrence and cover of the bryophyte species were recorded on the basal trunk by placing micro-plots of 20 × 20 cm within 150 cm height from the base. The sampling positions on the trunk were divided into six height zones, and within each zone three microplots were positioned on different directions. Therefore, the study was performed on total 270 microplots (18 on each host trunk). The physical parameters of each tree such as bark roughness, and diameter at breast height (DBH) were documented. Bark roughness was recorded on a 5-point scale (1, smooth; 2, rather smooth; 3, medium; 4, rather rough; 5, rough). Sampling of epiphytes and underlying bark was done following a method modified from Bargali et al. (2014). A few plants or plant parts of all visibly distinguishable bryophytes were collected for identification. Location coordinates and altitude of the study sites were recorded using a GPS device (Garmin, USA). The taxonomic identity of the collected mosses was determined based on the morpho-anatomical features of gametophytes and sporophytes using the keys and standard manuals described in the monograph 'Mosses of Eastern India and Adjacent Regions' (Gangulee 1969-80). The climate data of the study site were extracted from WorldClim 2.1 at a spatial resolution of 1 km^2 (Fick, Hijmans 2017).

Analysis of bark pH

The pH of bark was determined following a method described by Gustafsson and Eriksson (1995). The epiphyte remains were removed from the bark samples. Each sample (100 mg) was cut into pieces, then soaked in 10 mL of 25 mM KCl solution for 10 min and the pH was determined by using a digital pH meter (Systronics, India).

Analysis of bark total cations

The di-acid digestion method was followed for the estimation of total Ca, Mg, Na, and K content. Dried and coarsely ground bark (1 g) was mixed with 10 ml of acid mixture containing HNO_2 and $HClO_4$ (9:4, v/v) by swirling. The mixture was kept on a low-heat hot plate for digestion, and was then heated at a higher temperature until the cessation of production of red NO_2 fumes. Volume

of the contents was reduced by evaporation till the liquid became colourless. After cooling, 20 mL of deionized water was added and the solution was filtered through Whatman No. 1 filter paper. An aliquot of the filtrate was used to determine the total Ca, Mg, Na, and K content using an atomic absorption spectrophotometer (AAS model: PinAAcle 900F, Perkin Elmer, USA).

Analysis of total phenolics and flavonoids

The bark samples were dried at 65 °C for 72 h, and were then ground into powder. An amount of 50 to 500 mg powdered bark was refluxed with 10 mL of methanol diluted with distilled water (4:1, v/v). The extract was filtered and the filtrate was used for the estimation of total phenolics and flavonoids.

Total phenolic content was determined following the method of Singleton and Rossi (1965). A volume of 1 mL of the methanol extract was added to 4 mL of ethanol and 0.5 mL of Folin-Ciocalteau reagent diluted with distilled water (1:1, v/v). The mixture was incubated for 30 min followed by the addition of 2 mL 5% Na_2CO_3. The mixture was placed in a boiling water bath for 1 min followed by immediate cooling. Absorbance of the developed colour was measured at 650 nm using a UV-visible spectrophotometer (UV-1700 Pharmaspec, Shimadzu). Different concentrations of gallic acid were used to prepare the calibration curve and the content was expressed as mg of gallic acid equivalent g^{-1} dry weight.

The method proposed by Chang et al. (2002) was followed to determine the total flavonoid content. An aliquot of extract was mixed with 1.5 ml of methanol and 0.1 mL of 10% $AlCl_3$ followed by the addition of 0.1 mL of 1M CH_3COOK. The mixture was incubated at room temperature for 30 min and absorbance of the developed colour was measured at 415 nm. The standard curve was prepared using different concentrations of rutin and the content was expressed as mg rutin equivalent g^{-1} dry weight.

Data analysis

Effectiveness of sampling effort at the study site was evaluated through construction of a species accumulation curve or sample- based rarefaction curve using 20 × 20 cm microplots with 95% confidence intervals (Gotelli, Colwell 2001). The method plots the cumulative species number as a function of the number of examined samples. The rarefaction method calculates the species richness by sampling individuals. The abundance of a taxon was calculated as the mean percentage cover of that species. The importance value index (IVI) of each species was calculated from relative frequency (RF) and relative cover (RC) using the formula (Printarakul, Meeinkuirt 2022):

$$IVI = RF + RC.$$

RC and RF were estimated using the following equations:

$$RC = C_i / \Sigma C \times 100,$$

where C_i is percent cover of i-th taxon, ΣC is total percent cover of all taxa; and

$$RF = F_i / \Sigma F \times 100,$$

where F_i is number of micro-plots with occurrence of the i-th taxon, ΣF is total occurrence of all taxa.

Species diversity at the study site was determined with the Shannon-Wiener diversity index (H') from the cover estimates using the following formula (Shannon, Weaver 1963):

$$H' = \Sigma^{s}_{i=1}(p_i) \times \ln(p_i),$$

where p_i is the proportion of total cover contributed by the i-th species. Species richness was determined using the non-parametric richness estimators, the bias-corrected Chao and the first order jackknife (Colwell, Coddington 1994).

The bark samples collected from each height zone were considered as replicate samples for analysis of chemical parameters, and the values were represented as mean ± SD. For each height zone of a trunk, the mean values of the chemical parameters were obtained from the samples of the three sites of the respective height zone. Spearman's rank correlation between the environmental variables was calculated to determine the relationship between the variables, and any observed correlation was considered as significant at $p < 0.05$ (Hollander, Wolfe 1973). To evaluate the effect of bark physicochemical factors on the abundance and composition of epiphytes, canonical correspondence analysis (CCA), a multivariate ordination technique, was performed considering the studied attributes as variables (Ter Braak 1988). Correlation with the variables was determined by inflation factors where high inflation factors indicated a stronger association. The CCA results were tested using Monte Carlo permutation to check the significance of the model. Multiple regression analysis with backward elimination of independent variables was carried out to predict the key host trait influencing the total cover of mosses and cover of individual species. In this process, the variable exhibiting highest p value was excluded, and the regression step was repeated until all the remaining variables showed significant effect ($p < 0.05$). The species showing more than 50% frequency percentage were considered for the analysis. For all the statistical analyses the PAST 4.03 software package was used (Hammer et al. 2001).

Results

Species composition and richness

The species accumulation curve depicted that sufficient sampling effort was conducted in the study (Fig. 1). While the tip of the curve showed a slight upward trend, it was relatively flat, and therefore, can be considered to have reached the plateau. Epiphytic bryophytes were almost uniformly distributed on basal trunks of phorophytes, above which abundance decreased. The mosses were present as scattered colonies with different life forms,

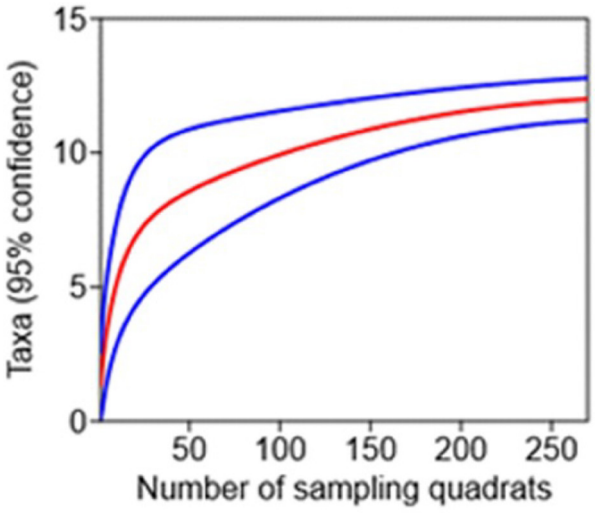

Fig. 1. Species accumulation curve showing number of species added per added quadrat.

which were occasionally associated with leafy liverworts.

A total of twelve mosses were identified, which belong to either Dicranales or Hypnales (Table 1). *Syrrhopodon confertus*, a member of Calymperaceae, was the predominant moss which exhibited maximum cover (20.50%) with 93.38% frequency of occurrence. Colonies of this moss were observed in the form of a uniform carpet in most of the microplots, occasionally occurring as a single dominant species. Among the other acrocarpous mosses, *Dicranodontium asperulum* and *Leucobryum humillimum* were considerably frequent on host trunks with 46.69 and 40.02% frequency, respectively. The members of Hypnales were not very abundant compared to the Dicranales members. *Pylaisiadelpha amblystega*, a member of Sematophyllaceae, was the most frequent having 1.17% cover. Compared to the mosses, the liverwort composition was less diverse, which was primarily represented by *Bazzania* sp. A significant difference between total moss and liverwort abundance was recorded by the Mann-Whitney U test ($p < 0.001$). The occurrence of the two plant groups was negatively correlated at a significance level of $p < 0.001$ ($r^2 = 0.12$) (Fig. 2). However, *Bazzania* sp. was the second most frequent bryophyte species, showing 12.57% mean cover. The dominance of *Syrrhopodon confertus* was apparent from its importance value (IVI > 1), which was much higher than the other frequent taxa – *Dicranodontium asperulum, Pylaisiadelpha amblystega*, and *Leucobryum humillimum* (Table 1).

Among the identified taxa, *Syrrhopodon confertus, Leucobryum humillimum, Dicranodontium asperulum, Taxiphyllum taxirameum*, and *Pylaisiadelpha amblystega* were recorded at all the height zones from base to 120 cm height (Fig. 2). *Sematophyllum subhumile* and *Erythrodontium julaceum* were not observed within 40 and 80 cm from base, respectively. In contrast, *Anisothecium spirale* occurred only near the base of the tree trunk (below 40 cm). The abundance of *Syrrhopodon confertus* decreased from base (27.49%) to the 100 cm height zone (13.7%). Similarly, cover of *Dicranodontium asperulum* was highest at base of tree (9%), which gradually reduced above 40 cm. Abundance of *Campylopus fragilis* var. *pyriformis* also reduced from base to upper height zones. No such trend was apparent from the cover data of other mosses.

Statistical estimates exhibited nearly similar values to the observed species richness on the phorophytes. The first order jackknife and chao 2 values were computed as 12.49 and 11.66, respectively. Low diversity of mosses was also apparent from the Shannon diversity index, which was recorded as 0.91. The concentration of species dominance (Simpson index) was calculated as 0.39. The low species diversity was due to presence of considerably low species number and dominance by *Syrrhopodon confertus*.

Table 1. Species composition and their abundance on *Cryptomeria japonica* at the study area. Mean values are given together with SD

Taxon	Family	Life form	Mean coverage percentage	Frequency percentage	Importance Value Index
Syrrhopodon confertus Sande Lac.	Calymperaceae	Dense cushion	20.50 ± 19.99	93.38	1.07
Leucobryum humillimum Cardot	Dicranaceae	Large cushion	0.47 ± 1.32	40.02	0.15
Campylopus fragilis var. *pyriformis* (Schultz) Agst.	Dicranaceae	Large cushion	0.89 ± 3.44	6.67	0.05
Anisothecium spirale (Mitt.) Broth.	Dicranaceae	Small cushion	0.01 ± 0.03	6.67	0.02
Dicranodontium asperulum (Mitt.) Broth.	Dicranaceae	Large cushion	2.65 ± 4.55	46.69	0.25
Taxiphyllum taxirameum (Mitt.) M. Fleisch.	Hypnaceae	Smooth mat	0.09 ± 0.19	20.01	0.07
Bryosedgwickia aurea (Schwägr.) M. Fleisch.	Hypnaceae	Smooth mat	0.02 ± 0.07	6.67	0.02
Isopterygium micans (Sw.) Kindb.	Hypnaceae	Smooth mat	0.03 ± 0.11	13.34	0.04
Brotherella amblystega (Mitt.) Broth.	Sematophyllaceae	Smooth mat	1.17 ± 2.94	33.35	0.15
Sematophyllum subhumile (Müll. Hal.) M. Fleisch.	Sematophyllaceae	Smooth mat	0.67 ± 1.19	33.35	0.13
Entodon rubicundus (Mitt.) A. Jaeger,	Entodontaceae	Smooth mat	0.12 ± 0.46	6.67	0.03
Erythrodontium julaceum (Hook. Ex Schwägr.) Paris	Entodontaceae	Thread-like mat	0.003 ± 0.010	6.67	0.02

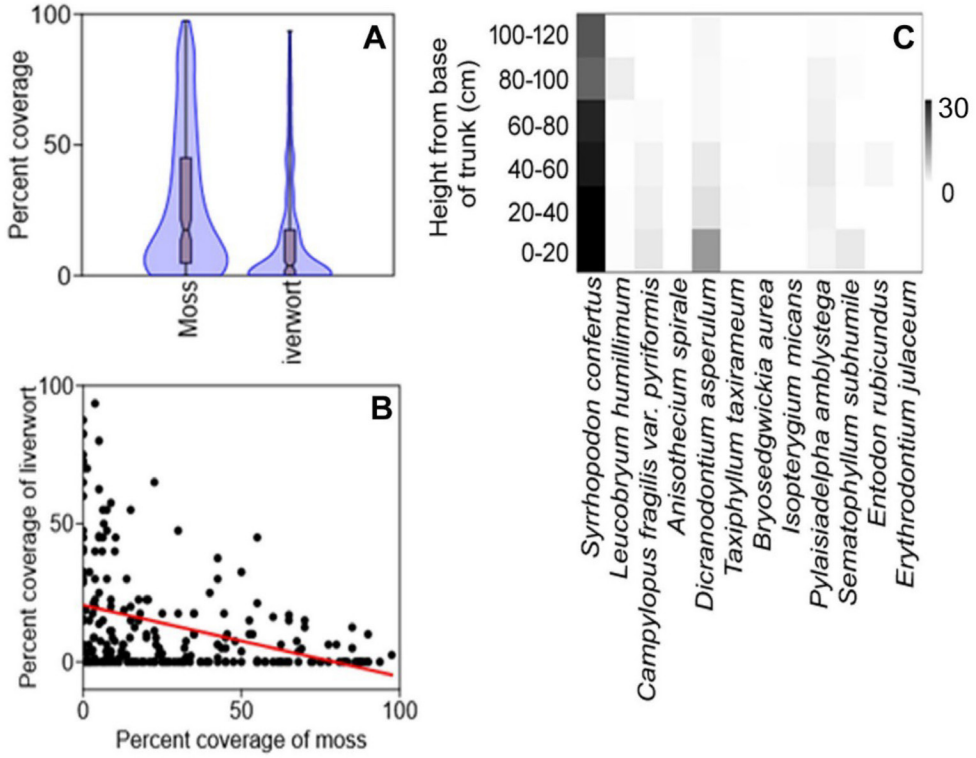

Fig. 2. Comparison of percent cover of total mosses and liverworts on the basal trunks (A). Correlation of percent cover of mosses and liverworts on basal trunks ($p < 0.001$) (B). Matrix plot showing mean percent cover of the mosses on different height zones of tree trunks. Intensity of the colour corresponds to abundance of the moss with scale ranging from 0 to 30% (C).

Physicochemical features of host bark-

Bark of all the host samples were moderately rough and was placed within the same ordinal scale. Therefore, this parameter was not considered as a variable for further statistical analyses. Diameter at breast height (DBH) ranged from 1.6 to 2.7 m with an average value of 2.09 m (Table 2). Unlike the data from different phorophyte individuals, the chemical parameters from different height zones of basal trunk did not exhibit any significant difference. Bark was slightly acidic with pH value ranging from 5.2 to 6.3. The bark samples collected from base (0 to 20 cm) and 100 to 120 cm zone exhibited a pH range from 5.4 to 5.8 and 5.7 to 5.9, respectively. Among the cations, Ca content was found to be highest (39.45 mg g^{-1} DW), whereas Mg content was lowest (3.05 mg g^{-1} DW). These two values were also positively correlated at the significance level of $p < 0.05$. The Ca content of the basal bark samples (0 to 20 cm zone) ranged from 40.11 to 58.24 mg g^{-1} DW, while the value varied from 26.64 to 44.29 mg g^{-1} DW at 100 to 120 cm height zone. Mg content of samples from 0 to 20 cm and 100 to 120 cm ranged from 3.04 to 3.14 mg g^{-1} DW and 2.98 to 3.10 mg g^{-1} DW, respectively. Na content ranged from 0.63 to 37.35 mg g^{-1} DW and K content varied from 2.61 to 17.48 mg g^{-1} DW. Flavonoid content was found to be consistent with the bark phenol content with a significant positive correlation ($p < 0.001$) (Fig. 3). Mean phenolics and flavonoid content was recorded as 1.81 and 1.13 mg g^{-1} DW, respectively.

Table 2. Host traits of *Cryptomeria japonica* used as variables for the study. DBH, diameter at breast height

Variables	Mean value	SD	Maximum	Minimum
DBH	2.088667	0.318588	2.7	1.6
pH	5.756	0.333505	6.27	5.2
Ca content (mg kg^{-1} DW)	1905.95	745.6067	3722.5	1025.5
Mg content (mg kg^{-1} DW)	152.53	3.10222	159.125	145.9
Na content (mg kg^{-1} DW)	216.3833	484.402	1867.5	31.25
K content (mg kg^{-1} DW)	311.1667	171.9134	873.75	130.5
Flavonoid content (mg g^{-1} DW)	1.130489	0.748014	2.343	0.247667
Phenolics content (mg g^{-1} DW)	1.814356	1.010703	3.283	0.718333

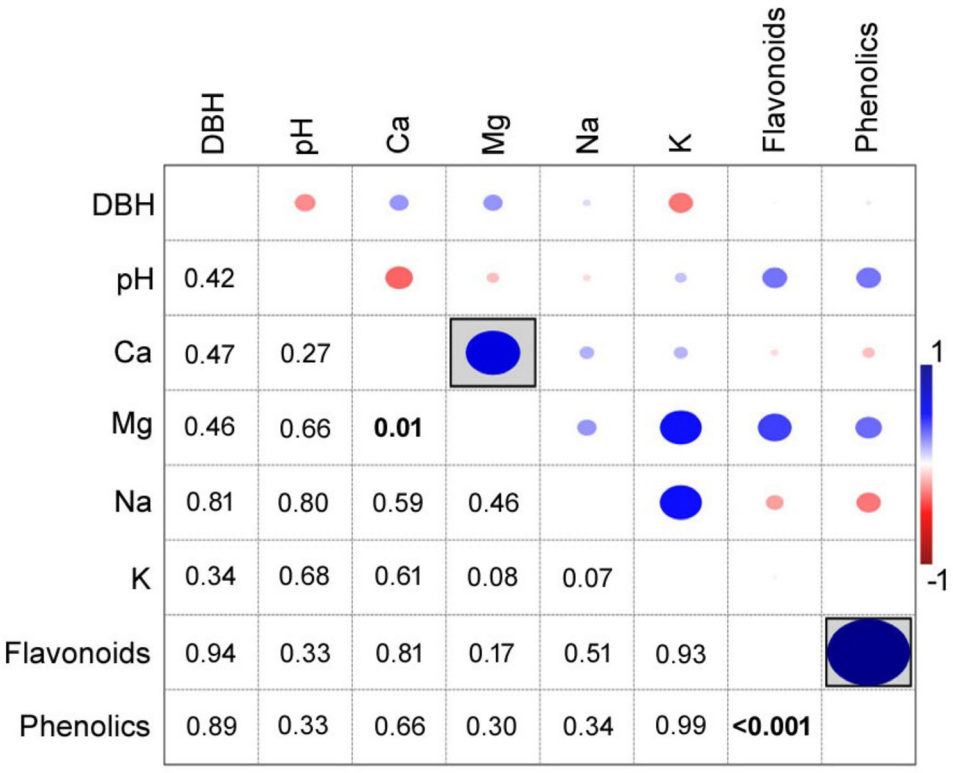

Fig. 3. Correlation matrix of the variables measured by Spearman's rank correlation coefficient (r s). The upper half of the diagonal represents the correlation coefficient where size of the circles corresponds to the strength of the correlation and colour scale range from –1 to +1; blue and red colour of circles signify positive and negative correlation respectively. The lower half of the diagonal represents p values showing significant correlation between Ca and Mg contents ($p = 0.01$) and between total phenolics and flavonoids ($p < 0.001$).

Relationship between moss assemblage and host factors

The study aimed to predict the influence of host chemical parameters within and between the phorophytes. Assemblages of mosses on the six height zones of a plant had no significant correlation with the host parameters. To understand the relationship between the host chemical parameters and the abundance of mosses, CCA was used as an ordination method where cover of mosses on individual host plants and their respective parameters were considered. The proportion of variance accounted for by the first and second axes was 51.3 and 23.3%, respectively, which explained 74.6% of the total variance (Fig. 4). All inflation factors were less than 5. DBH was found to be aligned with the first axis of the plot as a higher fraction of this variable was explained by this axis. Bark phenolic and flavonoid content was also found to be associated with the first axis with a low significance level. Bark K, Na, and Mg content and bark pH were associated with the second axis. The potassium content exhibited the strongest correlation. *Leucobryum humillimum, Sematophyllum subhumile*, and *Pylaisiadelpha amblystega* were found to be more associated with the first axis and their cover can be assumed to be influenced by the DBH. *Taxiphyllum taxirameum* was found to be aligned almost equally with both axes. The dominant moss *Syrrhopodon confertus* was found to be aligned at the negative sides of both axes, which indicated that the chemical factors may have negative impact on its abundance.

Relationship between individual species abundance and host factors

As *Syrrhopodon confertus* was the most frequent (> 50%) epiphyte in the study area, cover of only this moss was considered for multiple regression analysis. Such selection was also previously performed by Gustafsson and Eriksson (1995). The analysis predicted bark pH as the key host chemical factor that influenced the abundance of this species. In the first step of regression, bark magnesium content was excluded due to its least significance ($p = 0.59$). Bark pH was observed as significant contributor in the regression model ($p < 0.05$), after eliminating DBH, total Na, K, Ca, phenolics, flavonoid contents in the subsequent steps (Table 3). Therefore, the cover of *Syrrhopodon confertus* was found to have negative correlation with bark pH value at a significance level of $p < 0.05$ ($r^2 = 0.34$; $t = -2.57$). A negative correlation was also observed between the overall moss cover and bark pH ($p < 0.05$) (Fig. 5). A similar regression method was followed to search key predictor variables depicting the distribution of the dominant epiphytic liverwort *Bazzania* sp. which was recorded on almost 87% of the studied trees. The regression model revealed that the cover of this liverwort

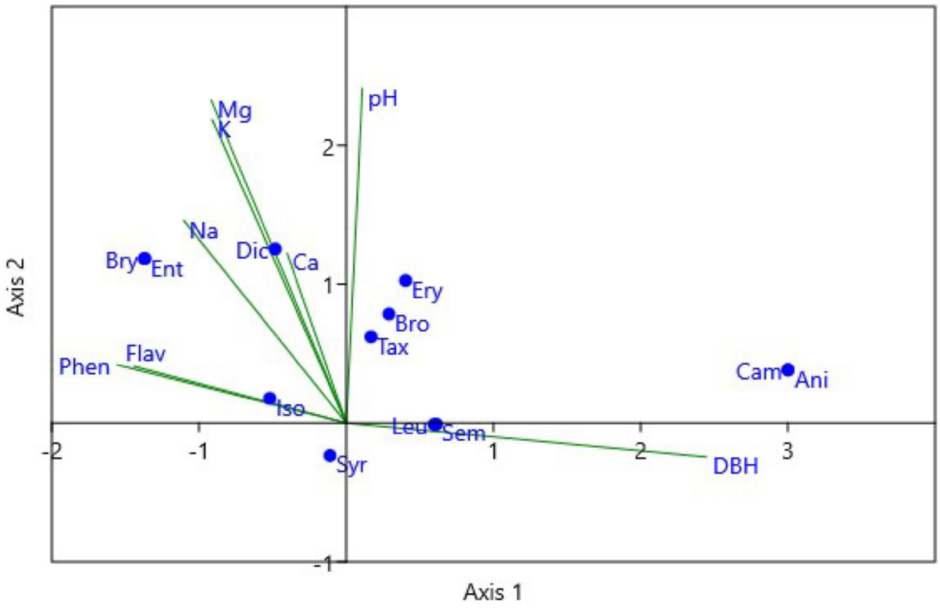

Fig. 4. CCA biplot dhowing correlation of percent cover of epiphytic mosses of basal tree trunks as dependent variables with host physicochemical traits as independent variables. Syr, *Syrrhopodon confertus*; Ent, *Entodon rubicundus*; Ani, *Anisothecium spirale*; Cam, *Campylopus fragilis* var. *pyriformis*; Tax, *Taxiphyllum taxirameum*; Bry, *Bryosedgwickia aurea*; Iso, *Isopterygium micans*; Did, *Dicranodontium didymodon*; Leu, *Leucobryum humillimum*; Sem, *Sematophyllum subhumile*; Bro, *Pylaisiadelpha amblystega*; Ery, *Erythrodontium julaceum*; DBH, diameter at breast height; Phen, phenolics content; Flav, flavonoid content.

was significantly influenced by bark pH and Mg content ($p < 0.05$). Both variables were positively correlated with the abundance of this species.

Discussion

The complex interaction between the phorophytes and non-vascular epiphytes residing on them can be explained by the bryological richness and comparison between the abundance of coexisting species. In spite of being exposed to favourable climatic conditions, considerably low species richness at the basal zone of trunk was quite apparent in the present study. The basal trunk surprisingly exhibited homogeneous existence of *Syrrhopodon confertus* as the dominant species. Features of host trees are crucial factors for controlling the composition of epiphyte bryophytes and are generally related to the tree species. Generally, exfoliating barks are not suitable for colonization of epiphytes, including bryophytes (Wolf 1993; Hamalainen et al. 2023). Low species richness on the basal trunk can be explained by the exfoliating bark of mature *Cryptomeria* trunks. Another explanation for low species richness can be dispersal limitation, which is experienced by the mosses established on trunks. The trunks of closed canopy forests are exposed less to the wind, which restricts the dispersal ability of the small propagules of epiphytic mosses (Berdugo et al. 2022). Difference in vertical distribution of mosses was not apparent in the present study except for *Dicranodontium asperulum* and *Campylopus fragilis* var. *pyriformis*, which mostly restricted near the base.

Table 3. Statistical summary of multiple regression model for percent coverage of *Syrrhopodon confertus* as dependent variable with host traits as independent variables for prediction of significant variables following backward elimination process

Backward elimination step	Multiple *R*	Multiple R^2	Multiple R^2 adjusted	*F* value	*P* value	Eliminated environmental variable
1	0.87	0.75	0.42	2.28	0.16	–
2	0.86	0.74	0.48	2.84	0.10	Total Mg content
3	0.86	0.73	0.53	3.68	0.05*	DBH
4	0.80	0.64	0.44	3.23	0.06	Total Na content
5	0.79	0.63	0.48	4.21	0.03*	Total K content
6	0.72	0.52	0.39	4.01	0.04*	Total Ca content
7	0.64	0.41	0.31	4.18	0.04*	Total phenolics content
8	0.58	0.34	0.29	6.60	0.02*	Total flavonoid content

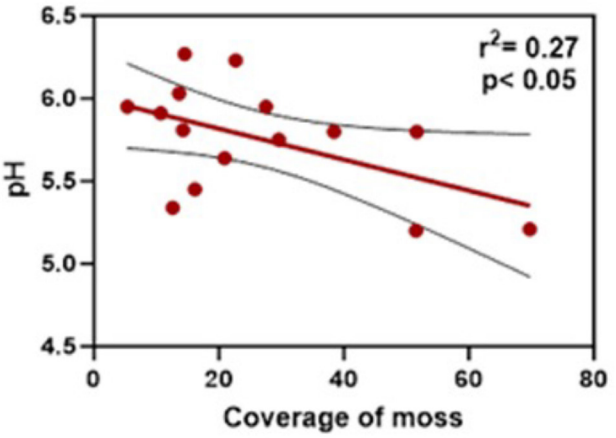

Fig. 5. Correlation of total moss percent cover and cover of Syrrhopodon confertus with the phorophyte bark pH.

Bark of the basal part of a mature trunk is more cracked having favourable microhabitats and these parts usually have slightly higher pH. All these criteria enhance the accumulation of bryophytes (Gustafsson, Erikson 1995). Moreover, the bryophytes occur more in the shady and humid microhabitats at the basal trunk (Ranius et al. 2008).

The influence of bark physicochemical factors on the abundance of epiphytic mosses was apparent in the statistical interpretation. CCA demonstrated that the investigated mosses have distinct combinations of requirements. Among the studied variables, bark acidity, DBH, K content were observed to have considerably greater impact on moss assemblages. Some of the major predictions were negative influence of bark pH on colonization of *Syrrhopodon confertus*, positive effects of cations on abundance of *Dicranodontium asperulum*, and higher abundance of *Leucobryum* on trunks with higher DBH. Previously, Mitchell et al. (2021) also proposed that the host preference of epiphytes can be explained by acidity, nutrient content, and diameter of the host. A higher occurrence of liverworts was observed on the trunks of *Cryptomeria* having greater diameter (Mukhia et al. 2019). The trunks with larger DBH can provide more area for epiphyte occupancy. Greater trunk circumference enhances the possibility of dispersal of spores on trees, thereby increasing the number of colonies (Wiklund, Rydin 2004). Although there are some previous reports suggesting the effect of trunk diameter on species richness and diversity (Kiraly et al. 2013), no such assumption can be made in the present study.

Among the chemical parameters, bark pH was observed as the most significant factor. The acidity of phorophyte bark was previously reported as a remarkable determinant for species composition and abundance (Gabrie, Bates 2005; Larsen et al. 2007). Each bryophyte has a specific substratum pH requirement, and most bryophytes prefer neutral to sub-neutral pH (Pereira et al. 2014). The existing literature suggests lower species richness on bark with acidic pH, as on *Fraxinus* and *Salix* (Bates et al. 1997; Mezaka, Znotiņa 2006). The pH range of the investigated *Cryptomeria* bark also varied within the acidic range, although the values were close to neutral. Total moss cover as well as cover of *Syrrhopodon confertus* was negatively correlated with bark pH. The result can be corroborated with previous reports that suggested relatively greater occurrence of mosses on bark with sub-neutral pH (Bates 1992; Fojcik et al. 2015). Habitat preference of another species of *Syrrhopodon*, *Syrrhopodon fimbriatulus,* was found to be influenced by host bark pH and it is adapted to tolerate desiccation by the intense crisping of the leaves (Reese, Bartlett 1982).

A view exists that spore germination may be inhibited or delayed on conifers as an effect of lower bark pH (Wilklund, Rydin 2004). This may not be applicable for *Syrrhopodon confertus* compared to the other mosses as the moss exhibits higher occurrence of vegetative propagation. In general, tall acrocarpous mosses and large pleurocarpous mosses are expected to have greater tolerance than small acrocarpous mosses as the latter have relatively more contact with the substrate. So, for small acrocarps, the substrate properties are more important during early establishment (Cleavitt 2001). Moreover, large acrocarps such as *Syrrhopodon confertus* and *Dicranodontium asperulum* disperse their large vegetative fragments or whole detached plants to continue growth on the substrate.

Existing reports on the correlation between bark pH and bryophyte species richness are quite contradictory (Kubesova, Chytry 2005; Hydbom et al 2012). Some bryophytes prefer weakly acidic substratum while some are true acidophilic (Tyler 2016). In the present study, the significant negative correlation between the relative cover of *Syrrhopodon confertus* and another dominant liverwort, *Bazzania*, suggested competition between the epiphytes for their occupancy on the basal trunk. The bark pH requirement can be predicted as the driving factor for their competitive colonization. *Bazzania* is considered as an acidophilic epiphyte which usually colonizes more on barks with low pH value (Studlar 1982). The greater abundance of the liverwort on more acidic bark predicted that barks with lower pH were preferred by the liverwort over *Syrrhopodon confertus*. Therefore, bark acidity controls the assemblages of epiphytes on trunks, besides influencing cover of the dominant moss on the lower trunk of *Cryptomeria japonica*.

Conclusions

The investigation provided a comprehensive outlook on the diversity and assemblages of epiphytic mosses on the basal trunk of *Cryptomeria japonica*, the predominant conifer in plantation forests of Darjeeling hills. The composition of epiphytic mosses was characterized by low species richness and dominance of *Syrrhopodon confertus*. Occupancy of *Syrrhopodon confertus* was found to be constrained by

liverworts. Bark acidity was identified as the key factor influencing the abundance of *Syrrhopodon confertus* along with its association with other dominant colonizers. The outcome provides insight into the community dynamics of non-vascular epiphytes colonizing on the basal tree trunks of *Cryptomeria* forests. The baseline knowledge on moss distribution may help to determine conservation priorities aimed at preserving moss diversity and measure the success of conservation efforts in plantation forests.

Acknowledgements

Authors are thankful to Acharya Prafulla Chandra Roy Government College for providing infrastructural facilities to perform experiments.

References

Alam A., Sharma V., Sharma S.C. 2011. Bryoflora of Ranthambhore Tiger Reserve, Rajasthan (India). *Arch. Bryol.* 106: 1–8.

Azuma W.A., Komada N., Ogawa Y., Ishii H., Nakanishi A., Noguchi Y., Kanzaki M. 2022. One large tree crown can be defined as a local hotspot for plant species diversity in a forest ecosystem: a case study in temperate old-growth forest. *Plant Ecol.* 223: 99–112.

Bansal P., Nath V., Chaturvedi S.K. 2011. Epiphytic bryophytes on *Thuja orientalis* in Nagaland, North-eastern India. Bangladesh J. Plant Taxon. 18: 163–167.

Bargali R., Awasthi V., Pande N. 2014. Ecological study of bryophytes on *Platanus orientalis* L. trees in Nainital (Western Himalaya). *Amer. J. Plant Sci.* 5: 3880–3888.

Bates J.W. 1992. Influence of chemical and physical factors on *Quercus* and *Fraxinus* epiphytes at Loch Sunart, western Scotland: a multivariate analysis. *J. Ecol.* 80: 163–179.

Bates J.W., Proctor M.C.F., Preston C.D., Hodgetts N.G., Perry A.R. 1997. Occurrence of epiphytic bryophytes in a 'tetrad'transect across southern Britain 1. Geographical trends in abundance and evidence of recent change. *J. Bryol.* 19: 685–714.

Berdugo M.B., Gradstein S.R., Guerot L., León-Yánez S., Bendix J., Bader M.Y. 2022. Diversity patterns of epiphytic bryophytes across spatial scales: Species-rich crowns and beta-diverse trunks. *Biotropica* 54: 893–905.

Blatt-Janmaat K., Neumann S., Schmidt F., Ziegler J., Qu Y., Peters K. 2023. Impact of in vitro phytohormone treatments on the metabolome of the leafy liverwort *Radula complanata* (L.) Dumort. *Metabolomics* 19: 17.

Callaway R.M., Reinhart K.O., Moore G.W., Moore D.J., Pennings S.C. 2002. Epiphyte host preferences and host traits: mechanisms for species-specific interactions. *Oecologia* 132: 221–230.

Chang C.C., Yang M.H., Wen H.M., Chern J.C. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.* 10: 178–182.

Cleavitt N. 2001. Disentangling moss species limitations: the role of physiologically based substrate specificity for six species occurring on substrates with varying pH and percent organic matter. *Bryologist* 104: 59–68.

Colwell R.K., Coddington J.A. 1994. Estimating terrestrial biodiversity through extrapolation. *Phil. Trans. Royal Soc. London B* 345: 101–118.

Coxson D.S. 1991. Nutrient release from epiphytic bryophytes in tropical montane rain forest (Guadeloupe). *Canad. J. Bot.* 69: 2122–2129.

Ezer T. 2017. Epiphytic bryophyte communities and succession on *Platanus orientalis* trees in Kadincikvalley (Mersin/Turkey). *Pak. J. Bot.* 49: 623–630.

Fick S.E., Hijmans R.J. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *Int. J. Climatol.* 37: 4302–4315.

Fojcik B., Chruścińska M., Nadgórska-Socha A., Stebel A. 2015. Determinants of occurrence of epiphytic mosses in the urban environment; a case study from Katowice city (S Poland). *Acta Mus. Siles. Sci. Natur.* 64: 275–286.

Gabriel R., Bates J.W. 2005. Bryophyte community composition and habitat specificity in the natural forests of Terceira, Azores. *Plant Ecol.* 177: 125–144.

Gangulee H.C. 1969–1980. *Mosses of Eastern India and Adjacent Regio*ns. Vol. I–III. Books and Allied Limited, Calcutta.

Gauslaa Y. 1985. The ecology of *Lobarion pulmonariae* and *Parmelion caperatae* in *Quercus* dominated forests in south-west Norway. *Lichenologist* 17: 117–140.

Glime J.M. 2007. *Bryophyte Ecology*. Volume 1. Physiological Ecology. Michigan Technological University and the International Association of Bryologists, Houghton.

González-Mancebo J.M., Losada-Lima A., McAlister S. 2003. Host specificity of epiphytic bryophyte communities of a laurel forest on Tenerife (Canary Islands, Spain). *Bryologist* 106: 383–394.

Gotelli N.J., Colwell R.K. 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecol. Lett.* 4: 379–391.

Gustafsson L., Eriksson I. 1995. Factors of importance for the epiphytic vegetation of aspen *Populus tremula* with special emphasis on bark chemistry and soil chemistry. *J. Appl. Ecol.* 32: 412–424.

Hämäläinen A., Fahrig L., Strengbom J., Ranius T. 2023. Effective management for deadwood-dependent lichen diversity requires landscape-scale habitat protection. *J. Appl. Ecol.* 60: 1597–1606.

Hammer O., Harper D.A.T., Ryan P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. *Paleontol Electron.* 4: 4.

Hollander M., Wolfe D.A. 1973. *Nonparametric Statistical Methods.* John Wiley and Sons, New York, USA.

Hydbom S., Odman A.M., Olsson P.A., Cornberg N. 2012. The effects of pH and distribution on the bryophyte flora in calcareous sandy grasslands. *Nord. J. Bot.* 30: 446–452.

Király I., Nascimbene J., Tinya F., Ódor P. 2013. Factors influencing epiphytic bryophyte and lichen species richness at different spatial scales in managed temperate forests. *Biodivers. Conserv.* 22: 209–223.

Kubesova S., Chytry M. 2005. Diversity of bryophytes on treeless cliffs and talus slopes in a forested European landscape. *J. Bryol.* 27: 35–46.

Larsen R.S., Bell J.N.B., James P.W., Chimonides P.J., Rumsey F.J., Tremper A., Purvis O.W. 2007. Lichen and bryophyte distribution on oak in London in relation to air pollution and bark acidity. *Environ. Pollut.* 146: 332–340.

Mežaka A., Znotiņa V. 2006. Epiphytic bryophytes in old growth forests of slopes, screes and ravines in north-west Latvia. *Acta Univ. Latv.* 710: 103–116.

Mitchell R.J., Hewison R., Beaton J., Douglass J.R. 2021. Identifying substitute host tree species for epiphytes: The relative importance of tree size and species, bark and site characteristics. *Appl. Veget. Sci.* 24: e12569.

Mukhia S., Mandal P., Singh D.K., Singh D. 2019. The abundance of epiphytic liverworts on the bark of *Cryptomeria japonica* in relation to different physical and biochemical attributes, found in Senchal Wildlife Sanctuary, Darjeeling, Eastern Himalaya. *BMC Ecol.* 19: 37.

Nath V., Pande N., Asthana A.K., Gupta R. 2012. Epiphytic moss flora of Pachmarhi Biosphere Reserve (MP): An important aspect of bryophyte diversity. *Natl. Acad. Sci. Lett.* 35: 195–200.

Paukov A., Teptina A., Ermoshin A., Kruglova E., Shabardina L. 2022. The role of secondary metabolites and bark chemistry in shaping diversity and abundance of epiphytic lichens. *Front. Forests Global Change* 5: 828211.

Pereira I., Mueller F., Moya Moraga M.R. 2014. Influence of *Nothofagus* bark pH on the lichen and bryophytes richness, Central Chile. *Gayana Bot.* 71: 120–130

Printarakul N., Meeinkuirt W. 2022. The bryophyte community as bioindicator of heavy metals in a waterfall outflow. *Sci. Rep.* 11: 6942.

Putna S., Mežaka A. 2014. Preferences of epiphytic bryophytes for forest stand and substrate in North-East Latvia. *Folia Cryptogam. Estonica* 51: 75–83.

Ranius T., Johansson P., Berg N., Niklasson M. 2008. The influence of tree age and microhabitat quality on the occurrence of crustose lichens associated with old oaks. *J. Veg. Sci.* 19: 653–662.

Reese W.D., Bartlett J.K. 1982. *Syrrhopodon fimbriatulus* C. Müll., and the family Calymperaceae (Musci), new to New Zealand; and notes on Calymperaceae from the New Zealand Island territories. *J. Bryol.* 12: 209–214.

Rose F. 1992. Temperate forest management: its effect on bryophyte and lichen floras and habitats. In: Bates J.W., Farmer A.M. (Eds.) *Bryophytes and Lichens in a Changing Environment.* Clarendon Press, Oxford, pp. 211–233.

Shannon C.E., Weaver W. 1963. *The Mathematical Theory of Communication*. University of Illinois Press, Urbana.

Shao Y., Wang S., Li Y., Chen Y., Zhao H., Wang J., Yuan Z. 2023. Importance of bark physicochemical properties in an epiphytic bryophyte community within a temperate deciduous broadleaf forest. *Diversity* 15: 688.

Singleton V.L., Rossi J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Amer. J. Enol. Viticult.* 16:144–158.

Strazdiņa L. 2010. Bryophyte community composition on an island of Lake Cieceres, Latvia: dependence on forest stand and substrate properties. *Environ. Exp. Biol.* 8: 49–58.

Studlar S.M. 1982. Host specificity of epiphytic bryophytes near Mountain Lake, Virginia. *Bryologist* 85: 37–50.

Tatsumi S., Ohgue T., Azuma W.A., Nishizawa K. 2023. Bark traits affect epiphytic bryophyte community assembly in a temperate forest. *Plant Ecol.* 224: 1089–1095.

Ter Braak C.J.F. 1987. The analysis of vegetation- environment relationships by canonical correspondence analysis. *Vegetatio* 69: 69–77.

Ter Braak C.J.F. 1988. Partial canonical correspondence analysis. In: Classification and related methods of data analysis: proceedings of the first conference of the International Federation of Classification Societies (IFCS), Technical University of Aachen, FRG, 29 June – 1 July 1987, North-Holland, pp. 551–558.

Tyler T., Olsson P.A. 2016. Substrate pH ranges of south Swedish bryophytes – Identifying critical pH values and richness patterns. *Flora* 223: 74–82.

Whitelaw M., Burton M.A.S. 2015. Diversity and distribution of epiphytic bryophytes on Bramley's Seedling trees in East of England apple orchards. *Glob. Ecol. Conserv*. 4: 380–387.

Wiklund K., Rydin H. 2004. Ecophysiological constraints on spore establishment in bryophytes. *Funct. Ecol.* 18: 907–913.

Wolf J.H. 1993. Diversity patterns and biomass of epiphytic bryophytes and lichens along an altitudinal gradient in the northern Andes. *Ann. Missouri Bot. Gard.* 80: 928–960.

Zechmeister H.G., Grodzińska K., Szarek-Łukaszewska G. 2003. Bryophytes. *Trace Metals and Other Contaminants in the Environment* 6: 329–375.

Received 16 April 2024; received in revised form 10 june 2024; accepted 10 August 2024