BJNRD

BJNRD (2024), 11(1): 1-13

Bhutan Journal of Natural Resources & Development

Article

www.bjnrd.org

Open Access

ISSN 2409–2797 (Print)
ISSN 2409–5273 (Online)

DOI: https://doi.org/10.17102/cnr.2024.91

Diversity and Distribution of Bryophytes along Pasaphu-Mochhu River Basin in Pasaphu, under Trashigang District

Thinley Dorji[1*] and Ugyen Dorji[2]

Abstract

The study presents an analysis of bryophyte species diversity and distribution along the Pasaphu-Mochhu river in Trashigang district. For this study, the river was stratified into three different zones on which a systematic random sampling was carried out. A total of 156 plots with dimension of 1 m x 1 m were studied with plot-to-plot distance of 50 m x 10 m. The study documented 28 bryophyte species, comprising 19 moss species, 8 liverworts, and 1 hornwort. The highest diversity was observed in the lower part of river (H'=3.317). *Brachythecium* sp. was found to be the most dominant species with an important index value (IVI) of 34.40. There was a significant difference in diversity between the strata. Bryophyte species diversity fluctuated with increasing altitude (p=0.94) and no significant relationship between canopy closure and bryophyte coverage was observed (p=0.61). In terms of substrate, bryophyte species were predominantly distributed on silt and rock. Specifically, mosses were mainly found on rock substrates, liverworts on silt, and hornworts exclusively on silt substrate. Mosses were present across a range of canopy closures, while liverworts and hornworts were located in areas with partial to dense canopy coverage. The study underscores the need for more comprehensive investigations to fully understand the effect of changing environments on the conservation of bryophyte habitats and species, thus safeguarding them from degradation. Extensive degradation of habitat could threaten and lead to the disappearance of sensitive species and thus more comprehensive study is recommended.

Keywords: Bryophyte, Liverwort, Mosses, Shannon diversity index

Introduction

Bryophytes are non-vascular and non-flowering micro group of plants that thrives in wide range of micro habitat across the world including moist environment such as forest, stream, river, rock, silt and trunk substrate. It is divided into three classes inclusive of Hepaticopsida (Liverwort), Anthocerotopsida (Hornworts) and Bryopsida (Mosses). They exhibit wide range of diversity in-terms of reproductive strategies and physical characteristics (Bowden *et al.,* 2006). With 25,000 species, bryophytes constitute the second-largest group of land plants after flowering plants. Bryophytes, the earliest land plants, play a vital role in ecosystems, with an estimated appearance around 480 million years ago. They are primarily found in moist, humid, or swampy environments such as damp rocks, forest floors, and tree trunks (Gunathilaka, 2019).

[1]Forestry Officer, Department of Forest and Park Services
[2]Department of Forest Science, College of Natural Resources, Royal University of Bhutan
*Corresponding author: thinley11999@gmail.com
Received: October 29, 2023
Accepted: June 26, 2024
Published online: June 30, 2024
Editor: Yonten Dorji

These plants lack leaves and stems, and instead, they have leaf and stem-like structures referred to as phylloids and axes, respectively. Rather than roots, bryophytes use rhizoids to carry out root functions. Their cells can efficiently absorb moisture from the ground or directly from the atmosphere. Consequently, bryophytes thrive in damp soils. These plants are typically quite small, ranging from a few millimeters to several centimeters in size. The smallest bryophyte is *Zoopsis* sp. (5 mm), while *Dawsonia* sp. (50-70 cm) stands as the largest bryophyte (Juyal, 2015).

Bhutan falls within the eastern Himalayan biodiversity hotspot, where climatic conditions are conducive in supporting a wide variety of bryophytes, although the region remains relatively unexplored (Dorji, 2021). The National Biodiversity Centre (NBC) has noted the presence of many non-vascular plant species, such as sphagnum mosses, liverworts, and hornworts, but no comprehensive inventory of this plant group has been conducted (NBC, 2014).

Past studies have indicated that ectohydric and pleucarpous bryophytes serve as good indicators of environmental pollution and are known as hoard of remedies as it can contains medicinal properties (Chandra *et al*., 2017). With these known importance, more intensive study is required to gain a better understanding of their distribution patterns, community structure, and responses to ecological factors along environmental gradients (Jiang *et al*., 2018). The lack of intensive study and documentation of bryophytes over the globe and particularly in Bhutan, has impeded their identification, utilization, and the assessment of potential conservation threats. Nevertheless, it is not too late to collect, document, and characterize these plants, studying their ecological significance (Molder *et al*., 2015). With this in mind, the primary focus of this study is to determine the diversity and distribution of bryophytes along an altitudinal gradient across various microhabitats. As Pasaphu-Mochhu River passes through different altitudinal range with diverse micro-habitat including grazed area, virgin forest and degraded area due to anthropogenic activities with varied canopy closure it proves to be a diverse site for research. This research aims to study the diversity and distribution in different microhabitat along Pasaphu-Mochhu River, thus laying a fundamental baseline for future studies on bryophytes in Bhutan.

Methods and Materials

Study area

The study was conducted along the Pasaphu-Mochhu river in Kangpara Gewog under the Trashigang district. The selection of this study site was based on the presence of different microhabitats as Pasaphu-Mochhu river passes through different environment, which are expected to contribute to variations in the diversity, distribution of bryophytes and it was selected due to accessibility and representativeness of broader habitat. Notably, this research is the first of its kind in our country, and the choice of the study area was made without any potential bias. Often, potential research areas in remote locations are overlooked by researchers, but this study area was selected to explore the diversity and distribution of bryophytes in a remote region. The area is situated at an altitude of 1400 to 1600 masl and is characterized by a cool broad-leaved forest. It receives a mean annual rainfall of 1200 mm (Dzongkhag Administration, 2018).

Materials

The sampling involved manual collection, which included hand-picking, and a knife was used when samples were firmly attached to the substrate. For the sample identification, a magnifying lens with 10-20x magnification and a scale were used. The coordinates and elevation of the study plots were recorded using a GPS device. To reduce moisture content in the samples, tissue paper was used. The collected samples were then carefully placed into paper envelopes for preservation. A data collection sheet was used to assign a unique sample ID and document the specific microhabitat where the samples were found.

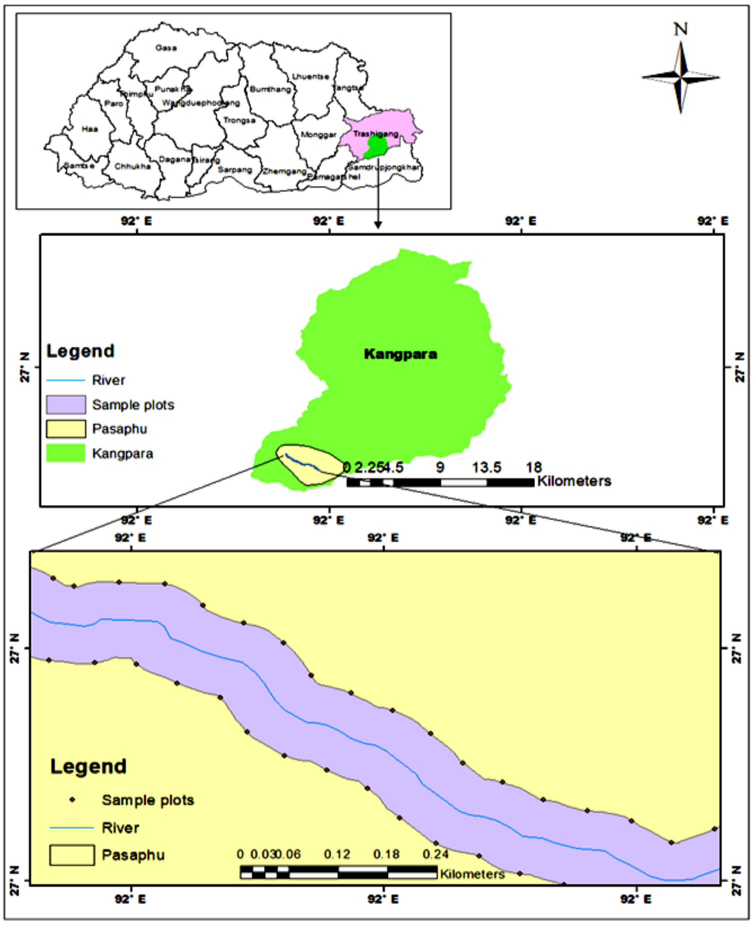


Figure1. The location of study site

Sampling design

The river was stratified into three parts comprehensives of headwater, middle river and lower reaches to study the distribution of bryophytes in different micro-habitat based on the level grazing, anthropogenic disturbances and silt deposition. To ensure a comprehensive assessment, a buffer zone extending 50 m from the river's edge, following Parker (2018), was established on both sides, as bryophytes within the vicinity of river remains moist due the incessant moisture from river. Generally floristic habitat sampling is deployed in terms of bryophytic research to cover an area as thoroughly as possible as diversity and distribution can be altered by potential microhabitat (Glime, 2017). Within this buffer zone, a systematic grid were laid out. The grid consisted of individual plots, each measuring 50 cm x 50 cm. The distance between adjacent plots was set at 10 m and 50 m, as illustrated in Figure 2 (adapted from Vasquez *et al*., 2019).

After laying all plots systematically over the three strata of river (ArcGIS 10.8), total number of plots to be studied along the entire river length was calculated using Solvin's formula and was be verified using 30% sampling thumb rule:

$$n = \frac{N}{1 + Ne^2}$$

Equation 1

n= Number of samples
N= Total number of populations
e= error margin

The total number of sample/ numbers of plot from each stratum was then obtained through proportionate calculation to the stratum's population size using the formula provided below:

$$\text{No. of plots in each stratum} = \frac{\text{Required sample number (Eq. 1 ans)} * \text{No. of plot in each strata}}{\text{Total number of plots in all stratum}}$$

Equation 2

After calculating the proportionate number of samples in each strata of river in association to the required sample size of entire river length, the sample plot was laid randomly and data collection over that plots was conducted.

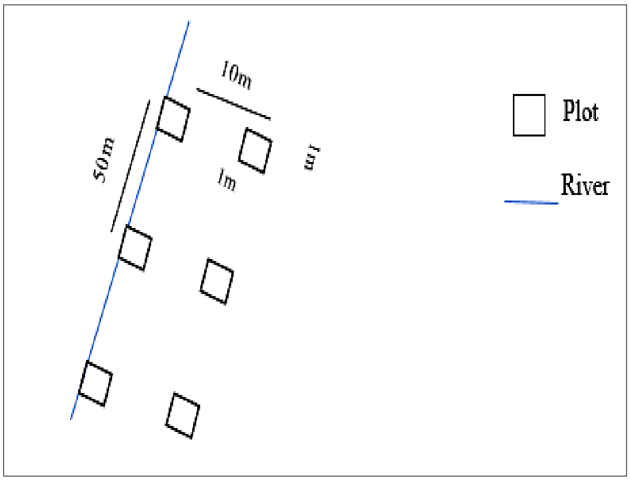


Figure 2. Sample plot

Data collection

Scientific gathering is necessary for various purposes, such as specimen identification and taxonomic research in herbarium collections. While larger species were typically identified in the field using a 10-20x hand lens, smaller species were identified based on microscopic characteristics. Therefore, reference collections of specimens are crucial in bryology studies, but it's important to follow proper collection and processing procedures to generate viable specimens for research.

In sample plots, bryophytes within a single small patch was counted as one patch, and if there are two patches in a sample plot, it was recorded as 2 bryophyte patches (including mosses, liverworts, and hornworts). This approach aids in calculating species dominance to determine the important index value (IVI). The coverage/ dominance of each patch was recorded in the form of percentage to calculate dominance ratio (DR). Concurrently, the frequency of each species across the entire study area was documented to calculate relative frequency (RF). IVI was then calculated by summing up dominance ratio and relative frequency.

$$IVI=DR\%+RF\% \qquad \textit{Equation 3}$$

Bryophyte inhibits a number of different substrates such as tree, dead wood, rock and ground forest. Different species tend to occupy and utilize different portion of resource continuum. The competitive exclusion principle predicts that species avoid competition by occupying different niches, creating a spatial pattern that represents habitat partitioning corresponding to habitat heterogeneity. The substrates in this paper comprises mainly of four division inclusive of rock, silt, tree trunk (live) and fallen log (dead) (Vasquez *et al*., 2019) as these substrates were found prominent in the study area and provides smallest landscape unit. The silt deposited by the bank of river with dead log, trunk of a tree and rock provides diverse habitat for bryophytes to thrive.

The distribution pattern was thus assessed based on the type of substrate (rock, log, trunk, and tree) on which the bryophytes grow along

Packing

Buck and Thiers (1996) argue that among plant species, bryophytes are the easiest to collect. They can often be gathered by hand since they lack roots. However, some species that are firmly attached to their substrate may require scraping with a knife to obtain all the plant parts necessary for identification. When collecting bryophytes, it is beneficial to search for sporophytes, as they are useful for identification purposes.

These bryophytes were collected with approximately 1-3 cm of the substrate since this can often provide diagnostic information (Whitehouse, 1966; Porley, 2008). Individual species within a collection were packed separately. An A4 sheet of paper was folded into a standard envelope measuring 10-12 x 14 cm (Figure 3).

In cases where specimens are quite wet, such as with Sphagnum, it's advisable to gently press them to remove most of the water before packing them into double or triple-thickness packages. When transporting and storing ground-dwelling species, it's often better to store them in rigid containers to prevent a mixture of soil particles and plant fragments.

Collecting specimens for scientific purposes is typically highly selective and seldom endangers the survival of the species. For robust species, gathering enough to fill a 12 x 8 cm package should generally be adequate.

Data labeling and drying

The information recorded were similar to that of other plants, encompassing habitat details (such as the type of tree or rock the species is found on), the characteristics of the surrounding environment such as canopy closure, substrate and specific location information, including GPS coordinates. It was important to note that many bryophyte species exhibit high clonality, with a single protonema capable of producing multiple gametophytes after the germination of a single spore.

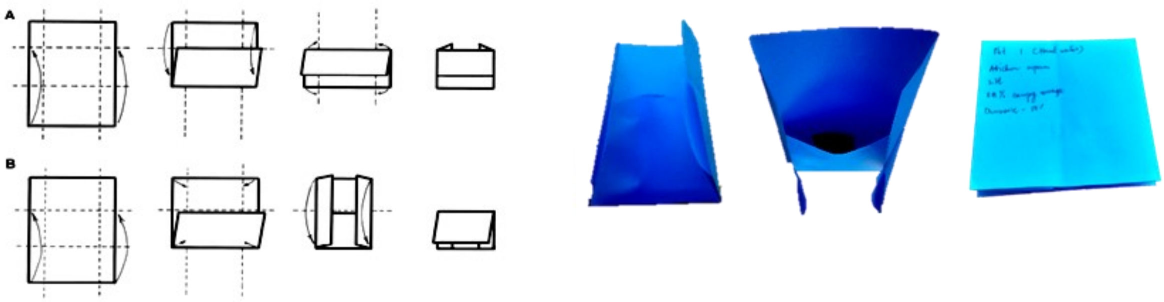


Figure 3. Standard envelopes with procedure on packing the bryophytes with prototype.

The collected specimens were promptly dried to prevent fungal damage. Typically, the packets were allowed to air-dry. Once dried, the bryophyte samples were carefully mounted onto A3-sized paper using water-soluble glue

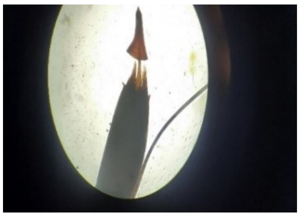
Identification

Firstly mosses, liverwort and hornwort were identified at division level by careful observation in the arrangement of leaves, presence of thallose and horn-like structure respectively. Bigger and familiar species were identified in field level using 10x (Loupe-2) hand lens and mosses and liverwort identification field guide from Britain and Ireland. Clear photographs of unidentified species were taken using HD zoom camera app available in android phone- and then packed with codename (Liverwort-1, 2, Mosses-1, 2 & Hornwort-1, 2. and so on). Photographs were uploaded in relevant social media group such as Bryophytes of Bhutan, British Bryological Society, Bryophytes of Ireland and Cumbria Bryophytes by giving the details of locality, habitat, elevation and substrate type. The unidentified package of bryophytes were identified using binocular microscope after wetting and mounting the specimen under the guidance of Dr. Karma Wangchuk of Sherubtse College. The field photographs (gametophyte, sporophyte) and lab photographs (operculum, calyptra, capsule, phyllids, and peristome) were send to expert for confirmation of the specimen. Some of the specimens identified is shown in Figure 4.

Leafy liverwort: *Lophocolea bidendata*

Thallose liverwort: *Wisnerella denudata*

Hornwort: *Anthoceros* sp.



Capsule (Operculum & peristome) of *Porella* sp.

Phyllid of *Bryum* sp.

Observing under binocular microscope

Figure 4. Identification of some specimens.

Data analysis

Ecological indices, including Margelef's richness, Shannon diversity, and Pielou's evenness, were calculated using Microsoft Excel 2013. A correlation test was performed to investigate the relationship between species count and elevation (Monika *et al*., 2021). Regression analysis was carried out to assess the impact of canopy closure on bryophyte coverage. For data visualization, ggplot was employed to create correlation plots, while scatter plots were generated for regression analysis and violin plot to depict the coverage distribution of mosses, liverworts, and hornworts based on canopy closure percentages using R version 4.13. Shannon diversity, Pielou's evenness, Margelef's similarity index, and Sorensen's similarity index were used to analyze the distribution of bryophytic species across various substrates using Microsoft Excel version 2013. Data visualization was used to understand and highlight the trend i.e., pie charts to illustrate the percentage distribution of mosses, liverworts, and hornworts using Microsoft Excel version 2013.

Table 1. Diversity of mosses, liverwort and hornwort in the study area

Species	Family	Dominance ratio
Pogonatum sp. (P.Beauv)	Polytrichaceae	4.33
Plagiomnium sp. (T. J. Koponen)	Mniaceae	12.66
Brachythecium sp. (Schimp)	Brachytheciaceae	17.93
Calyptothecium sp.	Pterobryaceae	1.69
Thuidium sp. (Bruch & Schimp)	Thuidaceae	10.49
Racophilum sp. (P.Beauv)	Racophilaceae	0.33
Leucobryum sp. (Hampe)	Leucobryaceae	1.35
Meteoriopsis squarrosa (Hook.) M. Fleisch	Meteoriaceae	7.1
Bryaceae	Bryaceae	4.39
Aerobrydium filamentosum (Hook. Ex Harv)	Meteoriaceae	2.53
Campylopus sp. (Brid,. 1819)	Leucobryaceae	1.5
Fissidens sp. (Hedw)	Fissidentaceae	2.87
Pterobryopsis sp.	Pterobryaceae	5.24
Papillaria sp.	Meteoriaceae	0.38
Hypnum sp. (Hedw)	Hypnaceae	2.19
Atrichum sp. P. Beauv.	Polytrichaceae	1.69
Philonotis sp. (Brid)	Bartramiaceae	1.18
Bryum sp. (Hedw)	Bryaceae	1.35
Mnium sp. (Hedw)	Mniaceae	2.19
Conocephalum conicum (L.) Underw.	Conocephalaceae	1.52
Dumortiera hirsuta (Sw.) Nees.	Dumortieraceae	3.55
Porella sp. (Linnaeus, 1753)	Porellaceae	3.55
Plagiochasma appendiculatum Lehm. & Lindenb.	Aytoniaceae	1.52
Lophocolea sp. (Dumort)	Lophocoleaceae	3.05
Wisnerella denudata (Mitt.) Stephani.	Wisnerellaceae	1.35
Frullania sp. (Raddi)	Frullaniaceae	1.69
Lophocolea bidendata (L.) Dumort.	Lophocoleaceae	2.36
Anthoceros sp. (Linnaeus)	Anthocerotaceae	1.16

Results and Discussion

Species diversity

A total 28 species of bryophyte under 20 different families were recorded along the Pasaphu-Mochhu river basin. The different bryophytic species were observed along the entire 6 km study area that comprises of three different stratums. The bryophyte species were under the families Polytrichaceae, Mniaceae, Brachytheciaceae, Pterobryaceae, Thuidaceae, Racophilaceae, Leucobryaceae, Meteoriaceae, Fissidentaceae, Hypnaceae, Bartramiaceae, Conocephalaceae, Dumortieraceae, Porellaceae, Aytoniaceae, Lophocoleaceae, Wisnerellaceae, Frullaniaceae and Anthocerotaceae. From the total of 20 different families, the highest number of bryophyte species was in

Meteoriaceae family (10.71%, n=3), followed by Polytrichaceae Mniaceae, Bryaceae, Pterobryaceae, Leucobryaceae, Lophocoleaceae with (7.14%, n=2), and each followed by Brachytheciaceae, Thuidaceae, Bartramiaceae, Racophilaceae, Fissidentaceae, Hypnaceae, Conocephalaceae, Dumortieraceae, Porellaceae, Aytoniaceae, Wisnerellaceae, Frullaniaceae, Anthocerophyta (3.57%, n=1) each (Table 1, 2 &3).

Table 2. Important Value Index of bryophytes

Species	RF%	DR%	IVI
Pogonatum sp.	3.61	4.33	7.95
Plagiomnium sp.	16.28	12.66	28.95
Brachythecium sp.	16.74	17.66	34.4
Calyptothecium sp.	1.8	1.66	3.47
Thuidium sp.	11.31	10.33	21.16
Racophilum sp.	1.35	0.33	1.69
Leucobryum sp.	1.35	1.33	2.69
Meteoriopsis squarrosa	6.33	7	13.33
Bryaceae	2.71	4.33	7.04
Aerobrydium filamentosum	1.35	2.5	3.85
Campylopus sp.	0.45	1.5	1.95
Fissidens sp.	3.61	2.83	6.45
Conocephalum conicum	2.71	1.5	4.21
Anthoceros sp.	3.16	1.16	4.33
Dumortiera hirsuta	2.26	3.5	5.76
Porella sp.	4.07	3.5	7.57
Pterobryopsis sp.	4.52	5.16	9.69
Plagiochasma appendiculatum	0.45	1.5	1.95
Papillaria sp.	1.35	0.33	1.69
Hypnum sp.	1.35	2.16	3.52
Atrichum sp.	1.8	1.66	3.47
Philonotis sp.	0.9	1.16	2.07
Lophocolea sp.	2.71	3	5.71
Wisnerella denudata	0.45	1.33	1.78
Frullania sp.	0.9	1.66	2.57
Bryum sp.	2.26	1.33	3.59
Mnium sp.	1.8	2.16	3.97
Lophocolea bidendata	2.26	2.33	4.59
Total	100	100	200

Important Value Index

The Important Value Index (IVI) is a common ecological assessment technique used to depict the importance of the species in the ecology. IVI helps prioritize conservation of least dominant species since it is associated with dominance of the species. The IVI is calculated using frequency and dominance only, as it is impossible to get the basal area and individual count of bryophytic species (Fajri and Romaidi, 2019). The IVI of the 28 species in 156 plots showed that the most dominant species was *Brachythecium* sp. with an IVI value of 34.408 (Table 4). The highest IVI (34.408) is largely associated to higher values of frequency and dominance relative to other species in the area sampled. There were 2 species (*Racophilum* sp. *and Papillaria* sp.) with the lowest IVI value of 1.6907 due to low frequency and dominance. The high IVI of *Brachythecium* sp. and other species maybe due to the ability of those species to grow in varied substrate in all three strata of river i.e., silt, rock, log and trunk. *Brachythecium* sp. species are terrestrial, epiphytic and lithophytic plants that are distributed around the world (Barton, 1905). The low IVI of *Racophilum* sp. and *Papillaria* sp. maybe due to the infirmity of those species to grow in varied species and are mostly confined to rock and trunk substrate in the study area. *Papillaria* sp. According to Streimann (2012,) the growth of *Papillaria* sp. is restricted to trunk and log with drier habitat with adequate canopy coverage.

Shannon diversity index, Pielou's evenness and Margalef's richness

Shannon diversity index, Pielou's evenness and Margalef's richness was calculated for three strata of river inclusive of lower reaches, middle river and head water. It was mainly calculated to examine the diversity and the distribution of species in the sampled area. The highest diversity (3.317), richness (8.854) and evenness (0.995) were recorded in lower reaches indicating maximum and even distribution of species. The lowest diversity (2.63), richness (6.652) and evenness (0.789) (Figure 5) was observed in head water, which could be due to unfavorable site and micro-habitat for the propagation of bryophytes.

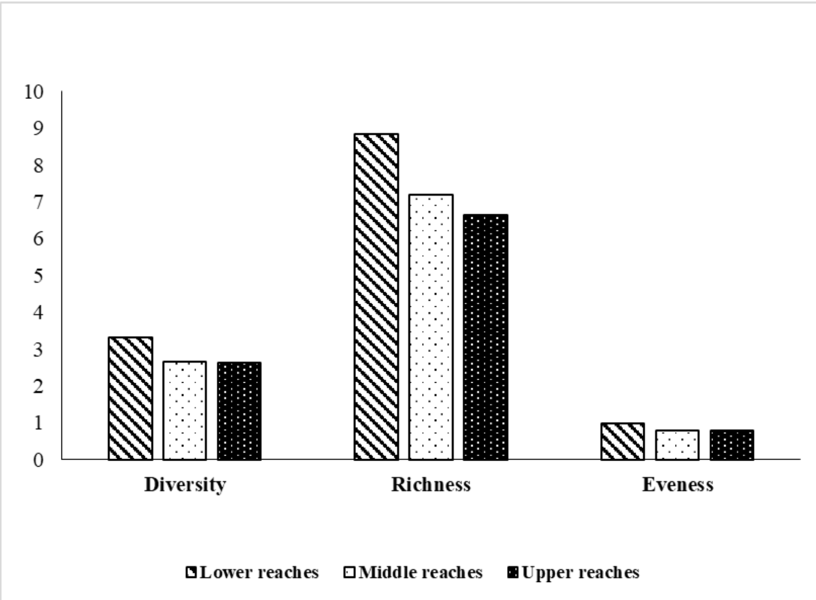


Figure 5. Diversity, evenness and richness

Relative frequency and dominance ratio

Brachythecium sp. was the most dominant species (DR = 17.66) relative to other species and most encountered species in the sampled area with the relative frequency of 16.28. *Papillaria* sp. and *Racophilum* sp. was the least dominate species with dominance ratio of 0.3 and *Campylopus* sp., *Plagiochasma appendiculatum* and *Wisnerella denudata* was the least encountered species with relative frequency of 0.45 (Figure 6). The least dominance of *Papillaria* sp. and *Racophilum* sp. could be due to the selective preference of habitat over the trunk of tree. The least encountering of *Plagiochasma appendiculatum* and *Wisnerella denudata* could be due to the selective preference of habitat over slopes of rocky outcrops. According to Chantanaorrapint and Sridith (2014), it was found that *Plagiochasma appendiculatum* grows on rocky outcrop with calcareous soils avoiding direct sunlight and competition from other plants.

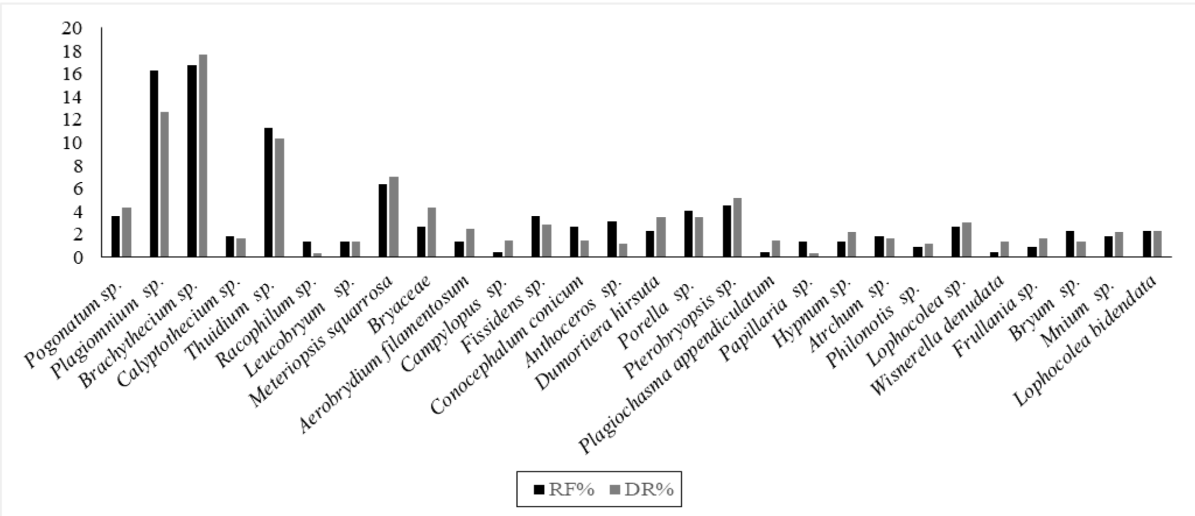


Figure 6. Relative frequency and dominance ratio of different bryophyte species

Comparison between different strata of river

According to the test, there was significant difference (*f* [2,165] =3.48), (*p*=0.03) in diversity among the different strata of river basin. Paired comparison of diversity using Bonferonni post hoc test indicated significant difference between upper strata (M=0.524, SD=0.464) and middle strata (M=0.324, SD=0.341) with the *p* value of 0.03. This be due to the confined area in upper strata than the middle strata and due to the presence of diverse microhabitat over the middle strata.

Distribution of species

Overall distribution of bryophyte based on substrate

The Shannon diversity and evenness was observed highest in silt indicating that the distribution was more preferred to silt than any other substrate. Though maximum of the collected bryophyte species were found to be growing on rock, the most preferred substrate was found to be silt based on the evenness (0.90) and diversity (2.43). The least preferred substrate was log with lowest diversity (1.64), evenness (0.91) and richness (35.26). We can conclude that bryophyte species was mostly distributed on silt substrate and least on log as shown in Table 5. It might be because of presence of diverse micro-habitat present on silt near the river banks. According to the paper by Turner and Pharo (2005), the distribution of bryophyte can be greatly affected by the substrate type on which it grows. They concluded that the bryophyte species were mostly distributed over fallen branches, logs and silt.

Table 3. Overall distribution of bryophytes based on substrate

Index	Substrate		
	Log	Rock	Trunk
Shannon Diversity, H'	1.64	2.31	1.69
Pielou's Evenness, J'	0.91	0.74	0.77
Margalef Richness, Dmg	35.26	550.44	114.86
Sorensen's Similarity, QS	0.17		

Distribution of mosses, liverwort and hornwort based on substrate

Basically, the distribution of moss, liverwort and hornwort were studied based on the substrate (rock, silt, trunk, log) on which it's thriving. Generally, mosses are more affined to rock substrate (23%) followed by substrate trunk (20%) and liverworts species are more distributed to log (10%) substrate, followed by silt (6%). Similarly, hornwort species is confined to silt substrate and it's not found in other substrate in my study area. The confined distribution of hornwort to silt could be due the absence of moist micro-environment in other substrate (Figure 7). Similarly, it is reported that mosses are mostly grown over rock, trunk, and forest floor (Abdullahi, 2018). Simpson (2010) stated that liverworts are mostly adhered to wet silt and substrate with moist surface. Posey (2022) also reported that hornworts are mostly found growing on damp areas including silt, rock and sand. From above observation it is evident that mosses exhibit broad habitat tolerance, thriving in both moist and dry environments, while liverwort possess moderate dry tolerance and hornwort i.e., *Anthoceros* sp. is hygrophilous, requiring moist habitat. The above observed variation may be due to the absence of fleshy morphology in mosses and leafy liverwort, the presence of cuticles on thallose of liverwort and entire morphology of fleshy thallose in hornwort (*Anthoceros* sp.) respectively. Thus, it is concluded that if the habitat degradation along the river intensifies, it may cause threat to the restricted species particularly *Anthoceros* sp.

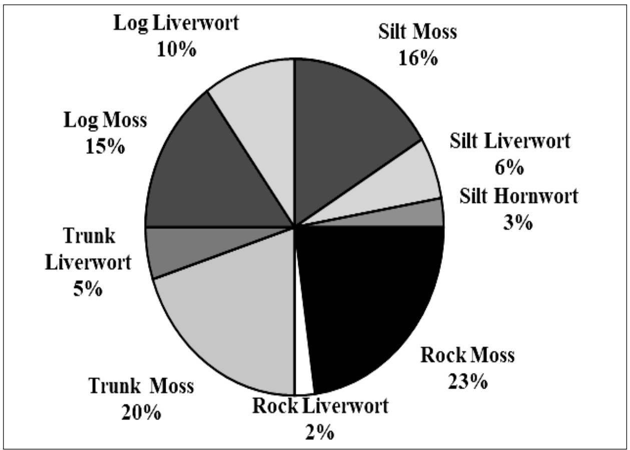


Figure 7. Distribution of mosses, liverwort and hornwort in different substrate

Elevation and species count

The non-parametric spearmen correlation result showed negative and no significant relationship between the numbers of species and elevation (r=0.04, p = 0.958) a show in Figure 8. Usually, the relationship between elevation and species diversity of bryophyte is either increasing, decreasing or else a humped relation (Sun *et al*., 2013). Altitude factor probably do not influence in species diversity. The humped relation was observed in convergence and Sun *et al*. (2013) observed with increase in the species diversity count after first hump, which might be due to the presence of different micro-habitat and less disturbances from grazing cattle and human activities. Sun *et al*. (2013) found humped relationship between bryophyte species and elevation, as there was increase in the species diversity after the first hump (r=0.605, p=0.01). The increase in the diversity count after first hump was due to the abrupt change of vegetation community from forest to open alpine shrub land.

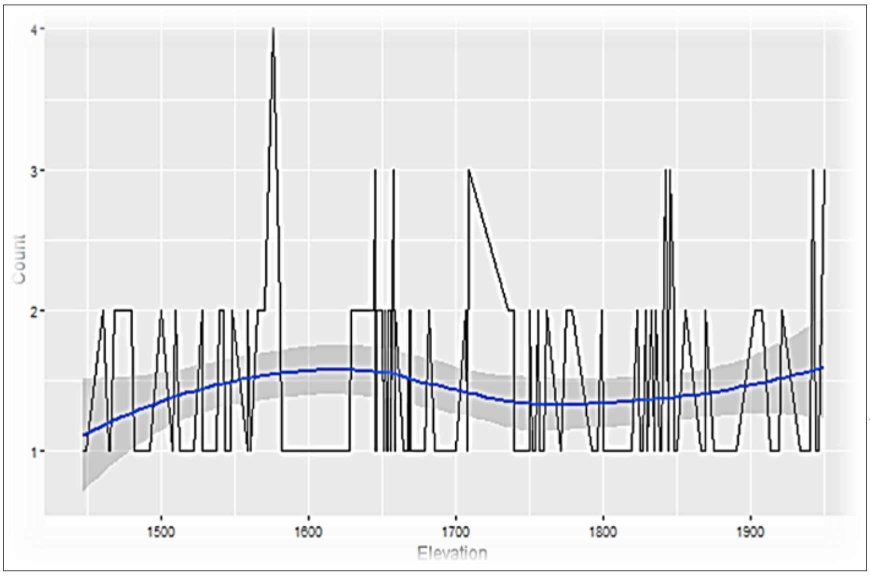


Figure 8. The correlation between altitude and bryophyte species count

Over all relationship between bryophyte coverage and canopy closures

A test of regression was conducted to examine how canopy closure affects the bryophyte coverage in the sampled area. The result showed no significant relationship between coverage and canopy closure (r=0.27, p=0.61). This might be due the disturbances of forest floor by grazing cattle and other anthropogenic activities that actually affects the growth and coverage of bryophytic species as bryophytes are disturbance sensitive species. As per sun *et al*. (2013) they found out a positive significant relationship (r=0.91, p=0.05) between the canopy and the bryophyte coverage. It could be because their study was conducted in a forest where it had less anthropogenic disturbances.

Distribution of moss, liverwort and hornwort based on canopy closure

Distribution of mosses, liverwort and hornwort were studied based on the canopy closure. Above graph displays that, mosses are widely found growing under varied canopy cover ranging from open (0%) to close (100%) with the average canopy closure of 44.15%. On the other side, liverworts and hornworts was present mostly in area with partial (20%) to close (100%) canopy closure (Figure 9), with the average canopy closure of 60% and 58.33% respectively. It can be concluded that mosses can adapt and thrive in varied range of habitat ranging from dry to moist habitat, whereas liverwort and hornwort was found in moist habitat. According to Gradstein and Yanez (2020) study, it was also reported that liverworts and hornwort are mostly found thriving in moist areas with high canopy closure (50-100%).

Bryophyte as ecological indicator

Generally leafy liverworts and pleucarpous mosses are sensitive to pollution and are used as ecological indicator. Although no in-depth study was done to evaluate and analyze the level of pollution in the study area, however according to an article by Wielgolaski (1975), ectohydric leafy liverwort and pleucarpous mosses are used to determine the pollution as they are sensitive to pollutants. The high frequency and dominance of leafy liverwort (*Lophocolea bidendata,* 2.26, 2.33) and pleucarpous moss (*Thuidium* sp.11.31, 10.33) were present along the entire river length, from which it can be concluded that there was no major pollution.

Figure 9. Distribution of hornwort, liverworts and mosses based on canopy closure

Conclusion

From the findings of the study, it can be concluded that there was high diversity of bryophyte along Pasaphu-Mochhu River under Trashigang with 28 species of bryophytes (19 were mosses, 8 were liverworts and 1 was hornwort species). A total of 6 bryophytes with medicinal values were documented, of which 3 were mosses and other 3 were liverworts. No new species were recorded from the study area. Of all the most dominant species found was *Brachythecium* sp. A decreasing trend of species diversity with altitude was observed probably due to the absence of good canopy closure in higher altitude. No influence of canopy closure on the coverage of bryophyte as the studied area was under the disturbances of grazing cattle and anthropogenic activities. This study could serve as a baseline information on the general knowledge of bryophyte, however there is a need for in-depth account study because there could other possible environment factor that affect bryophytes. Also, future researchers are suggested to conduct the study during the dry season with enrichment of different minute parameters such as precipitation, slope, aspect, soil type, moisture level, temperature and soil. Further, findings of bryophyte diversity require long term but very limited attempted to study or continue with the same topic, thus dire in the need of long-term study to ensure the good quality outcome. This paper also underscores and calls for similar research to be conducted over different forest type and river basins to observe the comparative differences and their variation in diversity and distribution.

References

Abdullahi, S. (2018). Occurrence and distribution of moss plant in relation to substrate type in selected parts of north western Nigeria. *FUW Trends in Science & Technology Journal,* 3(1): 292 – 294

Azuela, G. N., Sariana, G. L., & Pabualan, P. M. (2010). Diversity and ecological status of bryophytes inMt.

Kitanglad, Bukidnon. *Asian Journal of Biodiversity,* 1(1), https://doi.org/10.7828/ajob.v1i1.102
Barton, W. P. C. (1905). Flora of North America. 28, 404. https://floranorthamerica.org
Bates, W. J., & Farmer, M. A. (1992). Bryophytes and lichens in a changing environment. *Forestry, 66(1), 1993.* https://www.academia.oup.com
Binet, A. (2014). Living: bryophytes have certainly evolved. <https://www.reflexions.uliege.be>. Accessed 1 March 2024.
Bruno, J. S., Caram, J. S., Dias, N. S., & Cavalcanti, K. P. (2018). Bryophyte richness of soil island on rocky outcrops is not driven by island size or habitat heterogenic. *Acta Butinica Brngilica,* 32(2), 161-168. https://doi.org/10.1590/0102-330620179660281
Buck, W. R., & Thiers, B.M. (1996). *Guidelines for collecting bryophytes. In: ALEXIADES, M.N. (Ed.). Selected Guidelines for Ethnobotanical Research: A Field Manual*. New York Botanical Garden, New York: 143-146.
Callaghan, D. A. (2008). Scale dependency and area of occupancy: Tortula freibergii in north-west England. *Journal of Bryology* 30 (5), 279-282.
Chandra, S., Chandra, D., Barh, A., Pankaj, Pandey, R. K., & Sharma, I. P. (2016). Bryophytes: Hoard of rem edies, an ethno-medicinal review. *Journal of Traditional and Complementary Medicine.*7 (1), 94-98.https://doi.101016/j.jtcme.2016.01.007
Chantanaorrapint, S., & Sridith, K. (2014). The genus *Plagiochasma* (Aytoniaceae, Marchantiopsida) in Thailand. *Cryptogamie Bryoligie.* 35 (2), 137-132.
David, A. D., & Angel, B. (2019). Bryophyte community along a tropical urban river respond to heavy metal and arsenic pollution. *Water,* 11(4), 813. https://doi.org/10.3390/w1104813
Dorji, L. (2021). Diversity and conservation challenges of bryophytes in Merak and Sakteng Gewogs (semi-nomadic communities) under Trashigang District, Bhutan. The *Rufford Foundation.*
Dzongkhag Administration. (2018). *Trashigang Dzongkhag.* <https://trashigang.gov.com>. Accessed 23 March 2023.
Edward, C. U. (2001). Maryland Bryophytes. *Journal of Bryophytes,* 20(2), 101.
Evans, A. S., Halpern, B. C., & McKenzie, D. (2012). The contribution of forest structure and substrate to bryophyte diversity and abundance in mature coniferous forests of the Pacific Northwest. *The Bryologist,* 278-294.
Fariji, T. M., & Romaidi. (2018). Mosses diversity of Tumpak Sewu waterfall, Lamujang, East Java. *Journal Biologi,* 6 (4), 152-158. https://doi.org/10.18860/elha.v6i4.5883
Gecheva, G., & Yurukova, L. (2013). Quality monitoring by aquatic bryophyte. *Journal of Environmental Chemistry for a Sustainable World,* 3(9), 415-448. https://doi.org/10.1007/978-94-007-6836-9_9
Glime, J. M. (2017). Field Taxonomy and Collection Methods. Chapt. 1-1. In: Glime, J. M. Bryophyte Ecology, 3. https://digitalcommons.mtu.edu/bryophyte-ecology/.
Goffinet, B & Shaw, J. A. (2008). *Bryophyte Biology*. Cambridge University Press: United Kingdom.
Gradstein, R. S., & Yonez, L.S. (2020). Liverwort diversity in Polylepis Pauta forest of Ecuador under different climatic conditions, *Neotropical Biodiversity*, 6 (1), 138-146. https://doi:10.108023766808.2020/1809273
Gunathilaka, L, K. (2019). A review of bryophytes, evaluation value and threats. *International Journal of Scientific and Research Publication,* 9(5), 384-389. https://doi.org/10.29322/USRP-9.05.2019.P8946
Juyal, P. (2015). Biology and diversity of algae, bryophyta and pteridophyta. *Journal of Bryophyta,* 30(3), 47.
Jiang, T., Yang, X., Zhong, Y., Tang, Q., & Su, Z. (2018). Species composition and diversity of ground bryophytes across a forest edge-to-interior gradient. *Sci Rep*, 8, 11868. https://doi.org/10.1038/s41598-018-30400-1
Kumar, P. V., Afroz, A., & Rawat, K. K. (2013). Assessment of liverworts and hornworts flora of Nilgiris, Western Ghats (India). *Polish Botanical Journal*, 58(2), 525-537. https://doi.org/102478/pbj-2013-0038
Lepp. H. (2012). *Australian bryophytes.* https://www.anbg.gov.au/bryophyte/references/reference-34.html.
Long, D. G. (1994). Mosses of Bhutan II*. A checklist of the mosses of Bhutan, *Journal of Bryology*, 18(2), 339-364. https://doi.org/10.1179/jbr.1994.18.2.339
Long, D. G., Gurung, R. B., & Pradhan, R. (2009). *The rediscovery and conservation status of Bazzania bhutanica in Bhutan.* <http://biodiversity.bt/biodiv/content/documents/document-e64c7ad8-293b-4d97-83e7-

3b5f1d8d48eb/779.pdf>. Accessed 23 December 2023.

Marko, S., Aneta, B., & Dragoligab, G. (2001). Bryophyte as a potential source of medicinal compound. *Journal of Academia,* 40(2).

Molder, A., Schmidt, M., Schonfelder, E., & Engel, F. (2015). Bryophyte as indicator of ancient woodlands in Schleswig-Holstein (Northern Germany), *Ecological Indicators,* 54, 12-30. https://doi.org/10.1016/j.ecolind.2015.01.044

Monika, S., Jan, E., &Ewa, S. (2021). Diversity and composition of moss guilds on uprooted trees in central European mountain forests: effects of uprooting components and environment variables. *Annuals of Forest Science,* 45(78).

National Biodiversity Centre. (2014). *National Biodiversity Strategies and Action Plan of Bhutan*, Thimphu. Royal Government of Bhutan: Ministry of Agriculture and Forests.

Parker, S. (2018). *Aos Protocol and Procedure: Aquatic Plant, Bryophytes, Lichen and Microalgae Sampling*. <https://www.data.neoscience.org>. Accessed 23 December 2023.

Parveen, R. (2014). *Bryophytes.* <https://magadhuniversity.ac.in/download/econtent/pdf/BRYOPHYTES.pdf>. Accessed 24 December 2023.

Porley, R. (2008). *Arable Bryophytes, a field guide to the mosses, liverworts and hornworts of cultivated land in Britain and Ireland*. Wild Guides, Old Basing: 140 pp.

Posey, L. (2022). *Hornworts: Types, Characteristics & Facts.* <https://www.annforsci.biomedcentral.com>. Accessed 1 January 2024.

Sabovijevic, S. M., Aneta, S. D., & Sabovijevic, D. M. (2020). Bryophytes: Introductory Chapter. *Journal of Intech Open.* https://doi.org/10.5772/intechopen.91420

Simpson, G. M. (2010). *Evolution and Diversity of Green and Land Plants*. Plant systematics. Academic press.

Stagg, B., Donkin, M. E., & Smith, A. M. (2015). Bryophytes for Beginner: The usability of a printed dichotomous key versus multi-access computer-based key for bryophyte identification. *Journal of Biological Education,* 49(3), 274-2876. https://doi.org/10.00219266.2014.93900

Stieperaere, H., Heylen, O., & Podoor, N. (1997). Differences in Species Composition of the Bryophytes layer of some Belgium and Dutch pinewoods with and without the invading hepatic. *Journal of Bryology,* 19(3), 425-434. https://doi:10.1179/jbr.1997.19.3.425

Stotler, J. B., & Began, B. E. (2007). *Morphology of mosses.* <http://www.mobot.org/plantscience/bfna/F27/27-00BMorphology.pdf>.

Streimann, H. (2012). Australian Mosses. <https://www.anbg.gov.au/abrs/Mosses_Online/Papillaria.pdf(2012)>.

Sun, S. Q., Wu, Y. H., Wang G. X., Zhou, J., & Yu, D. (2013). Bryophyte species richness and composition along and altitudinal gradient in Gongga Montain, China. *PLOS ONE* 8(3). https://doi.org/10.1371/journal.pone.0058131

Vitt, H. D., Finnegan, L., & House, M. (2019). Terrestrial Bryophyte and Lichen Response to Canopy Opening in Pine-Moss-Lichen Forests. *Forests,* 10(233).

Whitehouse, H.L.K. (1966). The occurrence of tubers in European mosses. *Transactions of the British Bryological Society* 5(4), 103-116.

Wielgolaski, F. E. (1975). Biological indicators on pollution. *Urban Ecology I*(I), 63-79. https://doi.org/10.1016.0304-4009(75)90005-4

World Flora Online. (2013). *The Plant List.* <https://www.theplantlist.org>. Accessed 1 June 2023.

Zechmeister, H. G., Breure, M. a., & Markert, A. B. (2003). Trace Metal and other Contaminants in the Environment. *Research Gate,* 329.