Assessment of bryophyte diversity in selected localities of Assam, North East India: a quantitative approach

Priyanshu Srivastava, Vinay Sahu and Ashish K. Asthana*

CSIR-National Botanical Research Institute, Lucknow 226 001, India

A study on the quantitative estimation of diversity in 32 selected grids in the bryophyte-rich localities of Assam, North East India has been carried out. Assessment was done in 160 macroplots of 10 m × 10 m, randomly established in the forest and within each macroplot, five microquadrats (10 cm × 10 cm) were placed at different habitats, viz. saxicolous, terricolous and epiphytic. A total of 80 taxa belonging to 29 species of liverworts under 18 genera and 10 families, and about 51 species of mosses belonging to 27 genera and 13 families were assessed. In the study area, Lejeuneaceae and Fissidentaceae as well as *Cololejeunea latilobula* (Herzog.) Tixier and *Entodontopsis tavoyensis* (Hook ex Harv.) W. R. Buck & R. R. Ireland were the dominant families and taxa respectively. Four species were new additions to NE India, and seven taxa were reported for the Assam region. The present study elucidates the bryophyte species diversity and the species richness, and evenness of the region, which can further define their importance in the community.

Keywords: Bryophytes, diversity assessment, evenness, quantitative estimation, species richness.

BRYOPHYTES play a crucial role in sustaining plant community structure and are an important aspect of our ecosystem. In this study, we performed a quantitative assessment of bryophyte diversity in selected regions of Assam, North East India. This state has three physiographic divisions which favour the unique diversity of flora and fauna. The assessment was done by measuring different diversity indices and species abundance in the communities[1]. These indices mainly deal with the species richness (no. of species) and species evenness (how uniformly abundant the species are in a community)[2]. They provide information on the sampling data, species diversity and species importance in the community. There are numerous indices to evaluate species diversity between different communities. These diversity indices can be translated into information for bioresources inventory, mapping, livelihood and economic value. The present study broadens the spectrum of diversity indices by finding Simpson index, Shannon–Weiner index, important value index (IVI), species-area curve and Raunkier's law of frequency of these cryptogams (bryophytes) in Assam. In fact, the quantitative study can be further used to assess its importance in the community as these cryptogamic plants have helped maintain the community structure since the Ordovician period.

The present study is based on the earlier works on this aspect done by renowned workers. Significant contributions have been made to quantitative community analysis related to bryophyte ecology in Signy Island, Antarctica[3]. Lee and La Roi[4] worked on gradient analysis of bryophytes in Jasper National Park, Alberta, Canada. Later, Bates[5] deliberated upon the quantitative approaches in bryophyte ecology. Krebs[6] discussed the importance and problems associated with ecology. Wolf[7] studied diversity patterns and biomass of epiphytic bryophytes and lichens. Pharo and Beattie[8] described regional species richness and species-turnover of bryophytes and lichens of Australia. Detailed studies were made on the application of diversity indices to determine plant availability in traditional medicinal markets of South Africa[9]. Newmaster *et al.*[10] compared plot sampling and floristic habitat sampling to estimate bryophyte diversity. Mandl *et al*.[11] compared alpha and beta diversity patterns of ferns, bryophytes and macrolichens. Mokany *et al.*[12] contributed by combining alpha and beta diversity models. Hofmeister *et al.*[13] quantitatively assessed old forests related to cryptogam species richness.

Studies related to quantitative assessment of non-flowering plants are sporadic in India. Pande[14] studied the ecophysiological aspects of bryophytes in Nainital Hills, Uttarakhand. Tewari *et al*.[15] studied the epiphytic succession in three major tree species – *Cedrus deodara, Quercus floribunda* and *Quercus leucotrichophora* in a wet forest of Kumaun Himalaya, and found that total biomass and species number per unit area increased with trunk size. Awasthi *et al*.[16] studied bryophyte diversity on the stem surface of *Erythrina arborescens* and observed that 17 moss species grew on the tree bark while liverworts were not found. Bargali *et al*.[17] studied bryophyte vegetation associated with *Platanus orientalis* L. and compared it with other tree species of Nainital. They found that 15 species were common to the dominant trees of that locality, while 12 species were confined to *P. orientalis*. Studies on the habitat distribution of several acrocarpous mosses were also done[18]. Earlier studies from Assam revealed around 143 liverworts and hornworts[19]. Barukial[20] studied the ecological assessment of 162 taxa of bryophytes under 90 genera and 30 families with reference to their habitat. He estimated the frequency of diverse microhabitats: 52.3% (terricolous), 35.27% (epiphytic) and 8.72% (aquatic)[20].

Assam is situated south of the Eastern Himalayas. The climate of the state is tropical monsoon experiencing heavy rainfall with high humidity. Figure 1 is a map showing the localities.

The study was carried out in 32 grids of high and moderate priority in Assam from 10 October to 1 November 2018. In each grid, five random macroplots of 10 m × 10 m were laid, and within each macroplot, five quadrats of 10 cm × 10 cm were placed at different habitats, viz. saxicolous, terricolous and epiphytic. The quantitative data as percentage

*For correspondence. (e-mail: drakasthanaster@gmail.com)

cover were chosen. Further, plants were air-dried and transferred to brown paper packets. Plant samples were soaked and washed in tapwater for morphological and anatomical study and were mounted in 30% glycerine for further examination under the microscope. The specimens were deposited in the Bryophyte Herbarium, CSIR-National Botanical Research Institute, Lucknow (LWG). The vegetation data were estimated for density (total no. of individuals/no. of quadrats studied), relative density (RD = density of a given species × 100/sum of density), abundance (total no. of individuals/no. of quadrats in which the species occur), relative abundance (RD = abundance of a given species × 100/sum of abundance), frequency (total no. of quadrats in which the species occur/total no. of quadrats × 100), frequency class (according to Raunkier's law; class A = 0–20; class B = 21–40; class C = 41–60; class D = 61–80; class E = 81–100), relative frequency (RF = species occurrence × 100/count of all frequencies), IVI (RD + RF + RA), Simpson index[21] and Shannon–Weiner index[21].

Analysis of bryophyte specimens in the 32 grids revealed the occurrence of 80 species in the study area (Supplementary Table 1). The study has revealed four new records for NE India, viz. *Fissidens biformis* Mitt., *Fissidens orishae* Gangulee, *Fissidens zollingeri* Mont. and *Entodontopsis tavoyensis* (Hook ex Harv.) W. R. Buck & R. R Ireland and new records for Assam, viz. *Hydrogonium consanguineum* (Thw. et Mitt.) Hilp., *Lopholejeunea abortiva* (Mitt.) Stephani, *Oxystegus cylindrothecus* (Mitt.) Gangulee, *Philonotis leptocarpa* Mitt., *Riccia sorocarpa* Bisch, *Solmsiella biseriata* (Austin) Steere and *Thuidium venustulum* Besch. The data demonstrate a greater representation of mosses than liverworts in the study area. The most dominant family of liverworts in the study area was Lejeuneaceae with 11 taxa, followed by Jungermanniaceae with six species, while the dominant moss family was Fissidentaceae with 15 taxa, followed by Pottiaceae with eight taxa and Hypnaceae with seven species. Within the study area, species with maximum percentage cover were *Fissidens ceylonensis* Dozy & Molk., *Gemmabryum apiculatum* (Schwagr.) J. R. Spence & H. P. Ramsay, *Philonotis mollis* (Dozy & Molk.) Mitt. and *Riccia sorocarpa* Bisch. In terms of density, species such as *Fissidens ceylonensis* (20), *Gemmabryum apiculatum* (20), *Philonotis mollis* (20), *Ectropothecium cyperoides* (Hook. Ex Harv.) A. Jaeger (19.1) and *Solenostoma hyalinum* (Lyell) Mitt. (19) were densely populated. According to Raunkier's law, *Entodontopsis tavoyensis* and *Erpodium glaziovii* Hampe belonged to class E (most frequent), while *Barbula indica* (Hook) Spreng., *Cololejeunea latilobula*, *Fissidens virens* Thwait ex Mitt., *Fissidens zollingeri* Mont., *Lopholejeunea* sp1, and *Garckea phascoides* (Hook.) Müll. Hal. belonged to class D. The species were placed in their respective classes according to Raunkier's law. The study depicts the maximum number of species in class A (41 taxa), followed by classes B (19 taxa), class C (12 taxa), class D (6 taxa) and class E (2) taxa (Figure 2). The most abundant species in the study area were *Fissidens ceylonensis* (100), *Gemmabryum apiculatum* (100), *Philonotis mollis* (100) and *Riccia sorocarpa* (100). The Simpson index showed a high richness value of 0.98 in terms of species abundance and evenness, while Shannon–Weiner index showed more richness and evenness with a value of 3.8. The study area has rich bryophyte species diversity as the number of species increases

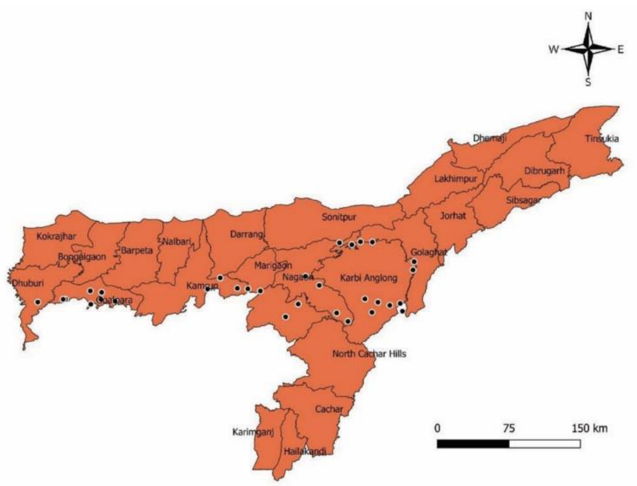

Figure 1. Map showing the study localities in Assam, North East India.

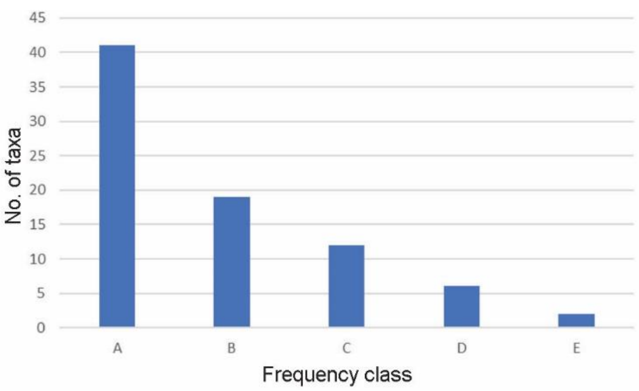

Figure 2. Distribution of bryophyte species in their respective classes.

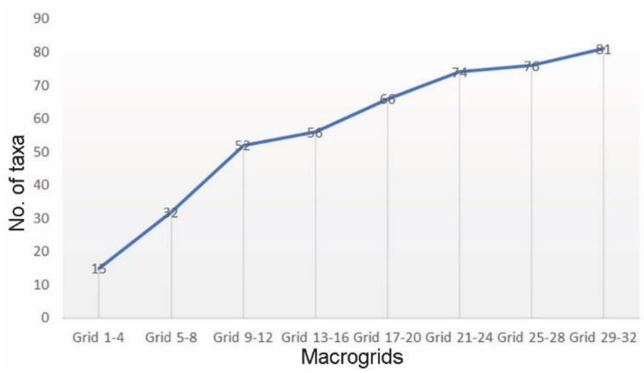

Figure 3. Species area curve.

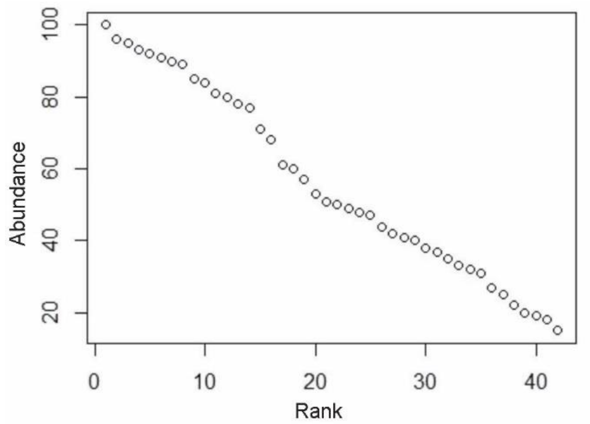

Figure 4. Whittaker plot or rank abundance curve.

(Figure 3). IVI has been used in this study because it reveals the ecological importance of a species in a given community. It also helps prioritise species conservation, whereby species with a low IVI value need higher conservation priority than those with a high IVI value. The high IVI value exhibited by many species is likely due to their higher relative frequencies, densities and abundance compared to other species (Supplementary Table 1). The presence of many species with low IVI values reveals that they are rare in the study area and need immediate conservation measures. *C. latilobula* occurred in about 71 microquadrats (10 cm × 10 cm), followed by *E. tavoyensis*, which occurred in about 60 microquadrats. The dominant species occurring in several localities were *C. latilobula* recorded from 15 sites, followed by *E. tavoyensis* reported from 13 sites, *Archilejeunea minutilobula*, *Calymperes tenerum* and *Fissidens zollingeri* encountered from eight sites each and *Erpodium glaziovii*, *Fissidens virens* and *Garckea phascoides* recorded at seven sites each. The sites having rich species diversity were Pudum Pukhuri, with 15 species belonging to 14 genera and 12 families; Lokhra Hills, with 13 species belonging to 12 genera and 11 families and Tegharia waterfall, with 12 species belonging to 10 genera and 9 families. Localities like Hathikhuli, Naambor Habi and Balijana revealed the occurrence of 11 taxa of liverworts and mosses. Whittaker plot or rank abundance curve depicts species richness and evenness (Figure 4). Species are ranked according to their abundance (no. of individuals), i.e. the most abundant species is given rank 1, the second most abundant species is given rank 2, and so on. Species richness can be observed as the number of species and species evenness, i.e. a steep gradient indicates low evenness among species and a shallow gradient indicates that the study site has high evenness as the abundance (no. of individuals) of species was same. Species such as *Fissidens ceylonensis*, *Gemmabryum apiculatum*, *Philonotis mollis* and *Riccia sorocarpa* were given rank 1 as they had the same abundance values. Likewise *Hyophila involuta* (rank 2), *Ectropothecium cyperoides* (rank 3), *Solenostoma hyalinum* (rank 4), *Fissidens crispulus* var *robinsonii* (Broth.) Z. Iwats. (rank 5), etc.

The present study has added some new findings to NE India[22] and Assam. Using a different approaches to assess the bryophyte diversity, it has been found that the species are dominant in some localities compared to others. Simpson index (0.98) provides data regarding species abundance and evenness, whereas Shannon–Weiner index (3.8) indicates richness and evenness. Species area curve clearly indicates the rich bryophyte diversity in the study area as the number of species is on the increase. Furthermore, IVI values predict important species, i.e. taxa with a low IVI value need higher conservation priority compared to those with a high IVI value.

1. Ludwig, J. A. and Reynolds, J. F., *Statistical Ecology – A Primer on Methods and Computing*, John Wiley, Toronto, Canada, 1988, pp. 107–202.
2. Magurran, A., *Ecological Diversity and its Measurement*, Chapman & Hall, Princeton, New Jersey, 1988, pp. 1–179.
3. Gimingham, C. H., Quantitative community analysis and bryophyte ecology on Signy Island. *Philos. Trans. R. Soc. London, Ser. B*, 1967, **252**, 251–259.
4. Lee, T. D. and La Roi, G. H., Gradient analysis of bryophytes in Jasper National Park, Alberta. *Can. J. Bot*., 1979, **57**, 914–925.
5. Bates, J. W., Quantitative approaches in bryophyte ecology. In *Bryophyte Ecology* (ed. Smith, A. J. E.), Chapman & Hall, Dordrecht, the Netherlands, 1982, pp. 1–44.
6. Krebs, C. J., Overexploited populations can collapse. In *The Message of Ecology*, Harper & Row, New York, USA, 1988, pp. 1–195.
7. Wolf, J. H. D., Diversity patterns and biomass of epiphytic bryophytes and lichens along an altitudinal gradient in the northern Andes. *Ann. Mo. Bot. Gard*., 1993, **80**, 928–960.
8. Pharo, E. J. and Beattie, A. J., Bryophyte and lichen diversity: a comparative study. *Austral. Ecol.*, 1997, **22**, 151–162.
9. Williams, V. L., Witkowski, E. T. F. and Balkwill, K., Application of diversity indices to appraise plant availability in the traditional medicinal markets of Johannesburg, South Africa. *Biodivers. Conserv*., 2005, **14**, 2971–3001.
10. Newmaster, S. G., Belland, R. J., Arsenault, A., Vitt, D. H. and Stephens, T. R., The one we left behind: comparing plot sampling and floristic habitat sampling for estimating bryophyte diversity. *Divers. Distrib*., 2005, **11**, 57–72.
11. Mandl, N., Lehnert, M., Kessler, M. and Gradstein, S. R., A comparison of alpha and beta diversity patterns of ferns, bryophytes and macrolichens in tropical montane forest of southern Ecuador. *Biodivers. Conserv*., 2010, **19**, 2359–2369.
12. Mokany, K., Harwood, T. D., Overton, J. Mc. C., Barker, G. M. and Ferrier, S., Combining alpha and beta diversity models to fill gaps in our knowledge of biodiversity. *Ecology*, 2011, **14**, 1043–1051.
13. Hofmeister, J. *et al*., Value of old forest attributes related to cryptogam species richness in temperate forests: a quantitative assessment. *Ecol. Indic*., 2015, **57**, 497–504.
14. Pande, N., Ecophysiological studies of bryophytes in Nainital Hills. Ph.D. thesis, Kumaun University, Nainital, 1984.
15. Tewari, M., Upreti, N., Pandey, P. and Singh, S. P., Epiphytic succession on tree trunks in a mixed oak–cedar forest, Kumaun Himalaya. *Vegetatio*, 1985, **63**, 105–112.
16. Awasthi, V., Pande, R. and Pande, N., Bryophyte Diversity on *Erythrina arborescens* in Nainital. *Proc. Natl. Acad. Sci. India Sect. B*, 2013, **83**, 461–464.
17. Bargali, R., Awasthi, V. and Pande, N., Ecological study of bryophytes on *Plantanus orientalis* L. trees in Nainital (Western Himalaya). *Am. J. Plant Sci*., 2014, **5**, 3880–3888.

18. Gupta, R. and Asthana, A. K., Studies on species composition and diversity of acrocarpous mosses at Pachmarhi Wildlife Sanctuary, Madhya Pradesh (India). *Indian For.*, 2018, **144**(6), 588–591.
19. Singh, D. K., Singh, S. K. and Singh, D., *Liverworts and Hornworts of India: An Annotated Checklist*, Botanical Survey of India, Kolkata, 2016, pp. 1–439.
20. Barukial, J., A Bryofloristic ecological assessment of Assam, India. *Indian J. Fundam. Appl. Life Sci*., 2011, **1**(3), 98–106.
21. Magurran, A. E., An index of diversity. In *Measuring Biological Diversity*, Blackwell Publishing, Victoria, Australia, 2005, pp. 1–215.
22. Ellis, L. T. *et al.*, New national and regional bryophyte records. *J. Bryol*., 2019, **59**, 1–18.

ACKNOWLEDGEMENTS. We thank the Director, CSIR-National Botanical Research Institute, Lucknow for encouragement and providing the necessary facilities and the Department of Biotechnology, New Delhi for financial assistance. We also thank the Forest Department, Assam for help. Thanks are also due to Ethical Committee of CSIR-NBRI for providing manuscript no. CSIR-NBRI_MS/2020/05/12 for the present paper.

Received 19 May 2020; revised accepted 19 January 2023

doi: 10.18520/cs/v124/i7/848-851

Identifying ticks of genus *Hyalomma* using the *COI* gene from preserved old specimens – a significant approach for controlling zoonotic diseases[†]

Ankita Rajpoot[1], Shelley Acharya[2] and Archana Bahuguna[3,*]

[1]Maaty Biodiversity Conservation and Societal Research Organization, Dehradun 248 001, India
[2]Zoological Survey of India, Prani Vigyan Bhawan, M-Block, New Alipore, Kolkata 700 053, India
[3]Northern Regional Centre, Zoological Survey of India, 218, Kaulagarh Road, Dehradun 248 195, India

Ticks are vectors for a range of human and animal diseases. Accurate species identification is a crucial step for effective pest management, as each species plays host to specific parasites. Species identification based on morphological characteristics is prone to error in cryptic species. Molecular techniques have been used in recent times for accurate species identification; however, few studies are available on Indian tick species. The present study aims to bridge this gap in species identification of *Hyalomma* ticks from India using conventional morphological and recent molecular methods. We also studied the evolutionary relationships between species using a phylogenetic approach. The study included historical samples (N = 14) representing four species obtained from the National Zoological Collection of the Zoological Survey of India, Kolkata. Genetic analysis was done using universal barcoding with COI primers. The results indicate a 99–100% match between the genetic and morphological analyses for the four samples of *Hyalomma* species collected, i.e. *Hyalomma hussaini*, *Hyalomma aegyptium*, *Hyalomma kumari* and *Hyalomma anatolicum*. The findings were also supported by phylogenetic and evolutionary tree analyses. The present study is helpful in identifying tick species using integrated approach, interpreting evolutionary relationships between different species, and solving taxonomic problems.

Keywords: Accurate species identification, evolutionary divergence, genetic analysis, *Hyalomma*, phylogenetic tree, zoonotic diseases.

TICKS are arthropod (class Arachnida) vectors of various animal and human diseases (e.g. Rocky Mountain spotted fever, tick-borne relapsing fever, Q fever and Lyme disease) and significantly impact the health of human beings, livestock and wild animals[1,2]. They are divided into two major families – Ixodidae (hard ticks; n = 650 species) and Argasidae (soft ticks; n = 150 species) with a global distribution. The genus *Hyalomma* of family Ixodidae includes about 30 species and subspecies that inhabit areas with the long dry seasons in Asia, Europe and Africa[3]. *Hyalomma hussaini*, *Hyalomma aegyptium*, *Hyalomma kumara* and *Hyalomma anatolicum* are of significant human and animal health importance[3–6]. *Hyalomma* tick fauna of India is characterized by the presence of several distinct endemic species that have small-sized individuals. The genus includes several medically important species that act as vectors of Crimean–Congo hemorrhagic fever (CCHF) in parts of Europe, Asia and Africa[7–12]. In 2011, Gujarat was the first state to confirm CCHF in India[13] and *H. anatolicum* was being identified as the vector. Ticks are also pests of livestock such as camels, cattle, sheep and goat[14,15]. Therefore, accurate species identification plays a critical role in the management of tick-borne zoonotic diseases.

Conventionally species identification of ticks has primarily relied on morphological characters, which are typically restricted by sampling conditions, high hybridization rates and morphological similarity. Due to its adverse impact on human as well as livestock health, a rapid and dependable identification technique is necessary[16]. Over the past several years, molecular technology has evolved to provide methods for rapidly and accurately identifying tick species[17–19]. DNA barcoding-based approach has been widely accepted for its efficacy in discriminating ticks at species level[19,20]. Several molecular markers have been utilized to resolve the problems related to taxonomy of the family Ixodidae[21–23].

[†]The data that support the findings of this study are available in GenBank (https://www.ncbi.nlm.nih.gov/genbank/; accession number MW587123-MW587126).
*For correspondence. (e-mail: archana.bahuguna65@gmail.com)