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continued on the back inside cover

Cover: Orange Oakleaf *Kallima inachus* with colour pencils and watercolor wash by Elakshi Mahika Molur adapted from a workshop by Lenin Raj.

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COMMUNICATION

OPEN ACCESS

The study of biogeographic patterns of the genus *Parmotrema* in Wayanad District, Kerala with a new record in India

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Abstract: This research focuses on Wayanad District within the Nilgiri Biosphere Reserve of the Western Ghats, a renowned hotspot for lichen diversity. A thorough investigation documented 10 distinct *Parmotrema* species, with one newly identified species (*Parmotrema clavuliferum*). Each species was comprehensively described, encompassing their morphological, chemical, and biogeographical characteristics. The core objective of this study revolves around conservation and sustainable utilization of this valuable bioresource. This research contributes to our understanding of lichen ecosystems, particularly in regions facing diverse threats, and underscores the importance of the Wayanad District within the broader context of biodiversity conservation.

Keywords: Antimicrobial agents, biogeographical characteristics, conservation, hotspot, lichen diversity, morphology, Nilgiri Biosphere Reserve, secondary metabolites.

Editor: Anonymity requested.

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Author contributions: BJ has designed and conceptualized the present study, performed the field collection, and formal data analysis, and prepared the original draft. ETS has participated in the field collection and contributed to the review and editing of the draft. VTJ has participated in the field collection and contributed to the review and editing of the draft. HP has participated in the field collection, prepared QGIS maps and spatial data analysis, and contributed to the review and editing of the draft. NSP has provided proper guidelines for the research and supervised the field collection, data analysis and the preparation of the final manuscript. All authors read and approved the final manuscript.

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INTRODUCTION

Lichens are fascinating organisms, formed by the symbiotic association between algae and fungi, functioning together as a single organism (Honegger 1991). Lichens contribute to 8% of global biodiversity and approximately 19,387 species that comprise 995 genera, 115 families, 39 orders, and eight classes (Lücking et al. 2016). The Indian subcontinent has approximately 3,028 lichens (Awasthi 2000; Sinha 2021) and Kerala has 27% of this diversity (Purushothaman et al. 2021; Anilkumar et al. 2022; Sequeira et al. 2022). All the members of the genus *Parmotrema* A.Massal. are foliose lichens. The ventral of the thallus has green or pale green or ash color and the margins may or may not have cilia and pored epicortex. The dorsal side has brown, tan or black color and the lower margin is tan, brown or white in color. The margin of the dorsal side is generally devoid of rhizines. This erhizinate condition of the dorsal side is used as a key character to separate the lichens of the genus *Parmotrema* A.Massal. from other foliose lichens in the family Parmeliaceae. All the members in this genus have distinct cortex and medulla. The upper cortex is maculated due to the extension of medullar fungal hyphae to the cortex, which can be identified by the regions devoid of phycobiont (Spielmann & Marcelli 2009, 2020; Mishra & Upreti 2017). All the members of the genus *Parmotrema* are rich in pharmaceutically important secondary metabolites. Atranorin is a commonly occurring compound with anti–microbial properties against bacteria *Bacillus cereus, B. subtilis, Staphylococcus aureus, S. faecalis, Proteus vulgaris*, the fungi *C. albicans and C. glabrata* as well as the mycobacterium, *M. aurum*. In addition to this Atranorin has anticarcinogenic properties (Sroka et al. 2017). Salazinic acid is another secondary metabolite of the genus *Parmotrema* and has antimicrobial and cytotoxic properties. Lecanoric acid is a bioactive compound and has antiproliferative activity against HeLa cells (IC50 = 123.97 µg/ml). Lecanoric acid is a potential antioxidant and can be used as a molecular scavenger against free radicals **(**Zambare & Christopher 2012).

Lichens are generally sensitive to habitat, host (John 1992), environmental factors, latitude, climate (Fryday 2000) and environmental pollution (Larsen et al. 2007). The present study is focused on the assessment of biogeographic patterns of distribution of the genus *Parmotrema* in the rapidly urbanizing zones of Wayanad District within the Nilgiri Biosphere Reserve of the Western Ghats, for the lichen diversity status assessment and future reference. This is the first study that analyzes the ecology and population aspects of the genus *Parmotrema* in the Wayanad District of Kerala, in a scenario of lichens facing challenges of extinction due to endemism, ever increasing pollution, urbanization, & lack of studies and endeavors to protect them.

Study area

Wayanad (Figure 1) is a small hilly district in Kerala with an area of 2,131 km^2, located at 11.685^{0}N, 76.132^{0}E with the highest tribal population of about 1.25 Lakh, consisting of 17% of the total tribal population of the state. Wayanad has a salubrious climate with a mean rainfall of 2,786 mm and the elevation varies 700–2,061 m.

MATERIALS AND METHODS

The diversity assessment of lichens of the genus *Parmotrema* in Wayanad was based on 460 specimens of lichens collected from October 2021 to October 2022. The specimens were systematically identified using the keys of macro lichens of Awasthi (1976, 1991, 2007), Divakar & Upreti (2005), and Mishra & Upreti (2017). Out of the 460 samples, 258 samples belong to the genus *Parmotrema* and these specimens were further studied using a Leica MC170 stereo microscope for morphological studies and a Leica DM750 compound microscope for anatomical studies. The secondary metabolites in the thallus were also considered for species delimitation. The compounds were preliminarily determined through spot tests and thin-layer chromatography (Orange et al. 2001). Thin-layer chromatography was performed using solvent system A (toluene: dioxane: acetic acid = 180:45:5) (Nayaka 2014). Nomenclature was confirmed with the database Index Fungorum (http://www.indexfungorum.org). All the morphological characters noted were compared with the morpho-taxonomic accounts of Mishra & Upreti (2017) and Spielmann & Marcelli (2009). All the specimens were systematically processed and deposited at the herbarium of KSCSTE-Malabar Botanical Garden & Institute for Plant Sciences (MBGH).

The distribution maps were prepared using open source QGIS 3.16 software and the ecological studies were conducted using narrow frequency grid (sampling ladder) (Scheidegger et al. 2002). The study was carried out in 16 sample sites of Wayanad District and these sites were grouped into three zones based on elevation. The geographical parameters and zonal classification is given in Table 1. A total of 60 quadrats were laid randomly for

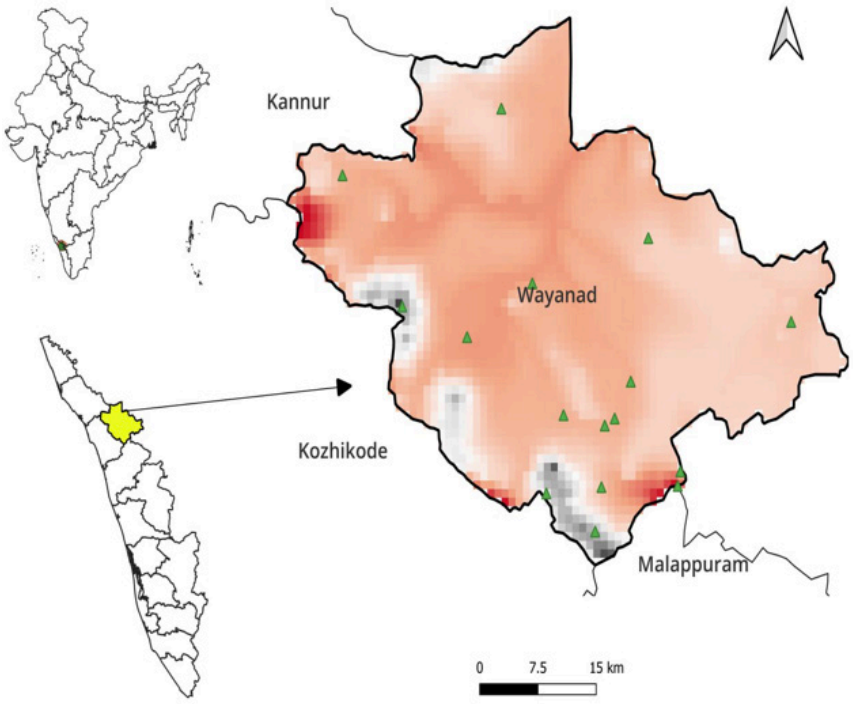

Figure 1. Study area - Wayand, southern Western Ghats showing sample sites.

the collection of ecological data (20 quadrats were used for each zone). The quadrat analysis of the study area is listed in Table 2. The alpha diversity of lichen habitats was assessed using the Shannon-Weiner index (Shannon & Weiner 1949), Simpson's index (Simpson 1949) and evenness. The following equations are used to calculate the alpha diversity of 16 lichen habitats of Wayanad.

Frequency and relative frequency were calculated as,

$$\text{Frequency} = \left(\frac{\text{Number of quadrats in which a species occurs}}{\text{Total number of quadrats sampled}}\right) * \mathbf{100}$$

$$\text{Relative Frequency} = \left(\frac{\text{Frequency of the individual species}}{\text{Total frequency of all the species}}\right) * \mathbf{100}$$

Density and relative density were calculated as,

$$\text{Density} = \left(\frac{\text{Total number of individuals in all the quadrats}}{\text{Total number of quadrats studied}}\right)$$

$$\text{Relative density} = \left(\frac{\text{Total number of individuals of the species in all quadrats}}{\text{Total number of individuals of all species in all quadrats}}\right) * \mathbf{100}$$

The importance value index (IVI) was used for the assessment of the ecological distribution of species in the ecosystem (Curtis & Mc Intosh 1950; Misra 1968) of lichens in different zones. IVI is the sum of relative frequency and relative density (Phillips 1959).

Shannon-Wiener Index (H') (1949) of species richness is based on the total number of species and the total number of individuals of each species in a sample. This index represents the average degree of uncertainty in predicting to which particular species, an individual randomly chooses from the sample.

$H' = -\sum [(ni/N) \ln (ni/N)]$

Where,

Ni = number of individuals of i^{th} species

N = total number of individuals of all species

H' = index value

Simpson's index (D) (1949) is used to measure the degree of concentration when individuals are classified into types.

$$\mathbf{D} = \sum_{\mathbf{i=0}}^{\mathbf{s}} \left(\frac{\mathbf{ni(ni-1)}}{\mathbf{N(N-1)}}\right)$$

Where,

ni = number of individuals in the i^{th} species

N = total number of individuals of all species

D = index value

Evenness of the region expresses the Shannon-Weiner function (H'), relative to the maximum value that H' can be obtained. Evenness reaches a maximum when all the species in the sample have the same number of individuals.

$$\mathbf{E} = \left(\frac{\mathbf{H'}}{\mathbf{H'max}}\right)$$

Table 1. Geographical factors of the study area.

	Location	Zone	Elevation (m)	Latitude (° N)	Longitude (° E)
1	Vaduvanchal	Zone 1 Altitude below 800 m	400	11.5210364	76.2393654
2	Chennalode		690	11.6631996	75.98487763
3	Arijermala		719	11.7146403	76.06218148
4	Neelimala		725	11.5327544	76.23645906
5	Kolagapara		753	11.61955001	76.17823095
6	Karappuzha		766	11.58407531	76.15903076
7	Puthoorvayal		766	11.58748825	76.09855323
8	Periya		769	11.81961661	75.83803087
9	Meppadi		785	11.57728355	76.14728895
10	ChooralMalai	Zone 2 Altitude between 800–1,200 m	818	11.51799489	76.14309851
11	Thirunelli		851	11.88324358	76.02617202
12	Wayanad Wildlife Sanctuary		856	11.67626877	76.36811484
13	Irulam		890	11.75776861	76.19951054
14	Chembra	Zone 3 Altitude above 1,200 m	1,252	11.51220149	76.07799674
15	Vellarimala		1,389	11.47523043	76.13555719
16	Banasuramalai		1,911	11.69295873	75.90831713)

Table 2. List of species and occurrence in the study area.

	Location	List of species and occurrence									
		*Au**	*Ce**	*Cl**	*Cr**	*Ha**	*Pl**	*Pr**	*Re**	*St**	*Ti**
1	Vaduvanchal	-	-	9	-	-	-	1	-	-	14
2	Chennalode	-	-	-	4	6	-	-	-	-	15
3	Arijermala	-	-	-	-	1	-	-	5	1	2
4	Neelimala	-	1	1	-	-	7	-	4	1	3
5	Kolagapara	1	-	-	-	-	1		-	1	6
6	Karappuzha	-	-	-	-	-	-	-	8	4	9
7	Puthoorvazhal	-	-	-	1	1	-	-	-	-	9
8	Periya	3	-	-	-	-	6	-	-	-	9
9	Meppadi	4	-	-	-	-	4	-	-	1	4
10	Chooral Malai	-	1	4	1	-	-	-	6	1	11
11	Thirunelli	-	-	1	1	-	-	-	16	6	9
12	Wayanad Wildlife Sanctuary	-	1	-	-	-	3	-	9	-	8
13	Irulam	1	-	-	-	-	2	-	-	1	4
14	Chembra	-	-	-	-	1	-	-	2	-	2
15	Vellarimala	-	-	-	-	1	-	-	2	-	4
16	Banasuramalai	4	-	-	-	3	-	-	-	-	6
	TOTAL	13	3	15	7	13	23	1	52	16	115

Au—P. austrosinense | Ce*—P. cetratum | Cl*—P. clavuliferum | Cr*—P. cristiferum | Ha*—P. hababianum | Pl*—P. planatilobatum | Pr*—P. praesorediosum | Re*—P. reticulatum | St*—P. stuppeum | Ti*—P. tinctorum.*

Where,

H' = Shannon-Wiener Index

H' max = Species Richness

RESULTS

The detailed description of various *Parmotrema* lichens collected from the study area are presented as 'Taxonomic studies' and detailed biogeographic features and distribution of species in the study area are described under 'Biogeography of the lichen genus *Parmotrema* in the study area'.

Taxonomic studies

The 10 species recorded from the study area are described here. *Parmotrema clavuliferum* (Räsänen) Streimann is reported here as a new record to India.

1) ***Parmotrema austrosinense*** (Zahlbr.) Hale, Phytologia 28(4): 335 (1974)

Index Fungorum Number: IF343014

Thallus foliose, corticolous, loosely attached to the substratum, 5–10 cm across; lobes rotund, each lobe 5 to 20 mm wide, margins ascending imbricate, sinuous; eciliate; upper surface pale green or grey colour, smooth, white-maculate, more or less rugose in the centre; soralia marginal, linear, soredia farinose to granular, sorediate margins are wavy and assenting imbricate, wide marginal zone ivory, tan or brown mottled; erhizinate shiny marginal zone; lower side centrally black; rhizines sparse in the centre part, simple; short; up to 1 mm long, medulla white. Apothecia rare, isidia absent (Image 3B).

Chemistry: Cortex K+ yellow; medulla K—, C+ rose red, KC+ red, P—

TLC: atranorin and lecanoric acid (Image 4(10)) Distribution: This taxon is found in elevations above 700m (Image 5e).

2) ***Parmotrema cetratum*** (Ach.) Hale, Phytologia 28(4): 335 (1974)

Index Fungorum Number: IF343018

Thallus foliose, corticolous or saxicolous, loosely adnate to the substratum, 7–20 cm across; lobes rotund, 5–10 mm wide, margin ciliate; cilia black with tapering end, simple to furcated, 1–3 mm long; upper side grey to darker green, densely white-maculate; maculae reticulate and fissured into a network (appearing as pseudocyphellae); isidia and soredia absent; lower side centrally black, marginal narrow zone ciliate; sparsely

Image 1. A—*Parmotrema reticulatum* (Taylor) M.Choisy | B—*Parmotrema clavuliferum* (Räsänen) Streimann | C—*Parmotrema cristiferum* (Taylor) Hale | D—*Parmotrema stuppeum* (Taylor) Hale. © Bibin Joseph.

rhizinate; rhizines restricted to the central part of the thallus, simple, black, 0.5–1 mm long; medulla white. Apothecia up to 10 mm in diameter, perforate; ascospores, colourless, simple, 13–17 × 6–10 µm. Pycnidia not seen (Image 3A).

Chemistry: Cortex K+ yellow; medulla K + yellow then red, C—, KC + red, P+ orange

TLC: atranorin, salazinic and consalazinic acids (Image 4(5)).

Distribution: In Kerala, this taxon is reported from Wayanad only (Image 5j).

3) *Parmotrema clavuliferum* (Räs.) Streimann, Bibliotheca Lichenologica 22: 93 (1986)

Index Fungorum Number: IF129346

Thallus foliose, corticolous, loosely attached with the substratum, 15–20 cm across; lobes dichotomously branched 5–15 mm wide, lobe margins ciliate rotund, margins entire ciliate, 0.3–1.5 mm long, black; upper side pale green or whitish-gray, dull to shiny, reticulatly maculate and cracked; soralia capitates and stalked, marginal, present in the laciniate lobes which appear as palmate, the lower side of the lacciniae white in colour; soredia found in a large cluster, granular, rotund; lower side centrally black narrow marginal zone, 2 mm wide, brown erhizinate; rhizines abundant, up to 1 mm; medulla white. Apothecia and pycnidia were not observed among the Wayanad specimens (Image 1B).

P.clavuliferum and *P. reticulatum* are similar in cortical and medullary chemistry in colour test and

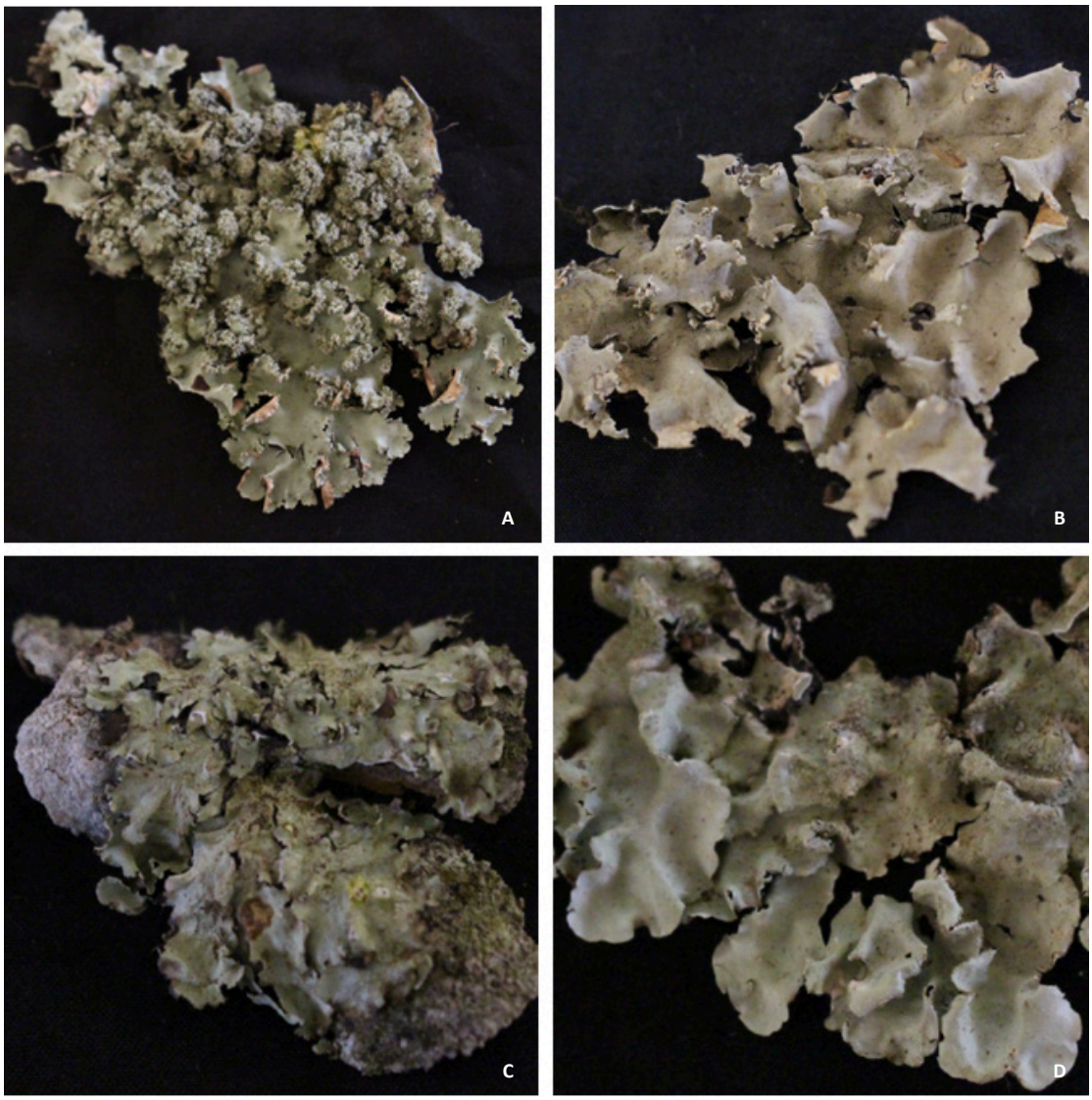

Image 2. A—*Parmotrema planatilobatum* (Hale) Hale | B—*Parmotrema hababianum* (Gyeln.) Hale | C—*Parmotrema praesorediosum* (Nyl.) Hale | D—*Parmotrema tinctorum* (Despr. ex Nyl.) Ha. © Bibin Joseph.

reticulate cracked upper side and differs in having palmate and elongate laciniae with capitates and stalked soralia; typically erhizinate and white lower side, lacking pigmentation by the former.

Chemistry: Cortex K+ yellow, KC–, C–, P+ yellow; medulla K + yellow then soon turning blood-red, C—, KC + red, P+ orange or deep yellow.

TLC: atranorin, salazinic acid (Image 4(2)).

Distribution: In India, this taxon is reported from Wayanad only (New to India)

4) *Parmotrema cristiferum* (Taylor) Hale, Phytologia 28(4): 335 (1974)

Index Fungorum Number: IF34303

Thallus foliose, corticolous rarely saxicolous, loosely attached to the substratum, large, spreading, 10–25 cm across; lobes rotund, laterally ascending, sinuous, 10–15 mm wide, emaculate; axils incised; margins entire, eciliate; upper side grey to pale grey, centrally brownish, cracked, soralia marginal on lateral lobules in the central part, crescent-shaped or confluent; soredia marginal to submarginal, rounded to confluent, sinuous and revolute, granular; lower side centrally black, wide marginal zone, 3–5 mm wide, brown, nude; rhizines sparse in the central part, short, coarse, up to 1 mm long; medulla white. Apothecia rare and pycnidia are absent in Wayanad specimens (Image 1C).

Chemistry: cortex K+ yellow; medulla K + yellow turning red, KC—, C—, P+ orange-red.

TLC: atranorin, salazinic acid and consalazinic acids

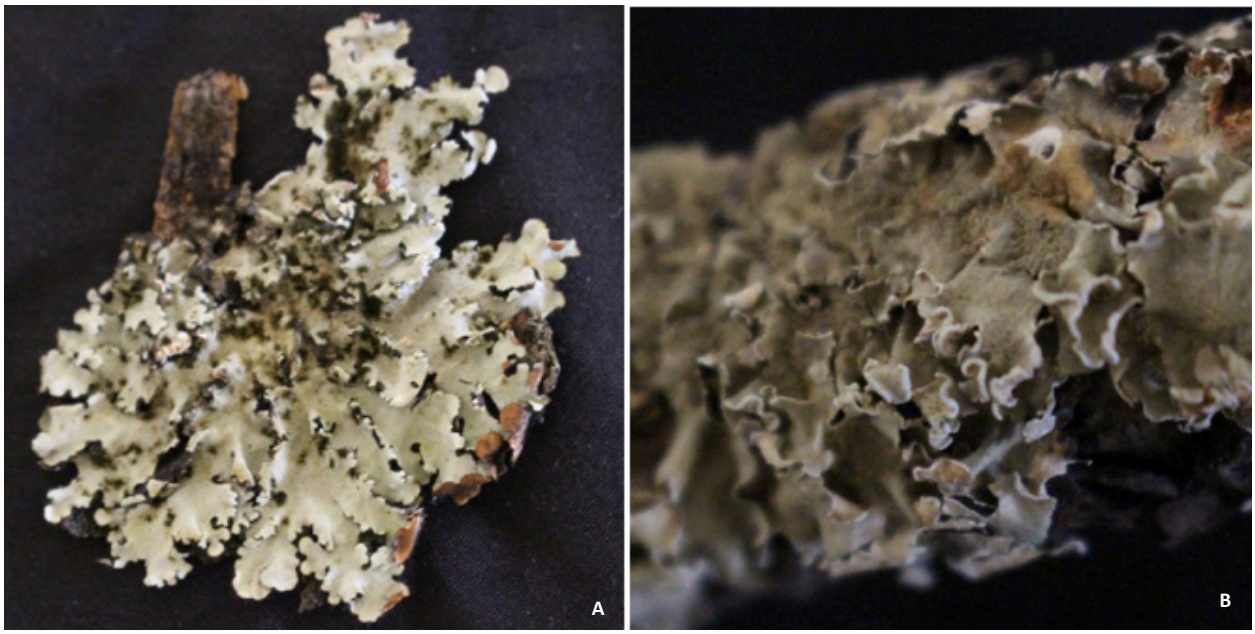

Image 3. A—*Parmotrema cetratum* (Ach.) Hale | B—*Parmotrema austrosinense* (Zahlbr.) Hale. © Bibin Joseph.

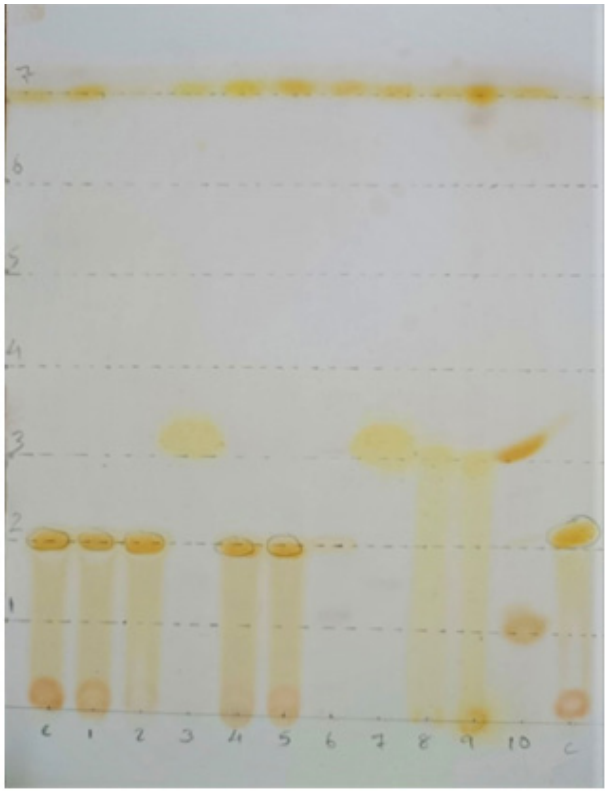

Image 4. Chromatogram of acetone extracts of lichens using solvent system A:
C—Standard (*Parmelia wallichiana*) | 1—*Parmotrema reticulatum* | 2—*Parmotrema clavuliferum* | 3—*Parmotrema hababianum* | 4—*Parmotrema stuppeum* | 5—*Parmotrema cetratum* | 6—*Parmotrema praesorediosum* | 7—*Parmotrema tinctorum* | 8—*Parmotrema cristiferum* | 9—*Parmotrema planatilobatum* | 10—*Parmotrema austrosinense*.

(Image 4(8)).

Distribution: This taxon is found in elevations above 600 m (Image 5h).

5) *Parmotrema hababianum* (Gyeln.) Hale, Phytologia 28: 336 (1974)

Index Fungorum Number: IF343060

Thallus foliose, corticolous, loosely attached to the substratum, 8–10 cm across; lobes rotund, 5–15 mm wide, margin crenate, sparsely ciliate; cilia simple, 0.5–2 mm long; upper side grey to brownish grey, smooth, faintly white-maculate to emaculate, sorediate; soralia marginal or submarginal; sorediate lobes revolute; lower side centrally brown-black; wide marginal zone ivory to brownish mottled, nude; rhizines sparse, uneven, present in scattered groups, simple, 1–2 mm long; medulla white. Apothecia and pycnidia are not seen in Wayanad specimens (Image 2B).

Chemistry: cortex K+ yellow; medulla K—, C—, KC+ reddish or purple P—.

TLC: atranorin and protolichesterinic acids (Image 4(3)).

Distribution: This taxon is found in elevations above 750m (Image 5g).

6) *Parmotrema planatilobatum* (Hale) Hale, Phytologia 28(4): 338. (1974)

Index Fungorum Number: IF343105

Thallus foliose, corticolous or saxicolous, closely to loosely attached to the substratum, 5–10 cm across;

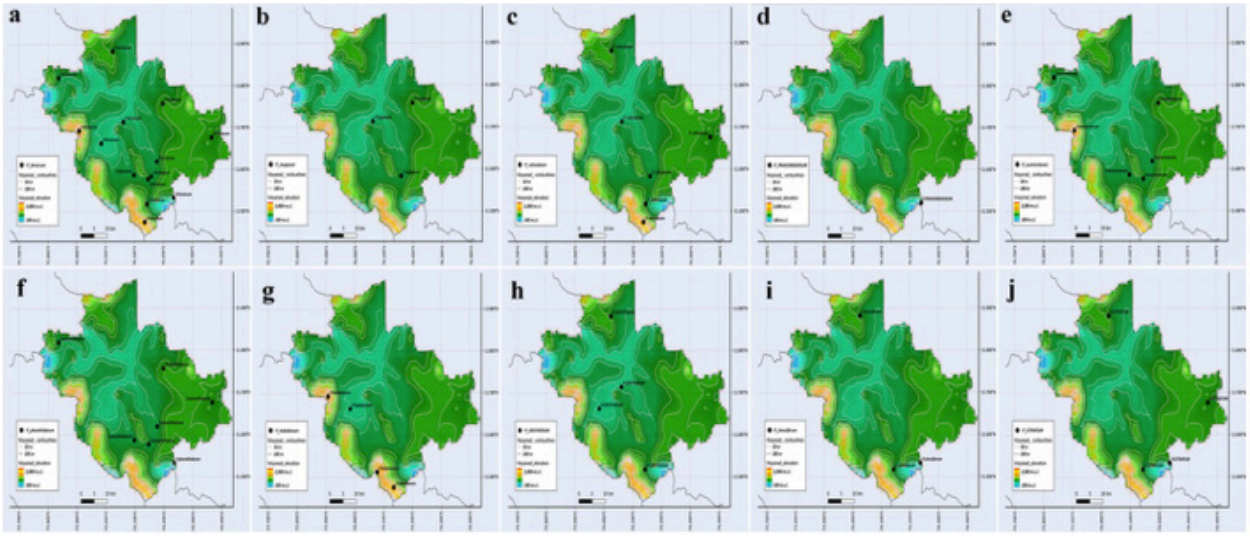

Image 5. QGIS distribution map of the genus *Parmotrema* in Wayanad: a—*Parmotrema tinctorum* | b—*Parmotrema stuppeum* | c—*Parmotrema reticulatum* | d—*Parmotrema praesorediosum* | e—*Parmotrema austrosinense* | f—*Parmotrema planatilobatum* | g—*Parmotrema hababianum* | h—*Parmotrema cristiferum* | i—*Parmotrema clavuliferum* | j—*Parmotrema cetratum*.

lobes rotund, 5–10 mm wide, apical margin entire or crenate, convolute, ciliate; cilia black, simple, 1–1.5 mm long; upper side grey, smooth, shiny, emaculate, with laminal to marginal isidia lacinulate thallus; lower side centrally black, marginal zone brown, nude; rhizines abundant, black, simple, 1–2 mm long; medulla white, with patches of K + purple pigment. Apothecia rare, up to 6 mm in diameter, disc imperforate, concave, pale brown; asci broad clavate, 38–42 × 20–25 µm, ascospores colourless, ellipsoid 15–18 × 7–9 µm. Pycnidia not seen (Image 2A).

Chemistry: cortex K+ yellow; medulla K—, C+ faint rose, KC+ red, P—

TLC: atranorin, gyrophoric acids and skyrin (Image 4(9)).

Distribution: This taxon is found in elevations above 750m (Image 5f).

7) ***Parmotrema praesorediosum*** (Nyl.) Hale, Phytologia 28(4): 338. (1974)

Index Fungorum Number: IF343106

Thallus saxicolous or corticolous, adnate, attached to the substratum, 3–10 cm across; lobes rotund, 5–10 mm wide, margins entire or crenate, sub erect and sorediate, eciliate; upper side grey to darker, emaculate, smooth, becoming slightly rugose and cracked in older parts, sorediate; soralia usually marginal, linear or crescent-shaped; soredia granular; lower side centrally black, narrow marginal zone lighter tan, nude; rhizines sparse, simple, short, 1–2 mm long; medulla white. Apothecia rare, short-stalked, 2–4 mm in diameter, disc imperforate, dark brown; asci clavate, 40–45 × 16–19 µm, ascospores simple, colourless, 15–21 × 7–10 µm. Pycnidia not seen (Image 2C).

Chemistry: cortex K+ yellow; medulla K—, C—, KC—, P—

TLC: atranorin, proto praesorediosic acid, praesorediosic and fatty acids (Image 4(6)).

Distribution: This taxon is found in elevations above 750 m (Image 5d).

8) ***Parmotrema reticulatum*** (Taylor) M. Choisy, Bull. Mens. Soc. Linn. Soc. Bot. Lyon. 21:175 (1952)

Index Fungorum Number: IF357464

Thallus foliose, corticolous or saxicolous, adnate loosely attached to the substratum, up to 10–20 cm across; lobes rotund, 5–15 mm wide, margin ciliate; cilia simple, black, 1–1.5 mm long; upper side grey to darker, smooth, densely white maculate; maculae eventually reticulately fissured, sorediate; soralia either capitate or marginal to submarginal on rounded or involute lobes; lower side centrally black, marginal zone white mottled or brown and nude or lower side black, rhizinate up to the margin; rhizines black, simple, 1–2 mm long; medulla white. Apothecia rare, up to 5 mm in diam., disc perforate or imperforate, brown; clavate asci; ascospores 8-spored, colorless, simple, 15–18 × 6–10 µm. Pycnidia not seen in Wayanad specimens (Image 1A).

Chemistry: Cortex K + yellow; medulla K + yellow then red, KC—, C—, P+ orange-red.

TLC: atranorin, salazinic and consalazinic acids

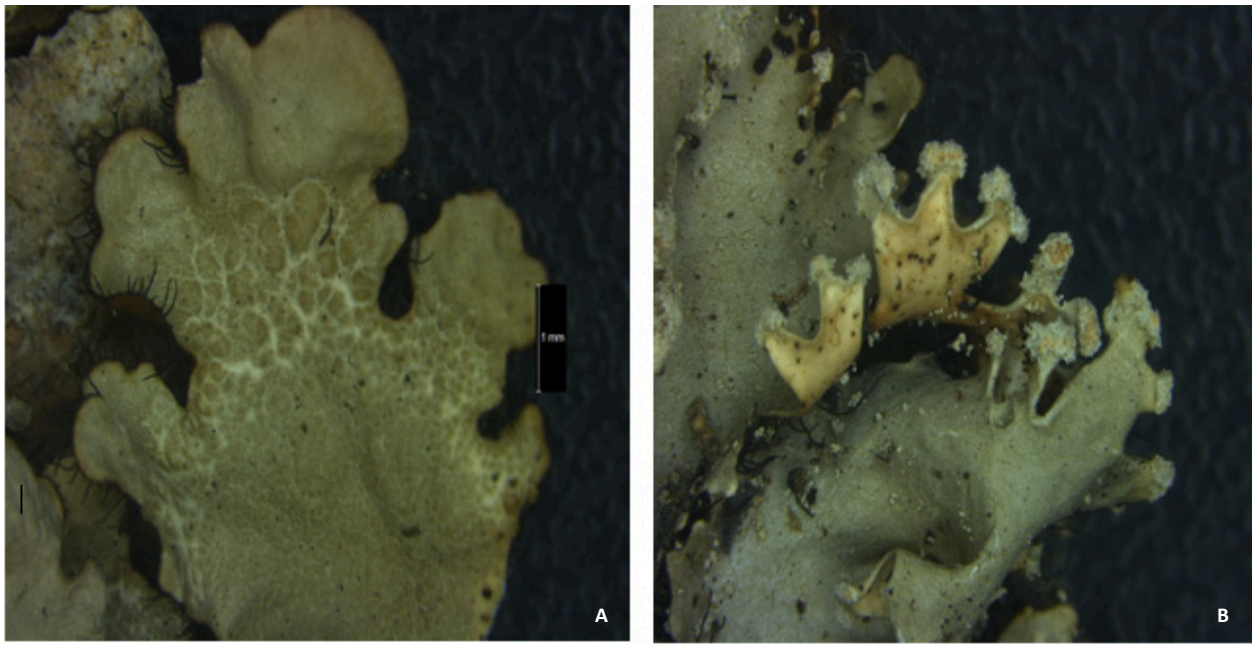

Image 6. *Parmotrema clavuliferum*: A—vegetative thallus with reticulate cracks and cilia | B—fertile thallus with variegated lacinules carrying soralia. © Bibin Joseph.

(Image 4(1)).

Distribution: This taxon is found in elevations above 600 m (Image 5c).

9) ***Parmotrema stuppeum*** (Taylor) Hale, Phytologia 28(4): 339 (1974)

Index Fungorum Number: IF343128

Thallus corticolous, rarely saxicolous, loosely adnate to the substratum, 10–15 cm across; lobes rotund, 10–20 mm wide, crenate-dentate, ciliate; cilia sparse to dense, simple, 1–3 mm long; upper side grey, dull, smooth, emaculate, cracked in older parts, sorediate; soralia marginal, on apices of dents in the central part, often confluent and submarginal; soraliate lobes involute; soredia farinose; lower side centrally black, wide marginal zone brown, nude or papillate; rhizines sparse, occur in patches in the central part, simple, 1–2 mm long; medulla white. Apothecia and pycnidia are not seen in Wayanad specimens (Image 1D).

Chemistry: Cortex K + yellow; medulla K + yellow turning red, C—, KC—, P+ orange-red.

TLC: atranorin, salazinic and consalazinic acids present in TLC (Image 4(4)).

Distribution: This taxon is found in above elevations 750 m (Image 5b).

10) ***Parmotrema tinctorum*** (Dèspr. *ex* Nyl.) Hale, Phytologia 28(4): 339 (1974)

Index Fungorum Number: IF343140

Thallus foliose, lobate, corticolous, saxicolous or terricolous, loosely attached to the substratum, membranaceous, 10–30 cm across; lobes irregular, 10–30 mm wide, apices rotund, margins entire to crenate, eciliate; upper side grey to pale green to mineral grey, emaculate; isidia granular to filiform becoming coralloid or rarely flattened; lower side centrally black, wide marginal zone, 3–6 mm, tan to brown, nude; rhizines sparse, dense at the centre, short 0.5–2.0 mm long; medulla white. Apothecia rare, not present in the specimens examined, up to 10 mm in diam., disc imperforate; asci clavate, 8 spored, ascospores simple, colourless, oval-ellipsoid 13–18 × 6–10 µm, epispore 1.5 µm thick (Hale 1965). Pycnidia not present (Image 2D).

Chemistry: Cortex K + yellow; medulla K—, C+ red, KC+ red, P—

TLC: Atranorin, lecanoric acid and traces of orsellinic acid (Image 4(7)).

Distribution: This taxon is abundantly present in all sample sites of the study area (Image 5a).

DISCUSSION

a) Study of species

In the comprehensive revisionary study of the lichen genus *Parmotrema* A.Massal. of India, Mishra & Upreti (2017) have provided a detailed morpho-taxonomic account of 53 species of *Parmotrma*. The

Table 3. Species diversity of the study area.

	Location	Shannon index (H)	Simpson's index (D)	Evenness (E)
1	Chembra	1.055	1.66	00.96
2	Karappuzha	1.05	0.31	00.95
3	Meppadi	1.285	00.21	00.927
4	Kolagapara	1.003	00.375	00.723
5	Vaduvanchal	00.815	00.441	00.742
6	Neelimala	1.512	00.208	00.844
7	Vellarimala	00.956	00.291	00.87
8	ChooralMalai	1.4	00.264	00.781
9	Thirunelli	1.227	00.314	00.763
10	Banasuramalai	1.058	00.285	00.963
11	Chennalode	00.942	00.443	00.74
12	Puthoorvayal	0.60	00.686	00.546
13	Irulam	1.213	00.22	00.875
14	Arijermala	1.149	00.275	00.829
15	Periya	1.011	00.33	00.920
16	Wayanad Wildlife Sanctuary	1.154	00.304	00.832

Table 4. Zone wise distribution of species.
The sixteen lichen habitats of the Wayanad District are divided into three zones based on elevation. The number of samples from each species collected from the study area is given in this table. The lichen habitats included in each zone were given in Table 1.

	Zone	*Au**	*Ce**	*Cl**	*Cr**	*Ha**	*Pl**	*Pr**	*Re**	*St**	*Ti**
1	Zone 1	8	1	10	*5*	*8*	18	1	17	8	71
2	Zone 2	1	2	5	2	0	5	0	31	8	32
3	Zone 3	4	0	0	0	5	0	0	4	0	12
	TOTAL	13	3	15	7	13	23	1	52	16	115

Au—P. austrosinense | Ce*—P. cetratum | Cl*—P. clavuliferum | Cr*—P. cristiferum | Ha*—P. hababianum | Pl*—P. planatilobatum | Pr*—P. praesorediosum | Re*—P. reticulatum | St*—P. stuppeum | Ti*—P. tinctorum.*

present study is adding *P. clavuliferum* (Räsänen) Streimann as the 54th species to the Indian lichen biota of the genus *Parmotrema*. The *P. clavuliferum* resembles *P. reticulatum* in its white to whitish-grey color, densely reticulate-maculate, often cracked upper surface abundantly sorediate margins, and simple cilia (Image 6A). They share the same chemistry (cortex with atranorin, medulla with salazinic acid). *P. clavuliferum* can be distinguished from *P. reticulatum*, the former having capitate soralia, sorediate lacinules at the lobe margins and the variegated lower side of the soredia (non–pigmented and white) (Image 6B). The distinctly stalked, capitate soralia protruding from long, slender, laciniate lobes is the characteristic feature of *P. clavuliferum*, whereas in *P. reticulatum* the soralia is in laminal to submarginal regions of the thallus (Moon et al.2001). This character can be used to segregate *P. clavuliferum* from *P. reticulatum*. Even though the erhizinate or nude and broad margins are the key characteristics of the genus *Parmotrema*, the broad lobes of *P. reticulatum* are typically densely rhizinate even close to the margin but the margins and clavulae of *P. clavuliferum* always devoid of rhizines (Bungartz & Spielmann 2019). The molecular studies of Ahn & Moon (2016) also provide evidence of the existence of *P. clavuliferum* as a separate species, not morphotypes. Spielmann & Marcelli (2009) reported *P. clavuliferum* from Brazil with filiform conidia and salazinic acid (K+ yellow turning blood red) as the secondary metabolite. Bungartz & Spielmann (2019) also recognised *P. clavuliferum* as separate species in their comprehensive inventory of all Galapagos lichens.

Table 4 shows that a total of 258 samples belonging to 10 different species is distributed through the three zones and among these zones; Zone 1 has a higher number of species and occurrences. The Zone 1, with nine lichen habitats has the maximum number of species in the genus *Parmotrema*, Zone 2 with four lichen habitats and Zone 3 has three lichen habitats. The Zone 1 has 10 species, Zone 2 has eight species and Zone 3 has four species of *Parmotrema*. From Table 5, *P. tinctorum* is the dominant species in all the zones, with the IVI value of 80.03, 71.346, and 88 respectively. *P. cetratum* is one species with a low IVI value and from Kerala, it is reported from Wayanad District only (Christy et al., 2022). Another species with a low IVI value is *P. praesorediosum* (IVI value 2.26), present only in zone 1, mainly due to its pollution–sensitive nature and hence can be used as a pollution indicator.

b) Biogeography and Ecological Studies

The diversity of species in the study area is termed alpha diversity. Simpson's Index of Diversity (D) is a measure of richness and relative abundance and also a measure of the dominance of the species in the population. Shannon-Wiener Index (H'), like the Simpson's Index, also measures the richness and abundance of the species. Evenness (E) gives us a picture of the relative abundances of the different species in the study area. Table 3 expresses the values of these indexes in the study area. Neelimala is the region with a high Shannon-Wiener Index (H'), Puthoorvayal is the region with a high Simpson's Index and Banasuramalai is the region with high species Evenness (E) with values of

Table 5. Ecological assessment of species occurrence in the three zones.

		Species	Number	Frequency	Density	Abundance	Relative density	Relative frequency	IVI
1	ZONE 1	*P. austrosinense*	6	30	0.4	1.33	5.4422	9.52	14.962
2		*P. cetratum*	1	5	0.05	1	0.7404	1.58	2.3204
3		*P. clavuliferum*	4	20	0.5	2.5	6.8027	6.34	13.14
4		*P. cristiferum*	4	20	0.25	1.25	3.40	6.34	9.74
5		*P. hababianum*	6	30	0.4	1.33	5.44	9.5	14.94
6		*P. planatilobatum*	8	40	0.9	2.25	12.24	12.69	24.93
7		*P. praesorediosum*	1	5	0.05	1	0.68	1.58	2.26
8		*P. reticulatum*	8	40	0.85	2.125	11.56	12.69	24.25
9		*P. stuppeum*	5	25	0.4	1.6	5.44	7.93	13.37
10		*P. tinctorum*	20	100	3.55	3.55	48.29	31.74	80.03
1	ZONE 2	*P. austrosinense*	1	5	0.05	1	1.16	2.43	3.59
2		*P. cetratum*	2	10	0.1	1	2.32	4.8	7.12
3		*P. clavuliferum*	3	15	0.25	1.66	5.81	7.31	13.12
4		*P. cristiferum*	2	10	0.1	1	2.32	4.8	7.12
5		*P. planatilobatum*	3	15	0.25	1.66	5.81	7.31	13.12
6		*P. reticulatum*	10	50	1.55	3.1	36.04	24.39	60.43
7		*P. stuppeum*	6	30	0.4	1.33	9.30	14.63	23.93
8		*P. tinctorum*	14	70	1.6	2.28	37.20	34.146	71.346
1	ZONE 3	*P. austrosinense*	2	10	0.2	2	16	13.33	29.33
2		*P. hababianum*	4	20	0.25	1.25	20	26.66	46.66
3		*P. reticulatum*	3	15	0.2	1.33	16	20	36
4		*P. tinctorum*	6	30	0.6	2	48	40	88

1.512, 0.60, and 0.963, respectively. All these areas show rich biodiversity. The higher values of alpha diversity indicate the wellbeing of the ecosystem. The data in Table 3 can be used as a baseline record of the ecological and population attribute of lichens of Wayanad. The increase in any of these values can be appreciated and negative change is the indication of depreciation of air quality and increasing pollution.

From the ecological perspective, lichens act as the first successors of barren rock, indicators of pollution, etc. The naturally healthy lichen biota of a region indicates the ecological well-being as well as lesser environmental pollution of the region. Information needs for biodiversity studies are many and varied. Any data that deals with biodiversity information has to be geographically based. The role of GIS is to integrate and analyze large varieties of spatial and attribute data for assessment and monitoring purposes of biodiversity. The QGIS data recorded in Image 5 can be used for the needs of today as well as tomorrow as a baseline date to understand the trends in changes of biodiversity of lichens due to pollution, urbanization and climate change.

CONCLUSION

Studying the lichen ecosystems in Wayanad, particularly those confronting multiple threats, holds the potential to contribute significantly to the conservation of the region's biodiversity. The diverse lichen species in this area are currently facing threats attributed to pollution stemming from automobile exhaust and the acidic residues of sulphides and nitrites, a consequence of various human activities. The current study underscores the significance of Wayanad district as an untarnished haven for lichens. Moreover, this study serves as a foundational dataset elucidating lichen diversity and bio-geographic patterns, specifically those of foliose lichens. It also offers a platform for evaluating the impacts of climate change and pollution on the biodiversity of Wayanad district and the broader Western Ghats region.

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