Songklanakarin J. Sci. Technol.
41 (5), 1037-1047, Sep. – Oct. 2019

http://www.sjst.psu.ac.th

Original Article

Distribution pattern, composition, and diversity of the Rubiaceae species along the altitudinal gradient of the Himalayas in Western Bhutan

Tobgay[1, 2*] and Kitichate Sridith[1]

[1] *Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, 90110 Thailand*

[2] *Daga Higher Secondary School, Ministry of Education, Dagana, Thimphu, 00975 Bhutan*

Received: 4 April 2018; Revised: 22 May 2018; Accepted: 31 May 2018

Abstract

The study conducted along the altitudinal gradient of 300-3900 m asl. recorded a total of 36 species (21 genera) and 46 species (29 genera) in study areas I and II respectively. The sighting of only one species each for the majority of genera, including *Pavetta* L., *Oldenlandia* L., and *Argostemma* Wall., which are described as the largest genera of Rubiaceae, was unusual. While the species with a narrow distribution range were found concentrated at the lower altitude exhibiting endemic nature, the herbaceous species exhibiting maximum distribution range were dominant at the higher altitudinal range. The low altitudinal hump-shaped distribution pattern was observed in both study areas. The higher diversity indicated by the Shannon-Wiener and Simpson diversity analysis was attributed to the moderate mean precipitation, temperature, and relative humidity. The altitude and litter thickness contributed the maximum to the species composition and distribution pattern based on canonical correspondence analysis.

Keywords: Rubiaceae, Bhutan, altitude, Himalaya, diversity

1. Introduction

Of around 13000 species belonging to 620 genera (Bremer & Erikson, 2009), Rubiaceae stands as the fourth largest angiosperm family (Block, Taylor, & Huysman, 2009). Rubiaceae is found distributed all across the regions of the world except Antarctica (Barbhuiya, Dutta, Das, & Baishya, 2014), exhibiting high species diversity and richness proliferation from low to mid-altitude in humid forests (Davis *et al.*, 2009).

In spite of its abundance, the diversity of Rubiaceae is checked by high endemism further aggravated by ecological sensitivity (Barbhuiya *et al.*, 2014), thus increasing the vulnerability to extinction, particularly the monotypic genera. The susceptibility of Rubiaceae is further augmented by the presence of 72% of genera with less than 10 species and 211 monotypic genera (Davis *et al.*, 2009). So, the perceived restricted distribution of Rubiaceae observed in secondary forests highlights the ecological sensitivity of the family and the immeasurable risk of extinction. This behavior of the family limits their diversification in the disturbed places.

Against the backdrop of such ecological traits of Rubiaceae, the Himalayan Kingdom of Bhutan, which is described as one of the ten biodiversity hotspots of the world (Myers, Mittermeier, Mittermeier, Fonseca, & Kent, 2000) hosting around 7000 vascular plant species comprising 82 endemic species (Wangchuk, 2005) have recorded only 55 genera and 141 species (Grierson & Long, 1999) of Rubiaceae in the Flora of Bhutan. Apart from its record in the Flora, research concerning the distribution pattern and ecology about the family has not been conducted to date in Bhutan. Such documentation is necessary to assess the changes in plant diversity and to the conserve the endemic and endangered species.

*Corresponding author
Email address: tobgaytob@education.gov.bt

Hence, the focus of the current study was to assess the distribution pattern, species richness, and the environmental factors that influence the family. In particular, the study aimed to (i) explore the distribution pattern of the species in the family, (ii) evaluate the species richness and diversity of the family, and (iii) assess the influence of environmental factors on the distribution pattern and species diversity.

2. Methods

2.1 Study areas

The survey was conducted in two study areas along the Himalayan range of western Bhutan showing altitudinal variations from 300 to 3900 m asl. (Figure 1). Area I is the area falling within Phuntsholing and Thujaydrak and area II is the area falling within Dagachu and Dagala.

These areas cover subtropical forests (200-1000 m), warm broad-leaved forests (1100-2000 m), cool broad-leaved forests (2100-2900 m), conifer forests (3000-3500 m), and subalpine forests (3500-3900 m) according to vegetation zonation done by Norbu *et al.* (2003). The survey focused on covering the maximum range of area as Rubiaceae were found to occupy a wide range of habitats (Bremer & Erikson, 2009). The two areas that showed high elevational gradients were chosen based on the differences in rock composition (Long, McQuarrie, Tobgay, Grujic, & Hollister, 2011), annual precipitation, temperature, and relative humidity (Table 1).

The climate data for the past ten years recorded at the weather stations located in the study areas were obtained from the National Center for Hydrology and Metrology, Thimphu, Bhutan. The mean annual precipitation, the mean maximum and minimum temperature, and the mean relative humidity of the study areas are shown in Table 1.

2.2 Landscape, study sites, and data collection

The study sites are characterized by deep river valleys, slopes, ridges, thick forests, thickets, and alpine vegetation. The degree of anthropogenic influences along the gradient in both the study areas is almost similar, characterized by the presence of roads, hydro-power plants, and settlements. Therefore, it provides a wide range of microhabitats from disturbed to intact for the plant species to occupy.

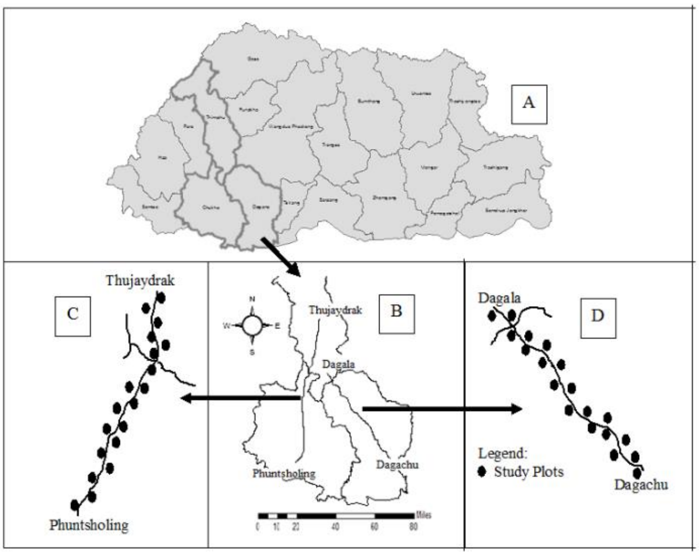

Figure 1. Map showing the study sites. A. Map of Bhutan; B. Map of Dagana, Chukha and Thimphu districts (study areas), C. Study area I (Phuntsholing-Thujaydrak), D. Study area II (Dagachu-Dagala).

Table 1. The annual mean temperature, rainfall and relative humidity calculated using the record of past ten years.

		Months											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Area I	T. Max	17.8	19.4	21.9	23.9	25.1	25.8	26.3	26.6	26.1	24.7	21.6	18.3
	T. Min.	3.8	6.0	8.4	10.9	13.2	16.3	17.1	17.2	16.3	12.4	8.1	5.1
	Rainfall	10.1	25.5	72.2	113.3	146.3	273.2	385.3	313.8	208.3	75.3	7.0	6.8
	RH	66.2	61.2	64.9	65.0	66.8	71.9	77.8	80.6	79.4	73.3	66.4	64.3
Area II	T. Max	17.3	19.2	22.2	24.4	25.9	27.4	26.2	26.4	25.9	25.0	21.6	18.5
	T. Min.	7.3	9.3	12.0	15.7	18.2	20.1	20.0	20.3	19.3	15.6	11.5	8.9
	Rainfall	10.4	13.6	28.9	53.1	119.3	22.4	416.5	280.1	197.3	99.2	9.6	2.5
	RH	75.8	75.9	73.9	75.2	79.5	86.8	89.6	88.9	86.6	81.2	78.4	78.0

The field visits were made twice a month from March to December 2017 to cover the entire flowering period. The species presence and absence data were obtained at every 200 meter elevation interval along the gradient in both the study areas. Consequently, at every 200 meter elevation, stands were marked as reference points for data collection and analysis. Along these lines, a total of 38 sites, 19 sites in each study area were marked. Nonetheless, the species encountered in other areas were also included in the nearest reference point (Vetaas & Grytnes, 2002) for the purpose of assessing the diversity and richness. The distribution range of each species was calculated following the method of Subedi, Bhattarai, and Chauudhary (2015).

The information on location, altitude, latitude, and longitude were gathered using Garmin GPS, the soil pH was recorded using the plant sap pH meter, and the litter thickness was measured by ruler (Zhang, Zu, & Li, 2013) for every plant specimen collected. The soil samples were collected and analyzed for carbon, nitrogen, phosphorus, and potassium at the National Soil Center, Simtokha, Thimphu, Bhutan.

The plant specimens were identified using the Flora of Bhutan (Grierson & Long, 1999), Flowers of the Himalaya (Polunin & Stainton, 1984), Flowers of the Himalaya: a supplement (Stainton, 1984) and other available taxonomic literature. The voucher specimens were prepared following the methods of Bridson and Forman (1992) and deposited at the National Herbarium, Serbithang, Thimphu, Bhutan.

2.3 Data analysis

The species diversity indices, evenness and richness were calculated using the Simpson diversity index (Simpson, 1949) and the Shannon-Wiener index (Shannon & Weaver, 1949). The metrological data and the species-altitude relationship data were analyzed through direct computation in Microsoft Excel 2007. The canonical correspondence analysis (CCA) was used to elucidate the effect of environmental variables, i.e. pH, carbon, nitrogen, phosphorus, potassium, litter thickness, and altitude, to the species composition. The Pearson correlation analysis was executed to analyze the relationships between environmental variables and the species richness. The analyses were performed using Statistical Package for the Social Sciences (SPSS) version 24.

3. Results

3.1 Species richness and diversity

A total of 36 species of Rubiaceae belonging to 21 genera and 46 species belonging to 29 genera were recorded from study areas I and II, respectively (Table 2A & B). Among them were 7 species which could not be identified due to the lack of complete floral characters. The species richness was higher for area II at 56.1% (46 species) against 43.9% (36 species) for area I of all Rubiaceae species recorded in the two study areas. The Simpson and Shannon-Wiener diversity and evenness analysis (Table 3) revealed that the species diversity and also the evenness were a little higher for area II.

Overall, the highest species recorded was for *Wendlandia* DC. and *Galium* L. taxa with five species each followed by *Ophiorrhiza* L. and *Mussaenda* L. taxa with four species each. The majority of the taxa consisted of one or two species only as detailed in Table 2A & B. The *Galium* L. taxa, specifically *Galium aparine* L., showed maximum diversification from sub-tropical forests through alpine forests based on the eco-floristic zonation by Ohsawa (1987). In total, 18 taxa were represented by only one species.

Both study sites showed similar variations in species life forms. In general the shrub contributed 39.1% to the composition while tree, herb, and climber constituted 13.4%, 36.6%, and 10.9% respectively.

3.2 Patterns of Rubiaceae species distribution along the altitudinal gradient

The Rubiaceae species shows varying degree of proliferation between 300 m to 3900 m asl. in both study areas along the altitudinal gradient. But the rate of proliferation along the gradient was not uniform and exhibited a hump-shaped pattern followed by a gradual decrease (Figure 2). The highest species recorded in both study areas was at about 1400 m asl. The species distribution pattern in area I exhibited a gradual decrease while study area II displayed a sharp decreasing pattern beyond 1500 m asl.

3.3 Impact of environmental factors on distribution pattern

The cumulative degree of variance explained by the first two dimensions of CCA is 69.5% for area I and 79.7% for area II. The chi-square values for area I and area II were 325.977 ($P<0.001$) and 312.634 ($P<0.001$), respectively. The quantitative explanatory variables, i.e. pH, carbon, nitrogen, phosphorus, potassium, altitude, and litter thickness for both study sites showed varying degrees of impact on the Rubiaceae species distribution pattern (Figure 3A & B).

In general, altitude and litter thickness showed greater influence on the species distribution pattern along the gradient. The degree of variance caused by altitude stands at 95.7% and 98.8% for areas I and II, respectively, caused significant influence on the pattern of species distribution. Cumulating to just 0.33% of variance in site I by carbon and 0.06% in site II by nitrogen, these two edaphic factors contributed least to the overall variation of species distribution pattern.

The Pearson correlation analysis supports the CCA, explaining a statistically significant negative relationship between species distribution pattern and altitude in both areas, $r(35)= -.37$ ($P=0.05$) for area I and $r(45) = -.36$ ($P=0.05$) for area II. The litter thickness at $r(35) = .31$ ($P=0.05$) for area I and $r\ (45) = .32$ ($P=0.05$) for area II on the other hand had shown a significant positive correlation to the species distribution pattern. The analysis also revealed varying degrees of correlation between different environmental factors. It is interesting to note the significant negative correlation between altitude and litter thickness (Table 4A & B).

Table 2A. Lists of Rubiaceae species recorded in study area I (Phuntsholing-Thujaydrak).

Scientific Name	Abb.[1]	Life form	Coordinates	Reference Points																			Voucher Number
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
				300	500	700	900	1100	1300	1500	1700	1900	2100	2300	2500	2700	2900	3100	3300	3500	3700	3900	
Argostemma sarmentosum Wall.	ArS.	H	26^{0}57’19.43”N 89^{0}35’37.72”E	X	X	X	X	X	X	X													Tgay55
Argostemma verticillatum Wall.	ArV.	H	26^{0}57’19.43”N 89^{0}35’37.39”E				X	X	X	X	X	X	X	X	X								Tgay54
Ceriscoides campanulata (Roxb.) Tirveng.	CeC.	T	26°58'40.04" N 89°35'34.40" E	X	X	X	X	X	X	X													Tgay61
Chassalia curviflora (Wall.) Thwaites	ChC.	S	26^{0}53’26.06”N 89^{0}32’15.46”E	X	X	X	X	X	X														Tgay65
Galium aparine L.	GaA.	H	27^{0}29’20.80”N 89^{0}34’28.96”E					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Tgay66
Galium asperuloides Edgew.	GaAs.	H	27°02'47.76" N 89°36'39.92" E											X	X								Tgay76
Galium craticulatum R.R. Mill	GaC.	H	27^{0}19’20.53”N 91^{0}07’46.67”E														X	X	X	X	X	X	Tgay77
Hedyotis scandens Roxb.	HeS.	H	26^{0}51’42.30”N, 89^{0}24’38.81”E	X	X	X	X	X	X	X	X	X											Tgay53
Hedyotis verticillata (L.) Lam.	HeV.	H	26^{0}57’16.30”N, 89^{0}35’39.43”E	X	X	X	X	X	X														Tgay72
Himalrandia tetrasperma (Wall. ex Roxb.) T. Yamaz.	HiT.	S	27^{0}01’39.80”N 89^{0}34’31.64”E					X	X	X	X	X	X										Tgay51
Ixora undulata Roxb.	IxU.	S	26^{0}51’22.80”N, 89^{0}24’04.20”E	X	X	X	X	X															Tgay57
Leptodermis amoena Springate	LeA.	S	27^{0}28’55.50”N 89^{0}36’59.20”E				X	X	X	X	X	X	X	X	X	X							Tgay80
Leptodermis kumaonensis R. Parker	LeK.	S	27^{0}29’13.40”N 89^{0}35’13.20”E												X	X	X						Tgay82
Leptodermis stapfiana H.J.P. Winkl.	LeS.	S	27^{0}29’13.40”N 89^{0}35’13.20”E						X	X	X	X	X	X	X	X	X	X	X	X			Tgay81
Luculia grandifolia Ghose	LuG.	S	27^{0}04’34.90”N 89^{0}32’42.40”E							X	X	X	X	X									Tgay83
Morinda angustifolia Roxb.	MoA.	S	26^{0}51’29.10”N 89^{0}24’15.90”E	X	X	X	X	X	X	X													Tgay33
Musaenda sp.	MuSp	S	26^{0}51’22.33”N 89^{0}23’49.24”E		X	X																	Tgay63
Mussaenda frondosa L.	MuF.	S	26^{0}58’41.42”N 89^{0}35’39.31”E						X	X													Tgay62
Mussaenda roxburghii Hook.f.	MuR.	S	26^{0}56’28.31”N 89^{0}35’50.59”E	X	X	X	X	X	X														Tgay46
Neohymenopogon parasiticus (Wall.) Bennet	NeP.	S	27^{0}03’32.20”N 89^{0}35’34.70”E				X	X	X	X	X	X											Tgay69
Neanotis ingrata (Wall. ex Hook.f.) W.H. Lewis.	NeI.	H	26^{0}53’46.60’’N 89^{0}26’12.40’’E				X	X	X	X	X	X	X	X									Tgay73

Table 2A. Continued.

Scientific Name	Abb.[1]	Life form	Coordinates	Reference Points																			Voucher Number
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
				300	500	700	900	1100	1300	1500	1700	1900	2100	2300	2500	2700	2900	3100	3300	3500	3700	3900	
Oldenlandia corymbosa L.	OlC.	H	26^{0}57’15.97’’N 89^{0}35’40.75’’E	X	X	X	X	X	X	X	X	X	X	X	X								Tgay71
Ophiorrhiza mungos L.	OpM.	H	27^{0}03’42.40’’N 89^{0}35’34.30’’E							X	X	X	X	X	X								Tgay44
Ophiorrhiza rugosa Wall.	OpR.	H	27^{0}03’36.60’’N 89^{0}35’33.40’’E								X	X	X	X	X	X	X	X	X	X			Tgay45
Pavetta subcapitata Hook.f.	PaS	S	26^{0}57’58.94’’N 89^{0}35’59.05’’E	X	X	X	X	X	X														Tgay48
Psilanthus benghalensis (B. Heyne ex Schult.) J.-F. Leroy	PsB.	S	26^{0}51’26.30”N, 89^{0}23’50.60”E	X	X	X	X																Tgay34
Rubia cordifolia auct.	RuC.	C	26^{0}56’14.27”N 89^{0}30’51.29”E								X	X	X	X	X	X	X	X	X				Tgay60
Rubia manjith Roxb. ex Fleming	RuM.	C	26^{0}58’45.88”N 89^{0}35’34.48”E					X	X	X	X	X	X	X									Tgay84
Rubia sikkimensis Kurz	RuS.	C	27^{0}02’11.28”N 89^{0}35’24.36”E		X	X	X	X	X	X	X												Tgay39
Rubiaceae sp1.	Sp1.	S	26°58'07.71" N 89°35'28.78"E				X	X	X	X													Tgay89
Rubiaceae sp2.	Sp2.	H	27^{0}03’58.60”N 89^{0}35’27.50”E										X	X									Tgay93
Spermacoce latifolia Aubl.	SpL.	H	26^{0}59’23.10”N 89^{0}56’08.90”E		X	X	X	X	X	X	X												Tgay79
Spermacoce mauritiana Gideon	SpM.	H	26^{0}57’23.84”N 89^{0}35’11.98”E	X	X	X	X	X	X	X													Tgay78
Wendlandia grandis (Hook.f.) Cowan.	WeG.	T	26^{0}58’04.83”N 89^{0}35’36.42”E	X	X	X	X																Tgay47
Wendlandia speciosa Cowan	WeS.	T	26°57'16.09" N 89°33'24.69" E								X	X											Tgay87
Wendlandia wallichi Wight & Arn.	WeW.	T	26°53'35.41" N 89°26' 42.07" E				X	X	X	X	X												Tgay86

Note. [1] = Abbreviation of species name, T=Tree, S=Shrub, H=Herb, C=Climber, X=Presence of species

Table 2B. Lists of Rubiaceae species recorded in study area II (Dagachu-Dagala).

Scientific Name	Abb.[1]	Life form	Coordinates	Reference Points																			Voucher Number
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
				300	500	700	900	1100	1300	1500	1700	1900	2100	2300	2500	2700	2900	3100	3300	3500	3700	3900	
Argostemma sarmentosum Wall.	ArS.	H	26059’22.00”N 89055’36.60”E	X	X	X	X	X	X														Tgay55
Argostemma verticillatum Wall.	ArV.	H	27004’39.50”N 89052’31.60”E			X	X	X	X	X	X	X	X	X									Tgay54
Catunaregam longisipna (Link) Tirveng.	CaL.	S	26056’16.90”N 90000’52.30”E	X	X	X																	Tgay52

Table 2B. Continued.

Scientific Name	Abb.[1]	Life form	Coordinates	Reference Points																			Voucher Number
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
				300	500	700	900	1100	1300	1500	1700	1900	2100	2300	2500	2700	2900	3100	3300	3500	3700	3900	
Chassalia curviflora (Wall.) Thwaites	ChC.	S	27004'34.90"N 89053'24.60"E	X	X	X	X	X															Tgay65
Galium aparine L.	GaA.	H	27010'35.21"N 89046'00.76"E				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Tgay66
Galium asperuloides Edgew.	GaAs.	H	27°05'04.36"N 89°051'7.15"E										X	X									Tgay76
Galium craticulatum R.R. Mill.	GaC.	H	27014'27.70"N 89044'11.53"E														X	X	X	X	X	X	Tgay77
Galium sp.	GaSp.	H	27004'28.20"N 89053'31.26"E					X	X														Tgay67
Hedyotis auricularia L.	HeA.	H	27004'08.00"N 89054'29.00"E	X	X	X	X	X	X	X	X												Tgay70
Hedyotis scandens Roxb.	HeS.	H	27004'42.80"N 89052'00.30"E	X	X	X	X	X	X	X	X												Tgay53
Hedyotis verticillata (L.) Lam.	HeV.	H	26059'27.30"N 89055'35.50"E	X	X	X	X	X															Tgay72
Himalrandia tetrasperma (Wall. ex Roxb.) T. Yamaz.	HiT.	S	27005'09.20"N 89051'30.40"E				X	X	X	X	X	X											Tgay51
Hymenodictyon flaccidum Wall.	HySp.	T	26° 56'48.30"N 90°00'53.78" E		X	X	X	X	X	X	X	X	X										Tgay69
Ixora undulata Roxb.	IxU.	S	6051'22.80"N 89024'04.20"E	X	X	X	X																Tgay57
Luculia gratissima (Wall.) Sweet	LuG.	S	27004'02.90"N 89052'04.40"E			X	X	X	X	X	X	X	X										Tgay85
Mussaenda glabra Vahl	MuG.	S	27000'02.30"N 89055'36.50"E	X	X	X	X	X	X	X													Tgay50
Mussaenda macrophylla Wall.	MuM.	S	26055'58.30"N 89059'36.90"E	X	X	X	X	X															Tgay49
Mussaenda roxburghii Hook.f.	MuR.	S	27003'48.40"N 89053'01.00"E	X	X	X	X	X															Tgay46
Mycetia longifolia (Wall.) Kuntze	MyL.	S	26059'26.30"N 89055'32.70"E	X	X	X	X	X	X	X	X	X	X										Tgay56
Neanotis ingrata (Wall. ex Hook.f.) W.H. Lewis	NeI.	H	27004'80.00"N 89054'29.00"E			X	X	X	X	X	X	X	X										Tgay73
Oldenlandia corymbosa L.	OlC.	H	26059'27.30"N 89055'35.50"E	X	X	X	X	X	X	X	X	X	X	X									Tgay71
Ophiorrhiza fasiculata D.Don.	OpF.	H	27°04'33.08" N 89°53'25.94" E	X	X	X	X	X	X														Tgay42
Ophiorrhiza mugos L.	OpM.	H	27003'59.32"N 89053'19.93"E						X	X	X	X	X	X									Tgay43
Oxyceros fasciculatus (Roxb.) T. Yamaz.	OxF.	S	27°01'47.82"N 89°54'24.48"E					X	X														Tgay59
Paederia foetida L.	PaF.	C	27004'36.00"N 89052'11.70"E	X	X	X	X	X	X														Tgay75
Pavetta subcapitata Hook.f.	PaS.	S	27004'35.40"N 89053'27.20"E	X	X	X	X	X															Tgay48

Table 2B. Continued.

Scientific Name	Abb.[1]	Life form	Coordinates	Reference Points																			Voucher Number
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
				300	500	700	900	1100	1300	1500	1700	1900	2100	2300	2500	2700	2900	3100	3300	3500	3700	3900	
Pentas Sp.	PeSp.	S	27005'09.20"N 89051'30.40"E								X	X											Tgay88
Psilanthus benghalensis (B. Heyne ex Schult.) J.-F. Leroy	PsB.	S	26056'52.10"N 90000'10.80"E	X	X	X	X																Tgay34
Richardia brasillensis Gomes	RiB.	H	27003'48.40"N 89053'01.00"E				X	X	X														Tgay58
Rubia cordifolia auct.	RuC.	C	27005'09.90"N 89051'31.40"E								X	X	X	X	X	X	X	X	X				Tgay60
Rubia manjith Roxb. ex Fleming	RuM.	C	27004'39.00"N 89052'06.20"E					X	X	X	X	X	X	X									Tgay84
Rubia sikkimensis Kurz	RuS.	C	27°00'43.34" N 89°55'19.52" E		X	X	X	X	X	X	X												Tgay39
Rubiaceae sp1.	Sp1.	S	27004'31.44"N 89053'31.69"E						X														Tgay89
Rubiaceae sp2.	Sp2.	S	26026'19.30"N 89055'49.10"E				X	X															Tgay90
Rubiaceae sp3.	Sp3.	T	26059'25.70"N 89055'33.90"E				X	X															Tgay91
Rubiaceae sp4.	Sp4.	S	26°56'48.30" N 90°00'53.78" E	X	X																		Tgay92
Spermacoce latifolia Aubl.	SpL.	H	27002'15.15"N 89054'11.05"E		X	X	X	X	X	X	X												Tgay79
Spermacoce mauritiana Gideon	SpM.	H	27002'15.44"N 89054'24.39"E	X	X	X	X	X	X	X													Tgay78
Spermadictyon suaveolons Roxb.	SpS.	S	26059'23.10"N 89056'08.90"E		X	X	X	X	X														Tgay74
Tarenoidea wallichii (Hook.f.) Tirveng. & Sastre	TaW.	T	27005'09.20"N 89051'30.40"E	X	X	X	X	X	X	X													Tgay68
Uncaria scandens (Sm.) Hutch.	UnS.	C	27003'48.40"N 89053'01.00"E	X	X	X	X	X	X														Tgay41
Uncaria sessilifructus Roxb.	UnS.	C	27003'53.50"N 89053'03.80"E	X	X	X	X																Tgay40
Wendlandia coriacea (Wall.) DC.	WeC.	T	27°04'04.13" N 89°53'32.86" E		X	X	X	X	X														Tgay36
Wendlandia heynei (Schult.) Santapau & Merchant.	WeH.	T	27004'01.20"N 89053'21.50"E	X	X	X	X	X	X														Tgay38
Wendlandia puberula DC.	WeP.	T	27°03'56.37" N 89°53'06.09" E				X	X	X	X	X	X	X	X	X								Tgay37
Wendlandia tinchoria (Roxb.) DC.	WeT.	T	27003'52.80"N 89053'17.50"E		X	X	X	X	X														Tgay35

Note. [1] = Abbreviation of species name, T= Tree, S= Shrub, H= Herb, C= Climber, X = Presence of species

Table 3. Species richness, diversity, and evenness of the two study areas analyzed using the Shannon-Wiener index and Simpson diversity index.

	Genera	Species Richness	Shannon's Diversity	Shannon's Evenness	Simpson's Diversity	Simpson's Reciprocal Index
Area I	21	36 (43.9%)	2.99	0.83	0.94	24.99
Area II	29	46 (56.1%)	3.47	0.91	0.96	27.04

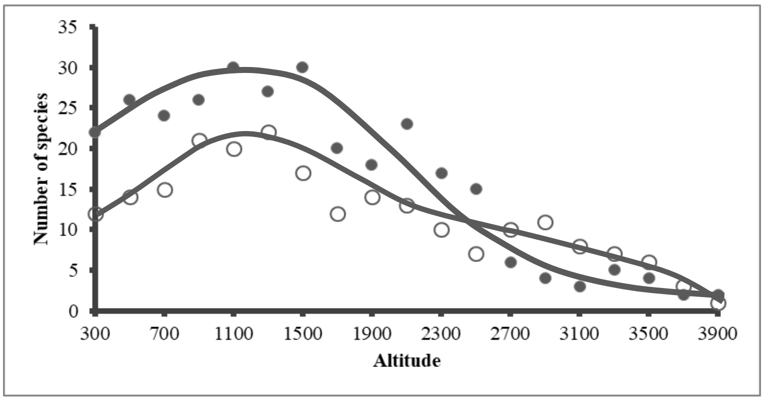

Figure 2. Species richness and distribution pattern of Rubiaceae along the altitudinal gradient. (○ Study area I and ● Study area II)

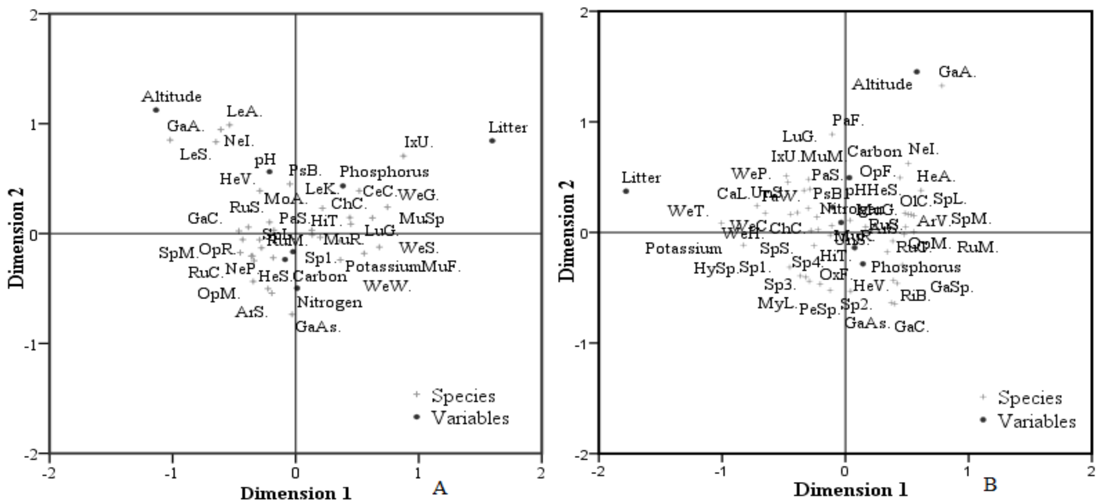

Figure 3. Figure displays CCA ordination results showing the relationships between environmental variables (●) and species (+). The distribution of species on the ordination diagram is related to environmental factors. The abbreviated species names are displayed in the figure and the full name list, author and distribution range is given in Table 2A & B. (A. Study area I and B. Study area II).

Table 4. Pearson correlation analysis among environmental variables (A. Study area I and B. Study area II).

	A	1	2	3	4	5	6	7	8
1	Species								
2	pH	.11							
3	Carbon	-.01	-.25						
4	Nitrogen	-.06	-.29	.65**					
5	Phosphorus	-.11	.36*	.02	-.11				
6	Potassium	-.23	.11	.52**	.59**	.32			
7	Altitude	-.37*	.01	.26	.06	.01	.19		
8	Litter	.31*	.01	.11	.18	.24	.24	-.33*	

N=36. * P<0.05; **P<0.01.

Table 4. Continued.

	B	1	2	3	4	5	6	7	8
1	Species								
2	pH	-.04							
3	Carbon	-.05	.08						
4	Nitrogen	.09	.09	.49**					
5	Phosphorus	.08	.11	.07	-.13				
6	Potassium	.04	.37*	.22	.46**	.44**			
7	Altitude	-.36*	-.14	.22	.04	-.19	-.16		
8	Litter	.32*	.21	.12	.12	-.11	-.03	-.31*	

N=46. * P<0.05; **P<0.01.

4. Discussion

4.1 Species diversity along the elevation gradient of the two study areas

The higher species diversity for study area II revealed by Simpson and Shannon-Wiener diversity and evenness analysis may be an indication of the prevalence of favorable conditions for greater species diversification in this area. While other factors may have had an influence on the higher species diversity, the moderate variation in temperature, rainfall, and relative humidity in study area II compared to the study area I may have generated an overreaching impact on the overall species diversity. It is evident from the maximum mean temperature of 26.6 °C and the minimum mean temperature of 3.8 °C in area I against a maximum mean temperature of 27.4 °C and minimum mean temperature of 7.3 °C in area II, to conclude that the temperature range was favorable in area II for greater physiological processes, growth, development, and diversification of plant species. Furthermore, moderate rainfall and relative humidity may have enabled better conditions for the diversification. Conversely, in area I, the extreme maximum and minimum temperatures coupled by high variation in precipitation and relative humidity along the gradient and across the year (Table 1) may have hindered the growth rate and diversification as plant growth is found to cease below 5 °C and above 30 °C even when other conditions were reliable (Haferkamp, 1988).

4.2 Patterns of Rubiaceae distribution along the altitudinal gradient

The predominance of Rubiaceae at the lower altitude exhibiting a peak at around 1400 m asl. conformed with what was proposed by Davis *et al.* (2009). Hosting all forms of life, the species composition at the lower altitudinal range of 300-1500 m asl. stands at 86% against 50% at the higher altitude range of 1600-3900 m asl. The dominance of herbaceous species at the higher altitude and the absence of tree species beyond 1900 m asl. and shrub species above 2700 m asl. hint at limited tolerances of these life forms to reduced temperature, precipitation, and relative humidity. On the contrary, the preeminence of herbaceous species at the higher altitude range may be fostered by the ability of herbaceous perennials to undergo dormancy during unfavorable periods and complete their life cycle within the short growing period.

Generally, the Rubiaceae taxa that represent only one or two species and the taxa showing a narrow distribution range, such as *Wendlandia grandis* (Hook.f.) Cowan., *Psilanthus benghalensis* (B. Heyne ex Schult.) J.-F. Leroy, and *Ixora undulata* Roxb., *Uncaria sessilifructus* Roxb., were concentrated at the lower altitude. The nature of the species might suggest the endemic character of these species and their susceptibility to extinction due to various disturbance regimes.

The detection of only one species for 18 taxa is unusual for the family which is described as the fourth largest angiosperm family. The sighting of only one species for *Pavetta* L., *Oldenlandia* L. and just two species for *Argostemma* Wall. was surprising as these three taxa are described as the top 20 largest genera in Rubiaceae (Davis *et al.*, 2009). Such findings may well be associated with current research artifacts or unfavorable conditions caused by topography, climate, and edaphic factors for these groups of taxa. The maximum distribution range demonstrated by *Galium* L., particularly the *Galium aparine* L., that ranged from 900 to 3900 m asl., confirmed the high resilience of this taxa to varying environmental conditions and topography. In contrast to species that show a narrow distribution range, such taxa are expected to express high tolerances to the effects of disturbance and global climate change.

4.3 Impact of environmental factors on the distribution pattern

The assessment of the impact of environmental factors on the species distribution pattern and composition revealed that each factor caused varying degree of influences on the total species composition (Figure 3A & B). The cumulative degree of variance explained by the first two dimensions of CCA at 69.5% for area I and 79.7% for area II substantiates the degree of variance caused by the environmental factors to the actual species distribution pattern. The chi-square values of 325.977 (P<0.001) for area I and 312.634 (P<0.001) for area II validates the statistical significance. Consequently, the environmental factors have fostered heterogeneity of conditions that shape the overall species distribution pattern and diversity.

Different Rubiaceae species were influenced differently by each environmental factor as revealed by the CCA ordination diagram (Figure 3A & B). But altitude and litter thickness, in most cases, showed a larger influence on the species distribution pattern along the gradient. The degrees of variance caused by altitude were 95.7% and 98.8% for areas I and II, respectively, causing significant influence on the pattern of species distribution and diversification. The overreaching impact of altitude on species composition could be related to its impact on productivity (Bruun, 2006) and biotic interactions (Lovett, Marshall, & Carr, 2006), which were reported to decrease coherently as the altitude increased. The Pearson correlation analysis strengthens the CCA findings and explains the significant negative relationship between species distribution pattern and altitude, r(35)= -.37 (P=0.05) for area I and r(45)= -.36 (P=0.05) for area II. This result is in accord with the inference of other similar research reports (Rahbek, 1995; Lomolino, 2001; Jamtsho& Sridith, 2017) where species diversity was reported to decline along with the altitudinal gradient.

The total degree of variance caused by litter thickness accounts for 94.8% in area I and 99.7% in area II on CCA ordination, thereby exhibiting a high degree of influence on the species diversity and distribution pattern. It is further validated by Pearson correlation analysis, r(35) = .31 (P=0.05) for area I and r (45) = .32 (P=0.05) for area II, showing statistically positive significance of litter thickness on the species distribution pattern. The correlation analysis also exhibited varying degrees of associations between different environmental factors (Table 4A & B) which have eventually brought a combined effect on the overall species distribution pattern and composition. So, the significant negative correlation between altitude and litter, r (35) = -.33 (P=0.05) for area I and r(45) = -.31 (P=0.05) for area II, supports the idea of one factor influencing the other and the combined control of all the factors on the overall species distribution pattern.

The other environmental factors like pH, carbon, nitrogen, phosphorus, and potassium did not show a statistically significant influence on the species composition. Nonetheless, their combined effect is important in determining species diversity as these nutrient elements play a key role in the growth and development of plant species in general.

5. Conclusions

The higher species richness and diversity observed at low to mid-altitude followed by gradual decline above 1500 m asl. was in concordance with the results of earlier research. The species richness and diversity were found to be influenced by favorable range of annual precipitation, temperature, and relative humidity. In general, altitude and litter thickness showed supremacy over other factors in shaping the distribution pattern of Rubiaceae along the elevational gradient. The representation of 18 taxa, including some of the taxa described as the largest, by one species in the current research requires further validation. The investigation into this gap may help to uncover the obscurity which was possibly caused by the current research design or the species diversity restriction of those taxa powered by disturbance regime or ecological factors.

Acknowledgements

The authors are grateful to the Department of Forests and Park Services, Ministry of Agriculture and Forests (MoAF), Bhutan, for the research permit; the National Soil Centre, Simtokha for their generous support in analyzing the soil samples; the National Centre for Hydrology and Metrology, Thimphu, Bhutan for providing the climate data; and the Graduate School, Prince of Songkla University, Hat Yai, Songkla, Thailand for providing financial support to the first author as well as the research funding of the project through Thailand's Education Hub for Southern Region of ASEAN Countries (TEH-AC) funding and the Thailand Annual Budget of the year 2016.

References

Barbhuiya, H. A., Dutta, B. K., Das, A. K., & Baishya, A. K. (2014). The family Rubiaceae in Southern Assam with special reference to endemic and rediscovered plant taxa. *Journal of Threatened Taxa*, *6*(4), 5649-5659.

Block, P., Taylor, C. M., & Huysmans, S. (2009). Third International Rubiaceae Conference: Introduction. *Annals of the Missouri Botanical Garden*, *96*(1), 1-3.

Bremer, B., & Erikson, T. (2009). Time tree of Rubiaceae: Phylogeny and dating the family, subfamilies, and trees. *International Journal of Plant Sciences*, *170*(6), 766-793.

Bridson, D., & Forman, L. (1992). *The herbarium handbook* (3rd ed.) Kew, England: Royal Botanic Gardens.

Bruun, H. H., Moen, J. V., Virtanen, R., Grytnes, J. A., Oksanen, L., & Angerbjorn, A. (2006). Effects of altitude and topography on species richness of vascular plants, bryophytes and lichens in alpine communities. *Journal of Vegetation Science, 17*, 37-46.

Davis, A. P., Govaerts, R., Bridson, D. M., Ruhsam, M., Moat, J., & Brummitt, N. A. (2009). A global assessment of distribution, diversity, endemism, and taxonomic effort in the Rubiaceae. *Annals of the Missouri Botanical Garden*, *96*(1), 68-78.

Grierson, A. J. C., & Long, D. G. (1999). *Flora of Bhutan. Including a record of plants from Sikkim and Darjeeling* (Vol. 2 Pt. 2). Edinburg, England: The Royal Botanic Garden Edinburgh.

Haferkamp, M. R. (1988). *Environmental factors affecting plant productivity*. Bozeman, MT: Montana Agricultural Experiment Station.

Jamtsho, T., & Sridith, K. (2017). Species composition of the vegetation along the Sherichu River, lower montane area of Eastern Bhutan. *Songklanakarin Journal of Science and Technology, 39*(3), 303-316.

Long, S., McQuarrie, N., Tobgay, T., Grujic, D., & Hollister, L. (2011). Geological Map of Bhutan. *Journal of Maps*, *7*(1), 184-192.

Lomolino, M. V. (2001). Elevation gradients of species–density: Historical and prospective views. *Global Ecology and Biogeography*, *10*, 3-13.

Lovett, J. C., Marshall, A. R., & Carr, J. (2006). Changes in tropical forest vegetation along an altitudinal gradient in the Udzungwa Mountains National Park, Tanzania. *African Journal of Ecology, 44*, 478-490.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Fonseca, G. A. B., & Kent, J. (2000). Biodiversity Hotspots for Conservation Priorities. *Nature*, *403*, 24. doi:10.1038/35002501

Norbu, C., Dorji, T., Dorj, T., Tamang, H. B., Tshering, K., & Hutcheon, A. (2003). A provisional physiographic zonation of Bhutan. *Journal of Bhutan Studies, 8*(8), 54-87.

Ohsawa, M. (1987). *Life zone ecology of the Bhutan Himalaya*. Chiba, Japan: Laboratory of Ecology, Chiba University.

Polunin, O., & Stainton, A. (1984). *Flowers of the Himalaya.* Oxford, England: Oxford University Press.

Rahbek, C. (1995). The elevational gradient of species richness: A uniform pattern? *Ecography*, *18*(2), 200-205. doi:10.1111/j.1600-0587.1995.tb00341.x

Shannon, C. E., & Weaver, W. (1949). *The mathematical theory of communication*. Urbana, IL: University of Illinois Press.

Simpson, E. H. (1949). Measurement of diversity. *Nature*, *163*, 163-688. doi:10.1038/163688a0

Stainton, A. (1984). *Flowers of the Himalaya: A supplement*. Oxford, England: Oxford University Press.

Subedi, S. C., Bhattarai, K. R., & Chauudhary, R. P. (2015). Distribution pattern of Vascular plant species of mountains in Nepal and their fate against global warming. *Journal of Mountain Science*, *12*(6), 1345-1354.

Vetaas, O. R., & Grytnes, J. A. (2002). Distribution of vascular plant species and endemic richness along the Himalayan elevation gradient in Nepal. *Global Ecology and Biogeography, II*, 291-301.

Wangchuk, S. (2005). Biodiversity at its best. In Department of Tourism, Royal Government of Bhutan (Ed.), *Bhutan-Land of the thunder dragon* (pp. 52-57). Thimphu, Bhutan: Department of Tourism, Royal Government of Bhutan.

Zhang, J. T., Xu, B., & Li, M. (2013). Vegetation patterns and species diversity along elevational and disturbance gradients in the Baihua Mountain Reserve, Beijing, China. *Mountain Research and Development, 33*(2), 170-178.