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Research Article

Community Composition of Beetles (Insecta: Coleoptera) along Elevational Gradients in Phulchowki Hill, Lalitpur, Nepal

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Abstract: Beetles are recognized as important bio-indicators of the ecosystem that can be used to determine species diversity, genetic diversity, and ecosystem diversity. We investigated the species composition and diversity of beetles in four seasons along elevational gradients in Phulchowki hill from June 2018 to May 2019. Sampling was done using pitfall traps in five sites located at 1500 m, 1800 m, 2100 m, 2400 m, and 2700 m altitude respectively. Overall, we documented 43 morphospecies under 37 genera and 12 families from the study area. Scarabaeidae was the most dominant family whereas *Onthophagus sp*. 2 was the most abundant species in our study. The Shannon-Weiner diversity index, species richness and abundance were highest at 1500 m. Furthermore, diversity and species richness were highest in the spring, whereas peak beetle abundance was observed in summer. Principal component analysis (PCA) was performed to analyze the distribution patterns of the beetle families along the elevational gradients. PCA revealed a strong association of the Carabidae family with 1500 m, 1800 m, and 2100 m altitude whereas the Scarabaeidae family was mostly associated with human-influenced areas such as 1500 m and 2700 m altitude. The generalized linear model (GLM) revealed that temperature had a major impact on the overall beetle composition. Our study could set the standards for the research community to carry out conservation efforts on beetle diversity at different elevational ranges in the hill region.

Keywords: Abundance, Biodiversity conservation, Pitfall traps, Seasonal variations, Species richness

1. INTRODUCTION

Beetles (Insecta: Coleoptera) are dominant worldwide, constituting nearly a quarter of all known fauna [1]. They form great biodiversity in different habitats and play significant roles in the functioning of the ecosystem [2]. They occur in all major habitats, except for the Polar and marine habitats, and are economically important as agricultural and household pests or predators [3]. About 400,000 species of beetles have been identified worldwide [4] representing 211 families [5], with many more species yet to be discovered [6]. Sixty-three beetle families have been formally recorded from Nepal [7].

Beetles are recognized as important bio-indicators of the ecosystem that can be used to determine species diversity, genetic diversity, and ecosystem diversity [8]. A high diversity of beetles often indicates high diversity of other elements in an ecosystem [9]. Therefore, it is critical to understand global diversity and distribution patterns for assessing the status of overall biodiversity in the present crisis of mass extinction [10-12]. Furthermore, ground layer beetles show a wide range of distribution patterns in terms of geographical regions, climatic conditions, and vegetation patterns [13, 14] and are sensitive to environmental change [15-17]. Abundance, species richness, and composition of ground beetles are affected by the presence of tree canopy, leaf litter, and prey abundance in the forest [18] along with different habitat forms such as deciduous forest, which is important for maintaining rare species diversity [15].

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Environmental conditions change more quickly with altitude than with latitude, so mountain areas are thought to be an ideal location for investigating the relationship between biodiversity patterns and climatic components within spatial constraints [19-21]. Mountains provide steep environmental gradients [22] that contribute to high species diversity and draw the attention of conservationists [23]. The environmental variables, evolutionary factors and land use patterns collectively determine the biodiversity of montane ecosystems [24]. Moreover, beetle species composition also varies along elevational gradients [25, 26]. Species richness tends to decline with increasing elevation [27, 28] or peaks at mid-elevation [29-31]. The temperature has been identified as the primary predictor of species richness along with a few other factors such as relative humidity, soil nutrients, local habitat features, vegetation patterns, and available areas, all of which influence species diversity [32-34].

Phulchowki is the highest hill located in the mid-mountain region of Nepal. It offers a range of geographical slopes that support the inhabitation of a wide variety of flora and fauna. Despite the study area being recognized as a biodiversity hotspot, the beetle assemblages along elevational gradients had yet to be investigated. To understand the overall biodiversity of the mountain ecosystem, a shift in focus on the understudied beetles' community was necessary. Therefore, we carried out this study to: (i) assess species richness and abundance of beetles in the study area; (ii) compare the composition of beetle assemblages along elevational gradients of Phulchowki hill; (iii) determine the seasonal variation and (iv) investigate the relationships between the beetle community and environmental variables (temperature and humidity). The main purpose of this study was to explore the community structure of beetles associated with ecological and environmental components of the mid-hill region. Moreover, this research will help to conduct further studies and implement conservational strategies for the Coleopteran diversity in Nepal.

2. MATERIALS AND METHODS

2.1 Study Area

Phulchowki hill (Latitude: 27°35'00" N and Longitude: 85°24'00" E) is situated in the Lalitpur district of Nepal. Its elevation ranges from 1500 m to 2762 m. Forest is covered by shrubs, herbs and trees and therefore represents a diverse floral assemblage. The Phulchowki hill is characterized by three distinct evergreen broad-leaved forest types: mixed *Schima-Castanopsis* forest at the base (1500 m - 1800 m), Oak- Laurel forest (1800 m - 2400 m) and evergreen oak forest (above 2000 m) [35]. Phulchowki is 4281 ha in size, one-third of which is managed as a community forest (1368 ha), and the rest (mainly on and around the summit) is a national forest [36]. The elevational gradients ranging from 1500 m to 2700 m were chosen for the study (Fig. 1).

2.2 Study Design

The study area was divided into five sampling sites maintained at 1500 m (27°35'18" N, 85°22'47" E), 1800 m (27°34'53" N, 85°22'57" E), 2100 m (27°34'44" N, 85°23'30" E), 2400 m (27°34'38" N, 85°23'54" E) and 2700 m (27°34'16" N, 85°24'13" E). A survey was conducted from June 2018 to May 2019 that covered four different seasons (summer, autumn, winter, and spring). The pitfall trap method was used to collect beetles at each site [37]. Ten pitfall traps were set up in each sampling site within a 300 sq. m area. Each trap was spaced three meters apart from the others and five meters away from the forest fragment border. The traps were then filled with one-fourth of water and a few drops of ethylene glycol as preservatives [38]. All of the traps were set up from 11 am to 4 pm on the same day and then left for one week. These traps were kept in the same place during all seasons. The specimens from each trap were separated from debris and unwanted particles and preserved at 70 % ethyl alcohol. Then labels with site and sample numbers were marked on the vials. Further lab works were conducted at the Department of Zoology, Amrit Campus.

2.3 Identification and Categorization of Specimens

The specimens were sorted into their respective families and assigned to morphospecies based on their morphological features. Different taxonomic keys were used for the identification of specimens up to the family and morphospecies levels [39-42]. We further compared these specimens with labeled beetle specimens available at Natural History

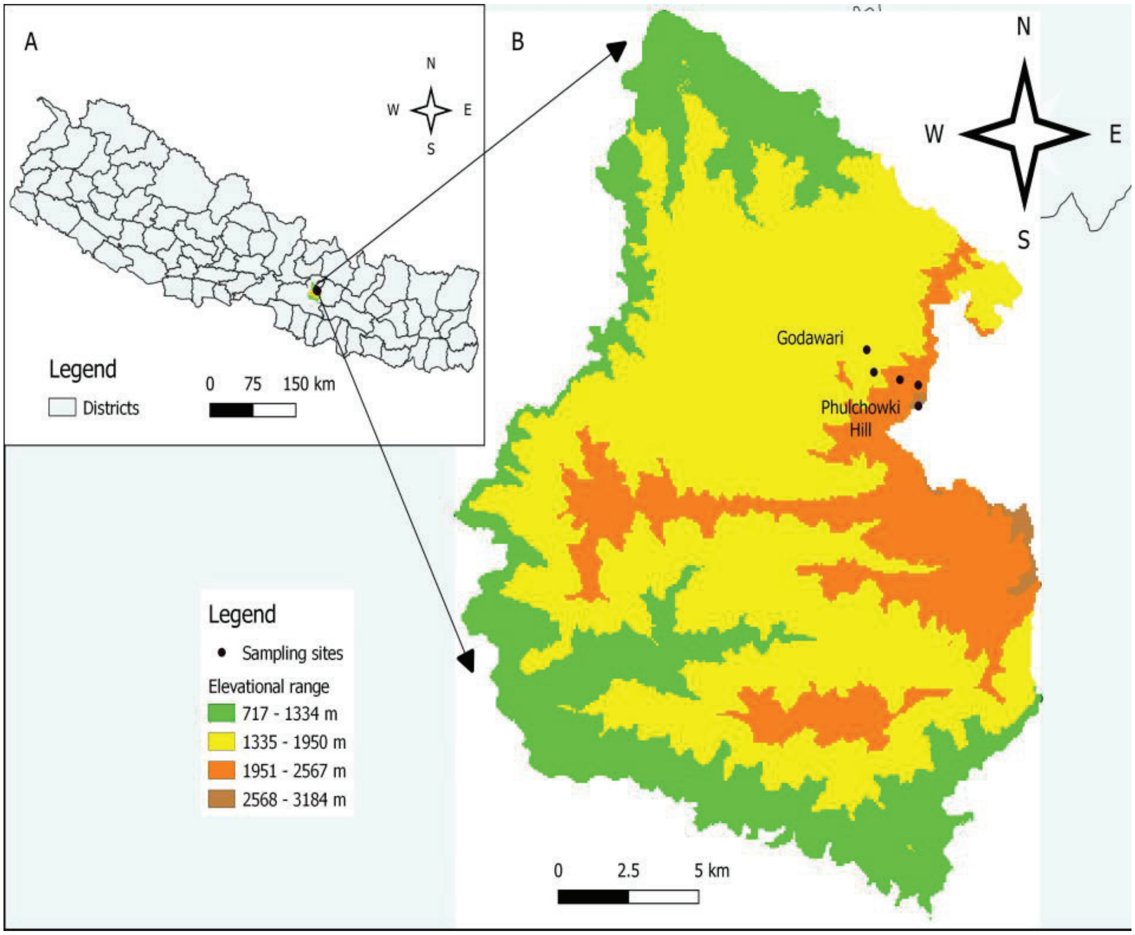

Fig. 1. Map of the study area. A) Map of Nepal showing Lalitpur district; B) Elevation map of Lalitpur district showing sampling sites located at Phulchowki hill

Museum, Swoyambhu, Kathmandu to identify them.

2.4 Data Analysis

Shannon-Weiner (H´) and Pielou´s evenness (J) were estimated for calculating the species diversity of the beetle in study area [43, 44]. Bray Curtis's analysis for hierarchical clustering using the single linking method was used to analyze the similarities among the beetle assemblages. Principal component analysis (PCA) was done to analyze the distribution patterns of beetles among the altitudinal gradient. The data were normalized before analysis. Furthermore, we evaluated the averages of environmental variables i.e. temperature and humidity from our recorded data. These data were measured by ourselves during the sampling period in the study field using a digital thermo-hygrometer (HTC-2). The relationship of temperature and humidity with the species richness and abundance of beetles was tested by generalized linear modeling (GLM) [45]. Data were analyzed using the vegan package [46] in R software version 3.6.1 [47].

3. RESULTS

3.1 Species Richness and Abundance of Beetles

Overall 237 beetle specimens were collected during the survey representing 43 morphospecies under 37 genera and 12 families (Scarabaeidae, Carabidae, Coccinellidae, Chrysomelidae, Silphidae, Staphylinidae, Curculionidae, Megalopodidae, Prionoceridae, Cantharidae, Cleridae, and Tenebrionidae) (Table 1). The Carabidae family had the most species (11) followed by Chrysomelidae (10), Scarabaeidae

Table 1. Beetle species recorded from different elevations in Phulchowki hill

S.No.	Family	Morphospecies	Presence (+) or absence (-) of morphospecies at different elevations					Abundance (%)
			1500 m	1800 m	2100 m	2400 m	2700 m	
1	Cantharidae Imhoff, 1856	*Athemus trimaculatus* Hope, 1831	+	-	-	-	-	1.27
2	Carabidae Latreille, 1802	*Abax* sp.	-	-	+	-	-	1.27
3		*Agonum* sp. 1	+	+	-	-	-	1.27
4		*Agonum* sp. 2	-	+	-	-	-	0.84
5		*Carabus* sp.	-	-	-	-	+	0.84
6		*Cicindela* sp.	-	+	-	-	-	0.42
7		*Craspedophorus* sp.	-	+	-	-	-	0.42
8		*Harpalus* sp.	+	+	+	-	-	2.11
9		*Nebria* sp.	+	+	-	-	-	8.86
10		*Platynus* sp.	-	+	-	-	-	0.42
11		*Pterostichus* sp. 1	-	-	+	-	-	0.84
12		*Pterostichus* sp. 2	-	-	+	+	+	2.11
13	Chrysomelidae Latreille, 1802	*Aplosonyx* sp.	-	-	-	+	-	0.42
14		*Callosobruchus chinensis* Linnaeus, 1758	-	-	-	-	+	1.27
15		*Chaetocnema* sp.	+	+	+	-	-	5.91
16		*Chrysomela* sp. 1	+	-	-	-	-	1.27

S.No.	Family	Morphospecies	Presence (+) or absence (-) of morphospecies at different elevations					Abundance (%)
			1500 m	1800 m	2100 m	2400 m	2700 m	
17		*Chrysomela* sp. 2	-	-	-	+	+	1.27
18		*Cryptocephalus* sp.	-	-	-	+	-	1.27
19		*Gonioctena* sp.	-	-	-	+	-	0.42
20		*Hoplasoma* sp.	+	-	-	-	-	0.42
21		*Hyphasis* sp.	-	+	-	-	-	0.42
22		*Lema* sp.	+	-	-	-	-	0.42
23	Cleridae Latreille, 1802	*Onychotillus* sp.	+	-	-	-	-	0.84
24	Coccinellidae Latreille, 1802	*Coccinella* sp.	+	+	+	+	+	13.08
25	Curculionidae Latreille, 1802	*Otiorhynchus* sp.	+	+	-	+	-	6.33
26		*Phyllobius* sp.	-	+	-	-	-	0.42
27		*Trypodendron* sp.	-	+	-	-	-	0.84
28	Megalopodidae Latreille, 1802	*Zeugophora* sp.	-	-	-	+	-	0.42
29	Prionoceridae Latreille, 1802	*Idgia melanura* Kollar and Redtenbacher, 1844	+	-	-	-	-	0.84
30	Scarabaeidae Latreille, 1802	*Anomala* sp.	-	+	-	-	-	0.42
31		*Cetonia* sp.1	+	-	-	-	+	1.27

S.No.	Family	Morphospecies	Presence (+) or absence (-) of morphospecies at different elevations					Abundance (%)
			1500 m	1800 m	2100 m	2400 m	2700 m	
32		*Cetonia* sp. 2	+	-	-	-	-	0.84
33		*Onthophagus gagates* Hope, 1831	+	-	-	-	+	1.69
34		*Onthophagus* sp. 2	+	+	+	+	+	30.80
35		*Phyllophaga* sp. 1	+	-	-	+	-	0.84
36		*Phyllophaga* sp. 2	-	-	-	-	+	0.42
37	Silphidae Latreille, 1806	*Nicrophorus* sp.	-	+	-	-	-	1.69
38	Staphylinidae Latreille, 1802	*Creophilus* sp.	-	-	-	+	-	0.84
39		*Ocypus* sp.	-	-	+	-	-	0.42
40		*Oxytelus* sp.	+	-	-	-	-	0.84
41		*Stenus* sp.	+	+	-	-	-	1.27
42		*Xantholinus* sp.	-	-	-	+	-	1.27
43	Tenebrionidae Latreille, 1802	*Gonocephalum* sp.	-	-	-	+	+	0.84

(Seven), Staphylinidae (Five), and Curculionidae (Three). The families Coccinellidae, Tenebrionidae, Cleridae, Prionoceridae, Megalopodidae, Cantharidae and Silphidae were represented by single species. A considerable difference in beetle abundance was observed during the study period. We recorded the Scarabaeidae family (36.28 %) as the most dominant family. Overall, *Onthophagus* sp. 2 (30.80 %), *Coccinella* sp. (13.08 %), *Nebria* sp. (8.86 %), *Otiorhynchus* sp. (6.33 %), and *Chaetocnema* sp. (5.91 %) were the most abundant species. *Onthophagus* sp. 2 and *Coccinella sp*. were recorded from all sites.

3.2 Composition of Beetles along the Elevational Gradients

The highest number of species (19) were collected at 1500 m elevation while the least number of species (Eight) were recorded at 2100 m elevation. On the other hand, the abundance of beetle was highest (88) at 1500 m elevation whereas the lowest abundance (20) was observed at 2700 m elevation (Fig. 2).

The Shannon-Weiner diversity index (H´) revealed the highest beetle diversity (H´=2.42) at 1500 m elevation whereas the lowest diversity (H´=1.72) at 2100 m and 2700 m elevations. The evenness index was recorded at maximum (J=0.92) at 2400 m elevation (Table 2).

PCA analysis for Coleoptera assemblages along the elevational gradients predicted the association of beetle families with particular elevations investigated. The first two principal components of the PCA biplot explained 87.9 % and 7.8 % of the total variation (Table 3). There was a strong correlation of species found at 1500 m and 1800 m elevations. A high correlation of 2100 m elevation was observed with 1500 m and 1800 m elevations. Similarly, 2700 m elevation also had a high correlation with 1500 m and 1800 m elevations. However, the beetle's composition of 2400 m elevation was differentiated from any of other elevations (1500 m, 1800 m, 2100 m, and 2700 m). There was a negative correlation of 2400 m and 2700 m elevations with principal component 1 (PC1) in which 2400 m showed a highly negative correlation with PC1. On the other hand, a positive correlation of 1500 m, 1800 m, and 2100 m was viewed with principal component 2 (PC2) (Fig. 3). Species of the Carabidae family were largely associated with 1500 m, 1800 m, and 2100 m elevations. Furthermore, the composition of the Scarabaeidae family was related to 1500 m and 2700 m elevations.

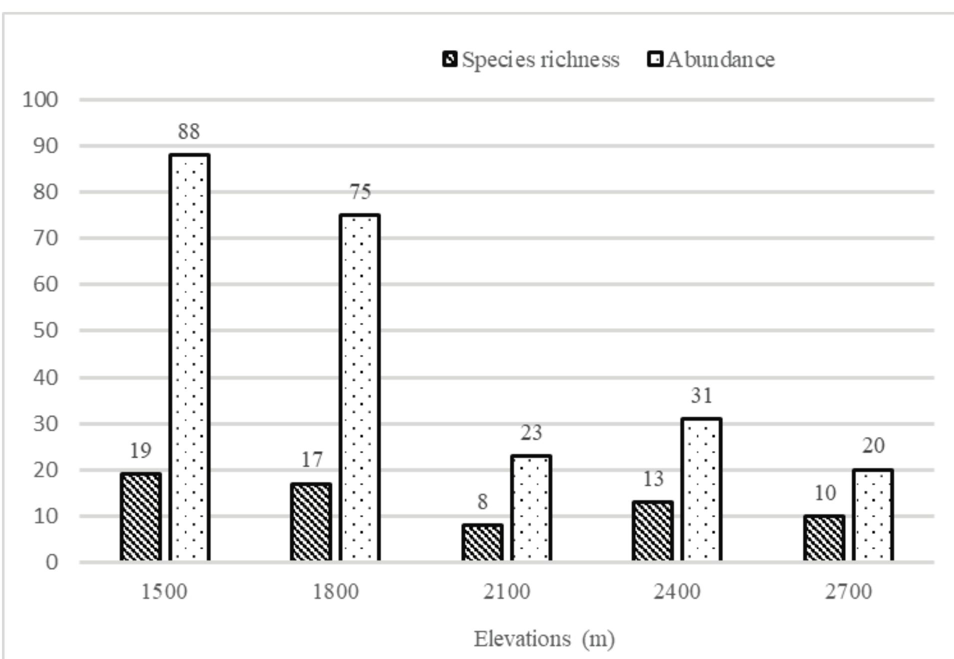

Fig. 2. Species richness and abundance of beetles along elevational gradients

Table 2. Main attributes of beetle assemblages in Phulchowki hill

Attributes	Elevations					Seasons			
	1500	1800	2100	2400	2700	Summer	Autumn	Winter	Spring
Number of families	9	7	4	9	5	7	7	3	10
Number of morphospecies	19	17	8	13	10	10	16	4	23
Shannon-Wiener index (H´)	2.42	2.18	1.72	2.36	1.72	1.25	2.38	1.03	2.50
Pielou index (J)	0.82	0.77	0.83	0.92	0.72	0.54	0.86	0.74	0.79

Table 3. Summary of Principal Component Analysis

Attributes	PC1	PC2	PC3	PC4	PC5
S.D.	2.0967	0.6249	0.34079	0.28529	0.12515
Proportion of Variance	0.8793	0.0781	0.02323	0.01628	0.00313
Cumulative proportion	0.8793	0.9574	0.98059	0.99687	1

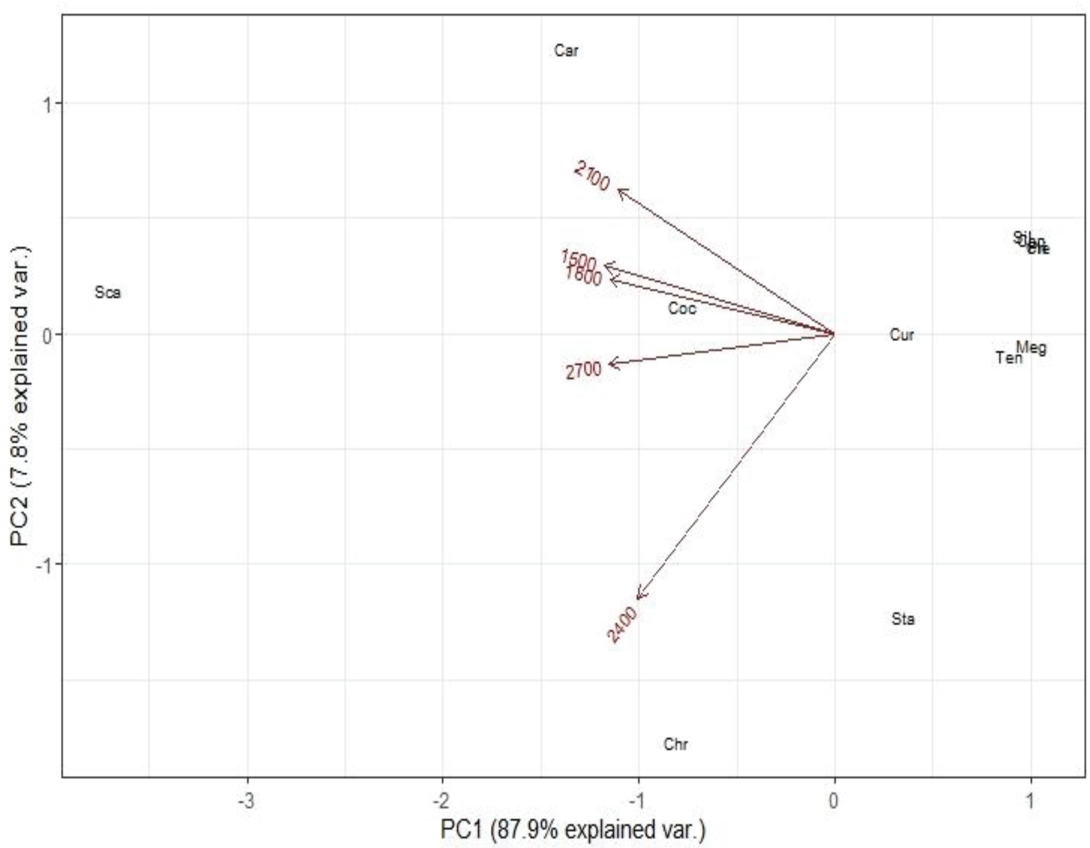

Fig. 3. Biplot of PCA for assemblages of Coleoptera families in five elevations

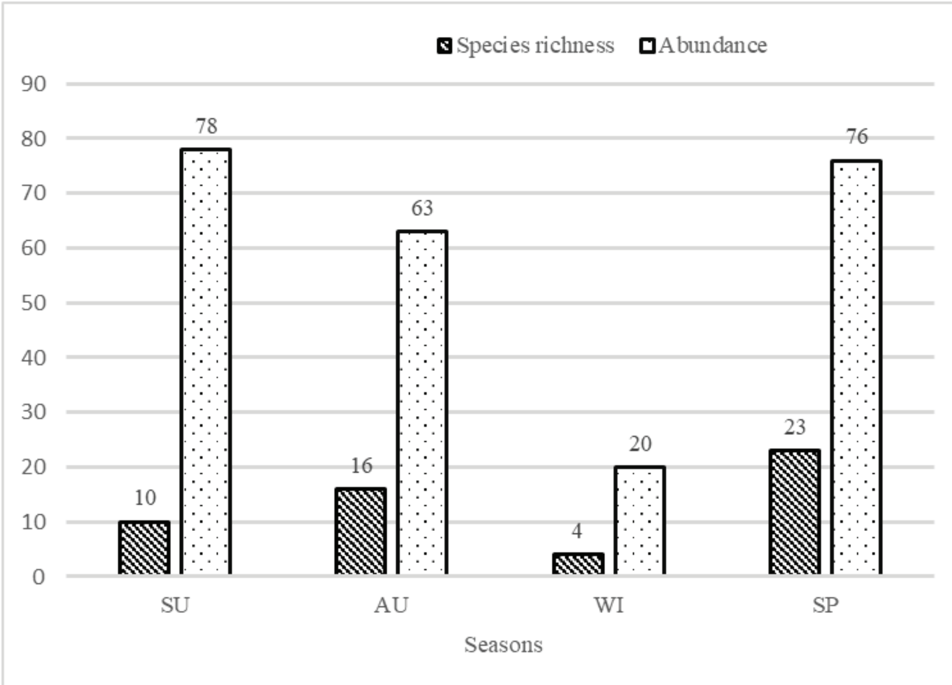

Fig. 4. Species richness and abundance of beetles in four seasons. Seasons symbols:- SU: Summer, AU: Autumn, WI: Winter, SP: Spring

3.3 Seasonal Variation of Coleoptera

Species richness was found to be the highest (23) in the spring season while the lowest number of species (four) were captured during the winter season. However, coleopteran abundance was highest (78) in the rainy summer season and least (20) in the dry winter season (Fig. 4). Furthermore, the Shannon-Weiner diversity index (H´) revealed that the beetle diversity peaked during the spring season (H´=2.5) while bottomed during the winter season (H´=1.03). In contrast to this, the Pielou´s evenness was more or less similar in winter and spring but was found to be maximum in autumn (J=0.86) (Table 2).

The hierarchical clustering dendrogram by cluster analysis depicted similarities in beetle composition between four different seasons such as summer, autumn, winter, and spring. A similar beetle composition was observed between the autumn, summer, and spring seasons. However, the beetle composition in the winter season was least similar to other seasons during the study (Fig. 5).

3.4 Relationships between Beetle Community and Environmental Variables

Environmental factors such as temperature and humidity were used as significant predictor variables (independent variable) whereas beetle abundance and species richness were used as the response variables in the General linear modeling (GLM). According to the results of analysis done using General linear modeling (GLM) with Poisson regression, there was an association between temperature and humidity with the beetle community throughout our study period in Phulchowki hill. It revealed that beetle abundance was significantly influenced by both temperature ($z = 8.211$, $p < 2e\text{-}16$) and humidity ($z = 3.827$, $p = 0.00013$) (Table 4). However, the species richness of beetle was significant with only temperature ($z = 2.263$, $p = 0.0236$). There was no significant impact on the species richness of beetles by humidity ($z = 1.707$, $p = 0.0879$) (Table 5). Overall, this finding indicated that the temperature [abundance ($p < 2e\text{-}16$) and species richness ($p = 0.0236$)] was the best predictor variable than

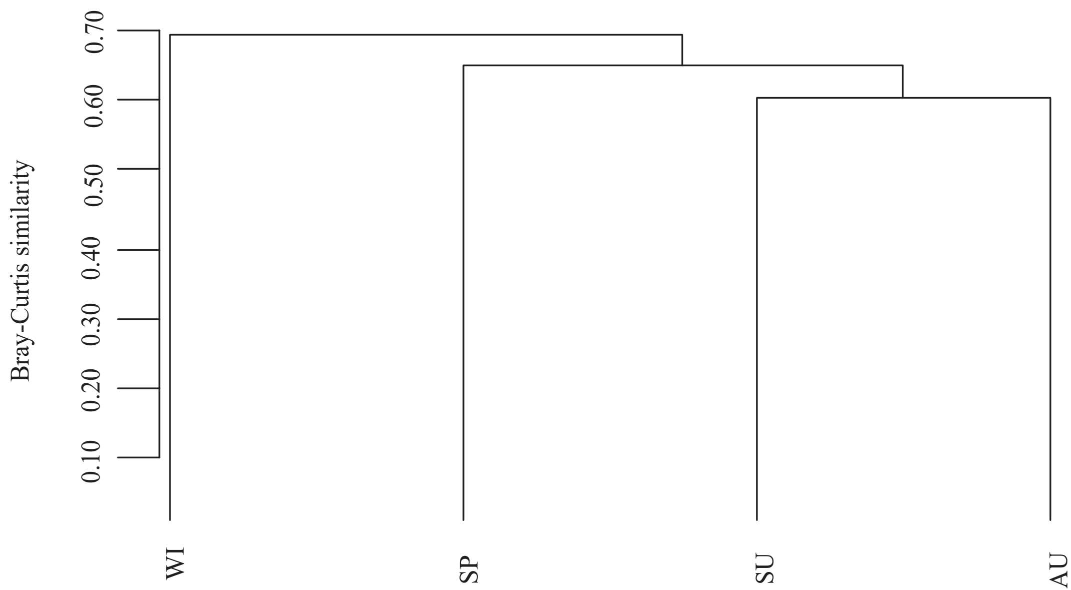

Fig 5. Cluster Dendrogram by Bray Curtis Analysis (single linkage) for beetle assemblages of seasons studied. Season symbols: SU-Summer, AU-Autumn, WI-Winter, SP-Spring

Table 4. Relation of beetle abundance with environmental factors (Generalized linear modeling with Poisson regression using log link function)

Factors	Estimate	Std. Error	z value	p-value
Temperature	0.28590	0.03482	8.211	< 2e-16
Humidity	0.05644	0.01475	3.827	0.00013

Table 5. Relation of species richness with environmental factors (Generalized linear modeling with Poisson regression using log link function)

Factors	Estimate	Std. Error	z value	p-value
Temperature	0.13008	0.05748	2.263	0.0236
Humidity	0.04381	0.02567	1.707	0.0879

humidity [abundance ($p = 0.00013$) and species richness ($p = 0.0879$)] as it greatly affected presence of beetle species along with their abundance and hereby shaped the patterns of beetle community in Phulchowki hill.

4. DISCUSSION

4.1 Species Richness, Abundance and Diversity of Coleoptera along Elevational Gradients

Family Scarabaeidae was most abundant in our study. Satheesha *et al*. [48] also reported Scarabaeidae to be the predominant family in their research from different habitat sites of Davangere University Campus, Karnataka, India. The occurrence of dung-producing mammals in forests could be the reason for the higher abundance of dung beetles [49]. Abundance and species richness, as well as beetle diversity, was observed maximum at the lowest elevation. Musthafa *et al.* [50] recorded the peak diversity at lower elevations. Species richness is considered an indispensable factor to estimate the biodiversity of an ecosystem. A greater number of species were associated with lower elevations than upper elevations. A similar species diversity pattern along increasing elevations at Genting Highland, Malaysia has been documented by Musthafa and Abdullah [8]. Likewise, Gebert *et al* [51] observed abundance to reach a peak at around 1500 m when abundances of beetle species were investigated from 870 m to 4500 m elevation. In addition, variables such as habitat, food availability, vegetation structure and leaf litter are responsible for the diversity and abundance of terrestrial insects [52-54]. Therefore, the availability of more food resources and mixed vegetation at lower elevations could be the reason for the maximum abundance of beetles.

The Carabidae family was predominantly associated with lower elevations such as 1500 m, 1800 m, and 2100 m. These elevations provided different geographical gradients and microhabitats such as caves, endogean, ant nests, termite tubes, leaf litter, tree bark, under logs, rocks, edge of small water bodies, small grassland areas, etc. Ground beetles displayed strong mobility between various ecosystems [55, 56] and their population and species diversity were positively correlated with habitat diversity [57-60]. Most species of Scarabaeidae were recorded from 1500 m and 2700 m elevations. Anthropogenic activities such as manufacturing industries, educational institutes, Godawari buspark, and construction projects around 1500 m elevation and the presence of military camps, temples and tourism-related activities at the top of the hill could be responsible for the existence of many dung beetle species. Musthafa and Abdullah [61] recorded maximum dung beetles in the same way from the high human-influenced area in a recent study. Dung beetle populations are used to determine the land use pattern and effects on biodiversity by human interactions [62].

4.2 Seasonal Variation of Coleoptera

Grouping of pairs by cluster analysis of seasons showed identical beetle assemblages in the summer, autumn, and spring season. However, beetle composition in winter was least similar to other seasons. The presence of favorable vegetation like tree canopies, suitable temperature, and excessive foraging materials contributed to the highest collection of beetle species during the spring season. The outcome of our study was closely related to Silva *et al*. [63], which also recorded the highest number of beetle species during the spring season in the forest fragments of Brazil. Furthermore, our study demonstrated the highest abundance of beetle in the summer season. We observed this result due to heavy rainfall and warm temperature during the summer season. The least abundance was reported in the winter season. Moreover, the dung beetle's composition was affected by the seasons as well and thus, does not occur uniformly throughout the year. The result was firmly associated with the finding of Jain and Mittal [49], which documented the highest abundance of dung beetles during wet summer in the forests of Haryana (India). Similarly, Wardhaugh *et al*. [64] reported the highest abundance of beetle during the wet summer season and the lowest during the dry winter season. Arya *et al*. [65] also recorded the most number of beetle individuals in the rainy season along the altitudinal gradient of Binsar Wildlife Sanctuary, Almora, Uttarakhand, India.

4.3 Relationships between Beetles Community and Environmental Variables

General linear model (GLM) results indicated that both beetle abundance and species richness were affected by temperature. This was further concluded by Moraes *et al*. [53] in their study of Carabid beetles in humid forests of southern Brazil. Contrary to this, the humidity only had a significant impact on the abundance of the beetle. However, no major effect was obtained on species richness due to humidity. The number of species presence greatly influenced the overall beetle diversity. The maximum species richness and beetle diversity were observed in the spring season when the temperature was highest. During cold winter, number of species and diversity were lowest. A study conducted by Nunes *et al*. [22] on dung beetles revealed the decline of dung beetle richness on decreasing temperature. As a whole, the temperature was the best predictor variable than humidity as it often determined the beetle community in Phulchowki hill. Wardhaugh *et al*. [64] also highlighted temperature as a major environmental variable to explain the total abundance and species diversity patterns. Likewise, Oliveira *et al*. [66] addressed the strong and significant correlations between Coleoptera abundance and temperature. Nevertheless, temperature and humidity were accountable for the beetle abundance and their activities [67]. This result was in accordance with the studies of seasonal variation of arthropods in the tropical region [68, 69]. Furthermore, Louzade and Lopes [70] reported the highest number of Scarabaeinae species during the hot summer season in the forests of Brazil. The study on the diversity and seasonal abundance of Scarabaeoid dung beetle in Central New Jersey showed a significant effect of temperature on the abundance of dung beetle captured each month in farm sites [71]. Similarly, Hernández and Vaz-de-Mello [72] found a positive correlation and a linear relationship between the species richness of beetles and the mean monthly temperature in the forest ($p < 0.01$).

There were a few potential limitations in our study. Some of them are mentioned as follows: (i) taxonomy problem for species-level identification of beetle specimens; (ii) difficulty in field visits and data collection due to heavy rainfall during the summer season; (iii) lack of prior research on beetles community in hills region of Nepal and (iv) lack of authoritative climatic data from concerning department.

5. CONCLUSION

This study from Phulchowki hill recorded a total of 237 beetle individuals representing 43 morphospecies belonging to 37 genera and 12 families. Beetle assemblages were more diverse at lower altitudes. The highly abundant Scarabaeidae in human-influenced areas such as 1500 m and 2700 m indicated the growing anthropogenic pressure in the hill region. The Carabidae was the most diverse family which were largely associated with 1500 m, 1800 m, and 2100 m altitudes. Overall, there was a high correlation of species composition between 1500 m and 1800 m elevations. Seasonally, the diversity peaked during the warm spring season and down during the cold winter season. Furthermore, the composition of beetles in the autumn and summer seasons was similar. Our results indicated that temperature had a strong influence on the composition of Coleoptera assemblage. This study in Phulchowki hill can be used to evaluate the elevational beetle diversity pattern in the mountain region in Nepal. It could further set the standards for the research community to carry out conservation efforts.

6. RECOMMENDATIONS

More information on beetle composition might be crucial as their species distribution patterns can be applied as a bioindicator. It can effectively determine the human impact on the mountain ecosystem and thereby help us to execute biodiversity conservation strategies in Nepal. Therefore, extensive research in a wide range of elevations in the mountain region is necessary for a better understanding of overall biodiversity.

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8. CONFLICT OF INTEREST

There is no conflict of interest among the authors.

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