SPECIES COMPOSITION AND FLORAL ASSOCIATION OF BUMBLEBEES (*Bombus* spp.) IN CHITWAN ANNAPURNA LANDSCAPE, NEPAL

THESIS SUBMITTED TO THE
CENTRAL DEPARTMENT OF ZOOLOGY
INSTITUTE OF SCIENCE AND TECHNOLOGY
TRIBHUVAN UNIVERSITY
NEPAL

FOR THE AWARD OF
DOCTOR OF PHILOSOPHY
IN ZOOLOGY

BY
KISHOR CHANDRA GHIMIRE
June 2024

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Institute of Science and Technology

DEAN'S OFFICE

Kirtipur, Kathmandu, Nepal

Reference No.:

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EXTERNAL EXAMINERS

The Title of Ph.D. Thesis: " Species Composition and Floral Association of Bumblebees (*Bombus* spp.) in Chitwan Annapurna Landscape, Nepal "

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18 June , 2024

(Dr. Surendra Kumar Gautam)
Asst. Dean

DECLARATION

Thesis entitled **"Species Composition and Floral Association of Bumblebees (*Bombus* spp.) in Chitwan Annapurna Landscape, Nepal"** which is being submitted to the Central Department of Zoology, Institute of Science and Technology (IOST), Tribhuvan University, Nepal for the award of the degree of Doctor of Philosophy (Ph.D.), is a research work carried out by me under the supervision of Associate Professor Dr. Daya Ram Bhusal of Central Department of Zoology Tribhuvan University and co supervised by Associate Prof. Sudeep Thakuri of Central Department of Environmental Science.

This research is original and has not been submitted earlier in part and full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.

…………………………..

Kishor Chandra Ghimire

18 June 2024

RECOMMENDATION

This to recommend that **Kishor Chandra Ghimire** has carried out research entitled **"Species Composition and Floral Association of Bumblebees (*Bombus* spp.) in Chitwan Annapurna Landscape, Nepal"** for the award of Doctor of Philosophy (Ph. D.) in **Zoology** under our supervision. To our knowledge, this work has not been submitted for any other degree.

He has fulfilled all the requirements laid down by the institute of Science and Technology (IOST), Tribhuvan University, Kirtipur for the submission of the thesis for the award of Ph. D. degree.

…………………………………

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[June, 2024]

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LETTER OF APPROVAL

Date: 18 June, 2024

On the recommendation of **Associate Prof. Dr. Daya Ram Bhusal and Associate Prof. Dr. Sudeep Thakuri,** this Ph. D. thesis submitted by Kishor Chandra Ghimire, entitled **"Species Composition and Floral Association of Bumblebees (*Bombus* spp.) in Chitwan Annapurna Landscape, Nepal"** is forwarded by Central Department Research Committee (CDRC) to the Dean, IOST, T.U.

……………………………………

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Kishor Chandra Ghimire

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शोधसार

भमरा विशेषत उच्च भेगमा पाइने प्राकृतिक र कृषि प्रजातिका वनस्पतिहका महत्वपूर्ण परागसेचक हुन् । यिनीहरूको संख्या विश्वबाट कम हुँदै गईरहेकोले, नेपालमा यिनीहरूको दीर्घकालिन संरक्षणको लागि प्रजाति प्रचुरता, विचरण गर्ने वनस्पति र वातावरणीय असरबारे अध्ययन आवश्यक छ । यो अध्ययनले चितवन अन्नपूर्ण परिद्दश्यमा पाईने भमरा प्रजातिको संरचना, र फूल फुल्ने विरुवासँग तिनीहरूको सम्बन्धको बारेमा अनुसन्धान गरेको थियो । यसको लागि २०१८ र २०१९ को धेरै फूल फुल्ने समयमा समुन्द्र सतहबाट ६००–३५०० मी .को उचाइसम्म कालीगण्डकी) मुस्ताङ तर्फ*)*, मरस्याङदी) मनाङ तर्फ*)* र बुढीगण्डकी) गोरखा तर्फ*)* का पहुचवाला वाटाहरू प्रयोग गरिएको थियो । ती वाटाहरू थरिथरिका वासस्थानको किसिम) कृषि क्षेत्र, घाँसे मैदान, जंगल र करेसाबारी (हुँदै गुज्रन्थ्यो । भमराहरू जम्मा गर्नको लागि अनुसन्धान कर्ता ती वाटाहरूमा विस्तारै हिडे त्यस अवधिमा जुन प्वाइन्टमा भमरा देखिन्छ त्यहाँ रोकिएर २० मी .दायाँ र २० मी .बायाँ गर्दै ३० मीनेट बिताएका थिए र भेटिएका भमराहरूलाई इन्टोमोलोजिकल जाली र हातको सहायताबाट छोपेर जम्मा गरिएको थियो । सम्पूर्ण हिड्ने प्रकृया ३ पटक माथि जाने र ३ पटक तल झर्ने गरी तय गरियो । वनस्पति र भमरा संजालको अध्ययनको लागि ३ वटा ट्रान्जेक्टमा २/२ वटाका दरले समुन्द्र सतहबाट १७०० मी .र २८०० मी .उचाइमा ४० मी x.४० मी .का ६ वटा प्लटहरू तयार गरिएको थियो र अध्ययन अवधिमा प्रत्येक प्लटमा १२ पटक भ्रमण गरियो । एक भ्रमणमा प्रत्यक प्लटमा ६० मीनेट विताइयो । त्यस अवधिमा जुन भमराले प्रजनन अंगलाई छुन्छ, त्यसलाई आधिकारीक भ्रमण मानी गणना गरियो । भमराहरू र फुलफुल्ने वनस्पतिका वर्गहरूलाई नेटवर्क प्रकृया द्वारा विश्लेषण गरियो जसमा उच्च तहमा १६ वटा भमरा प्रजातिहरू र तल्लो तहमा विरुवाका वर्गहरू) स्वदेशि र आयतित(ले प्रतिनिधित्व गरेको थियो । यस अध्ययन अवधि भर १६ वटा भमरा प्रजातिहरू पहिचान गरिएको थियो । यस्तै भमराहरूको सापेक्षित समृद्धि उचाइ, वासस्थान र खाद्यन वनस्पतीको प्रकार अनुसार फरक पाइयो । वी .हेमोरोइडालीस को सापेक्षित समृद्धि सबैभन्दा बढी =n १३४,२.**0**२९ %त्यस्तै दोस्रो र तेस्रोमा वी .फेस्टिभस =n १२९,१९.५७ %र वी .एग्जीमस =n१२४,१८.८४ %थियो तर वी .व्रानिस्को, वी .नोभस र वी .प्रेसस जस्ता प्रजातिको सापेक्षित समृद्धि =n ५% थियो । भमराहरूको समृद्धिमा उचाइले पारेको असर .R=0)२८७६, p=0.000७ (महत्वपूर्ण थियो । सबैभन्दा धेरै प्रजातिको विविधता मध्य उचाई) २००० मीं देखी ३००० मी .सम्म रेकर्ड गरिएको थियो । यसको कारण त्यस क्षेत्रमा फूलहरूको उपलब्धता अत्याधिक रहनु हो । त्यस्तै, गोरखा क्षेत्रको जंगली आवासमा =H)२.१८ (प्रजाति विविधता उच्च थियो त्यसपछि मुस्ताङको घाँसे मैदानमा=H) २.१० (रेकर्ड गरियो । समग्रमा मनाङ र मुस्ताङमा भन्दा गोरखा ट्रान्जेक्टमा प्रजातिहरूको विविधता उच्च थियो । चितवन अन्नपूर्ण परिद्दश्यमा भमराहरूको वितरण, विविधता र

प्रजाति संरचना मुख्यत, आवास मोजेक, अल्टीच्युडनल ग्रयाडिन्ट र होस्ट वनस्पतिको श्रेणीले प्रभाव पार्दछ । अध्ययन गरिएका तीन नदीहरूका वेसीनमा पूर्व देखि पश्चिम सम्मको वातावरणीय परिवर्तनले वनस्पतिका विविधतामा फरकपन ल्याएको छ यसले चितवन अन्नपूर्ण परिदृश्यमा पाइने भमरा प्रजातिको समुदाय संरचनामा महत्वपूर्ण सम्बन्ध राखेको छ । अर्को तर्फ पूर्वी भागमा बढी वर्षा हुने तर पश्चिमी भागमा कम वर्षा हुने भएकोले पश्चिमी भाग सुख्खा छ । त्यसकारण विभिन्न किसिमका परागसेचन मैत्रिक फूलहरूका प्रजातिहरू पूर्व क्षेत्र) बुढी गण्डकी क्षेत्रमा (बढी पाइन्छ त्यसकारण भमराका प्रजातिहरू पनि यो क्षेत्रमा बढी पाइयो । यो अध्ययनले भमरा प्रजातिहरूको फुलमा विचरण गर्ने सम्बन्धलाई करेस्पोन्डीङ एनलाइसीस लिनियर मीक्स मोडल र कल्सटर एनलाइसीस मार्फत वनस्पतीको फ्यामिली, फूलको रंग र वनस्पतिका वर्ग) स्वदेशी र आयतित (द्वारा मापन गरिएको थियो । यो अध्ययनले वनस्पतिका फ्यामिली र फूलको रंग निस्चित भमराहरूले फूलहरू रोज्ने महत्वपूर्ण जैविक कारण हुन् भन्ने देखाएको छ । भमरा जातिहरूको विचरण तिव्रता तल्लो र माथिल्लो उचाइका विन्दुहरूमा स्थानिय र आयतित वर्गका वनस्पतिहरूमा महत्वपूर्ण र निश्चित थियो । भमरा र वनस्पतिका वर्णहरूको संजालको विश्लेषण गर्दा स्थानिय वनस्पतिका वर्गहरूमा आयतित वनस्पतिका वर्गहरूमा भन्दा बढी भमराहरूको प्रतिक्रिया रहयो । यसले निश्चित भमरा प्रजातिहरू)जस्तै, वि .एसियाटिकस, वि .हेमोरोइडालिस, वि .टुनिक्याटस, वि .एग्जीमस(, का भमराहरू अधिक सामान्य हुन्छन् यस्ता जातिहरूको विसाल पोषणीय क्षेत्र हुन्छ र यिनीहरू विभिन्न थरीका वनस्पति हरू स्थानिय, आयतित दुवै विचरण गर्न सक्छन् । यो खोजले विभिन्न इलेभेसन ग्रयाडिन्टमा माहुरी प्रजातिहरूको पारिस्थीतीक भूमिका र विचरण प्रणाली माथि प्रकाश पारेको छ । यसले केही भमरा जातिहरू सामान्यपूर्ण हुने र विभिन्न प्रकारका वनस्पति र पारिस्थीतीक वनावटमा घुलमील गर्न सक्छन् । केही भमरा प्रजातिहरू विशेषज्ञ हुन्छन् र आफ्नो अन्तरकृयालाई विशेष भू–संरचना र विशिष्ट प्रकारका वनस्पतिका वर्गहरूमा केन्द्रित गरेको हुन्छन् । भमरा प्रजातिहरूको फूलको विचरणको फरक पनाले परागसेचन गर्ने बानीमा प्रभाव पार्दछ त्यसकारण चितवन अन्नपूर्ण परिदृश्यको पर्यावरणमा यिनीहरूको योगदान महत्वपूर्ण रहेको छ ।

ABSTRACT

Bumblebees (*Bombus* spp.) are important pollinators especially in high altitude flowering plants either natural or cultivated types, yet these bee communities are rapidly declining globally. Understanding abundance, species composition, distribution patterns, foraging plant species and responses to environmental factors is highly needed to understand the longterm conservation of these vital pollinators in Nepal. This research work was aimed to investigate the species composition of *Bombus* species and their foraging association with flowering plants present along the Chitwan Annapuna Landscape (CHAL). The field survey was carried out in the most flowering seasons of (April to November) of 2018 and 2019. Three walking accessible transects (600 to 3500 m asl.) along the Kaligandaki (Mustang site), Marshyandi (Manang site), and Budhigandaki (Gorkha site) river basins were followed for the collection of bumblebees. The transects passed through different types habitat such as agricultural, home gardens, grasslands, and forests. For the sample collection, researcher moved at a slow pace along the transect, 20 meters right and left. The entire walking process included three times up and three times down from April to November for two consecutive years. The total field collection was performed 108 days (April-May, July-August, October –November). Whenever a bumblebee was encountered, stopped at that point and spent 30 minutes for extensive searching around the point. All possible foraging workers of *Bombus* species were captured before continuing the walking along the transect. The specimens were collected by entomological sweeping net for hovering bumblebees and directly picked by hand-vials method for those bumblebee which are foraged inside the flower. For the plant-bumblebee network anlysis, six plots (plot size: 40m x 40m) were established along the three transects (2 plots in each transect). The upper point was established at the 2800 m where flowering plants were highly clustered during the survey time. Similarly, lower point was also selected on this basis of availability of flowering plants. The lower point was established at 1700 m asl. All possible foraging workers that visited to the floweres were collected within 60 minutes. During the collection, the number of bumblebee visitation in each flower was noted. A single plot was observed for 12 times(hrs) during study period. If bumblebees were in contact with the reproductive structure of a particular flower, it was considered as valid individual for the visitation count. The bumblebees and flowering plant categories (e.g. native type, non-native cultivated type, non-native naturalized type, and invasive type) was analysed by using bipartite network analysis where upper level of network was represented by 16 *Bombus* spp. and lower level was represented by categories of plants. In this study, a total of 16 *Bombus* spp. were identified during the whole study period. The relative abundance of *Bombus* species vary with altitude, habitat, transect sites and foraging plant types. The relative abundance of *B. haemorrhoidalis* was highest (n= 134, 20.29%), followed by *B. festivus* (n=129, 19.57%) and *B. eximius* (n=124, 18.84%). But, species like *B. branickii*, *B. miniatus*, *B. novus*, and *B. pressus* were the least abundant species with 1% relative abundance of each. The effects of elevation on bumblebee richness was found significant (R=

0.2876 p=0.0007) and the highest richness was recorded at the mid-elevation (2000-3000 m asl.), likely attributed to the increased availability of pollinator-dependent flowering plants within this range. The highest species diversity was recorded in forest habitat of Gorkha site (Shannon index H′= 2.18), followed by grassland habitat of Mustang site (Shannon index H′= 2.10). In overall, species diversity was comparatively higher in the habitats of Gorkha transect site than Manang and Mustang sites. This indicates that the distribution, diversity, and species composition of bumblebees in CHAL primarily influenced by the habitat mosaic, elevation gradient and categories of host plants. The climatic variation that created alteration in vegetation dynamics among the three study river basins from eastern to western sites associated to the community composition and richness of *Bombus* species in CHAL. On other hand, drier sites in western rather than eastern sites of himalayn landscapes probably associated with rich vegetation dynamics that alinked with the higher diversity in Gorkha site (Budhigandki basins) rather than westen sites of this study. Overall result attributed to the higher mean precipitation in eastern parts of Nepal that followed by higher richness of flowring plants creating more opportunity for growing wider species of pollinator-friendly flowering plants in eastern sites of this study. Furthermore, the site specificity of some *Bombus* species would associated to the presence of specific flowering plant in that sites. This study examined the foraging relation of *Bombus* species by performing linear mix effective model, cluster analysis and corresponding analysis(CA) of plant families, flower colours and categories (invasive and non invasive) of plants. This study that the plant families and colour of flower were important biotic factors for the forging choice of particular *Bombus* species in this landscape. There was a significant variation in the foraging intensity of identified bumblebee species with native versus non-native categories of plants and it is distinct with lower and upper evelation points. While in the plant-bumblebee interactions network analysis, the highest interaction was indicated in the native flora rather than invasive types of plant categories. This attributes the plant specific foraging behavior of certain bumblebee (specialist) species (eg: *B. asiaticus, B. novus*) where as some species are more generalist (e.g: *B. festivus, B. tunicatus, B. haemorrhoidalis*). This suggests that some *Bombus* species have a broader dietary range and are capable of pollinating various types of plants, including both native and non-native species. In contrast, other *Bombus* species are more selective in their foraging habits, focusing their interactions on a single category of plants. These findings provide insights into the ecological roles and foraging behavior of different bee species in the different elevational gradients. Certain *Bombus* species are referred to as generalists, demonstrating the ability to engage with a diverse range of plant types and ecological settings, while others function as specialists, concentrating their interactions on a specific category of plants within a particular landform. These differences in foraging behavior can have implications for pollination dynamics and the ecological roles of these bee species in the chitwan Annapurna landscape.

Key Words: *Altitudinal gradients – foraging- invasive- interaction- landscape- pollinators*

LIST OF ABBREVIATIONS

BMNH	:	British Museum (Natural History)
CA	:	Corresponding Analysis
CHAL	:	Chitwan Annapurna Landscape
CITES	:	Convention on International Trade in Endangered Species of Wild Fauna and Flora
D.V.	:	Dorsal View
DNA	:	Deoxyribose Nucleic Acid
F.V.	:	Frontal View
GPS	:	Global Positioning System
IAPS	:	Invasive and Alien Plant Species
INV	:	Invasive
IUCN	:	International Union for Conservation of Nature and Natural Resources
L.V.	:	Lateral View
Masl	:	Meters Above Sea Level
NODF	:	Nestedness Based on Overlap and Decreasing Fill
SYN	:	Synonyms
USNM	:	US National Museum of Natural History, Washington DC, USA

LIST OF SYMBOLS

%	:	Percentage
♀	:	Female
In λ	:	Inverse Simpson's
H’	:	Shannon’s diversity index
χ2	:	Chi-square

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CHAPTER 1

1. INTRODUCTION

1.1 Background

Bumblebees are endothermic eusocial insects that originated from central Asia (Williams, 1985) where high fluctuation of temperature occurs. They are well adapted for their activities in the cool climatic condition than other bees so "they are capable for endothermy" (Heinrich, 1972b; Williams, 2007). These insects are categorized within the order Hymenoptera and the superfamily Apoidea and family Apidae. Some bumblebees are popularly recognized as 'cuckoo bumblebees' and are kept in an isolated subgenus, *Psithyrus*, of the genus *Bombus.* Their classification is based on two main factors: first, they are unable to collect pollen, and second, they reproduce by taking over the nests of true bumblebees instead of creating their own nests and raising their own worker bees. Black and yellow body hair, as well as frequent bands, are characteristics of bumblebees. However, the body color of some species can be red, orange, or entirely black. Another distinguishing feature is the soft hair covering their entire bodies, giving them a fuzzy appearance. The hind leg of female bumblebees is transformed into a corbicula, a shiny concave surface that is naked but surrounded by a fringe of hairs used to transfer pollen. This feature helps distinguish them from similar, large, fuzzy bees.

Bumblebees are more beneficial than honeybees due to high laborious nature, presence of long proboscis, efficiency in pollination even in low population, capacity of pollinate vigorously at very low temperature and light density (Allen-Wardell *et al.*, 1998; Goulson, 2010). Therefore, how body size and specific morphological traits impact on pollination efficiency of bumblebee is still vastly unknown. Bumblebees found in the warmer regions are smaller than cooler regions (Peat *et al.*, 2005; Goulson *et al.*, 2005). Similarly, Color patterns is one of the striking characters of Asian bumblebees that greatly vary among the different species (Williams and Osborne, 2009). Some species exhibit mimicry in color of other bumblebees (William, 2007).

In context of global bumblebee species status, a total of 250 species are recorded (Pedersen, 1996; Williams *et al*., 2008) while 34 species are recorded from Nepal (Williams *et al*., 2010), and 48 species from India. Whereas, in China, 130 species have been identified (William, 2022).

The distribution and diversity of *Bombus* spp. have been well-explored in the eastern and western parts of the USA, but there is limited species inventory in Southeast Asia and Africa. Geographically bumblebees are more specific, they are largely confined to northern hemisphere however some typical species are also found in South America and low elevation of south East Asia (Williams, 1998, Goulson, 2010). In Himalayan region, bumblebees they have high species richness and lagerly abundant. These are generally found at an altitude of 1000-5600 masl in Himalayas (Williams, 1985). In the mountains of central and eastern Asia, there is a greater diversity of species (William, 1994). Whereas greater richness of species is associated with flower rich meadows of upper forest and sub-alpine regions of Europe (Goulson *et al*., 2008; Williams, 1991). Some bumblebees (*B. humilis* and *B. sylvarum*) have been disappeared from the warm region as they could not shift to new habitat specially to the northward (Carvell, 2002).

1.2 Nesting behavior of Bumblebees

A solitary queen establishes a small annual colony of 50-1500 bumblebees during the spring in each year. The queen which hibernates in winter in a small underground cavity, known as a hibernaculum, is responsible for formation of the colony (Koch *et al*., 2012).

After awaking from hibernation, queen actively seeks a suitable site to construct a nest. During this period, she ceases to gather nectar from flowers (Goulson, 2010). Bumblebees have the tendency to construct their nests in various cavities such as underground including rodent burrows, holes found in building foundations, or even stacks of firewood. The specific choice of nesting location may vary among different bumblebee species. (Richards, 1978; Svensson *et al*., 2000; Kells & Goulson, 2003; Osborne *et al*., 2008). After finding a suitable nesting site the queen prepares nesting space by building a honey pot in which she collects the pollen for developing brood (Borror *et al*., 1981). The queen lays and incubates the eggs on the pollen lumps (Heinrich, 1972a). Unless there is a need for additional food gathering, the queen primarily resides within the nest. Approximately after four weeks, the queen's initial eggs get hatched into adult workers which perform various tasks, including foraging, maintenance and cleaning of nest, and caring for the brood (Borror *et al*., 1981; Koch *et al*., 2012). There is a noticeable size difference among many species of bumblebees, with foragers typically being larger than bees that remain in the hive (Colville, 1890; Sladen, 1912; Richards, 1946; Cumber, 1949; Brian, 1952; Free, 1955; Goulson *et al*., 2002). The smaller workers have weaker or deformed wings and a less worn coat due to their limited flight activity (Free & Butler, 1959). As the colony expands during the summer, these workers assist the queen in producing a clutch of male offspring, followed shortly by new queen bees. Young queen abdomen is largely full of fat so she is heavier than worker (Cumber, 1949; Richards, 1996). The reproductive bumblebees depart the hive in search of appropriate males after the old queen passes away in autumn. And just after breeding, male bees die while the queen heavily feeds before creating her own hibernulum where she enters a dormant stage duration the winter. There are differences between *Bombus* species in terms of colony size, number of gynes (new queens), and life cycle timing. (Koch *et al*., 2012).

They prefer to forage primarily on perennial plants (Fussell & Corbet, 1992; Mand *et al.,* 2002; Potts *et al.,* 2009), though, they have also been observed foraging in biennial and annual plants. Bumblebees are the most efficient pollinator in some fruits such as large cardamom (Sinu & Sivanna, 2011), and species like *B. haemorrhoidalis* and *B. breviceps* are chief pollinators of crops (Daka *et al*., 2011). In the agricultural as well as natural ecosystems, bumblebees are the important pollinators (Williams, 1994; Velthuis & van Doorn, 2006; Losey & Vaughan, 2008; Chacoff *et al.,* 2010; Bommarco *et al*., 2012) as they perform buzz pollinator for seed set production or in the breeding of seed (Osborne & Williams, 1996; Corbet *et al*., 1991; Carreck & Williams, 2002; Free, 1993; Sabir *et al*., 2011). In various regions of the world, *B. terrestris* is reported to be highly effective pollinators in commercial greenhouse tomato farming, and later most of the tomato growers were used this species instead of honeybees. And they quickly became established as a standard pollinator in greenhouse tomato farming in Europe as well (Goulson, 2010). In India, *Bombus* spp. usually visit crops and horticulture from March to December and species like *B. asiaticus*, *B. albopleuralis,* and *B. simillimus* significantly pollinate Leguminaceae and Solanaceae during blooming period of field crops. The flower morphology is a vital factor for the foraging attraction of bumblebee. Many researchers suggested flower colour and morphology is one of the important factor for the foraging preference of these insect fauna (Menzel, 1967; Menzel & Backhaus, 1991; Heinrich *et al.,* 1977; Menzel & Shmida, 1993). In other hand, the morphological traits of bumblebee well adapted for the pollination. On the basis of tongue length, *Bombus* spp. select their specific host flower in such a way that bumblebees with long tongues prefer to forage plants with deep nectar flowers, making them specialized pollinators for deep nectar flowers (Prys-Jones 1982; Goulson & Hanley 2004; Kawakita *et al*., 2004; Goulson *et al*., 2006). The nesting site selection is one of the important factor for the bumblebee life history success. The suitability of nesting site ultimately influences on the abundance, diversity and distribution of bumblebees. However, the nesting ecology of the bumblebee is vastly unknown however they general trend indicated that they build their nest close to the suitable foraging host that commonly longer seasonality (Free, 1993). Bumblebees of cold alpine regions exhibit low temporal division of labor among different castes in the colony as queen remains active throughout the year to supply resources to the colony (Byron, 1981), unlike the bumblebees of montane and sub-alpine regions where the foraging frequency of queen is significantly declines after emergence of workers. Similarly, there is "intraspecific niche overlap" between workers and queen in higher altitudes as the time for flowering is shorter.

1.3 Conservation issues for Bumblebee

The abundance of important pollinator, bumblebees is declining from the world these days (Westrich, 1996; Buchmannn & Nabhan, 1996; Kevan & Phillips, 2001; Potts *et al*., 2010; Bommarco *et al*., 2012), mainly in past 50 years (Goulson *et al*., 2005). The relationship beween bumblebees and their host plants, their foraging behavior and ecology are still unknown. On the other hand, bumblebees are seriously threatened by pesticides, alien competitors, infections, and habitat degradation (Williams & Osborne, 2009; Cameron & Sadd, 2020). The need for their conservation is realized due to factors like climate change, habitat loss, agriculture intensification, and pathogens (Thompson & Hunt, 1999; Yang, 1999; Goulson *et al*., 2008; Williams & Osborne, 200; Gill *et al*., 2012; Laycock *et al*., 2012; Whitehorn *et al*., 2012; Arbetman *et al*., 2017; Graystock *et al*., 2013; Morales *et al*., 2013). Therefore, detail study is needed for conservation in the natural ecosystem. To reduce the negative effects of environmental changes and consequent uncertain outcomes, we must protect diversity of bumblebees as far as possible by extensive research. To attain this, we must determine the bumblebees are the most endangered species (Williams & Osborne, 2009). Similarly, altitude influences plant-bumblebee's interaction networks and it significantly impacts behavior of bumblebees (Miller-Struttmann & Galen, 2014).

The Himalayas range is one of the largest hotspots for *Bombus* spp., and is home to more than one-fifth of all bumblebee species reported (62/250: updated from Williams, 1998). In a global scale, the maximum number of species is recorded in the sub-alpine grasslands. Bumblebees are not present however in the lowland plains, the Western Ghats mountains, the Himalayan foothills below roughly 300 masl., and the lowland plains of the Indian sub-continent. The Himalayan region exhibits a diverse range of climatic conditions. In the eastern part, the climate remains stable with an abundance of annual rainfall, while the western part experiences significant annual temperature fluctuations and arid conditions (Williams *et al*., 2010; Rawat, 2017). These climatic differences give rise to distinct variations in fauna and flora. Few annual rainfall ecosystems are found in the western Himalayas where dry alpine grazing grasslands and broadleaf temperate forests are found in elevation places. (Rawat, 2017). Conversely, the eastern regions of Himalayas receive much higher annual precipitation, reaching up to 5,000 mm, leading to the development of moist alpine meadows and subtropical broadleaf forests at higher altitudes (Rawat, 2017).

The eastern Himalayas are recognized as a global biodiversity hotspot due to the abundant diversity of species (Myers *et al*., 2000). Extensive research attempts on composition of bumblebees have been carried out in both the western and eastern Himalayas (Williams, 1991; Williams *et al*., 2010, Saini *et al*., 2015). However, the central Himalayas comprising Sikkim and Nepal, be distinct as the regions with the greatest abundance of bumblebees (Williams *et al*., 2010; Saini *et al*., 2015). The distribution limits of the eastern and western bumblebee species are seen in Nepal, and the intersection of faunal zones is thought to be responsible for the high diversity of this insect species (Williams *et al*., 2010).

Chitwan Annapurna Landsape region, situated in the central Himalayas, displays remarkable diversity in terms of latitude, altitude, rainfall, topography, and climate. Abundant biodiversity in the region is strongly influenced by a diverse range of geographical and environmental factors. The region has a wide range of vegetation, from tropical deciduous wood forest to temperate forests, due to its diversified landscape, geographic position, climatic fluctuations, rainfall patterns, and variable elevations (Luitel *et al*., 2019). Therefore, there is an urgent requirement for fundamental data documentation, ecological information, and the conservation status assessment of insect pollinators, including bumblebees. Except Williams *et al*. (2010), there is no systematic study of the bumblebees in Nepal including the study area. There is less information regarding conservation status in IUCN red list category for these globally declining pollinator from Himalayan region including Nepal (Williams & Jepsen, 2017). Therefore, this study is crucial to assess the diversity of *Bombus* spp. Similarly, regarding CITES bumblebee species (if any) have not been updated from Nepal and this study will be base line information to identify bumblebee as under CITES appendices.

1.4 Rationale

Bumblebees are highly efficient pollinators for many wild and cultivated plants because they can operate at extremely low temperatures, where no other pollinators can fly (Saini et al., 2012). This is due to their thermoregulatory abilities, which allow them to maintain a thoracic temperature above the ambient temperature when in flight. Bumblebees can keep their thorax warm and active even when the external temperature drops as low as 5°C (41°F) and can continue flying and foraging in temperatures up to around 30°C (86°F) (Williams, 2022). This remarkable temperature range is possible because bumblebees generate heat through rapid muscle contractions in their thorax, enabling them to warm up their flight muscles and remain active in conditions that would halt other insects. CHAL is majorly occupied by agricultural habitats and enriched with natural vegetation where the efficient pollinator like bumblebee is important for their reproductive success. CHAL harbors several plant families, including the Solanaceae and Leguminosae as the major cropping plants where bumblebee is leading pollinator (Bhattarai *et al.*, 2006, Bhusal *et al.*, 2019, Rajbhandari *et al.*, 2021). Furthermore, they are the important pollinators for numerous crops: *Cichorium endivia, Lycopersicum esculentum Raphanus sativa, Solanum melongena, Brassica oleracea B. napus* (Bhattarai *et al.*, 2006, Gurung, 2019, Rajbhandari *et al.*, 2021). Beside this, the significant decline in bumblebee populations, posing a substantial threat to biodiversity and ecosystem services across various regions, including the Himalayan area (Goulson, 2003; Ollerton, 2017; Williams & Osborne, 2009; Biesmeijer *et al*., 2006). Bumblebee declines are caused by a number of factors, including diseases, loss of habitat, use of pesticide, climate change, and agricultural intensification. (Edwards & Williams, 2004; Williams & Osborne, 2009; Cameron *et al*., 2011). In this context, the rapidly degraded ecosystem of CHAL is need to be measured in term of pollinator richness, important indicator species for pollination success and structure of plant–pollinator interaction. This is vital to ecosystem resilience and agroecosystem conservation in this landscape. Therefore, plant pollinator interaction and to understand the rare and common bumblebee species for the reproductive success of any important plant is highly essential from this region. The decreasing tendencies and worldwide extinctions of bumblebees highlight the significance of accurately identifying *Bombus* species, knowing their distribution, and investigating their foraging behavior, both in Nepal and the present study area. The taxonomic importance of bumblebee in this region is further significant to be studied since little information on the species richness and ecology of bumblebees in Nepal (Williams, 1991) has known yet. Although the documentation of 34 bumblebee species in different regions of Nepal (Williams *et al.*, 2010), a notable gap persists in the national-level species records, including those from the CHAL. Species like *Bombus pressus*, *B. branickii*, *B. abnormis*, *B. personatus*, and *B. grahami* identified as rare, have been recorded in various parts of Nepal (Williams *et al*., 2010). However, there is vastly lacking in the scientific and systematic database preparation and understanding of the ecology of bumblebees in the country. The taxonomic study of bumblebee (*Bombus* spp.) is challenging since they exhibit distinctive feature of colour pattern (Williams, 2007). As in CHAL, information regarding the ways in which the conversion of natural habitats into new anthropogenic environments affects species and the means by which the species survives in these modified ecosystems is necessary for the proper conservation of bumblebees in agricultural habitat. The conservation of this important insect in the CHAL is under stress due to the rapidly changing agricultural landscape and the growing use of pesticides (Thapa, 2015). The Himalayan belt is a hotspot for many species of bumblebees and their taxonomic richness, the study on bumblebees remains limited (Bhusal, 2020; Williams, 2009; Williams *et al*., 2010; Saini *et al*., 2015; Streinzer *et al*., 2019). For the time being, these are vulnerable to climate change and other human-induced pressures. Therefore, it is urgently needed to create inventory of species and their distribution ecology and plant –bumblebee interaction. Such condition has highlighted the need for a comprehensive exploration regarding the taxonomic richness and distribution patterns and interaction of bumblebee with local plants in this landscape. The connection of bumblebees with foraging plants, their abundance and richness with altitude is urgently needed under rapidly expanding invasive flora in this landscape. Hence the objective of this study was to explore the taxonomic status, foraging ecology and plant –bumblebee interaction in CHAL.

Therefore, a complete inventory of the bumblebees becomes crucial across altitudinal gradients along CHAL river basins. The present study areas severely facing by habitat loss due to habitat fragmentation, forest firing, infra structure development and use of pesticides. Therefore, this work signifies to identify the *Bombus* species, their foraging relation in CHAL. This very first extensive survey work for bumblebee is urgently needed to produce species database, distribution map and identification of important foraging plants of this fauna.

1.5 Objectives

1.5.1 General objective

- ❖ To explore species abundance, diversity and floral preference of bumblebees (*Bombus* spp.) along three river basins (Marsyangdi, Kaligandaki and Budigandaki) of Chitwan Annapurna Landscape, Nepal

1.5.2 Specific objectives

- ❖ To investigate the species abundance and diversity of bumblebees in study area.
- ❖ To examine the foraging preference of *Bombus* species with their associated flowering plants.
- ❖ To evaluate the interaction network between *Bombus* species with foraging plants in study area.

1.6 Organization of the thesis

In this thesis, every research questions are solved in a separate chapter. There are many species of bumblebee which play an important role in pollination in various ecology especially at high altitude region. Thus in this thesis taxonomy, abundance, diversity, relation with different plants and the interaction of different species of bumblebee are focused. These are vital pollinator of natural ecosystem.

Chapter 1

This chapter provides the outlines general introduction of thesis, highlights objectives in depth, description of study area and rationales of study.

Chapter 2

This chapter provides the taxonomy of different species of bumblebee recorded from Chitwan Annapurna landscape. Worker based identification keys Seven sub-genera and 16 species were recorded.

Chapter 3

In chapter three, the abundance, species richness and diversity of bumblebees across various elevations were examined. In our investigation, we observed a significant impact of elevation on bumblebees, indicating a mid-domain effect in their distribution across different altitudes.

Chapter 4

This chapter discusses the foraging behavior of *Bombus* species in the Chitwan Annapurna Landscape, comparing their frequency on native and non-native plants. The study revealed that *Bombus* species demonstrate preferences for particular plant families and flower colors, providing insights into their foraging behavior within both native and non-native plant.

Chapter 5

This chapter explores how plant-pollinator networks change with elevation in the Chitwan Annapurna Landscape. The results show that bumblebee species exhibit different foraging preferences, with many favoring native plants over nonnative ones, and specific bumblebee species tending to be more specialized in their flower choices.

Chapter 6

This study in the Chitwan Annapurna Landscape examined the diversity and foraging behavior of Bumblebee (*Bombus* spp.) at different altitudes. According to the research, species richness and diversity were highly impacted by elevation, with the mid-elevation region (2000–3000 m asl) having the maximum species richness. Native plants were preferred by *Bombus* species, and the study highlighted the importance of these plants for conservation. The study also observed complex plant-bumblebee interactions, with some species being specialists while others were generalists. Chapter six presents a comprehensive overview of the entire thesis, encompassing a summary of the research conducted. Moreover, the main conceptual framework illustrated in Figure 2.

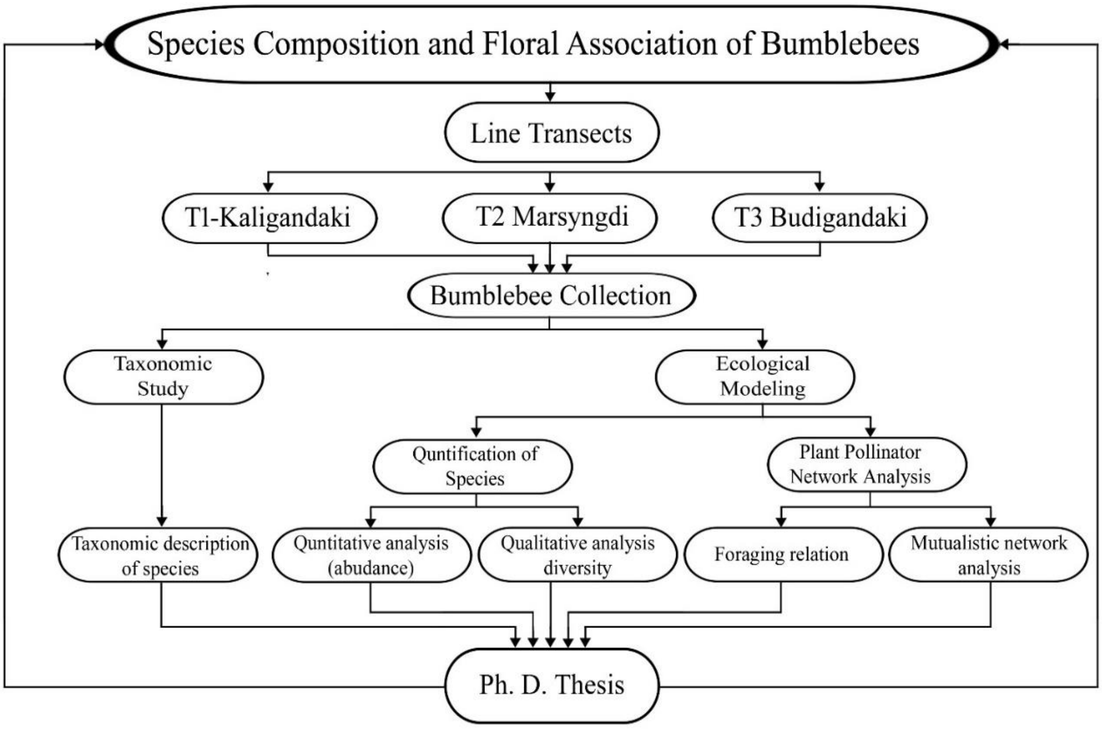


Figure 1: Illustrates the conceptual framework for the thesis

CHAPTER 2

2. SPECIES IDENTIFICATION OF BUMBLEBEES

2.1 Introduction

Bumblebees (Hymenoptera-Apidae) are social insects and regarded as the most efficient pollinators (Saini *et al*., 2012) for native plants and agricultural crops (Semida & Elbanna, 2006). They play a vital role in conserving numerous endangered native plant species (Milberg & Bertilsson, 1997) and facilitating the reproduction of various crops, such as clover, blueberries, and tomatoes (Heinrich, 2004). Unlike other pollinators which cannot fly at lower temperatures, they show remarkable ability to forage (Allen-Wardell *et al*., 1998; Saini *et al*., 2012). Bumblebees have ability to pollinate with high speed and can open the pollen sacs with vibrating as buzzing behavior which makes them effective pollinator.

Bumblebees with shorter tongue lengths, unable to access nectar in deep corolla, exhibit robbing behavior (Free & Williams, 1976; Inouye, 1980). Globally, 250 species with 15 subgenera are known (Williams *et al*., 2008) whereas 34 species with 10 subgenera are recorded from Nepal (Williams *et al*., 2010). In Arunachal Pradesh, (India), Streinzer *et al*., (2019) accompanied first systematic investigation of bumblebee distribution in the eastern part of Himalaya. Saini *et al*., (2015) identified 21 species of bumblebees belonging to six sub-genera at an altitudinal range of 233 to 4,260 masl in India. Altogether, the recorded number of *Bombus* spp. of India was 48. Williams *et al.,* (2017) identified 130 bumblebee's species from China, which represents about half of the total species reported from the world. There are 44 different species of bumblebees in the Tibetan Plateau, according to Williams *et al*., (2009). Similarly, an *et al*., (2014) reported a total of 76 species of bumblebees from north China. Out of 250 species, 45 species are categorized as "cuckoo bumblebees" and are classified under the separate subgenera *Psithyrus*.

Bumblebees are relatively larger than the majority of bee species and are covered in thick piles. So, they are capable of endothermy (Heinrich, 2004). Bumblebees look colorful which is characterized by black and yellow body hair often in bands. Bumblebees display a wide range of color patterns, with some having orange or red body hair, while others may be entirely black. The variability in color patterns within species and the similarity of these patterns among different species pose challenges for taxonomists in accurately identifying the bumblebee species. For the classification of bumblebees, species are particularly challenging. This is due to the fact that bumblebees' noticeable and complex variation in the color patterns of their hair has frequently resulted in quite varied perceptions of the species by various people. Vogt, (1911), broad geographical variation within one (polytypic) species among different regions (Vogt, 1911; Reinig, 1935; Tkalcu, 1968, 1989; Hines & Williams 2012) and frequently combined with color pattern convergences among sometimes distantly related species within one region (Reinig, 1935; Tkalcu, 1968, Willams, 2007; Hines & Williams 2012) are just a few examples of colour-pattern variation. All of these variations have some genetic component to them, but they are further complicated by variations brought on by things like aging-related color fading from sunlight exposure, hair loss from abrasion, and occasionally failure of the normal hair color to develop due to things like thermal or other shocks to pupae during development. Because of this complexity, experts on bumblebees have long disagreed on the criteria that should be applied to identify species (Radoszkowski, 1884) and the concepts that result from this (Williams *et al*., 2015).

There is an urgent need of basic data documentation, ecological information and conservation status of insect pollinators including bumblebees. Except little work (Williams *et al*., 2010) there is no systematic study of the bumblebees in Nepal including the study area. There is less information regarding conservation status in IUCN red list category and CITES for these globally declining pollinator from Himalayan region including Nepal (Williams & Jepsen, 2017). The lack of knowledge and documentation of bumblebee species in this region may lead to the ichance of disappearance and extinction of numerous bumblebee species. So, this study is very crucial to assess the diversity of bumblebee’s species. Further, the outcomes of this research will be useful for disseminating and promoting taxonomic knowledge and drawing the particular attention about importance of bumblebees as their loss will have negative significances on various habitats.

Limited information is available concerning the bumblebee species and their geographical distribution, along with their associations to the host plants and species richness in the Kaligandaki, Marsyngdi, and Budigandaki river basins within the CHAL region. Thus, establishing baseline information on the composition of species and distribution of the genus *Bombus* throughout the three river basins systems of Central Himalaya was one of the primary objectives of this study.

2.2 Materials and Methods

2.2.1 Study area

This research was done along an altitudnal gradient in Chitwan Annapurna Landscape majorly focusing on main three river basins [Fig. 3 (a)]: Kaligandaki (Mustang site 28°87′ 65.15″ N - 83°79′ 47 65″ E), Marshyandi (Manang site, 28°57′ 52. 62″ N - 84°18′ 66. 28″ E) and Budhigandaki (Gorkha site, 28°18′ 36.44″ N - 84°85′15.79″ E). The bumblebee collection was done followed by these riverbasins starting from 600 to 3500 m asl in each basins. These river valleys are hotspots of biodiversity with various habitat types, including forest, agriculture, grassland, and human settlements [Fig. 3 (b)]. These regions exhibit a range of microclimatic variations, each distinct in its ecological characteristics, including microclimatic types and vegetation structures. These elements play crucial role in establishing appropriate environments for a variety of species within bee communities. Additionally, these river valleys serve as vital migratory routes for birds and provide essential habitats for several endangered species, such as the Himalayan black bear (*Ursus thibetanus*), snow leopard (*Panthera uncia*), and red panda (*Ailurus fulgens*) (Adhikari, 2019; Chetri, 2019). Moreover, these valleys include significant protected areas, especially the Annapurna Conservation Area, in Nepal. The study locations contain a wide range of subtropical climate with high rainfall in the south (below 1000 m) to a temperate climate in the mid-hills (1000–4000 m) and an alpine/arctic climate with low rainfall above 4000 m (Chetri, 2017). Over four million people live in the CHAL, where they primarily depend on forest resources and ecosystem services for their livelihoods and overall well-being. The region also possesses a significant cultural heritage. These days, the area faces significant risks from growing human activity, fast migration and settlement, invasive alien plant species (IAPS) degrading ecosystems, and climate change effects. Significant biodiversity loss in the region is very likely as a result of these causes.

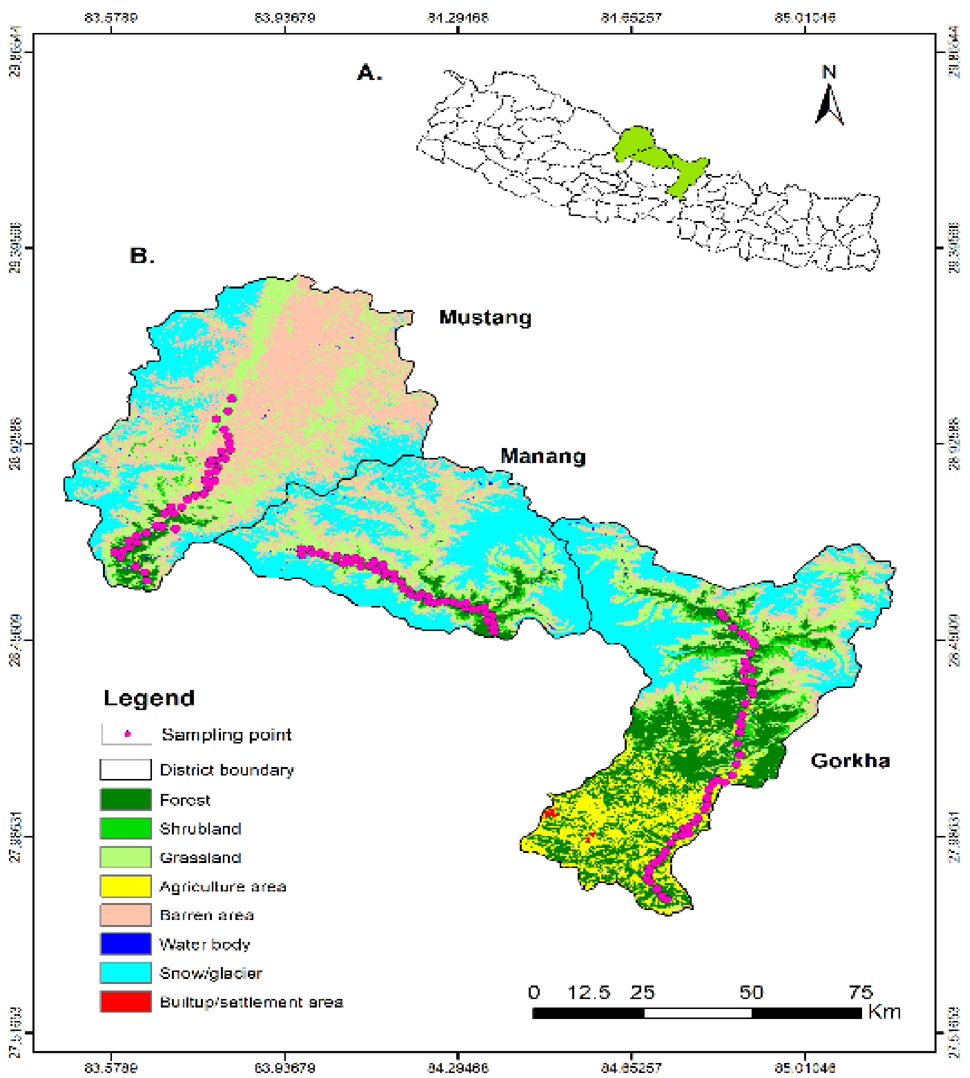


Figure- 2: Study site

2.2.2 Sampling techniques

The survey was conducted in the peak flowering seasons of (April to November) of 2018 and 2019. For the collection of bumblebees, three walking accessible transects (600 to 3500 m asl.) along the Kaligandaki, Marshyandi, and Budhigandaki River basins were followed. The transects passed through different habitats: agricultural areas, forests, grasslands, and home gardens. The *Bombus* specimens (hovering workers) were collected by entomological sweeping net and some specimens were also directly collected by hand picking method in collecting vials. The hand picking method was applied for those bumblebee which are situated inside the flower while collecting.

Three researchers moved at a slow pace along the transect, following 20 meters right and left of transect. The collected specimens were carefully transferred to collecting vial having cotton bulb with ethyl acetate. The complete walking routine (performed three times upward and three times downward) took place from April to November over two consecutive years (2018 and 2019). The entire field collection covered 108 days, covering the months of April to May, July to August, and October to November.Whenever a bumblebees were sighted, we stopped at that point and spent 30 minutes for extensive searching around the point along the transect. Meanwhile, some *Bombus* specimens were also opportunistically collected along the transect beside the targeted collection i.e. 30 minutes at a particular point (Ghimire *et al*., 2023; Saini *et al*., 2015).

For the plant-bumblebee network anlysis, six plots (plot size: 40m x 40m) were established along the three transects (2 plots in each transect). The upper point was established at the 2800 m where most flowering plants were highly clustered during the survey. Similarly, lowere point was selected on the basis of highest clustered of flowering plants. The lower point was established at 1700 m asl. The bumblebees were observed and captured all possible foraging workers within 60 minutes by three people. During the collection, the number of bumblebee visitation in each flower was noted. The plot observation was carried out 12 times during ascending and descending the trastsects in sampling months of the collection years. If a bumblebee visited multiple flowers on the same infloresence each was considered a visit and valid for data collection The bumblebees and flowering plant categories was analysed by using bipartite network analysis

2.2.3 Identification

Pubescence’s colour, the ocello-ocular area, the inter-ocellar distance, the length of malar space, the sculpturing of the labrum, and the length of antennal segments 3, 4, and 5, along with the number of mandibular teeth, the seventh tergum, and the sternum in females, have been identified as reliable, consistent, and clear taxonomic characteristics for bumblebee taxonomy (An et al., 2014, Williams et al., 2010, Saini et al., 2015, and Williams, 2022), During the species identification process, the identified species can be referenced using the information provided by the Natural History Museum, accessible at the following link: https://www.nhm.ac.uk/research-curation/research/projects/bombus/or.html

2.3 Results: Taxonomic Characters

2.3.1 Subgenus: *Alpigenobombus*

The mandible lacks a broad, rounded posterior end; instead, it features six evenly spaced, substantial triangular teeth that may experience wear over time. On the hind basitarsus, the longest erect hairs are situated close to the front edge of the outer side, generally measuring as long as or even longer than the narrowest portion of the basitarsus (Williams *et al*., 2008).

Bombus (*Alpigenobombus*) *grahami* (Frison, 1933) (Appendix 3 Photo plate: 1)

Taxonomic features

Type species: *Bremus (Alpigenobombus) grahami (*Frison, 1933): 334 (obsolete combination of *Alpigenobombus beresovskii*), Type locality: China: (USNM) (Wang, 1982):427.

B. grahami can be diagnosed by the following characteristics: Mandible distally having six large pointed teeth, the thoracic dorsum exhibits grey-white pubescence intermixed with black, the oculo-malar distance is less than half the basal width of the mandible, ratio of length of molar space (MS) and base of mandible at its base (BMB) = 1.7:2, ratio of antennal segment 3:4:5= 2:1:2 (see Williams *et al.*, 1991, William 2010, Saini *et al.*, 2015 for detailed diagnosis).

Materials examined (n=5):

Upperkerunja, (Gorkha)- 84°89'73“E, 28°23’73“N, 3000 m asl, ♀5, Barpak, (Gorkha), 84°90'85“E, 28°22'81“ N, 2191 m asl, ♀5, Laprak, (Gorkha), 84°81'87“, 28°19'63“, 2468 m asl, 2♀, Chame, (Manang), 84°78'79”, 28°20'49”, 2623 m asl, 4♀, Lete, (Mustang), 84°79'36“, 28°20'44“, 2241m asl, 10♀, (Collection: Kishor)

Distribution-

Worldwide: Sikkim, Tibet, Central and Southwest China, 2600-2800 m asl (William *et al.*, 2010, An *et al.*, 2014 and Saini *et al.*, 2015)

Bombus (*Alpigenobombus*) *breviceps* (Smith, 1852) (Appendix 3: Photo plate: 1)

Taxonomic features

Type species: *Bombus breviceps* (Smith, 1852): 44 (obsolete combination of *Bombus simulus*), Type locality: China: Zhejiang (BMNH), (Sakagami, 1972):163, (Wang, 1979):1379

B. breviceps can be diagnosed by the following characteristics: The first two abdominal tergites are black, creamy white, and the third to fifth abdominal tergites are brick red in color. The head is covered in thick pubescence, with the exception of the molar space, the clypeus, the labrum, the area laterals to and in front of the ocelli, and the narrow stripes on the inner and post orbits. heavily infuscated wings. Between prominent lateral tubracles, the labrum has a deep median furrow and a basal transverse depression. The distal half of the meta basitarsus' posterior margin is concave, and the proximal margin's thick pubescence (the auricle) extends to the projection's outer surface as a few sparse hairs, ratio of length of molar space and base of mandible at its base MS: BMB= 1.3:3.5, ratio of antenal segment 3:4:5= 1.5:.1:1.3 (Smith, 1852, Williams 1991, William, 2022, Saini *et al.,* 2015).

Material examined (n=6):

Gumda, (Gorkha), 84°88 22E, 28°23’90” N, 1709 m asl, ♀ 5, Gumda, (Gorkha), 84° 89’ 99” E, 28°23’50” N, 1702 m asl, ♀ 4, Barpak, (Gorkha), 84°79’10” E, 28°02’910” N, 3500 m asl, ♀ 12, Gumda, (Gorkha), 84^{0}84’10” E, 28°18’63” N, 2082 m asl, ♀ 2, Gumda, (Gorkha), 84°88’23” E, 28°23’78” N, 2040 m asl, ♀ 1, Dharapani, (Manang) 84°83’76” E, 28°18’88” N, 2017 m asl, ♀ 1, Kabre, (Manang), 84°81’44” E, 28°20’14” N, 2570 m asl, ♀ 4, Timang, (Mustang), 84°88;23” E, 28°23’78” N, 1656 m asl, ♀ 5, (Collection Kishor)

Distribution-

Worldwide: Himanchal Pradesh, Sikkim, Arunaanchal Pradesh, Myanmar, Tibet, Meghalaya, Central and Southwest China, Laos, Thailand, Vietnam, 980-3400 m asl (William, *et al.,* 2010, An, *et al.,* 2014 and Saini, *et al.*, 2015).

2.3.2 Sub genus - *Bombus*

The mandible has a notch near its back corner, which is nearly as deep as it is wide. This notch separates a robust posterior tooth that may experience wear over time. The central part of the clypeus always appears prominently swollen and protruding, creating a noticeable contrast with the deep depressions on either side, running parallel to the labral margin. The clypeus features large punctures spread evenly with a few smaller ones. The hind basitarsus exhibits a consistently strong, curved margin along its rear edge (Williams *et al*., 2008).

Bombus (*Bombus*) *tunicatus* (Smith, 1852) (Appendix 3 Photo plate: 1)

Taxonomic features

Type species: *Bombus tunicatus* (Smith, 1852):43 (obsolete combination of *Bombus vallestris*), Type locality: North India (BMNH) Tkalcu, (1974) a:334.

B. tunicatus can be diagnosed by the following characteristics: worker characterized by a black head, mesonotum, and third abdominal tergite. Additionally, they display a white pronotum and metanotum, along with a brick-red coloration on abdominal tergites 4 and 5. The thorax and abdomen's visible areas are consistently covered in pubescence. The thorax is marked by prominent lateral tubercles and a basal transverse depression that extends apically, forming a deep median furrow between them. The labrum possesses a lamella that extends beyond the apical border, and its anterior edge is complete. The ratio of the length of the molar space to the base of the mandible at its base is 2:4.5 (MS: BMB), while the antennal segments 3, 4, and 5 have a ratio of 1.5:1.1:1.1. (see Tkalcu, 1974, Williams *et al.,* 2010, Saini, *et al.,* 2015 for detailed diagnosis).

Material examined (n=5)

Boksekhola, (Manang), 84°22’97” E, 28°53’30” N, 2132 m asl, ♀ 2, Danque, (Manang), 84°22’97” E, 28°52’99” N, 2270 m asl, ♀ 3, Khokhethati, (Mustang), 83°59’99” E, 28°51’76”N, 2515 m asl, ♀ 5, Barpak, (Gorkha), 83°59’99”E, 28°51’74”N, 2515 m asl, ♀ 12, Gumda, (Gorkha), 83°59’91”E, 28°51’76”, 2536 m asl, ♀ 8, Laprak, (Gorkha), 83°59’91”E, 28°51’75”N, 2536 m asl, ♀ 4, (collection Kishor)

Distribution –

Worldwide: Himanchal Pradesh, Pakistan, Afghanstan, Kashmir, Sikkim, Uttaranchal, 2829-3650 m asl (William, *et al.,* 2010, An, *et al.,* 2014 and Saini, *et al.,* 2015).

2.3.3 Sub genus – *Melanobombus*

The distal half of the hind basitarsus has short, slightly feathery hairs spaced apart, which make the area between them glossy and easily visible. The central region of the clypeus is characterized by scattered large and small punctures, giving it an irregular and dull appearance. There is usually a band of punctures along the dorsal inner edge of the eye between the lateral ocellus and the inner margin. This band usually consists of very few small punctures, mostly separated by shining areas that are wider than the puncture widths. Additionally, the large unpunctured and shining area next to the ocellus is usually quite extensive, about three-quarters of the length separating the inner edge of the eye from the lateral ocellus (Williams *et al*., 2008).

Bombus (*Melanobombus*) *eximius*, (Smith, 1852) (Appendix 3 Photo plate: 1)

Taxonomic features

Type species: *Bombus eximius* (Smith, 1852): 47 (obsolete combination of *Bombus latissimus*), Type locality: India (Tkalcu, 1961):70

B. eximus can be diagnosed by the following characteristics: The basitarsi and mid and hind tibiae hairs are orange, whilst the thoracic pubescence is black. The coloring of metatasomal tergum 2 is black. The wings display a light orange-brown shade, and the mid basitarsus features a distal posterior corner forming an almost right angle, not sharply pointed. The hind basitarsus has a nearly straight posterior margin. The proximal width of the mandible and the oculo-molar distance are almost identical. The labrum has around half of its surface covered with an uneven but fairly straight lamella. ratio of length of molar space and base of mandible at its base MS: BMB= 1:1.2, antennal segment 3:4:5= 1.1:1.1:1.2 (see Smith, 1852, Williams, 1991, Saini *et al.*, 2015 for more diagnosis).

Material examined (n=7)

Lapu, (Gorkha), 84°88’88”E, 28°23’52”N, 1760 m asl, ♀ 12, Lapu, (Gorkha), 84°90’61”E, 28°23’18”N, 2353 m asl, ♀ 2, Lapu, (Gorkha), 84°90’72”E, 28°23’18”N, 2371 m asl, ♀ 6, Laprak, (Gorkha), 84°91’22”E, 28°22’63”N, 2400 m asl, ♀ 5, Laprak, (Gorkha), 84°91’43”E, 28°22’51”N, 2413 m asl, ♀ 3, Lapu, (Gorkha), 84°91’32”E, 28°22’55”N, 2423 m asl, ♀ 2, Laprak, (Gorkha), 84°91’14”E, 28°22’66”N, 2351 m asl, ♀ 13, Laprak, (Gorkha), 84°90’34”E , 28°23’53”N, 2279 m asl, ♀ 17, Lapu, (Gorkha), 84°90’54”E, 28°23’24”N, 2256 m asl, ♀ 1, Laprak. (Gorkha), 84°90’40”E, 28°23’42”N, 2092 m asl, ♀ 2, Laprak, (Gorkha), 84°90’44”E, 28°23’36”N, 2202 m asl, ♀ 3, Lapu, (Gorkha), 84°90’41”E, 28°23’27”N, 2284 m asl, ♀ 11, Laprak, (Gorkha), 28°23’5”N, 84°90’34”E, 2263 m asl, ♀ 1, Barpak, (Gorkha), 28°23’43”N, 84°90’41”E, 2237 m asl, ♀ 5, Laprak, (Gorkha), 84°90’35”E, 28°23’58”N, 2066 m asl, ♀ 5, Lapu, (Gorkha), 84°90’20”E, 28°23’52”N, 2043 m asl, ♀ 2, Lapu, (Gorkha), 84°90’03”E, 28°23’53’N, 2039 m asl, ♀ 1, Nesyang, (Manang), 84°89’98”E, 28°23’54”N, 2044 m asl, ♀ 2, Syarkhu, (Manang), 84°81’89”E, 28°19’60”N, 2258 m asl, ♀ 1, Thanchock, (Manang), 84°80”76”E, 28°20’04”N, 2351 m asl, ♀ 2, Danque, (Mustang), 84°80’52”E, 28°20’15”N, 2422 m asl, ♀ 11, Kokhethati, (Mustang), 84°794’2E”, 28°20’61”N, 2544 m asl, ♀ 3, Boksekhola, (Muatang), 84°80’67”E,28°20’11”N, 2351 m asl, ♀ 2, Danque, (Mustang), 84°81’42”E, 28°19’23”N, 2592 m asl, ♀ 2, Ghasha, (Mustang), 84°80’57”E, 28^{0}20’16”N, 2423 m asl, ♀ 3, Dana, (Mustang), 84°82’58”E, 28°19’07”N, 2424 m asl, ♀ 7, Kokhethati, (Mustanga), 84°83’89”E, 28°18’68”N, 2003 m asl, ♀ 5, Dharapani, (Mustang), 84°83’76”E, 28°18’88”N, 2013 m asl, ♀ 5 (Collection Kishor)

Distribution:

Worldwide: Sikkim, Darjeeling, Bengal, Arunachal, Meghalaya, Myanmar, Tibet, Center and Southwest China, Taiwan, Thailand, Vietnam. 914-2700 m asl (William *et al.,* 2010, An *et al.,* 2014& Saini *et al.*, 2015).

Bombus (*Melanobombus*) *festivus* (Smith, 1861) (Appendix 3 Photo plate: 2)

Taxonomic features

Type species: *Bombus festivus* (Smith, 1861): 152. (obsolete combination of *Bombus atrocinctus*), Type locality: India (Frison, 1935):356.

B. festivus can be diagnosed by the following characteristics: The pubescence is black in the thorax and white in the two abdominal tergite, except for the labrum, clypeus, and molar spaces, the head is covered in thick pubescence. thick pubescence is evenly present on the visible thorax and abdomen. Strongly infuscated wings, a labrum with a deep median furrow and a basal transverse depression (William, 2022). ratio of length of molar space and base of mandible at its base MS: BMB= 1:1, ratio of antennal segment 3:4:5= 1.5:1:1.25 (see Smith, 1870, Williams *et al.,* 2009, Saini *et al.,* 2015 for detail diagnosis).

Material examined:(n=8)

Ghasa, (Mustang), 83°64’61”E, 28°51’76”N, 1472 m asl, ♀ 3, Ghasa, (Mustang), 83°64’69”E, 28°51’76”N, 1506 m asl, ♀ 6, Ghasa, (Mustang), 83°64’69”E, 28°51’76”N, 1506 m asl, ♀ 1, Lower Kerunja, (Gorkha), 84°81’30”E, 28°19’93”N, 1690 m asl, ♀ 1, Lower Kerunj, (Gorkha)a, 84°82’26”E, 28°19’36”N, 1732 m asl, ♀ 1, Lete, (Mustang), 83°64’28”E, 28°51’76”N, 1948 m asl, ♀ 10, Syarkhu, (Manang), 83°64’28”E, 28°51’76”N, 1948 m asl, ♀ 4, Lete, (Mustang), 84°22’973E, 28°52’56”N, 2492 m asl, ♀ 10, Lete, (mustang), 83°61’38”E, 28°51’76”N, 2559 m asl, ♀ 10, Nesyang, (Manang), 83°61’13”E, 28°51’76”N, 2595 m asl, ♀ 4, Syarkhu, (Manang), 84°22’97”E, 28°55’16”N, 2632 m asl, ♀ 5, Ghasa, (Mustang), 84°22’97”E, 28°52’97”N, 2633 m asl, ♀ 2, Ghasa, (Mustang), 2638 m asl, 84°22’97”E, 28°52’86”N, ♀ 2, Ghasa, (Mustang), 84°22’97”E, 28°52’62”N, 2660 m asl, ♀4, Larjung, (Manang), 84° 22’97”E, 28°51’35”N, 2691 m asl, ♀ 2, Larjung, (Manang), 84°22’97”E, 28°51’45”N, 2693 m asl, ♀ 4, Upper Kerunja, (Gorkha), 84°24’08”E, 28°55’04”N, 2724 m asl, ♀ 2, Lower Kerunja, (Gorkha), 84°24’08”E, 28°55’04”N, 2724 m asl, ♀ 4, Upper Kerunja, (Gorkha), 84°24’08”E, 28°55’04”N, 2724 m asl, ♀ 11, Gumda, (Gorkha), 84°24’91”E, 28°54’97”N, 2724 m asl, ♀ 2, Laprak, (Gorkha), 84°22’97”E, 28°55’58”N, 2774 m asl, ♀ 2, Laprak, (Gorkha), 84°22’97”E, 28°51’35”N, 2774 m asl, ♀ 4, Upper Kerunja, (Gorkha), 84°22’97”E, 28°55’58”N, 2774 m asl, ♀ 11 (Collection Kishor)

Distribution:

Worldwide: Sikkim, Darjeeling, Bengal, Arunachal, Meghalaya, Myanmar, Tibet, Center and Southwest China, Taiwan, Thailand, Vietnam, 914 -2700 m asl (William *et al.,* 2010, An *et al.,* 2014 & Saini *et al.,* 2015).

Bombus (*Melanobombus*) *miniatus* (Bingham, 1897) (Appendix 3 Photo plate: 2)

Taxonomic Features

Type species: *Bombus miniatus* (Bingham, 1897): 552. (obsolete combination of *Bombus stenothorax*), Type locality: BMNH (Tkalcu, 1974):338

The following features can be used to diagnose *B. miniatus:* The black band between the wing bases has a lot of whitish hairs mixed up with it and T1 being cream or grey-white, T5 having some white hair, the head exhibits pubescence with short, branched yellow hairs, and there are pale thoracic bands along with a yellow hue on T1. There is a black stripe between the wing bases on the pubescence of the thoracic dorsum, along with anterior and posterior pale bands that appear either in a dull yellow or grey-white shade. Additionally, absence of short hairs on its outer surface of hind tibia, ratio of length of molar space and base of mandible at its base MS: BMB= 1.8:2.2, antennal segment 3:4:5= 2:.1.45:1.9 **(see** William, 1991, Saini *et al.,* 2015, William, 2022 for more diagnosis).

Material examined: (n=5)

Larjung, (Mustang), 84°82’09” E, 28°19’45” N, 2592 m asl, 5♀ (collection: Kishor)

Distribution:

Worldwide: Pakistan, Kasmir, Himanchal Pardesh, Uttaranchal, Sikkim, Tibet, Southwest China, 1900-4200 m asl (William *et al.*, 2010, An *et al.*, 2014 & Saini *et al.*, 2015).

2.3.4 Sub genus – *Orientalibombus*

The region between the ocelli and the eye, marked by extensive unpunctured and shiny regions, mostly lacks punctures. The punctures are limited to the outer quarter at the inner edge of the eye. With the exception of narrow strips of punctures between the center and side ocelli, this unpunctured region continues anteriorly in front of the three ocelli for a distance longer than the width of one ocellus. (Williams *et al*., 2008)

Bombus (*Orientalibombus*) *haemorrhoidalis* (Smith, 1852) (Appendix 3 Photo plate:2)

Taxonomic features

Type species: *Bombus haemorrhoidalis* (Smith, 1852) (obsolete combination of *Bombus assamensis)*. Type locality: Eastern China: Chusan (BMNH) (William, 1991): 58; (Burget *et al.*, 2009). 458, 461.

Bombus haemorrhoidalis can be diagnosed by the following characteristics: The worker's abdomen has a yellow color, while the head, thorax, and abdominal tergum 3 are all black. The color of the first and second abdominal tergites, as well as the fourth and fifth, is brick-red. The pubescence is uniformly distributed and the wings show a noticeable darkening. The head is completely covered with pubescence, with the exception of the malar space, clypeus, the area lateral to and in front of the ocelli, and thin stripes on the inner and post-orbits. The labrum is distinguished by its large lateral tubercles, broad lateral lamella extending over half of its basal width, and a deep median furrow formed by an apical basal transverse depression. Ratio of length of molar space and base of mandible at its base MS: BMB= 4:3.5, antennal segment 3:4:5= 2:.1.5:1.75 (see Smith, 1852, William 1991, Saini *et al.*, 2015, Williams, 2022 for more diagnosis)

Material examined (n=134)

Yeya, (Gorkha), 84°88'19"E, 28°23'49"N, 1407 m asl, ♀ 4, Yeya, (Gorkha), 84°88'30"E, 28°23'58"N, 1518 m asl, ♀ 1, Yeya, (Gorkha), 84°88'99"E, 28°23'68"N, 1539 m asl, ♀ 5, Yeya, (Gorkha), 84°88'31"E, 28°23'57"N, 1571 m asl, ♀ 1, Yeya, (Gorkha), 84°88'82"E, 28°23'52"N, 1709 m asl, ♀ 2, Dharapani, (Mustang), 84°88'99"E, 28°23'68"N, 1816 m asl , ♀ 5, Dharapani, (Mustang), 84°88'99"E, 28°23'84"N, 1870 m asl, ♀ 2, Lower, kerunja(Gorkha), 84°89'19"E, 28°23'77"N, 1906 m asl, ♀ 1, Lower kerunja, (Gorkha), 84°89'47"E, 28°23'70"N, 1973 m asl, ♀ 10, Lower kerunja, (Gorkha), 84°88'59"E, 28°23'82"N, 1594 m asl, queen 2, Lower kerunja, (Gorkha), 84°88'8"E, 28°23'52"N, 1760 m asl, ♀2 , Thumi, (Gorkha), 84°88'31"E, 28°23'25"N, 1700 m asl, queen 1, Barpak, (Gorkha), 84°90'68"E, 28°22'91"N, 2349 m asl, queen 10, Barpak, (Gorkha), 84°90'69"E, 28°23'04"N, 2326 m asl, queen 1, Barpak (Gorkha), 84°90'36"E, 28°23'52N, 2090 m asl, queen 2, Barpak, (Gorkha), 84°90'39"N, 28°23'37"N, 2097 m asl, queen 1, Barpak, , (Gorkha), 84°90'35"E, 28°23'50"N, 2126 m asl, queen 1 Baluwa, (Gorkha), 84°85'01"E, 28°13'35"N, 597 m asl, ♀ 2, Barpak, (Gorkha), 84°81'34"E, 28°20'11"N, 1906 m asl, ♀ 2 Barpak, (Gorkha), 84°81'33"E, 28°19'96"N, 2067 m asl, ♀ 6, Barpak, (Gorkha), 84°81'86"E, 28°20'16"N, 2083 m asl, ♀ 12 , Barpak, (Gorkha), 84°81'47"E, 28°20'05"N, 2093 m asl, ♀ 1, Barpak, (Gorkha), 84°80'94"E, 28°20'12", 2366 m asl, ♀ 1 , Laprak, (Gorkha), 84°82'22"E, 28°19'30"N, 2505 m asl, ♀ 12, Laprak, (Gorkha), 84°82'09"E, 28°19'45"N, 2592 m asl, ♀ 2, Laprak, (Gorkha), 84°80'70"E, 28°20'15"N, 2590 m asl, ♀ 2, Laprak, (Gorkha) 84°80'62"E, 28°20'04"N, 2614 m asl, ♀ 6, Barpak, (Gorkha), 84°80'94"E, 28°20'12"N, 2284 m asl, queen 10, Nesyang, (Manang), 84°82'09"E 28°19'45"N, , 2443 m asl, ♀ 2, Laprak, (Gorkha) 84°80'62"E, 28°20'04"N, 2739 m asl, ♀2, Chame, (Manang), 84° 80'62"E, 28°20'04"N, 2360 m asl, ♀4, Barpak (Gorkha), 84°84'84"E, 28°18'31"N, 2020 m asl, ♀6, Nesyang, (Manang), 84°82'93"E, 28°19'01"N, 2060 m asl, ♀ 6, Kabre, (Mustang), 84°83'75"E, 28°18'75"N, 2081 m asl, ♀ 6, Dharapani, (Mustang), 84°22'97" E, 28°51'75"N, 1904 m asl, ♀ 5, Bokekhola, (Mustang), 83°66'17"E, 28°51'76"N, 1386 m asl, ♀ 6, (Collection Kishor)

Distribution:

Worldwide: Pakistan, Uttaranchal, Darjaling, Bengal, Sikkim, Bhutan, Arunaanchal Pradesh, Meghalaya, Tibet, Southwest China, Laos, Thailand, Vietnam, 850-3400m asl, (William *et al.,* 2010, An *et al.,* 2014 & Saini *et al.,* 2015).

2.3.5 Subgenus – *Psithyrus*

In contrast to the mid tibia, the hind tibia doesn't widen on the outer surface. It maintains a consistent and gently curved shape, and it is covered in moderate to long, sturdy hairs evenly. These hairs on the edge are often not well-defined and do not create a pollen basket (corbicula). The inner distal border of the hind tibia lacks a row of sharp spines, known as rastellum. Finally, S6 (the sixth abdominal segment) features ventro-lateral keels or ridges (Williams *et al*., 2008).

Bombus (*Psithyrus*) *branickii* (Radoszkowski, 1893) (Appendix 3 Photo plate: 2)

Taxonomic features

Type species: *Bombus branickii* (Radoszkowski, 1893):241(obsolete combination of *Psithyrus branickii*), Type locality: USSR, Kirgiziya, (Tkalcu, 1969b)

B. branickii can be diagnosed by the following characteristics: The tubercles on the labrum are moderately noticeable, and the inner corner is angled. The thorax and malar space are yellow, while the pubescence on the head and abdominal tergum 4 is black. While abdominal tergites 2 and 3 are black with yellow posterior edges, abdominal tergum 5 is brick red. The wings are a pale shade of brown, ratio of length of molar space and base of mandible at its base MS: BMB= 2.5:1.1 reported 2.5:1.15], ratio of antenal segment 3:4:5= 1.8:.1:1.4 (see Tkalcu, 1969; Williams *et al*, 2010; Saini *et al.,* 2015 for more diagnosis).

Material examined: (n=5)

Upper Kerunja, (Gorkha): 84°22’97” E, 28°54’97” N, 2660 m asl, ♀5. (Collection Kishor)

Worldwide: Tajikistan, Kazakstan, Afaganstan, Himanchal Pardesh, Pakistan, Sikkim, Tibet, Kashmir, Southwest and Northwestern China, Mongolia, Russia, 3700 m asl, (William *et al.*, 2010, An *et al.*, 2014 & Saini *et al.*, 2015).

Bombus (*Psithyrus*) *cornutus* (Frison, 1933) (Appendix 3 Photo plate: 3):

Type species: *B. cornutus* (Frison, 1933):338 (obsolete combination of *Psithyrus cornutus*) Type locality: India (ZSI) (William ,1991):45

Taxonomic features

B. cornutus can be diagnosed by the following characteristics: The labrum having an acute median projection, the outer surface of the hind tibia being hairy and convex. Thoracic dorsum pubescence bearing wide yellow or grey-white stripes, ratio of length of molar space and base of mandible at its base MS: BMB= 2:2.4, antennal segment 3:4:5= 2:.1.2:2.5 (see Tkalcu 1989, William 2022, Saini *et al.*, 2015 for detail diagnosis).

Material examined (n= 5):

Laprak (Gorkha) 83°59’91” E, 28°51’76” N, 3000 m asl, 10♀, Upper Kerunja 84°22’97” E, 28°54’22” N, 2724 m asl, 5 ♀ (collection: Kishor)

Distribution:

Worldwide: Himanchal Pradesh, Uttaranchal, Central and Southwest China 3200-3390 m asl, (William *et al.*, 2010, An *et al.*, 2014 & Saini *et al.*, 2015).

B. (*Psithyrus*) *novus* (Frison, 1933) (Appendix 3 Photo plate 3)

Type species: *Bombus novus* (Frison, 1933):340 (obsolete combination of *Psithyrus novus* subsp. *nepalensis*). Type locality: India Kashmir, Kolkatta ZSI (Burger *et al.*, 2009):460.

Taxonomic features

B. novus can be diagnosed by the following characteristics: the labrum has a wide central furrow. lateral keels of S6 not protruding beyond T6 in dorsal side, the convex and hairy outer region of the hind tibia, head is covered in pubescence, and abdominal tergites 3-4 display a black coloration. The pronotum, mesonotum, metanotum, and the first and second abdominal tergites are yellow in color. Absdominal tergum 5 has a brick-red posterior band and a black anterior portion., MS: BMB= 3:4, antennal segment 3:4:5= 1.5:.1:1.2 (see Tkalcu, 1974, William, 1991, Saini *et al.*, 2015 for more diagnosis).

Material examined (n=5)

Laprak, (Gorkha) 83°59’91” E, 28°51’76” N, 2536 m asl, 5♀ (Kishor)

Worldwide: Himanchal Pardesh, Pakistan, Kashmir, 3000-4300m asl, (William *et al.*, 2010, An *et al.*, 2014 & Saini *et al.*, 2015).

Bombus (*Psithyrus) turneri* (Richards, 1929) (Appendix 3 Photo plate: 3)

Type species: *Bombus turneri* (Richards, 1929):141 (obsolete combination of *Psithyrus turneri*). Type locality: BMNH India, (William, 1991):52

Taxonomic features

B. turneri can be diagnosed by the following characteristics: The labral lamella is a large, rounded triangle that emerges strongly from the labrum. There are a few yellow hairs randomly scattered both anteriorly and posteriorly in the black pubescence of the thoracic dorsum (see (Richard, 1929) for detail diagnosis). Females can be identified (from *B. haemorrhoidalis, B. albopleuralis, B. breviceps, B. rotundiceps*) based on the convex and hairy outer surface of the hind tibia (William, 2022), ratio of length of molar space and base of mandible at its base MS: BMB= 3:2.5, ratio of antennal segment 3:4:5= 1:5: 1.4: 1.2 (Saini *et al.*, 2015).

Material examined (n=6):

Gumda, (Gorkha), 84°81’52” E, 28°19’96” N, 2270 m asl, ♀ 5, Boksekhola, (Mustang), 83°60’45” E, 28°51’76”N, 2592 m asl, ♀ 4, Thumi, (Gorkha), 84°81’52”E, 28°23’61”N, 2096 m asl, ♀ 1, Thumi, (Gorkha), 84°81’52”E, 28°20’02”N, 2127 m asl, ♀ 4, Chame, (Manang) 84°90’38”E, 28° 23’61”N, 2211 m asl, ♀ 1, Timang, (Manang)

84°82’09”E, 28°19’46”N 2191 m asl, ♀ 7, Thanchock, (Manang), 84°82’05”, 28°19’50”N 2550 m asl, ♀ 3, Boksekhol, (Mustang), 83°60’45”E, 28°51’76”N, 2550 m asl, ♀ 5 (Kishor)

Distribution:

Worldwide: Arunachal Pardesh, Central and Southwest China, 2250 m asl (William *et al.,* 2010, An *et al.,* 2014 & Saini *et al.,* 2015).

2.3.6 Sub genus - *Pyrobombus*

There's a strip of punctures running along the inner eye margin dorsally for most of its length. However, these punctures are quite scarce, mainly consisting of a few scattered large punctures, separated by broader shining areas that are wider than the puncture widths. The sizeable large unpunctured and shiny area next to the ocellus is usually extensive, spanning over three-quarters of the distance from the inner edge of the eye to the lateral ocellus. Alternatively, in cases with numerous densely packed small punctures, these small punctures cover the entire area between the eye and the depression surrounding the lateral ocellus (Williams *et al*., 2008).

Bombus (*Pyrobombus*) *lepidus* (Skorikov, 1912) (Appendix 3 Photo plate: 3)

Taxonomic features

Type species: *Bombus lepidus* (Skorikov, 1912): (obsolete combination of *Bombus nursei*), Type locality: China: Qinghai (ZISP); Williams, 1991:75, (Wang, 1982):1426.

B. lepidus can be diagnosed by the following characteristics: The pronotum, head, metanotum, and abdominal tergites 1 and 2 are yellow, whereas the mesonotum, malar space, and tergum 3 are black. An orange-red color is shown by abdominal tergites 4-5. An equal-length shallow median depression divides the cut off, bean-shaped lateral tubercles on the front border of the labrum in the center. Apart from the raised part of the lateral tubercle, the rest of the labrum is macro-perforated. ratio of length of molar space and base of mandible at its base MS: BMB= 2:2.5, ratio of antenal segment 3:4:5= 1:0.5:0.7 (see Skorikov, 1912, Williams *et al.,* 1991, William 2022, Saini *et al.,* 2015 for more diagnosis).

Material examined: (n=6)

Thanchock, (Manang), 84°22’97” E, 28°52’86” N, 2633 m asl, ♀ 3, Larjung, (Manang), 84°22’97” E, 28°52’84” N, 2655 m asl, ♀ 1, Lower Kerunja, (Gorkha), 84°22’97” E, 28°54’36” N, 2669 m asl, ♀ 10, Lete, (Mustang), 84°22’97"E, 28°51’35”N, 2691 m asl, ♀ 6, Boksekhola, Mustang, 84°22’97”E 28°54’09”, 2722 m asl, ♀ 4 (Collection Kishor).

Distribution:

Worldwide: Himanchal Pardesh, Pakistan, Sikkim, Tibet, Southwest China, 2850-3871 m asl (William *et al.,* 2010, An *et al.,* 2014 & Saini *et al.,* 2015).

Bombus (*Pyrobombus*) *parthenius* (Richards ,1934) (Appendix 3 Photo plate: 4)

Taxonomic features

Type species: *Bombus parthenius* (Richards, 1934):89 (obsolete combination of *Bombus signifier*), Type locality: Myanmar (BMNH); (Williams & Cameron 1993):89, 175.

B. parthenius can be diagnosed by the following characteristics: The hind tibia displays a dark brown exoskeleton on the outer surface, particularly proximally, often accompanied by numerous long and short hairs near the joint with the femur, which is also dark brown. The ocello-oculo area features a broad strip of punctures on the eye's inner border. The mandibles exhibit only two anterior teeth and a broadly rounded distal margin. The pubescence is entirely yellow, except for the black malar space. The mesobasitarsus has a sharply pointed distoposterior corner. Ratio of length of molar space and base of mandible at its base MS: BMB= 2:1.3, ratio of antennal 3:4:5 segment = 1.2:1:1.1 (see Richards, 1934, Wiiliam, 2022, Saini *et al.,* 2015 for more diagnosis).

Material examined: (n=5):

Lete, (Mustang), 84°82’05”E, 28°19’50”N, 2191 m asl, 5♀, Timang, (Manang) , 84°82’05”E,28°19’50’N, 2315 m asl, 5♀, Upperkerunja, (Gorkha), 84°80’44”E, 28°20’14”N, 2554 m asl, 5♀, (Collection Kishor)

Distribution:

Worldwide: Uttaranchal, Himanchal Pardesh, Sikkim, Bhutan, Tibet, 1300—4070 m asl (William *et al.,* 2010, An *et al.,* 2014 & Saini *et al.,* 2015).

Bombus (Pyrobombus) pressus (Frison, 1935) (Appendix 3 Photo plate: 4)

Taxonomic features

Type species: *Bombus pressus* (Frison, 1935): 342. (obsolete combination of *Bremus pressus*), Type locality: ZS, Munich (Tkalcu, 1974).

B. pressus can be diagnosed by the following characteristics: T6 posteriorly having the apex deeply divided (more apparent for large individuals). A distinct black hair band is in the space between the bases of the wings. The mandibles exhibit only two anterior teeth and a broadly rounded distal margin, while the labrum features a median longitudinal groove. The distal posterior corner of the hind tibia never formed a spine; instead, it was always shorter than its basal width., ratio of length of molar space and base of mandible at its base MS: BMB= 2:1.9, antennal segment 3:4:5= 1.4:1:1 (see William, 1991, William, 2022, Saini *et al.,* 2015 for detail diagnosis).

Material examined: (n=5)

Laprak, (Gorkha), 84°79’20” E, 28°20’62” N, 3500 m asl, 5♀, (Collection Kishor)

Distribution:

Worldwide: Utaranchal, Darjaling, Bengal, Sikkim, 3250-3950 m asl (William *et al.,* 2010, An *et al.,* 2014 and Saini *et al.,* 2015).

Bombus (*Pyrobombus) rotundiceps* (Friese, 1916) (Appendix 3 Photo plate 4)

Taxonomic features

Type species: *Bombus rotundiceps* (Friese, 1916):108 (obsolete combination of *B. montivolanoides*), Type locality: Myanmar (BMNH); (Williams & Cameron, 1993):89, 175.

B. rotundiceps can be diagnosed by the following taxonomic features: The pubescence on the first three abdominal tergites is a combination of black and dingy yellow, while the last three are characterized by a brick-red color. With the exception of the clypeus, the area laterals to and in front of the ocelli, the malar space, and the small stripes on the inner and post-orbits, the specified areas are covered with pubescence. The mid tibia and hind tibia feature long, bright orange hairs. Thick and even pubescence is observed on both the thorax and abdomen. The labrum exhibits prominent lateral tubercles that are slightly bluntly elevated, and a deep median furrow between them creates a lamella that overhangs the apical border by displacing the ridge between them. ratio of length of molar space and base of mandible at its base MS: BMB= 2:3, antennal segment 3:4:5= 2:1:1 (see Tkalcu 1974, Saini *et al.*, 2015 for more diagnosis).

Material examined (n=6):

Dana, (Mustang), 84°78'37"E, 28°02'04"N, 3500 m asl, ♀ 5, Thanchock, (Manang), 84°82'84"E, 28°18'97"N, 2098 m asl, queen 3, Timang, (Manang), 28°23'52", 84°90'368", 2090 m asl, queen 3, Gumda, (Gorkha), 84°82'05"E, 28°19'50"N, 1822 m asl, ♀ 5, Gumda, (Gorkha), 84°82'84"E, 28°18'97"N, 1852 m asl, ♀ 3, Thumi, (Gorkha), 84°88'80"E 28°23'58"N, 1731 m asl, ♀ 4, Thumi, (Gorkha), 84°88'52"E, 28°23'98"N, 1656 m asl, ♀ 4, Thumi, (Gorkha), 84°82'05"E 28°23'98"N, 1660 m asl, ♀ 1, Thumi,(Gorkha), 84°82'05"E, 28°19'50"N, 1470 m asl, ♀2, Gumda, (Gorkha), 84°82'05"E, 28°23'98"N, 1320 m asl, ♀5 Thanchock, (Manang), 84°90'36"E, 28°23'52"N, 2632 m asl, ♀ 4, (Collection Kishor)

Distribution:

Worldwide: Himanchal Pardesh, Utaranchal, Sikkim, Darjaling, Bengal, Meghalaya, Myanmar, Southwest China, 762-1350 m asl (William *et al.*, 2010, An *et al.*, 2014 & Saini *et al.*, 2015).

2.3.7 Subgenus - *Sibricobombus*

The proximal end of the hind basitarsus has a posteriorly directed extension that is covered with a dense cluster of relatively long, branching hairs on the inside. These hairs spread outward, forming an upright brush of elongated, branching hairs. Between these hairs, this brush frequently hides the basitarsus's external surface. Moving to the cheek or oculo-malar area, you'll find a central region marked by a diagonal line of small punctures (Williams *et al*., 2008).

Bombus (*Sibricobombus*) *asiaticus* (Morawitz, 1875). (Appendix 3, Photo plate: 4)

Taxonomic features

Type species: *Bombus asiaticus* (Morawitz, 1875). (obsolete combination of *Bombus regeli*), Type locality: USSR Tadzhikistan, (Dalla Torre, 1996): 512.

B. asiaticus can be diagnosed by the following characteristics: The distoposterior corner of the mesobasitarsus is bluntly pointed. The pronotum, metanotum, and abdominal tergum 1 have a dirty yellow color, whereas the head, mesonotum, and abdominal tergites 2–5 are black. There is no pubescence on the clypeus, and the abdomen and thorax are thoroughly covered with heavy pubescence. The metatibia's external surface is smooth and shiny, and its posterior border narrows in the distal half of the metabasitarsus. Ratio of length of molar space and base of mandible at its base MS: BMB= 3:2.2, antennal segment 3:4:5= 3.1:4.:4.9 (see Dalla Torre, 1896, Wang, 1982, Saini *et al.,* 2015 for full diagnosis).

Material examined: (n=6)

Upperkerunja, (Gorkha), 84°79’63” E, 28°20’39” N, 3020 m asl, ♀10, Laprak, (Gorkha), 84°78’83” E, 28°20’53” N, 3020 m asl, ♀ 10, Laprak, (Gorkha), 84°815’66” E, 28°199’51” N, 2614 m asl, ♀ 5, Gumda, (Gorkha), 84°80’71” E, 28°20’18”N, 2536 m asl, ♀1, Thumi, (Gorkha), 84°81’18” E, 28°20’07” N, 2066 m asl, ♀1, Barpak, (Gorkha), 84°88’23” E, 28°23’78” N, 2468 m asl, ♀1, Barpak, (Gorkha), 83°59’91”6 E, 28°51’76” N, 2536 m asl, ♀ 1. (Collection: Kishor)

Distribution:

Worldwide: Pakistan, Afaganstan, Kirkistan, Kasmir, Himanchal Pardesh, Uttaranchal, Sikkim, Tibet, Southwest China, 3000-3900 m asl (William *et al.,* 2010, An *et al.,* 2014 & Saini *et al.,* 2015).

2.4 Discussion

During several field surveys carried out in the Gorkha, Manang, and Mustang regions within the Budigandaki Marsyngdi, and Kaligandaki, river valleys in the CHAL, a total of 16 bumblebee species were identified across an altitudinal range spanning from 600 meters to 3500 meters. The central Himalayan range, acknowledged as a hotspot for biodiversity and a region of considerable conservation importance, has served as the site for the pioneering systematic research on bumblebee diversity (Myers *et al*., 2000). The first extensive survey of bumblebee distribution in the eastern region was carried out in Arunachal Pradesh, India, by Streinzer *et al.,* (2018). This study identified 21 species from six subgenera at elevations ranging from 233 meters to 4,260 meters. According to Saini *et al.*, (2015), the total number of bumblebee species in India is 48. In contrast, Williams *et al.*, (2017) recorded 130 bumblebee species in China, representing half of the estimated global species count. A different study (Williams *et al.*, 2015) examined 44 species of bumblebees as well as the Tibetan plateau's climate and glaciers (An *et al.,* 2014). 76 species were discovered during a field investigation conducted by the same team in North China between 2005 and 2012. A specimen from London also added to the total number of species, making a total of 77 species representing 10 subgenera.

Bumblebees can be distinguished by the colors of their hair, although relying solely on these colors for species identification can be misleading due to their variability. However, a more reliable approach involves recognizing subgenera based on differences in exoskeleton shape and sculpturing, facilitating effective identification. Despite this, variations in color patterns within specific regions, such as the Himalaya, can still offer valuable insights into species identification (Gauld & Bolton, 1988; Michener, 2007). The process of identification encompasses characteristics around the mouth, including the oculo-malar region, clypeus, and labrum. Notably, the malar area, a specific feature, can be measured by comparing the distance between the eyes and the jaw to the width of the jaw. This measurement correlates with the length of the tongue (proboscis), influencing flower choices for feeding. Interestingly, in species with longer tongues, the malar area's shape may different among individuals of different sizes within the similar species due to size-related changes (Sakagami, 1972). Despite potential variations within and between species, these characteristics remain valuable, although they can be influenced by wear and tear. The antennal segments' shape is considered for identification, particularly when noticeable differences exist. On the head, the size and position of the three ocelli vary, and the ocello-ocular area's sculpturing, between the lateral ocellus and the compound eye, is one of the most changeable features. The thorax provides key identification features on the legs, especially in the hairless area surrounded by pollen-carrying fringes. Noticeable developments at the far back corner of the mid basitarsus are observed in some species groups but may not be distinctly different in closely related species (Sakagami & Ito, 1981). The sculpturing of the hard posterior sclerites in the abdomen is a major feature, where metasomal segments are important. A rounded central boss, keels, or changes in the form of the apex are examples of sculpturing variants on female metasomal tergum 6 (T6) and sternum 6 (S6) that are frequently noticeable (William, 2022).

B. grahami and *B. breviceps* (subgenus *Alpigenobombus*) lack large, rounded terminal on the rear of the mandible is a common trait. The six big, pointed teeth on the distal side of *B. grahami*'s mandible set it apart from other members of its subgenus. However, *B. breviceps* is distinguished from other subgenus members by having black and creamy white first two abdominal tergites, and brick red third to fifth tergites (Smith, 1852, Williams, 1991). *B. eximius, B. festivus,* and *B. miniatus* (subgenus *Melanobombus*) share a common characteristic of a noticeable and shiny surface between the short, slightly feathery hairs in the distal half of the hind basitarsus.

B. eximius stands out from other species in the same subgenus as its mid and hind tibiae, as well as the basitarsi, contain orange hair, and thoracic pubescence is black. additionally, metatasomal tergum 2 is black (Smith, 1852). *B. festivus* distinguishes itself from other species in the same subgenus by having a basal transverse depression and a deep median furrow in the labrum (Smith, 1870). Furthermore, *B. miniatus* differs from another species in the same subgenus by its head pubescence, which includes yellow short branching hairs, pale thoracic stripes, and yellow T1 (William, 1991). *B. branickii, B. cornutus, B. novus,* and *B. turneri* (subgenus *Psithyrus*) commonly known as cuckoo bumblebees. The common characteristics among these species is that, the hind tibia containing moderate to long sturdy hairs maintains a consistent and gently curved shape without broadening on the outer surface.

Distinguishing features within this subgenus include: *B. branickii* stands out from other species due to moderately prominent labrum tubercles with the inner corner obtusely angled (Tkalcu, 1969). *B. cornutus* differs from other species in the same subgenus by having thoracic dorsum pubescence with broad yellow or grey-white bands (Tkalcu, 1989). *B. novus* distinguishes itself from other species as the labrum has a wide central furrow (Williams, 1991). *B. turneri* differs from other species in the same subgenus by the prominently jutting labral lamella, forming a wide and rounded triangle from the labrum (Richard, 1929).

B. lepidus, *B. parthenius*, *B. pressus*, and *B. rotundiceps* have been documented within the *Pyrobombus* subgenus. These species share a common characteristic that along the majority of the inner eye border, there is a strip of punctures on its dorsal side. Distinguishing features among these species include: The upper middle part of the thoracic scutum of *B. Lepidus* is as large as the tegula and is smooth without punctures near the back of the long groove in the middle (Skorikov, 1912, Williams *et al.*, 1991), *B. parthenius* often has a dark brown exoskeleton on the outside of the anterior part of the hind tibia. and is often covered with both long and short hairs near the joint with the femur, which is also dark brown (Richards, 1934, Wiiliam, 2022), mandibles of *B. pressus* have only two front teeth and a broadly rounded end, while the labrum has a groove running down its middle (William, 1991), The external side of the upper tibia of *B. parthenius* usually has a dark brown exoskeleton (Tkalcu, 1974).

Due to global warming, the increasing temperature has altered drastic faunal distribution patterns of organisms including bumblebees. For example, *B. haemorrhoidalis* was reported from an average altitude of 1622 m asl (Williams *et al.*, 2010) and 1850 m asl (Saini *et al*., 2015). But in this study, the same species was collected at 2,189 m asl. Similarly, *B. pressus* in this study was collected at lower altitude (3,000 m asl) than that of 3658 m asl. (Williams *et al*., 2010) and 3329 m asl. (Saini *et al*., 2015). This indicates the high possibility of generic and Subgenric shifting of bumblebees. It needs further investigation to make a valid claim, however.

It is apparent that color patterns of bumblebees greatly vary. As such patterns are remarkably similar among different species, and also, in some cases a single species exhibits dissimilar color patterns in different geographical regions, color patterns may not be always much reliable means of identification tool in bumblebees (Williams *et al.*, 2015). In such cases, alternative DNA barcoding may be an appropriate tool of identification. Previous studies have also claimed the possibility of cryptic nature in color patterns and morphology of Bumblebees (Williams *et al*., 2015).

2.5 Conclusion

The extensive field surveys in the central Himalayan range, specifically in the Gorkha, Manang, and Mustang regions, revealed a diverse bumblebee fauna comprising 16 species across an altitudinal range of 600 m to 3500 m. The significant conservation priority should be given to this region, recognized as a biodiversity hotspot. The study contributes for global understanding of bumblebee diversity, complementing previous researches in different geographic locations (e.g. India and China). The study acknowledges the variability and potential cryptic nature, suggesting DNA barcoding as an alternative identification tool in such cases.

CHAPTER 3

3. COMMUNITY DYNAMICS OF BUMBLEBEE ACROSS ELEVATION GRADIENTS AND HABITAT MOSAICS

Abstract

The study on the species composition of bumblebees (*Bombus* spp.) in the Chitwan-Annapurna Landscape was done from April - November 2018 and 2019. Opportunistic surveys were used to collect bumblebee samples. Walking transects were employed in accessible sites along the Kaligandaki, Marshyandi, and Budhigandaki river valleys, covering different habitats, including agricultural regions, forests, grasslands, and home gardens. Sixteen *Bombus* species were identified from the sampling areas, among them, the most dominant species was *B. haemorrhoidalis*, accounting for 20.29% of the relative abundance, closely followed by *B. festivus* at 19.57% and *B. eximius* at 18.84%. Instead, *B. pressus, B. miniatus, B. branickii*, and *B. novus* were the least prevalent species, with relative abundance 1% for each. A significant relationship between elevation and bumblebee richness was observed. The richness of species was highest in the mid elevation. Similarly, the forest habitat in the Gorkha site exhibited the highest species richness and diversity (n=12, Shannon index H'= 2.18), followed by the grassland habitat in the Mustang site (n=11, Shannon index H'= 2.10). In comparison, the habitats in the Gorkha site exhibited higher species diversity when compared to those in Manang and Mustang. The altitudinal gradients in the study area lead to significant variations in microclimatic conditions and vegetation dynamics across different habitats. These variations significantly impact the abundance, species richness, and diversity of bumblebees. The greatest species richness was found in the CHAL area's mid-elevation ranges of 2000–3000 m asl. This is probably because there are more foraging flowering plants in this region that depend on pollinators. Anthropogenic activities and the structure of land cover together with altitudnal gradient are major factors influencing the composition of bumblebees' species, their distribution, and diversity in the CHAL. These factors play a vital role in shaping the bumblebee community in this region. It is strongly recommended that decision-makers develop their conservation strategies within a socio-ecological framework.

Keywords: *Bombus* spp.; Gorkha; habitats; pollinators; species diversity

3.1 Introduction

The mountainous landscape is susceptible to alterations in both land cover and climate (Huber *et al.,* 2005; Beniston, 2006). Earlier studies (Acquaotta *et al.,* 2014) have revealed that the alpine and sub- alpine region around the earth are facing the impact of climate change including Asian bumblebees (Acquaotta *et al.*, 2014; Williams *et al.,* 2015; Naeem *et al.,* 2019). Mountain ecosystems face difficult conditions, as demonstrated by the high concentration of abiotic stresses and spatial complexity seen in the mountain landscape. The increase in average temperature in mountainous areas has a significant effect on abundance, distribution, and diversity of insects, and these differences are observed along elevational gradients (Williams *et al.,* 2015; Widhiono *et al*., 2017). It is challenging to predict the temporal and spatial patterns of distribution and diversity of bees across various latitude due to microhabitat characteristics and types of host plants available (Beck *et al.,* 2017). Bumblebees are typically located at high altitudes and hold a crucial role in the pollination of flowering angiosperms (Obeso, 1992; Bingham & Ranker, 2000; Xu *et al.*, 2009; An *et al*., 2014; Streinzer *et al*., 2019). These insects are well adapted to temprate and cold environments at higher latitudes and elevations (Williams, 2007).

The altitudinal distributions of *Bombus spp*. have been widely documented in various regions worldwide; however, the precise reasons for this variation remain incompletely understood (Martinet *et al.,* 2015; Oyen *et al.,* 2016). This deficiency of understanding is particularly pronounced when it comes to altitudinal gradients within complex landscape structures (Osborne *et al.,* 2008; Miller–Struttmann *et al*., 2014; Oyen *et al.,* 2016; Minachilis *et al*., 2020). They are significantly influenced by habitat types, alterations in land use, and the composition as well as availability of appropriate nesting places (Kells & Goulson, 2003; Williams & Osborne, 2009; Saini *et al.*, 2012; Williams *et al.,* 2016). In addition to environmental factors, the thermal tolerance limit of bumblebees (Hatfield & LeBuhn, 2007; Burkle & Alarcon, 2011; Morales *et al.*, 2013; Scaven & Rafferty, 2013) is also a major limiting factor that determines their range distribution along elevation gradients (Oyen & Dillon 2018). The impact of habitat type along elevation gradients, which affects the accessibility of flowering host plants, has been recognized as a factor that can influence the diversity and abundance of bumblebees in various regions worldwide (Bhattacharya *et al.,* 2003; Hopwood, 2008;

Redhead *et al.*, 2016). The density and dispersal of bumblebees are notably influenced by the quality of habitats, particularly when contrasting natural habitats with agricultural ones (Hatfield & LeBuhn, 2007; Iles *et al.*, 2018). Understanding the complex interactions among bumblebee abundance and diversity in various habitats, altitudinal gradients, and landscapes poses a challenge, as suggested by previous research (Carvell, 2002; Pywell et al., 2005; Biesmeijer *et al*., 2006; Carvell *et al*., 2006; Fourcade *et al*., 2019). Numerous research studies have explored the influence of various habitat types on the diversity and abundance of bumblebees, examining the impact of habitat loss on species composition, especially within the genus *Bombus* (Carvell *et al.*, 2006; Goulson *et al.*, 2008; Decourtye *et al.*, 2010; Morandin & Kremen, 2013; Scaven & Rafferty, 2013; Krimmer *et al.*, 2019; Bhusal, 2020). The global decline of populations of bumblebee can be attributed to many factors, including increased food stresses, decreased floral diversity, habitat loss, use of pesticide, and pathogens (Goulson *et al.*, 2015; Cameron *et al.*, 2016). However, the magnitude of these influences differs among various bumblebee species (Cameron & Sadd, 2020).

The understanding of how bumblebees respond to different types of habitat in the Himalayan Mountains remains limited. The region is experiencing significant environmental changes due to increasing temperatures, use of pesticide, habitat loss and agricultural intensification, (Pandit & Grumbine, 2012; Verma & Arya, 2021). While bumblebee species composition has been studied extensively in the western and Indian parts of the Himalaya (Williams, 1991; Saini *et al.*, 2015; Williams, 2022), research in the eastern and central Himalaya is lacking (Williams *et al*., 2010; Streinzer *et al.*, 2019). In the central Himalaya, there is a need for additional research on taxonomic diversity, susceptibility to climate change, the impact of various habitat types, and other human-induced pressures (Williams *et al.*, 2010; Bhusal, 2020). Moreover, bumblebee abundance, species richness, and distribution patterns along altitudinal gradients in this region have been understudied.

The Chitwan Annapurna Landscape (CHAL) in the mid-Himalayas is an important area known for its unique flora and various habitat types. However, this landscape is facing significant threats from climate change, habitat loss, invasive plant species, infrastructure development, landslides, erosion, and pesticide use in agriculture (Shrestha *et al.*, 2012; Pandit *et al.*, 2014; Mishra *et al.*, 2015; Maharjan *et al.*, 2019;

Shrestha *et al.,* 2019; Adhikari *et al.,* 2022a; Bhusal *et al.,* 2020; Adhikari *et al.,* 2022b). Bumblebees play a crucial role as pollinators, preserving the genetic diversity of higher-elevation plants (Bhusal, 2020). In spite of their significance, few studies have been conducted on the diversity, distribution, and abundance patterns of bumblebees across elevation and habitat gradients in the CHAL. Thus, this study aims to investigate the diversity, species richness, and composition of *Bombus* species in the Kaligandaki, Marshyandi, and Budhigandaki River basins within the CHAL

3.2 Materials and Methods

3.2.1 Site description and field design

The investigation was accompanied along the elevation gradient of the Chitwan Annapurna Landscape (CHAL), encompassing altitudes ranging from 600 to 3500 m asl. The study primarily focused on three major river basins (Fig 1). The Kaligandaki basin is located at the Mustang site (28° 87' 65.1” N - 83° 79' 47.6” E), the Marsyangdi basin at the Manang site (28° 57’ 52.6” N - 84° 18' 66.2” E), and the Budigandaki basin at the Gorkha site (28° 18' 36.4" N - 84° 85' 15.7" E). The river basins function as biodiversity hotspots, including diverse habitats such as agriculture, forests, grasslands, and human settlements. These regions exhibit diverse microclimates and ecological conditions, providing a range of microclimate types and vegetation structures that support various bee colonies. Furthermore, these river valleys are vital migration corridors for birds and provide habitat for endangered animals such as red pandas, snow leopards, and Himalayan black bears (Adhikari *et al.,* 2019; Chetri *et al*., 2019). The Chitwan Annapurna Landscape possess a significant cultural heritage, sustaining a population of over 4 million people who depend extensively on forest resources and for their general well-being and means of survival, these people heavily depend on ecosystem services. The Chitwan Annapurna Landscape possesses a significant cultural heritage, sustaining a population of over 4 million people who heavily depend on forest resources for their general well-being and means of survival. These people depend on extensively on ecosystem services. Unfortunately, this region has faced substantial challenges in recent years due to increasing human activities, rapid migration and settlement, ecosystem degradation caused by invasive alien plant species (IAPS), and the consequences of climate change. These factors have collectively led to a remarkable decline in biodiversity within the area. Unfortunately, in recent times, this region has faced notable challenges stemming from increased human activities, rapid migration and settlement, degradation of ecosystems due to invasive alien plant species (IAPS), and the effects of climate change. These reasons mutually led to a remarkable decline in biodiversity within the area.

3.2.2 Bumblebee surveying and identification

Three walking transects were established along the Budhigandaki in Gorkha, the Marshyandi in Manang, and the Kaligandaki in Mustang for conducting the bumblebee survey. At the time of field survey, three researchers moved at a slow pace along the transect, following opportunistic sampling on 20 meters right and left, passing through different habitats. Sweeping nets (entomological nets) were used for bumblebee collection, and upon capture, collected samples were quickly killed by ethyl acetate. The surveys were done during the highest flowering season from April to November 2018/2019. The entire process of surveying these walking transects was repeated three times per year: (April-May, July-August, October –November). When a bumblebee was observed, the transect walk was halted for 30 minutes to search for and collect any visible foraging workers present at the location. Location data, including geographic coordinates, elevation, and habitat type, was recorded at every sampling station by using a GPS device (Garmin eTrex 10). Bumblebee abundance was estimated by counting the individuals collected at each of the 138 sampling points along the transect during a 30-minutes period. To prevent the mold growth during transportation to the laboratory, specimens were kept in airtight containers with few layers of tissue paper and treated with small quantity of ethyl acetate. Collected specimens were analyzed in the lab for species identification and richness across the elevation and types of habitat such as agriculture, grassland, home garden, forest. The study employed following definition for habitat types: agricultural habitat referred to farm land areas, grassland encompassed open spaces with scattered flowering herbs, inside forests were considered as grassland, home garden was classified as areas within human settlements like front yards and backyards, and forest habitat was characterized by areas covered with trees along with bushes and herbs. Bumblebees were collected at 138 sampling points across three sites along elevation gradients. Species richness was determined within 22 distinct elevation bands, with each band spanning 100 meters and covering the range from 1350 to 3550 meters above sea level. The survey was conducted when there was no rain and high wind speed at the time in between 9 am to 6 pm. After collection, the specimens were dry-mounted and labelled using standard entomological pins. Subsequently, they were preserved and deposited at the CDZ, TU, Nepal. Detailed examination of the specimens was conducted using a stereoscopic microscope. To identify the species accurately, published identification keys such as those by (Williams *et al*., 2010, Saini *et al*., 2015, & Williams, 2022) were utilized. These reference materials provide specific criteria and characteristics for distinguishing between different bumblebee species, aiding in the accurate identification process.

Similarly, the unknown flowering plants were also collected from the sampling plots and prepared herbarium which were identified from National Herbarium, Godawari, Lalitpur.

3.2.3 Data analysis

Data analysis was performed with the R programming language, specifically version 4.0.3, developed by the R Core Team in 2022. Mean elevation of the 16 identified species of bumblebee from the collected specimens was examined, and the comparison between mean elevation within the Whole Sampling Area (WSA) and at individual sites was done. In order to understand the correlation between elevational gradients and the abundance of recorded bumblebee individuals along the study transects, we employed a Generalized Linear Model (GLM) with a quasi-Poisson error structure. This choice was made as a basic Poisson model revealed signs of overdispersion. The analysis of species richness was restricted to study points located above 1300 meters above sea level (asl). Cross-tabulation was utilized to ascertain the relative abundance of each of the 16 species of bumblebee in the specimens that were collected in order to do the correspondence analysis and diversity analysis. This involved examining the interrelationships among bumblebee species, sites, and habitats to know the distribution patterns and ecological preferences of them. Subsequently, diversity analysis was conducted utilizing the R package "vegan" (Oksanen, 2007). The inverse Simpson's index (In λ) and Shannon's diversity index (H') were analyzed. Additionally, Correspondence Analysis (CA) was used to find the association of abundance of bumblebee with various habitats. The "FactoMineR" R package (Le et al., 2008) was used to do the analysis.

3.3 Results

3.3.1 Site Based species composition

The total 656 bumblebee individuals were collected, and 16 different species belonging to the *Bombus* genus were identified. Each *Bombus* species' relative abundance was examined across the sample area within the three specific sites (Table 1).

The results showed that *B. haemorrhoidalis* (20.29%) was greatest in terms of relative abundance, with *B. festivus* (19.57%) and *B. eximius* (18.84%) following closely. But with just 1% of the overall abundance, *B. branickii*, *B. miniatus, B. novus*, and *B. pressus* were the least abundant species.

Table 1: Relative abundance of *Bombus* species in whole sampling area (WSA) and proportion of each species occurrence among 3 sites (GOR: Gorkha site; MAN: Manang site; MUS: Mustang site). The number in parenthesis indicates the number of individuals. (Ghimire *et al.,* 2023)

Sites	WSA (%)	GOR (%)	MAN (%)	MUS (%)
Bombus asiaticus	4.35(29)	100(29)	-	-
B. branickii	0.72(5)	100(5)	-	-
B. breviceps	5.07(34)	71.4(24)	14.3(5)	14.3(5)
B. cornutus	2.17(15)	100(15)	-	-
B. eximius	18.84(124)	65.4(81)	3.8(5)	30.8(38)
B. festivus	19.57(129)	48.1(62)	14.8(19)	37(48)
B. grahami	3.62(24)	40(10)	20(4)	40(10)
B. haemorrhoidals	20.29(134)	78.6(105)	14.3(19)	7.1(10)

B. lepidus	3.62(24)	40(10)	20(4)	40(10)
B. miniatus	0.72(5)	-	-	100(5)
B. novus	0.72(5)	100(5)	-	-
B. parthenius	2.17(15)	33.3(5)	33.3(5)	33.3(5)
B. pressus	0.72(5)	100(5)	-	-
B. rotundiceps	5.8(39)	62.5(24)	25(10)	12.5(5)
B. tunicatus	5.07(34)	71.4(24)	14.3(5)	14.3(5)
B. turneri	5.07(34)	28.6(10)	42.9(15)	28.6(9)

The Gorkha site exhibited the highest species diversity, with a representation of 15 different species. The most dominant species in this site were *Bombus asiaticus, B. haemorrhoidalis,* and *B. branickii*. Ten species were recorded from Manang site but *B. turneri* and *B. parthenius* were identified as dominant species. Eleven species were identified in Mustang site with *B. miniatus, B. lepidus*, and *B. grahami* were the dominant species (Figure 4 a). Additionally, habitats per site were used to examine the relative composition of *Bombus* species in the collection (Figure 4 b, c, and d). *B. asiaticus* appeared as the dominant species in the agricultural habitat, closely followed by *B. cornutus*. Especially, species like *B. branickii* were exclusively found in the agricultural habitat. On the contrary, species like *B. rotundiceps, B. parthenius, B. tuncatus, B. haemorrhoidalis,* and *B. festivus* exhibited greater dominance in forest areas. Both *B. miniatus* and *B. novus* were exclusively found in the forest habitat. *B. haemorrhoidalis, B. rotundiceps, B. lepidus*, and *B. turneri* were commonly recorded in the grassland, while *B. pressus* was specific in the grassland habitat. *B. eximius, B. breviceps, and B. haemorrhoidalis* were predominantly species found in home garden.

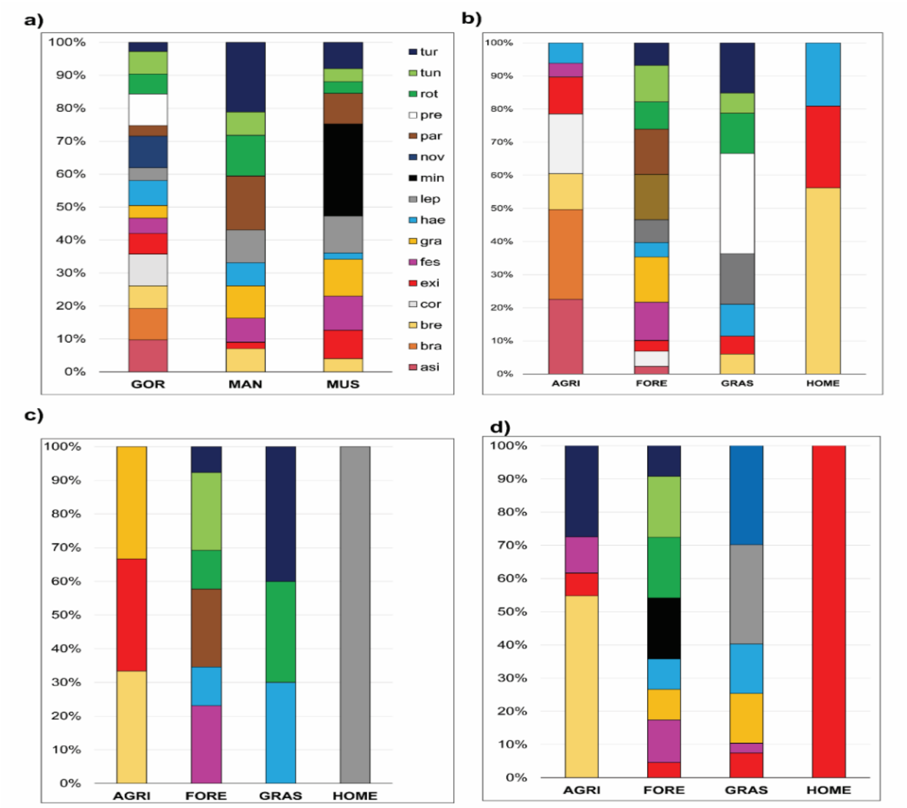


Figure 3: Species composition of bumblebee; a) Relative abundance by sites; b) Relative abundance of bumblebee in GOR: Gorkha site c) Relative abundance of bumblebee in MAN: Manang site d) Relative abundance of bumblebee in MUS: Mustang site **(**Ghimire *et al.,* 2023**)**

The composition of *Bombus* species at each site was also examined. Within the Gorkha site, *B. asiaticus* exhibited the highest dominance in the agricultural habitat, with *Bombus cornutus* following closely. In the forest habitat of this location, predominant recordings included *B. grahami*, *B. festivus*, *B. parthenius*, *B. tunicatus*, and *B. novus*. *B. pressus* was dominant species in the grassland habitat of the Gorkha site, which was followed by *B. turneri*. *B. pressus* and *B. turneri* were the predominant species found in the grassland ecosystem of the Gorkha site. Furthermore, *B. breviceps* was primarily represented in the habitat of home garden this site. The most prevalent species on the agricultural area at the Manang site were *B. breviceps, B. eximius, and B. grahami. B. parthenius, B. tunicatus,* and *B. festivus* showed greater dominance in the forest habitat.

Furthermore, the most prevalent species on the agricultural area at the Manang site were *B. breviceps*, *B. eximius*, and *B. grahami*. *B. parthenius*, *B. tunicatus*, and *B. festivus* showed greater dominance in the forest habitat. *B. lepidus* was unique to the home garden environment at the Manang site. *B. breviceps* and *B. turneri* were the most abundant in the agricultural site of the Mustang, but *B. miniatus*, *B. rotundiceps*, and *B.*

tunicatus dominated the forest habitat. Both *B. lepidus* and *B. parthenius* were found in the grassland environment of the Mustang site. Furthermore, records of *B. eximius* were made exclusively in this site's home garden environment.

3.3.2 Elevational records of *Bombus* species

Within the whole study area(WSA), *Bombus pressus* (300 m asl) was recorded in the highest mean elevation (Table 2) followed by *B. cornutus* (2700 m asl) and *B. Lepidus* (2673 m asl). *B. rotundiceps* (2121 m asl), *B. haemorrhoidalis* (2189 m asl), and *B. eximius* (2257 m asl) were the lowest mean elevation recorded species. Nevertheless, during a site-based analysis, variations were noted in their mean altitudinal records. In the Gorkha site, some species like *B. pressus* (3000 m asl), *B. grahami* (2734 m asl), and *B. cornutus* (2700 m asl) demonstrated higher mean elevation records. Conversely, in the Manang site, species such as *B. breviceps* (3500 m asl), *B. rotundiceps* (2799 m asl), and *B. lepidus* (2669 m asl) were observed at the highest mean elevations. In the Mustang site, *B. lepidus* registered the highest mean elevation (2706 m asl), followed by *B. festivus* (2597 m asl) and *B. tunicatus* (2270 m asl).

Table 2: Mean elevation of various *Bombus* species recorded in the different study sites. Whole study area (WSA), Budhigandaki River valley (GOR), Marshyandhi River valley (MAN), Kaligandaki River valley (MUS) (Ghimire *et al*., 2023)

Species	(WSA)	(GOR)	(MAN)	(MUS)
B. asiaticus	2556 ± 307	2556 ± 307	-	-
B. branickii	2660	2660	-	-
B. breviceps	2399 ± 677	1988 ± 388	3500	1709
B. cornutus	2700 ± 267	2700 ± 267	-	-
B. eximius	2257 ± 191	2245 ± 220	2202	2324 ±116
B. festivus	2443 ± 531	2337 ± 476	2395 ±593	2597 ±593
B. grahami	2452 ± 327	2734 ± 376	2191	2432 ±270

B. haemorrhoidalis	2189 ± 418	1880 ± 406	2351 ±277	2337 ±16
B. lepidus	2673 ± 34	2644 ± 15	2669	2706 ±21
B. miniatus	2592	-	-	2592
B. novus	2536	2536	-	-
B. parthenius	2353 ± 184	2554	2191	2315
B. pressus	3000	3000	-	-
B. rotundiceps	2121 ± 714	1833 ± 531	2799 ±991	1731
B. tunicatus	2309 ± 164	2527 ± 11	2132	2270
B. turneri	2353 ± 215	2550	2271 ±277	2240 ±41

3.3.3 Effect of elevation and landscape factors

The analysis examined the association between bumblebee abundance, richness, and species diversity in relation to landscape factors such as elevation, sites, and habitats. Remarkably, a significant impact of elevation on bumblebee abundance and richness was identified (Table 3).

Table 3: Summary of regression model (R^2 = 0.3876) illustrating the relationship between bumblebee species richness numbers and elevation (Ghimire et al., 2023)

Co-efficient	Estimate	Std. Error	t- value	P -value
Intercept	-4.220	2.010	-2.10	0.037
Elevation (m)	0.0020	0.0008	3.450	0.0007

In the model, species names were considered random factors along the altitudinal gradients while altitudinal gradients and habitat types were calculated as fixed factors. As a result of the investigation, a mid-domain effect was demonstrated by the trend of change in species richness from lower to higher elevations. Particularly, the mid-elevation zone ranging from 2000 m asl–3000 m asl had the highest species richness (Figure 5).

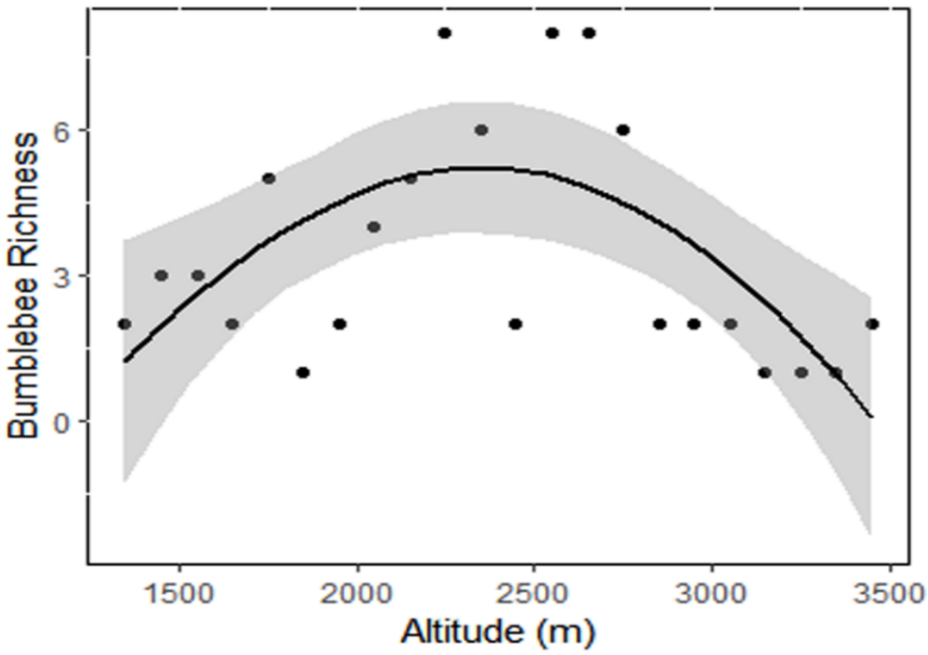


Figure 4: Species richness and altitudinal gradients

Likewise, the study aimed to investigate the relationship between habitat types and bumblebee abundance. In the corresponding analysis (CA), it was revealed that the primary axis accounted for the most significant variance (48%), closely followed by the second axis (43%), resulting in a cumulative variance of 91%.

Table 4: Correspondence analysis (CA) for the three axes with variance and cumulative variances (Ghimire *et al*., 2023).

	Axis-I	Axis-II	Axis- III
Eigenvalue	0.441	0.398	0.088
% of Variance	47.593	42.90	9.507
Cumulative % of Variance	47.593	90.493	100.

The percentage (%) contribution of different types of habitat to the abundance of *Bombus* spp. was found in two correspondence axes. The analysis showed that the species were clearly positioned and a significant number of *Bombus* species were specifically associated with the forest habitat, which is followed by the agricultural habitat (Figure 6).

Table 5: Abundance of bumblebees based on habitat types in corresponding axes (Ghimire *et al*., 2023)

Habitats	Axis-1 (%)	Axis-2 (%)
Agricultural	42.22	26.21
Forest	40.15	2.69
Grass land	2.32	63.57
Home garden	15.29	7.51

The primary contributors to the variance in bumblebee abundance along axis-1 were agriculture and forest habitats, with home gardens falling in between, and grassland habitat had the least contribution. Conversely, along axis-2, the highest variance (64%) in bumblebee abundance was attributed to the grassland habitat, while the forest habitat had the smallest contribution (3%) (Table 5).

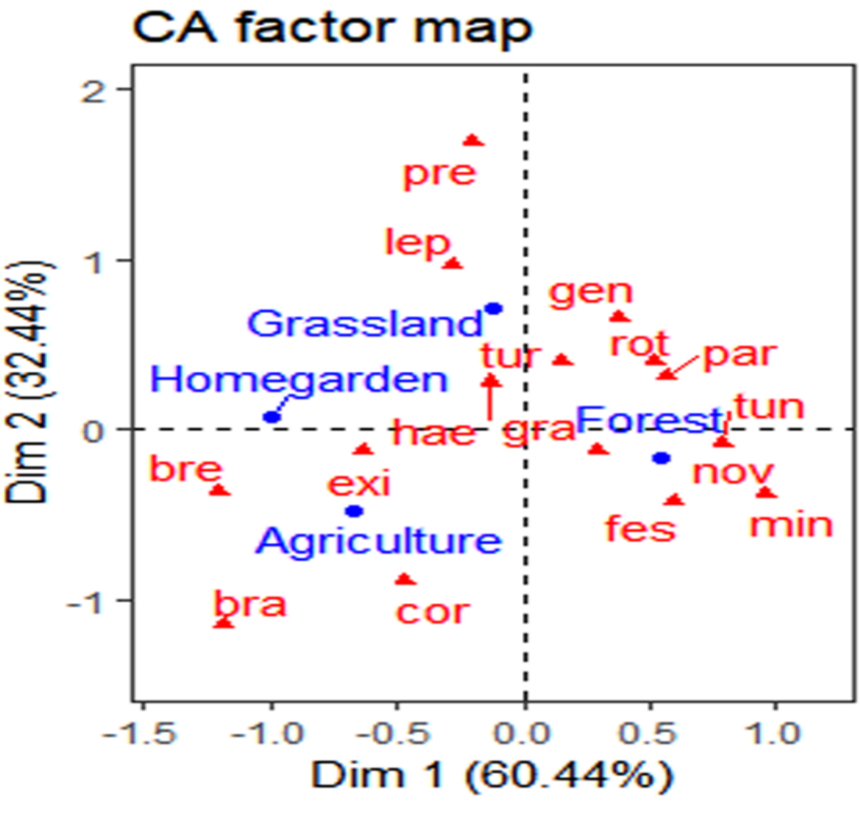


Figure 5: Correspondence analysis examining the relationship between *Bombus* species and various habitat types; AGRI: Agriculture, FORE: Forest-FORE, GRAS: Grassland, and HOME: Home Garden; asi: *B. asiaticus*, bra*: B. branickii*, bre: *B. breviceps*, cor*: B. cornutus*, exi: *B. eximius*, fes*: B. festivus*, gra: *B. grahami*, hae*: B. haemorrhoidalis*, lep*: B. lepidus*, min*: B. miniatus*, nov*: B. novus*, par*: B. parthenius*, pre*: B. pressus*, rot*: B. rotundiceps*, tun*: B. tunicatus*, tur*: B. turneri* (Ghimire *et al*., 2023)

Bombus. festivus, *B. grahami*, *and B. tunicatus* were the dominant species observed in forest. Some species like *B. branickii*, *B. cornutus*, and *B. asiaticus* were the most abundant in the agricultural land. In the grassland habitat, dominant species included *B. haemorrhoidalis*, *B. lepidus*, and *B. turneri*. Likewise, the majorly abundant species found in the home garden habitat were *B. haemorrhoidalis*, *B. eximius*, and *B. lepidus*.

The Shannon-Weiner index, Inverse Simpson, and species number were compared with the species diversity profiles. Across the sampling environments, the diversity indices showed a clear pattern of variation. Specifically, highest diversity profiles were observed in forest, then in grassland followed by agriculture, and home garden habitats (Figure 7).

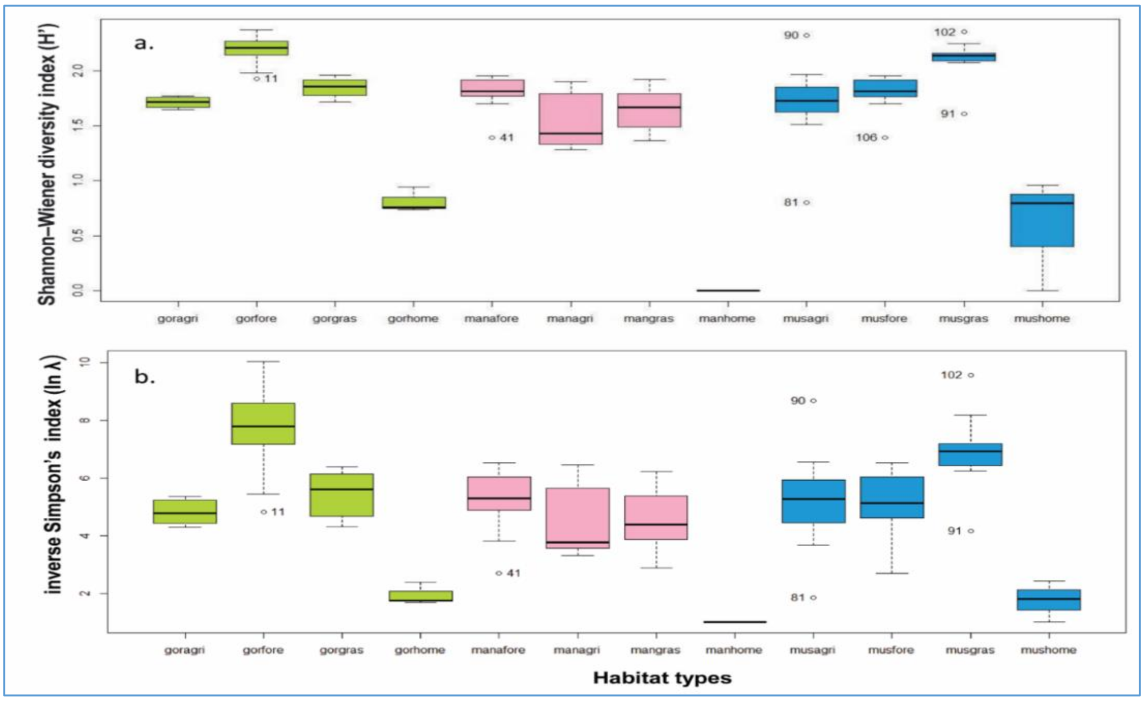


Figure 6: Species diversity profile of bumblebee: a) Shannon–Wiener diversity index (H') in four different habitats of three study sites; b. inverse Simpson's index (In λ). Here, goragri: Gorkha-agricultural habitat, gorfor: Gorkha-forest, gorgras: Gorkha Grassland, gorhome: Gorkha-homeland, managri: Manang-agricultural habitat, manfor: Manang forest, mangras: Manang Grassland, manhome: Manang homeland, musagri: Mustang agricultural habitat, musfor: Mustang forest, musgras: Mustang Grassland, mushome: Mustang homeland

Upon analyzing the data, it was observed that the highest species richness and diversity were documented in the forest habitat at the Gorkha site (S=12, Shannon index H'=2.18), followed by the grassland habitat at the Mustang site (S=11, Shannon index H'=2.10). Similarly, the Inverse Simpson index (In λ) showed higher values in the Gorkha site's forest (In λ=7.71), followed by the grassland at the Mustang site (In λ=6.92) and the forest at the Manang site (In λ=5.26)

3.4 Discussion

In this study, it was found that landscape characteristics, particularly elevation and habitat composition, influences the diversity and abundance of bumblebees. Elevation emerged as a main element affecting the distribution of bumblebees, while type of habitat and other landscape features also had an impact on the composition of species in bumblebee's community. Composition and diversity of bumblebees were notably impacted by the distinct landscape characters and vegetation dynamics present in the three river valleys in our study area. In this study, it was found that the proximate landscape factors along the elevational gradients play a crucial role as filtering factors due to the unique vegetation dynamics observed at different elevation levels. This observation is consistent with previous research conducted by Fourcade *et al.,* (2019), Sydenham *et al.*, (2015), Devoto *et al.*, (2014), Miller–Struttmann & Galen (2014), and Hoiss *et al.,* (2012). Additionally, it was observed that landscape dynamics, particularly related to climate and microclimatic factors, further contribute to the filtering process, as suggested by the work of Naeem *et al.,* (2019).

The study identified a mid-domain effect, where the geographic factors within the landscape, combined with suitable environmental conditions such as elevated habitat heterogeneity (Paudel & Sipos, 2014), likely played a role in creating a diverse range of foods available to bee communities. The observed pattern of distribution of species and abundance was probably influenced by these variables ((Wang *et al.,* 2009; Brehm *et al.,* 2007). Variation in species composition across different habitats along the altitudinal gradient in our study aligns with results of previous researches (Streinzer *et al.,* 2019; Saini *et al.,* 2012) explored in other regions of the Himalayan landscape and this consistency suggests that similar ecological patterns and processes may be operating across different regions within the Himalayas (Gautam *et al.,* 2017). Higher diversity profiles evident in forests and grasslands, as opposed to agricultural and human settlement areas, may be primarily attributed to anthropogenic disturbances, as indicated by (Agra *et al*. 2021). This factor likely impact the distribution of bumblebees in CHAL. Conversely, rapid decline of habitats and the increasing use of pesticides in agricultural regions may be influencing abundance and dispersal of specific bumblebees (Stout & Morales, 2009; Williams & Osborne, 2009; Williams *et al.,* 2016). Nonetheless, the CHAL's bumblebee population's diversity and abundance are significantly impacted by complex socio-ecological systems that are influenced by a number of environmental, social, and ecological factors (Timberlake et al., 2022). These factors include rapid changes in habitat utilization, increased pesticide application (Goulson et al., 2008), and changes in farming practices. General characteristics of the Himalayan landscape, such as the Chitwan Annapurna Landscape (CHAL), include a higher richness of flowering plants and relatively lower mean temperatures in the eastern river basin regions compared to the western ones. (Sharma *et al.,* 2008; Williams *et al.,* 2009; Poudel & Kotani, 2013; Saini *et al.,* 2015; Karki *et al.,* 2016).

During this study, *B. asiaticus*, *B. eximius, B. festivus, B. haemorrhoidalis, B. rotundiceps*, and *B. turneri*, displayed significant variations in elevation across different locations and even within the same area. These findings indicate that these bumblebee species have a wide range of elevational distributions, suggesting their adaptability to diverse environmental conditions and potentially reflecting their ability to utilize various habitats within the study region. Interestingly, *B. rotundiceps, B. haemorrhoidalis*, and *B. eximius*, most abundant species observed were found at relatively lower average mean elevations, consistent with findings reported from other areas in the Himalayas (Williams *et al.,* 2009; Streinzer *et al.,* 2019). The presence and distribution of specific *Bombus* species along elevational gradients may be influenced by the thermal tolerance limits specific to each species along those gradients. (Martinet *et al.,* 2015; Oyen *et al.,* 2016; Dudley *et al*., 2017; Vray *et al.,* 2019). Across the three study sites and in comparison to previous research findings, species such as *B. breviceps* showed significant differences in their mean altitude range (Winfree *et al.,* 2009; Williams *et al.,* 2009; Streinzer *et al.,* 2019). Some species of bumblebees showed a high degree of specificity within the study region, as evidenced by their limited elevation range or restriction to certain places. For example, only the eastern location included *B. asiaticus, B. branickii, B. cornutus, B. pressus,* and *B. novus*, whilst *B. miniatus* was only detected at the western site. These species might have developed adaptations to distinct microhabitats, as they are confined to a specific and limited altitudinal range within our study sites. The distribution of these species is possibly impacted by environmental variables including their thermal limits and the availability of more generalized food plants (Carvell, 2002; Saini *et al.*, 2015; Diaz–Forero *et al.*, 2013; Goulson *et al.*, 2015). Environmental factors, such as wind, significantly affect foraging behavior in bees, reducing their foraging efficiency and increasing their reluctance to take flight. Habitat destruction and fragmentation, along with chemical stressors like pesticides and fertilizers, and non-chemical stressors such as parasites, are key drivers of the decline in pollinating insects (Bhusal *et al*., 2019). Variables like temperature, rain, light, and wind can potentially alter foraging behavior (Reber *et al.*, 2015). Additionally, changes in pollinator activity are influenced by weather conditions. Understanding these factors is crucial for predicting the future impacts of climate change on bumblebees. Wind, in particular, can affect foraging behavior both directly and indirectly by causing flowers to shake (Potts et al., 2010). Similarly, body size variation (Goulson *et al.*, 2005) and intra and inter specific competition (Oliveira *et al.*, 2017) may also play an important role for the foraging behavior and selection of specific flowering plants by bumblebee species.

The altitudinal range of numerous species of bumblebee in the Himalayan region may be influenced by significant factors, including species range shifts caused by recent effects of global warming (Telwala *et al.*, 2013). Emerging evidence indicates that the CHAL is experiencing significant land cover changes, which ultimately impact the vegetation dynamics within the region (Adhikari *et al.*, 2022b). These alterations could have consequences for the composition and diversity of entire bee communities, encompassing bumblebees. Moreover, the existence of host plants specific to particular species in certain habitats, along with the accessibility of appropriate nesting sites, is likely a crucial factor influencing distribution of bumblebees in CHAL. The mid-elevation zones (2000-3000 m) of the river basins under investigation exhibit a higher species richness, which is indicative of a better availability of food resources, particularly appropriate host plant species. Presence of dominant foraging plants that depends on pollinators, particularly in mid-elevation region, may play a vital role for contributing to the highest richness of species of bumblebees. Some bumblebee-attracting plants dominantly found in this region include *Biden pilosa Solanum melongena, Cucurbita pepo, Cirsium wallichii*, *Reinwardtia indica*, *Vigno mungo, Rubus ellipticus, Berberis asiatica, Impatiens scabrida, Anaphalis busua, Rosa* *brunonii, Vigna unguiculata, Osbeckia nepalensis,* and *Diploknema butyracea* were identified. The study emphasizes the significant influence of environmental variation in distribution of bumblebees, and I propose conservation action plans that involve preserving areas with high richness of species. The overall results suggest that the diversity in immediate landscape factors along elevation gradients, combined with other landscape characteristics like habitat features, especially the presence of appropriate host plants in specific habitats, is significant in influencing the composition of the bumblebee assemblage. Moreover, the characteristics of the habitat, specifically the existence of appropriate flowering plants, were identified as significant factors in shaping the composition of bumblebees (Winfree *et al.,* 2007; Redhead *et al.,* 2016). The complexity in landscape along the elevation gradient plays a significant role to determine species structure, distribution, and diversity of bumblebees in the CHAL.

3.5 Conclusions

The community composition of bumblebees in the region is significantly influenced by an important environmental filter, which is the variation in habitat heterogeneity along elevation gradients across different river basins. Significantly, the study areas exhibited higher number of species at mid-altitudinal regions, most likely due to favorable microhabitat structures and the availability of suitable food plants. It can be suggested that emerging risk factors, such as rapid biological invasion and climate change, hold the potential to alter bumblebee habitat communities along gradients. In the future, the movement of appropriate flowering plants in combination with shifting elevations will play a pivotal role as a limiting factor, affecting species diversity and community structure of bumblebees in this region. In coming years, the relocation of suitable flowering plants with changing elevations will emerge as a crucial limiting factor to determine species diversity and community composition of bumblebee species. This study significantly improves understanding of species distribution and composition in the mid-Himalayas of Nepal. It's important to note that sampling was limited to major river basins along elevation gradients, suggesting more species are likely to be found elsewhere in the CHAL. Furthermore, an investication is conducted to assess how socio-ecological factors influence the composition of bumblebee populations within this area, considering both local and regional perspectives. The study offers valuable recommendations for decision-makers to support their conservation strategies and efforts.

CHAPTER 4

4. DETERMINE THE FORAGING PREFERENCE OF *BOMBUS* SPP. WITH THEIR ASSOCIATED FLOWERING PLANTS

Abstract

There is limited information regarding the impact of vegetation and floral color on the foraging behavior of bumblebees, as well as their frequency on native and non-native flowering plants. Foraging frequency of bumblebees on native and non-native flowering plants and foraging preferences, plant families and flower colors was compared with in the Chitwan Annapurna land scape (CHAL). Specimens were collected along an accessible walking transect ranging between 600 to 3500 meters above sea level (asl) during April and November 2019.The transect covered diverse habitats, including agriculture, forest, grassland, and home gardens. The *Bombus* species were collected by the sweeping net while visible at the walking transect. Relative frequency of bumblebees varied significantly based on type of flowering plants including native, non-native cultivated, non-native naturalized, and invasive) and the color of the flower along the sampling route across altitudinal gradients. Some *Bombus* species exhibited a distinct foraging preference for specific plants within the study sites, indicating specialized flowering preferences. In this study, it was also explored that *Bombus* spp. displayed a tendency to forage exclusively on specific flower families and exhibited a preference for particular flower colors. This study presents insights into the foraging behavior of different bumblebees within native and exotic plant communities in CHAL. The choices made by these species are influenced by plant families, specific colors, and individual foraging preferences.

Keywords: Altitudinal gradient, categories, colour, floral preference,

4.1 Introduction

The global understanding of pollination services due to impacts of multi-level sptial structure has widely been explored. However, there is a lack of comprehensive documentation on the impact of the spatial relationship between bumblebee distribution and vegetation dynamics, particularly concerning floral structures within specific plant families. Typically, it is widely accepted that the flower selection habit of pollinator bee communities, particularly regarding colour of the flower within specific plant families, is influenced by innate behaviour and experiences gained during its life (Gumbert, 2000). Nevertheless, there has been an extensive ongoing discussion regarding the mechanisms by which *Bombus* species choose particular floral colors and exhibit a preference for visiting plants with specific floral characters. (Gumbert, 2000; Lunau & Maier, 1995; Junker *et al.,* 2013). They show natural preferences towards identifiable flower patterns, such as shape (Zeil, 1997) and symmetry (Giurfa, 1996). Bumblebees possess trichromatic color vision, characterized by photoreceptors that have peak sensitivity around 350, 440, and 540 nanometers. (Peitsch, 1992). Bumblebees naturally and instinctively utilize floral guides present in the natural flowers (Lunau, 1992) of specific colors. A recent suggestion suggests that color preferences among crucial bee pollinators are believed crucial factors with a restricted phylogenetic signal. This phenomenon assists in the coexistence of closely related species, especially in tropical-subtropical island environments (Tai, 2020), as well as within plant-pollinator networks (Ibanez, 2016).

The structure and scent of flowers have been recognized as significant factors in attracting various bumblebee species during foraging (Odell, 1999). Bumblebees are drawn to inflorescences, which serve as plant-level attractants, based on the abundance of flowers that produce pollen (Thairu & Brunet, 2015). Moreover, bumblebees display varied tongue lengths, a characteristic that, among other factors, impacts their preferences for particular forage plants (Fussell & Corbet, 1991). Therefore, tongue length is recognized as a significant factor contributing to niche differentiation within bumblebee communities (Hoelzel, 1989). Bumblebee species possessing longer tongue lengths can access the stigma of flowers with extended corollas, while those with shorter tongues may need to bite into the corolla and navigate to reach the stigma (Goulson & Williams, 2001). Several researchers have argued that the color preference of bumblebees is a general tendency rather than an absolute rule (Raine & Chittka, 2008, Brunet *et al.*, 2015). Nevertheless, there is a widely recognized tendency among bumblebees to favor flowers that exhibit blue-purple colors. (Raine *et al.,* 2006; Rausher, 2008); Dyer *et al.,* 2012). Similarly, research indicates that numerous bumblebee species show a preference for the pink flowers of *Impatiens sulcate* (Balsaminaceae) compared to the yellow-colored flowers of *Impatiens scabrida* (Balsaminaceae) when both plants coexist in the same area (Saini *et al*., 2012).

Moreover, multiple research studies have confirmed the strong preference of long-tongued bumblebees for specific plant families, such as legumes (Fabaceae) (Gulson *et al.,* 2005; Hulsmann *et al.,* 2015). Furthermore, Bhusal *et al.*, (2019) reported that bumblebees most frequently visited plant Cucurbitaceae, Fabaceae, and Verbenaceae. Distribution of plant species within these families significantly impacted number of species and their abundance of *Bombus* spp. in an urban site of Nepal. Generally, perennial plants serve as the primary forage resource for bumblebees (Fussell & Corbet, 1992; Leonhardt & Bluthgen, 2012; Dramstad & Fry, 1995). Bumblebees tend to forage on plants with higher protein content compared to less visited plant species (Somme, 2014; Vaudo *et al.,* 2016).

The decline in bumblebee populations worldwide is often linked to the loss or alteration of vegetation composition, shifts in natural habitats, and climate change (William & Osborn, 2009). Bumblebees' abundance and diversity in different habitats is significantly influenced by diversity and composition of plant species. Yet, the precise foraging preferences of bumblebees, encompassing their choices of host plants, plant families, and floral colors, remain mostly unexplored. This study aims to investigate whether a foraging relationship exists between flower color, plant families, and the foraging preferences of bumblebees.

Numerous studies have provided evidence of global declines in insect pollinators, including bumblebees (Goulson *et al.,* 2015; Potts *et al.,* 2016). This declination is due to an array of factors, including alterations in land use, loss of habitats, pesticide application, biological invasions, pathogens, and potentially climate change (Goulson *et al.,* 2005, 2015). Flower-visiting behaviors of insect pollinators, including bumblebees, play an important role in pollen dispersal and reproductive success in many types of plant species. The spatial distribution, diversity, and abundance of flower resources are also vital factors in maintaining the community composition of insect pollinators, including bumblebees (Osborne *et al.,* 1999; Walther-Hellwig & Frankl, 2000; Bhusal *et al.,* 2019). Numerous ecological factors and plant characteristics have been investigated to understand the relationship between bee communities and their foraging host plants, which can significantly impact the species richness, diversity, and bumblebees' abundance (Potts *et al.,* 2010). Effects of invasions by non native plant species on pollination and reproductive success of native plants are well described. Yet, the interdependent relationship between native plants and the pollination and reproduction of exotic plants has not been sufficiently investigated. Moreover, the mechanisms underlying the choice of flowers, time of foraging, and frequency of flowers visits by pollinators in relation to successful pollination are still limited in knowledge. Understanding the effects of plant invasions on insect pollinators and vice versa is crucial in a global scale in this context (Carvallo *et al.,* 2013).

Bumblebees navigate a complex and dynamic environment while foraging, where the nectar and pollen they obtain vary widely among plant species. Foraging decisions of bumblebees are influenced by various floral cues, including odors, flower color, morphology, and the spatiotemporal availability of host plants (Brunet *et al.,* 2015; Vaudo *et al.,* 2015). Existence of nonnative plants has a capacity to affect bumblebees and ecosystem functioning both directly and indirectly (Mack *et al.,* 2000). Earlier research has indicated that invasive plants typically diminish the abundance as well as diversity of local and native plants, potentially causing changes in communities of pollinators (Biesmeijer *et al.,* 2006; Geib & Galen, 2012; Geib *et al.,* 2015). Bumblebees are widely spread and efficient pollinators, crucially contributing to the reproductive success of various native plant species and local crops. Investigating the foraging relationships of bumblebees can be challenging, as it can be influenced by range shifts of native flora, changes in floral composition, and phenology under the current scenario of climate change (Ogilvie, 2017; Woodard, 2017; Suzuki-Ohno *et al.,* 2020). However, the precise mechanisms governing these interactions remain incompletely understood. The morphology and flower color, as well as the specific foraging behavior of bumblebee species, are important factors in determining their flower choices (Inouye, 1980; Simonds & Plowright, 2004; Raine & Chittka, 2005). Distinctive features of genuine pollinators, such as pollen baskets on hind legs, jaws, and an elongated tongue for extracting pollen grains of anthers and soaking them by regurgitated nectar drops, are adaptations that contribute to the success of pollination. The foraging behavior and preferences of bumblebees for particular plant species, considering specific evolutionary features such as corolla’s shape and size and functional traits of plants are vital factors in anticipating reproductive success (Ordano *et al.,* 2008; Córdoba & Cocucci, 2011).

Various ecological factors, including the diversity and abundance of accessible flora for bumblebees and the distance between breeding placees and foraging plants, are also significant determinants affecting foraging preferences of various *Bombus* spp. (Walther-Hellwig & Frankl, 2000; Goulson & Stout, 2002). However, there is a lack of research on the foraging patterns of bumblebees, particularly with regards to native and exotic flowering plants across altitudinal gradients of Hindu Kush Himalayas of the central Nepal (Bhusal, 2020). In this context, understanding the foraging choices of bumblebees is crucial, especially considering the limited historical and current occurrence data from this region.

The foraging patterns of bumblebees, particularly alpine bumblebees, can be influenced by floral abundance and density (Shibata & Kudo, 2020). There may be a functional relationship between flower characteristics, color, and specific bumblebee species. The existence of host plant families substantially impacts *Bombus* species, even within both open and closed floral structures, amidst the influence of urbanization, and changes in land use (Hulsmann *et al.*, 2015; Bhusal *et al.*, 2019). The advantages of incorporating native and non-native plants into landscapes, particularly concerning pollinator management, are becoming increasingly debated. In general, there is a widely held belief that native plants generally contribute to higher faunal diversity and biomass Non-native species frequently exhibit specific benefits over native plant species, including rapid development and multiplication rates, greater ecological forbearance, or higher effective spreading mechanisms compared to exotics plantings (Burghardt et al., 2010). Exotic species often possess certain benefits over native counterparts, including accelerated development and multiplication rates, increased ecological tolerance, or more effective dispersal mechanisms (Sladonja *et al.*, 2018). The choices of pollinators can be impacted by the connections between flower characters and pollen rewards, potentially leading to choice of specific flower characters by particular bumblebee species (Brunet *et al.*, 2015). The tendency of native fauna to prefer native flora is frequently attributed to their extensive history of coevolution.

Plants have developed various physical and chemical mechanisms to attract pollinators, and insects that have coevolved with these plants are more likely to have specialized behavioral and physiological adaptations (Tallamy, 2004). The idea that plants and pollinators have evolved together over time could be one reason why pollinators seem to like native plants more than non-native ones. Bumblebee foraging responses depend on the color, morphology, and adaptations of both the flower and the bumblebee itself. Bumblebees extensively forage on multiple plant species, responding to categorical and measurable variations in floral resources.

Studying impacts of non-native plant species on bumblebee communities can be challenging. Exotic plants might offer novel resources for resident pollinator communities, but their ability to attract pollinators successfully can be hindered if they have specialized floral morphologies (Bode *et al.,* 2020). Some non-native plants can provide benefits, including increased pollination services with economic and environmental advantages, while others can have adverse effects on native pollinators (Stout & Morales, 2009). The effects of invasive exotics on both specialists and generalists' bees might vary; these variations depend on the landscape setting. The success of invasion can be limited by the availability of pollinators, but a plant that can attract a wide range of pollinators in various situations may become an effective exotic species.

It was hypothesized in this study that the foraging preference of Bumblebees (*Bombus* species) varies with native versus non-native plant species and aims to determine whether there is a foraging relationship between the color of the flower and plant families with the foraging preference of bumblebees in the human-dominated heterogeneous Chitwan Annapurna landscape (CHAL) Nepal.

4.2 Materials and Methods

4.2.1 Study area

This study was focused in Chitwan Annapurna Landscape (CHAL) of central Nepal, specifically covering an altitudinal range from 600 to 3500 meters above sea level. The study focused on three river valleys: Kaligandaki (west part), Marsyangdi (mid part), and Budhigandaki (east part) (figure. 1). These locations encompass various habitat types, comprising agriculture, forest, grassland, and human settlements. The study area is well-known for its rich biodiversity and includes the Annapurna Conservation Area, serving as a vital migratory route for birds and offering a habitat for endangered species like *Pantherd uncia*, *Ailurus fulgens*, and *Ursus thibetanus*. (Oli *et al*., 1994; Adhikari *et al*., 2019; Chetri *et al*., 2019) This area is well known for its vegetation, *including Rhododendron arboreum, Michelia champaca, Schima wallichii, Primula species, Gentiana species, Meconopsis species, Anemone species, Aconitum species, Saxifraga species, Potentilla species, Pedicularis species, Bauhinia variegate* (Sharma *et al.,* 2008). This landscape is also culturally significant, supporting a large population that heavily relies on resources of forest and services the ecosystem provides, particularly for agriculture production. However, the area is facing multiple risks including climate change, infrastructure development, and land-use change, which are contributing to the attack of invasive plant (Pandey *et al.,* 2020; Bhusal *et al.,* 2020). These invasions may disrupt the native floral composition, leading to reproductive challenges, competition for resources, and potential physiological and morphological abnormalities that ultimately impact the pollination process. In the long term, this competition can negatively affect the maintenance of plant-pollinator interactions and ecosystem services within landscape.

4.2.2 Bumblebee surveying and identification

Field surveys were carried out across the entire flowering season from April to November 2018/2019. Three walking transects were set up along the river valley sites (Kaligandaki, Marsyangdi, and Budhigandaki) in the study area to conduct extensive surveys. These transects covered an altitudinal range from 600 to 3500 meters above sea level. During the surveys, the presence of foraging bumblebees at various points along the walking routes was carefully observed. When a bumblebee was detected, the surrounding flowering plants were closely examined, and the bumblebee species present at the site was recorded. The surveys took place from 9 am to 6 pm, specifically during the morning and afternoon when there was no rainfall and low wind speeds. To capture the bumblebees, an entomological net was used, and they were killed by using ethyl acetate. Throughout the survey, details on the habitat types (agriculture, human settlement, and forest), altitude, and GPS location of the collection points were noted.

The collected samples were carefully kept in airtight vials having tissue layers and about 2-3 drops of ethyl alcohol for prevention of mold growth during transportation. Later, the samples were labeled by insects' pins (size-3, diameter 0.5mm, Length-38mm) as dry-mounted and placed in the Central Department of Zoology, Tribhuvan University (TU), Kathmandu, Nepal. A total of 656 worker individuals were collected, and each specimen was observed under a stereoscopic microscope for identification. To identify the bumblebee species, published identification keys specific to adjacent regions (Williams, 1991, 2022; Williams *et al*., 2009, 2010; An *et al*., 2014; Saini *et al.*, 2015) were utilized.

4.2.3 Data analyses

A two-way contingency analysis, also known as cross-tabulation, was conducted to find out relative frequency of bumblebees with relation to both plant families they forage on and the corresponding floral color. To assess the statistical significance, we used the Chi-square (X2) test for each contingency table, with a level of significance set at <0.05. Furthermore, the impact of bumblebee frequency in relation to the plant families they forage on was examined using a linear mixed-effects model in R. Prepared data were then subjected to cluster analysis (CA) and corresponding analysis (CA) to investigate the asociations between bumblebees and specific families of plants and flower colors. Relative frequency composition of the identified bumblebees was analysed.

Plant species were also identified and categorized into native (NAT) and non-native types. The non-native species were further classified as non-native cultivated (CUL), non-native invasive (INV), and non-native naturalized (NNU). Similarly, the habitat types (forest, home garden, agriculture, and grassland) were classified. The flowers were further categorized into open and closed types. The number of foraging observations of specific *Bombus* species with their host plants along the walking transects was analyzed using a linear mixed-effects model in R. Categories of plants type, habitat, and flower nature were used as explanatory variables. A two-way contingency table, or crosstab, was created to examine the association between *Bombus* species (rows) and the categories of plants and habitat types (columns). The level of significance for each contingency table was determined using the Chi-square (X2) test with a threshold of <0.05. The prepared data were then subjected to cluster analysis and correspondence analysis (CA) for investigating relationship between bumblebees and specific habitat types and plant categories in CHAL. Additionally, relative presence of bumblebees in different flowering plants was calculated. If a *Bombus* species was recorded more than 20 times in our samples, it was categorized as very common (VC); if it was recorded between 10 and 20 times, it was categorized as common (C); if it was recorded between 5 and 10 times, it was categorized as rare (R); and if it was recorded less than 5 times, it was categorized as very rare (VR).

4.3 Results

4.3.1 Relative frequency of Bumblebees with their host plant families

The cross-tabulation of the relative frequency of bumblebees and their host plant families was conducted using the collected samples (X–squared = 579.68, df = 448, p–value = 0.00002545). Certain *Bombus* species were observed exclusively in single plant families.

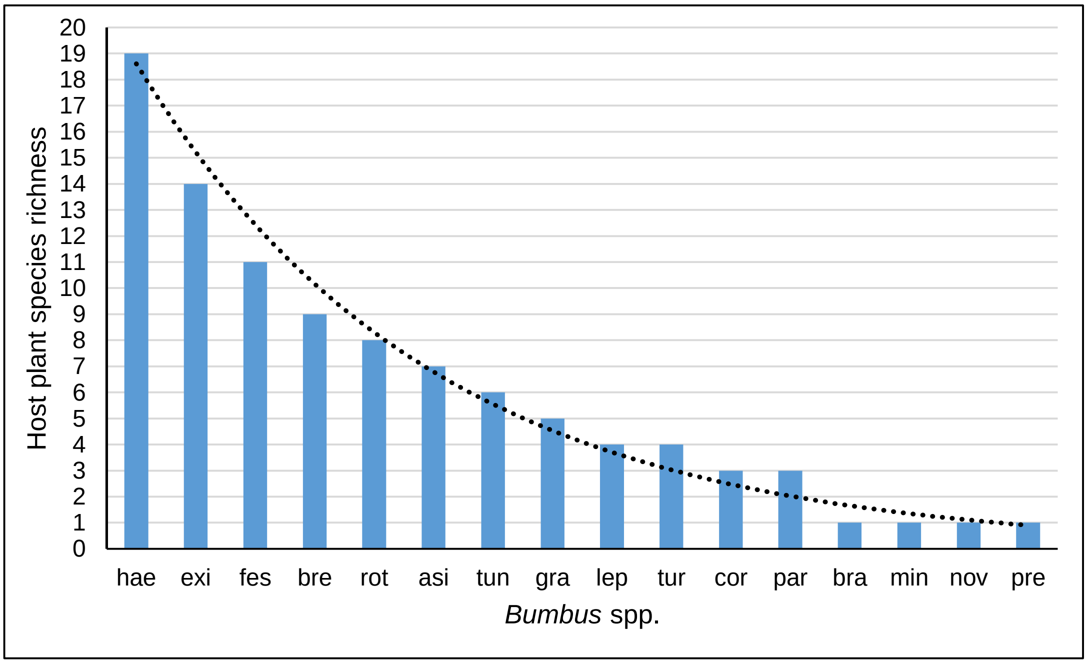


Figure 7: Foraging plant families of *Bombus* species. *Bombus* species: *B. asiaticus*(asi), *B. branickii* (bra), *B. breviceps*(bre), *B. cornutus*(cor), *B. eximius* (exi), *B. festivus*(fes), *B. graham*(gra), *B. haemorrhoidalis*(hae), *B. lepidus*(lep), *B. miniatus*(min), *B. novus*(nov), *B. parthenius*(par), *B. pressus*(pre), *B. rotundiceps*(rot), *B. tunicatus*(tun), *Bombus turneri*(tur),

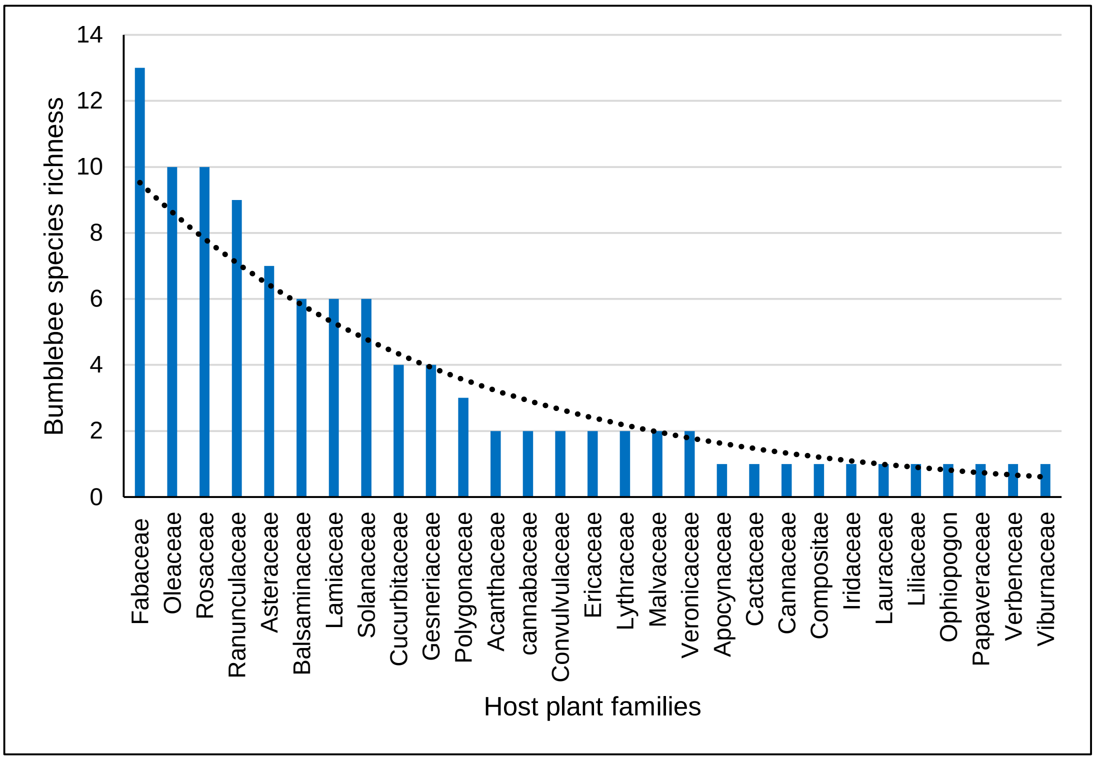


Figure 8: *Bombus* species richness with their foraging plant family. Identified from National Harbarium, Godawari, Lalitpur)

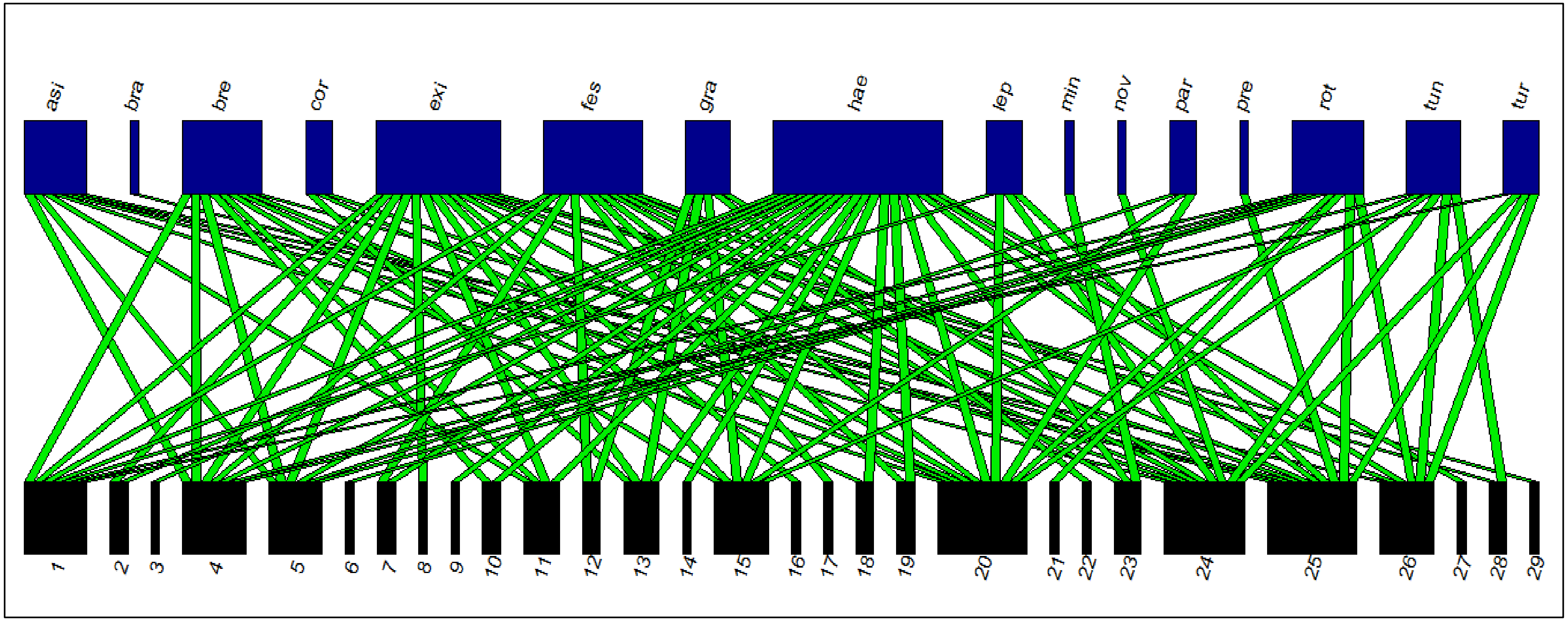


Figure 9: Bipertite diagram showing *Bombus* spp forage with their respective host plant families, upperleve *Bombus* species are= *Bombus asiaticus(*asi*), B. branickii* (bra*), B. breviceps(*bre*), B. cornutus(*cor*), B. eximius (*exi*), B. festivus*(fes*), B. graham(*gra), *B. haemorrhoidalis(*hae*), B. Lepidus*(lep*), B. miniatus(*min), *B. novus(*nov*), B. parthenius*(par*), B. pressus(*pre), *B. rotundiceps*(rot*), B. tunicatus(*tun*), B. turneri(*tur*).* Lowerlevel= The plant families are 1. Fabaceae, 2. Oleaceae, 3. Rosaceae, 4. Ranunculaceae, 5. Asteraceae, 6. Balsaminaceae, 7. Lamiaceae, 8. Solanaceae, 9. Cucurbitaceae, 10. Gesneriaceae, 11. Polygonaceae, 12. Acanthaceae, 13. Cannabaceae, 14. Convulvulaceae, 15. Ericaceae, 16. Lythraceae, 17. Malvaceae, 18. Veronicaceae, 19. Apocynaceae, 20. Cactaceae, 21. Cannaceae, 22. Compositae, 23. Iridaceae, 24. Lauraceae, 25. Liliaceae, 26. Ophiopogon, 27. Papaveraceae, 28. Verbenceae, 29. Viburnaceae (Ghimire & Bhusal, 2022)

Table 6: Summary of linear mixed model showing significant foraging relation of *Bombus* frequency and specific flower colors and families (Ghimire & Bhusal, 2022)

Fixed factors(Plant families and flower Colors)	Estimate	Std. Error	t- value
(Intercept)	10.11	1.72	5.87
Orange	–2.97	4.09	–0.72
Pink	–4.21	2.39	–1.75
Purple	0.01	1.77	0.01
Red	–2.22	2.22	–0.99
White	–1.43	1.66	–0.86
Yellow	–1.03	1.76	–0.58
Acanthaceae	–8.48	3.34	–2.53
Apocynaceae	–5.68	4.41	–1.28
Asteraceae	–4.39	1.28	–3.42
Balsaminaceae	–4.44	1.68	–2.62
Cactaceae	–3.68	4.41	–0.83
Cannabaceae	–4.006	3.58	–1.11
Cannaceae	–7.78	4.32	–1.8
Compositae	–7.28	4.32	–1.68
Convulvulaceae	–4.43	1.95	–2.26
Cucurbitaceae	–3.11	1.68	–1.84
Ericaceae	–3.03	3.14	–0.96
Gesneriaceae	–7.36	2.54	–2.88
Iridaceae	–6.69	4.43	–1.51
Lamiaceae	–4.93	1.83	–2.69
Lauraceae	–7.77	4.42	–1.75
Liliaceae	–6.81	4.48	–1.52
Lythraceae	0.32	2.43	0.13
Malvaceae	–4.75	2.55	–1.85
Oleaceae	–2.04	1.67	–1.22
Ophiopogon	–7.28	4.32	–1.68
Papaveraceae	–0.19	4.42	–0.04
Polygonaceae	–0.81	2.36	–0.34
Ranunculaceae	–3.79	1.77	–2.13
Rosaceae	–2.58	1.50	–1.72
Solanaceae	–2.32	1.36	–1.70
Verbenceae	1.20	2.21	0.54
Veronicaceae	–7.78	3.18	–2.44
Viburnaceae	–7.53	3.14	–2.39

The B. branickii species was exclusively recorded from the Rosaceae family. The *B. novus* was recorded from Polygonaceae. *B. miniatus* was collected only from the single Polygonaceae family and *Bombus pressus* was recorded only in the Rosaceae family of the plant during the field visit. On the other hand, *B. cornutus* was found foraging on plants from three families, including Ranunculaceae, Oleaceae, and Gesneriaceae. Similarly, *B. parthenius* was noted foraging on plants from three other families: Balsaminaceae, Lamiaceae, and Oleaceae. *B. lepidus* and *B. turneri* were reported in association with four plant families. *B. lepidus* was observed foraging on the Oleaceae, Ranunculaceae, Fabaceae, and Rosaceae, while *B. turneri* was seen foraging on the Ranunculaceae, Solanaceae, Fabaceae, and Rosaceae. *B. grahami* was documented foraging on plants from five different families: Gesneriaceae, Ericaceae, Papaveraceae, Lamiaceae, and Ranunculaceae. On the other hand, *B. tunicatus* was seen foraging on plants from six families, including Asteraceae, Oleaceae, Ranunculaceae, Solanaceae, Lamiaceae, and Verbenaceae. Both *B. asiaticus* and *B. rotundiceps* were observed foraging on plants from eight different families. *B. asiaticus* was documented on plants from Ranunculaceae, Solanaceae, Fabaceae, Rosaceae, Oleaceae, Asteraceae, Cucurbitaceae, and Balsaminaceae. Similarly, *B. rotundiceps* was recorded foraging on plants from Ophiopogon, Cactaceae, Solanaceae, Oleaceae, Fabaceae, Balsaminaceae, Rosaceae, and Asteraceae.

Additionally, *Bombus breviceps* was documented foraging on plants from nine different families, which include Convolvulaceae, Lythraceae, Oleaceae, Fabaceae, Balsaminaceae, Rosaceae, Asteraceae, Lamiaceae, and Cucurbitaceae. Finally, Bombus festivus was observed on plants from eleven different families: Cannabaceae, Ericaceae, Malvaceae, Liliaceae, Polygonaceae, Ranunculaceae, Solanaceae, Oleaceae, Fabaceae, Rosaceae, and Asteraceae.

In a similar fashion, *Bombus eximius* was predominantly noted foraging on plants from 14 different families, encompassing Acanthaceae, Gesneriaceae, Laureceae, Veronicaceae, Viburnaceae, Lamiaceae, Ranunculaceae, Oleaceae, Fabaceae, Balsaminaceae, Cucurbitaceae, Rosaceae, and Asteraceae. On the contrary, *Bombus haemorrhoidalis* was seen foraging on plants from an impressive 19 different families: Acanthaceae, Apocynaceae, Cannabaceae, Compositae, Genenculeceae, Iridaceae, Malvaceae, Polygonaceae, Verbeceae, Convulvulaceae, Lythraceae, Lamiaceae, Solanaceae, Oleaceae, Fabaceae, Balsaminaceae, Cucurbitaceae, Rosaceae, and

Asteraceae. Throughout the study, it was observed that the most frequently foraged families by *Bombus* species were Rosaceae, Oleaceae, Ranunculaceae, Fabaceae, and Asteraceae, while the least foraged plant families were Apocynaceae, Cactaceae, Cannabaceae, Iridaceae, Laureceae, Liliaceae, Ophiopogon, Papaveraceae, Verbenaceae, and Viburnaceae (Figure. 8, Figure. 9 and Figure. 10).

Cluster analysis (Figure. 11) was employed to categorize the plant families based on relative abundance of bumblebees (Pearson's Chi-squared = 579.68, df=448, $p < 0.00002545$). Consequently, 29 families of plants were successfully clustered.

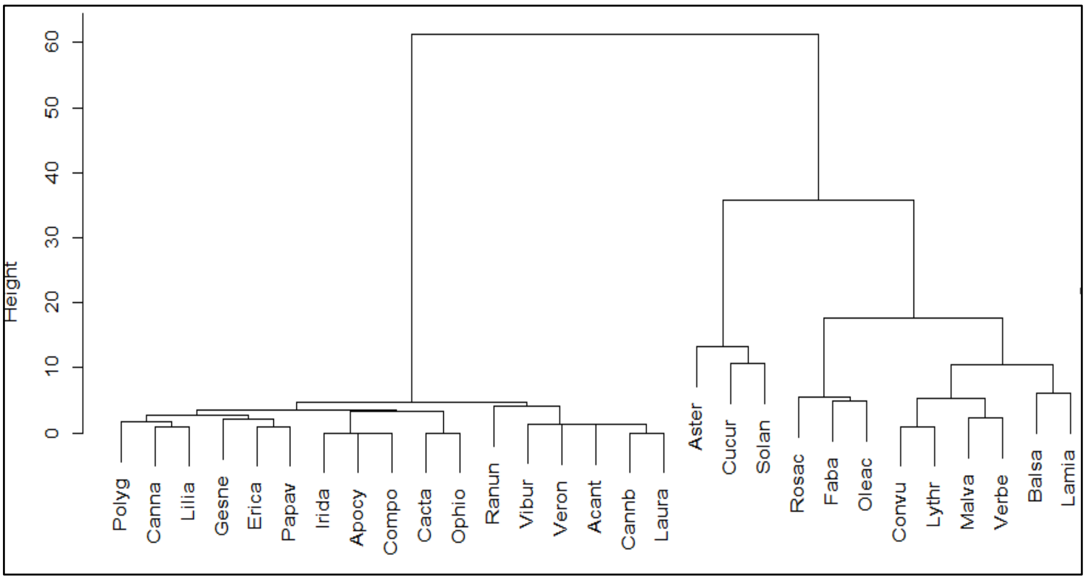


Figure 10. Cluster analysis (method=ward, distance=Euclidean) of plant families according to the relative abundance of *Bombus* species. Plant families are: *Fabaceae (Faba), Acanthaceae(Acant), Apocynaceae (Apocy), Asteraceae (Aster), Balsaminaceae, Cactaceae(Cacta), Cannabaceae (Cannb), Cannaceae (Canna), Compositae (Compo), Convulvulaceae (Convu), Cucurbitaceae (Cucur), Ericaceae (Erica), Gesneriaceae (Gesne), Iridaceae (irida),, Lamiaceae(Lamia), Lauraceae (Laura), Liliaceae (Lilla), Lythraceae (Lythr), Malvaceae (Malva), Oleaceae (Oleac), Ophiopogon (Ophio), Papaveraceae (Papav), Polygonaceae (polyg), Ranunculaceae (Ranun), Rosaceae (Rosac), Solanaceae (Solan), Verbenceae (Verbe), Veronicaceae (Veron), Viburnaceae (Vibur)* (Ghimire & Bhusal, 2022)

In the cluster analysis, some plant families relatively low abundance of bumblebees was Cannaceae, Compositae, Polygonaceae, Viburnaceae, Gesneriaceae, Liliaceae, Apocynaceae, Papaveraceae, Iridaceae, Cactaceae Veronicaceae, Ophiopogon, Ericaceae, Acanthaceae, Cannabaceae, Laureceae. In other Cluster was represented by

12 plant families in which the relatively highest abundance of bumblebees was recorded. It included the Asteraceae family's plant recorded the relatively highest abundance of bumblebees which was followed by Cucurbitaceae, Solanaceae, Balsaminaceae, Rosaceae, Fabaceae, Oleaceae, Ranunculaceae, Malvaceae, Verbeceae, Lythraceae, and Convulvulaceae.

4.3.2 The Relative frequency of *Bombus* species based on flower colour of their host plants

To analyze the color and color preference by bumblebees at foraging time, the particular color from where bumblebees and their frequency were recorded. The relative frequency observed during the field records of *Bombus* species with their host plant's flower having a particular color.

In the cluster diagram (Figure 12), it is apparent that most bumblebee species showed a preference for white, yellow, and purple-colored flowers. On the contrary, orange, red, blue, and pink colors were observed to be less favored among any species of *Bombus* in our study.

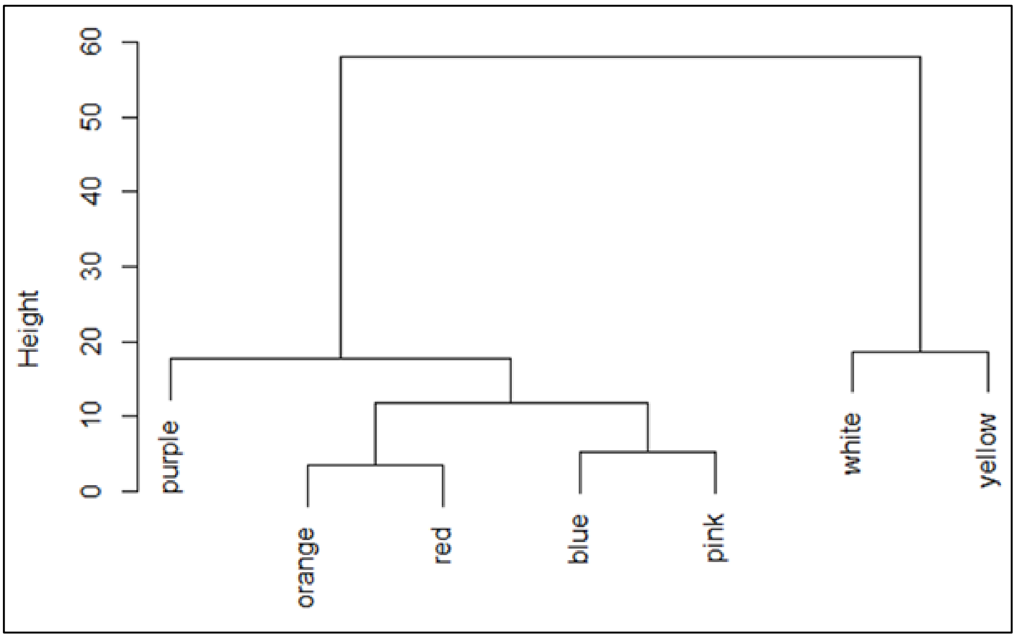


Figure 11: Cluster analysis (method=ward, distance=euclidean) of flower colors according to the relative frequency of bumblebees (Ghimire & Bhusal, 2022)

A corresponding analysis (Figure 13) was done to explore the relationship between *Bombus* species and floral color. The relative frequency of *Bombus* species was cross tabulated (Chi–square = 97.24112, $p<0.05$), within the floral color and the relative frequency of *Bombus* species.

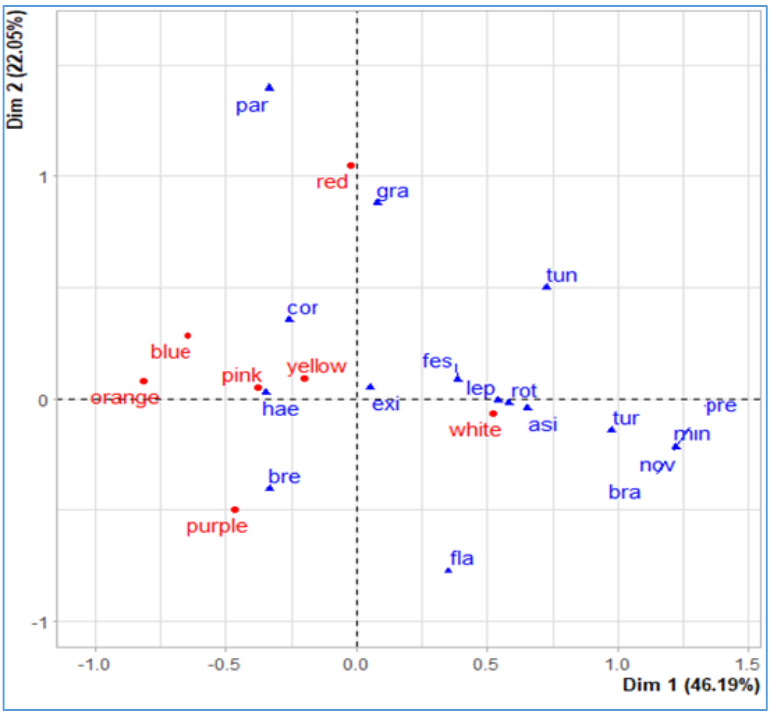


Figure 12: Correspondance analysis based on the relative frequency of *Bombus* species and flower color. The *Bombus* species are: *Bombus asiaticus*(asi), *B. branickii* (bra), *B. breviceps*(bre), *B. cornutus*(cor), *B. eximius* (exi), *B. festivus*(fes), *B. grahami*(gra), *B. haemorrhoidalis*(hae), *B.s Lepidus*(lep), *B. miniatus*(min), *B. novus*(nov), *B. parthenius*(par), *B. pressus*(pre), *B. rotundiceps*(rot), *B. tunicatus*(tun), *B. turneri*(tur). (Ghimire & Bhusal, 2022)

It was discovered that 12 species of bumblebees preferred white-colored flowers for foraging their nectar and pollen. The *Bombus cornutus*, *B. eximius*, and *B. haemorrhoidalis* preferred yellow flowers. The *B. grahami* favored red–colored flowers and *B. breviceps* liked to be forage on the purple flower but blue and orange flower host plants were less preferred by many species of bumblebees.

4.3.3 Relative abundance of Bumblebees in native and non native plants

All identified plants were categorized into native and non-native (INV, CUL, NNU) types.

The sum of the relative frequency observation within non-native floral versus native flora was determined.

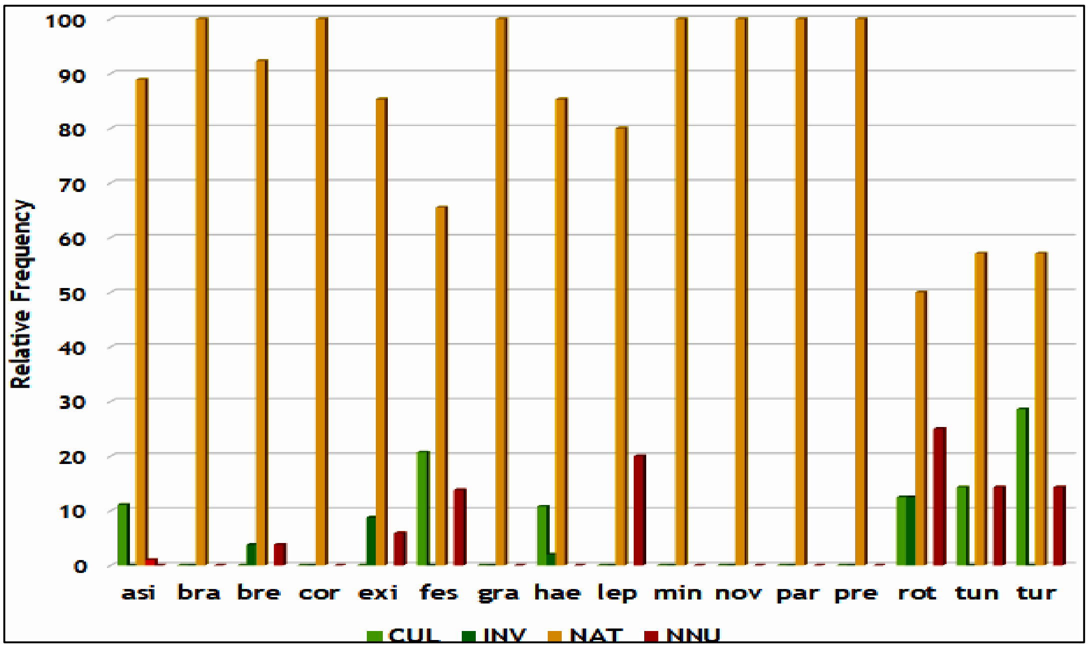


Figure 13: Relative abundance of *Bombus* species in different categories of plants: Native type (NAT), nonnative cultivated type (CUL), nonnative invasive type (INV) and nonnative naturalized (NNU); *Bombus asiaticus(*asi*), B. branickii* (bra*), B. breviceps(*bre*), B. cornutus(*cor*), B. eximius (*exi*), B. festivus*(fes*), B. grahami(*gra), *B. haemorrhoidalis(*hae*), B. Lepidus*(lep*), B. miniatus*(min), *B. novus(*nov*), B. parthenius*(par*), B. pressus(*pre), *B. rotundiceps*(rot*), B. tunicatus(*tun*), B. turneri(*tur*).* (Ghimire & Bhusal, 2022)

The frequency observations of identified bumblebees (X-squared = 40.383, df = 16, p-value = 0.006844) significantly varied with native (NAT) and sum of the non-native (CUL, INV, NNU) flowering plants (Figure 14). In the study most of the species were found to be foraged in native type of flora. Whereas, only one species for example: *B. haemorrhoidalis* was found to be foraged relatively higher in non-native group. Similarly, *B. rotudinceps* was found to be reported almost equally in both (native and non-native) types of flora (Figure 14).

Meanwhile, the frequency observation was conducted (Chi-square value = 50.141, df = 16, p-value = 0.00002177) within the categories of native and non-native plants (Figure 15). Similarly, cluster analysis of the categories of plants based on relative foraging frequency of identified bumblebees was performed, and corresponding analysis (CA) between foraging records of *Bombus* species versus categories of plants showed a well ordination (Figure 16). Most of the *Bombus* species were found to be ordinated towards with native types of plants rather than non-native types.

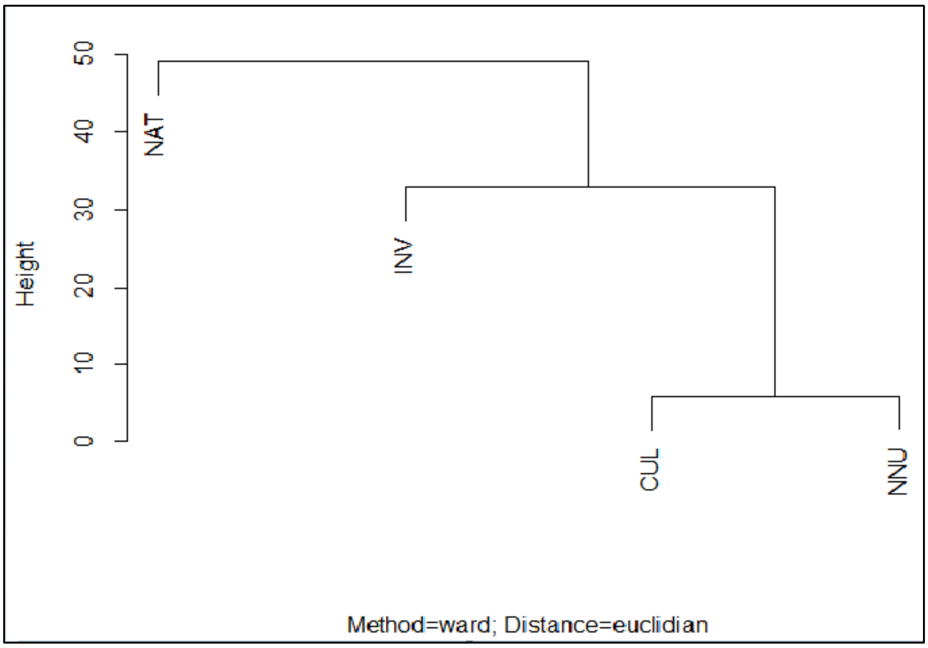


Figure 14: Cluster analysis plant categories of the basis of bumblebee foraging records (Ghimire & Bhusal, 2022)

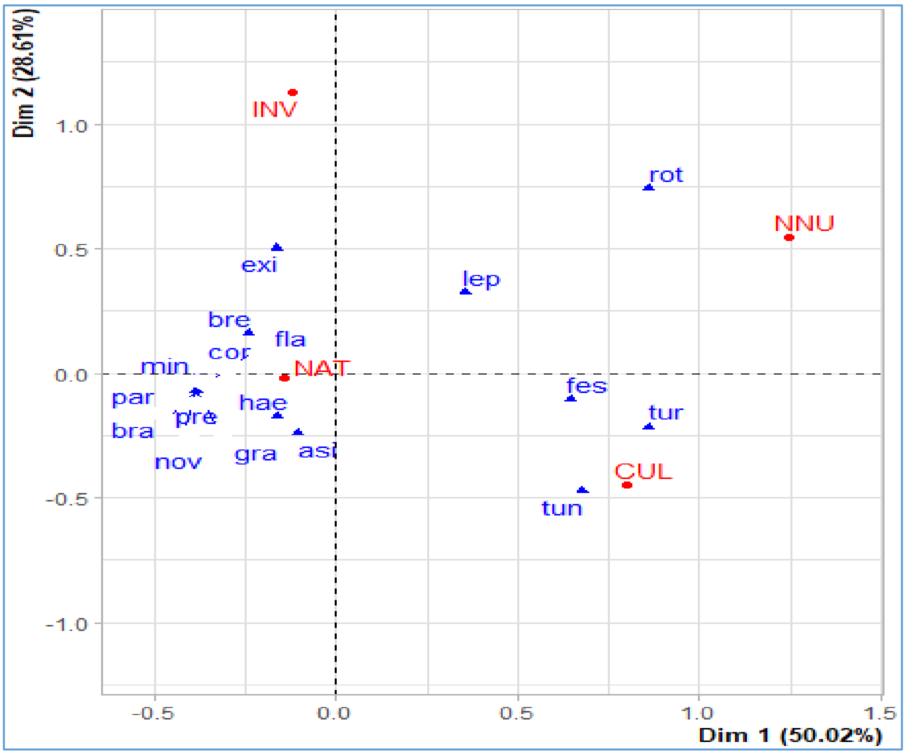


Figure 15: Corresponding analysis (CA) between plant categories and foraging records of *Bombus* species (Ghimire & Bhusal, 2023)

4.3.4 Effect of Category of host plants, habitat types and nature of plants on foraging frequency of *Bombus* spp.

To determine the effects of categories, habitat types (Forest, seminatural, agricultural, home garden), and the nature of flowers, a linear mixed model (lmm) was employed, fitted by maximum likelihood using 'lmerMod.' In this model, the frequency (n= 248) of observed *Bombus* species along the transect was fitted with the nature of the flower as a random factor, while category and habitat types were included as fixed explanatory factors (Table 7). There was significant variation of category of foraged plants whereas no significant variation was observed within habitat types.

Table 7: Summary of Linear mixed model between number of caught, plant categories and habitat fit by maximum likelihood ['lmerMod'] (Ghimire & Bhusal, 2022)

Fixed factors	Estimate	SD ±	t -value
(Intercept)	4.83	0.70	6.82
INV	1.92	1.73	1.12
NAT	2.04	0.99	2.05
NNU	4.28	1.20	3.54
Forest	-2.08	1.05	-1.97
Grassland	-1.85	1.10	-1.68
Home garden	-0.45	1.03	-0.44

4.3.5 Relative foraging frequency of *Bombus* species with flowering pant

The foraging preferences were analyzed based on the foraging records of *Bombus* species in various flowering plants within the collected samples. Based on the relative foraging records of *Bombus* species in different flowering plants during the study, an analysis categorized them as very common (VC), common (C), rare (R), and very rare (VR). It was observed that certain *Bombus* species were highly specific to particular plant species, while others were commonly found foraging on various kinds of flowering host plants. The highest relative frequency of bumblebees has been found foraging the host plants were *Anemone elongate, Jasminum humile, Rosa brunonii, Solanum tuberosum* and *Trifolium repens.* In this study, *B. asiaticus* was generally foraged on wide range of plant species such as *Anemone elongata*, *Solanum tuberosum*, *Rosa brunonii, Jasminum humile, Impatiens stenantha, Glycine max, Cucurbita sp., Cirsium arvense, Cirsium falconeri.* Similarly, *B. branickii* was seen to be foraged only on *Rosa brunonii* in our study areas. *B. brevicep* was like to forage on *Clinopodium spp., Cucurbita pepo, Cirsium falconeri*. Other species such as *B. cornutus* was seen on *Anemone elongata, Chirita bifolia, Jasminum humile* plant species. In this study *Bombus eximus* was observed to be foraged on *Jasminum humile* and *Bidens pilosa* plant species mustly. The *B. festivus* was seen to be foraged specially on *Jasminum* and *Trifolium* species. *B. grahami* was mostly foraged on *Anemone elongata, Chirita bifolia, Colqhounia coccinea, Corydalis casimiriana, Gaultheria fragrantissima* during our field study. *B. haemorrhoidalis* was most common on *Solanum viarum, Cuphea procumbens, Ipomoea purpurea species*. *B. lepidus* was foraged on *Jasminum humile, Anemone elongate* and *Cotoneaster frigidus* plant species. Observations revealed that *B. miniatus* was the only visible forager of *Aconogonum molle*, while *Bombus* novus was primarily observed foraging on Anemone elongata in the field areas. *B. parthenius* was seen on the host plant of *Jasminum humile, Colquhounia coccinea* and *Imatiens scabrida*. Similarly, we found, *Rosa brunonii* was specific foraging host plant for *B. pressus*. *Bombus* rotundiceps was commonly found to be foraged on *Trifolium repens, Jasminum humile, Ophiopogon* sp., *Rosa brunonii, Solanum tuberosum*. *B. tunicatus* was visited on *Anemone elongate* and *Anaphalis contorta* species. likewise, *B. turneri* frequently foraged on *Rosa brunonii, Solanum tuberosum* species.

4.4 Discussion

The results indicate that specific plant families with particular floral colors involve a vital part in the foraging preferences of specific species of *Bombus*. The foraging preferences varied among the *Bombus* species in the Chitwan Annapurna Landscape. The flower traits, including color and available resources, were identified as important parameters influencing the foraging preferences of *Bombus* species in the study areas. Rosaceae, Oleaceae, Ranunculaceae, Asteraceae, and Fabaceae are among the plant families that bumblebees most frequently visit. In contrast, the plant families with the least visitation by bumblebees included Apocynaceae, Verbenaceae, Laureceae, Cannabaceae, Iridaceae, Liliaceae, Cactaceae, Ophiopogon, Papaveraceae, and Viburnaceae. Previous studies have also reported distinct family-level variations in the foraging behavior of many *Bombus* species, with families such as Fabaceae and Verbenaceae being important for bumblebees (Hanley *et al.,* 2008; Westphal *et al.,* 2003). Additionally, flowers from the Rosaceae family were found to be highly visited by bumblebees (Somme, 2014). The specific visiting preferences of bumblebees are frequently associated with flower scent, serving as a crucial long-distance signal for native bees (Heinrich *et al.,* 1977; Dotter *et al.,* 2005). Moreover, floral traits and the availability of resources are likely crucial factors influencing the foraging selection and community dynamics of bumblebees (Potts *et al*., 2010). Bumblebee flower visitation rate has been shown to be influenced by morphological traits such as corolla and proboscis length. Compared to their long-tongued counterparts, bees with shorter probosci have been seen to feed more effectively on flowers with short corollas. The sustained availability of flower resources can influence the frequency of bumblebee visitation to specific plant families, such as Oleaceae, consequently affecting foraging preferences within bee communities (Backman & Tiainea, 2002; Pywell *et al.,* 2005). The structure of flowers, which includes both symmetrical and asymmetrical forms, plays a crucial role in nectar production and can significantly influence the foraging preferences of insect pollinators (Goulson et al., 2005; Reininghaus, 2017). A recent study proposed that variations in temperature across the floral surface, influenced by the floral architecture within the family, may also contribute to bumblebee-flower foraging preferences (Rands & Harrap, 2021).

A present study suggested that differences of temperature across the floral surface, influenced by floral architecture within the family, may also play a role in bumblebee-flower foraging preferences (Rands & Harrap, 2021). While most of the surveyed species did not exhibit selective preferences for specific families, foraging specificity was observed in most *Bombus* species within the study sites, which could be influenced by their phylogenetic relationship with certain flowers (Tai, 2020). Additionally, the phenological periods of plant species and the peaks of bumblebee colonies are major factors to consider (Reininghaus, 2017). Bumblebees face challenges in their foraging selection within the landscape due to season wise and space wise variations in the arrangement of plant resources. Floral resources exhibit diversity over time and space, as different plant species and habitats bloom at distinct periods, resulting in temporal turnover within the floral community, which may also differ across various locations (Simanonok & Burkle, 2014).

Concerning color preference, the study site revealed that purple, yellow, and white coloured flowers were repeatedly foraged floral colors, whereas pink, blue, orange, and red flowers were visited less frequently. It has been proposed that flower color is a pivotal trait influencing bee communities (Raine & Chittka, 2007; Schiestl & Johnson, 2013). This study observed differences in color preference among *Bombus* spp. This aligns with earlier research findings that color preferences in flower-visiting insects, including native bees, play a role in locating flowers for obtaining nutritional rewards (Giurfa, 1996; Raine & Chittka, 2007; Schiestl & Johnson, 2013). Colors appear to be particularly important for bumblebees in recognizing flowers (Menzel & Shmida, 1993; Spaethe *et al.,* 2001; Dyer & Chittka, 2004). The study found that bumblebees exhibited higher foraging intensity towards white and yellow flowers, suggesting a preference for more saturated and high-purity colors (Rohde *et al.,* 2013; Lunau *et al.,* 1996). Bumblebees have trichromatic color vision, including sensitivity to ultraviolet (UV) light (Spaethe *et al.*, 2001; Dyer & Chittka, 2004; Hempel *et al.*, 2014). The preference for white and yellow flowers by bumblebees may be attributed to the fact that these flowers are often melittophilous and have the ability to absorb UV light (Kevan *et al.,* 1996).

The overall foraging response of the bumblebee community showed significant differences between native and non-native flora. Certain *Bombus* species were found to forage exclusively on specific flowers within the study area, indicating their specific flower preferences. In this study, most *Bombus* species were observed foraging on native plants, while a smaller number of species were observed on invasive plants. It suggests that native floras are more beneficial for native pollinators compared to non-native plants. While there are few reports of native bees foraging on non-native plants (Williams *et al.*, 2011; Drossart *et al.,* 2017), it is still unclear if they select native or invesive plants. The study identified that the plant families most frequently visited by bumblebees were Rosaceae, Oleaceae, Ranunculaceae, Fabaceae, and Asteraceae. Conversely, plant families with the lowest visitation by bumblebees comprised Apocynaceae, Papaveraceae, Cannabaceae, Cactaceae, Lauraceae, Liliaceae, Verbenaceae, Ophiopogon, Iridaceae, and Viburnaceae. However, previous studies (Westphal *et al.,* 2003; Hanley *et al.,* 2008) have recommended that plants from certain families, such as Fabaceae and Verbenaceae, are particularly attractive to bumblebee workers. The foraging preference of *Bombus* species between natural and invasive flowers has been subject of ongoing debate, and it is likely influenced by various ecological and evolutionary factors. Floral characteristics, encompassing morphology, color, and scent, are believed to be linked to attract bumblebees to the foraging plants (Cnaani *et al.,* 2006; Fornoff *et al.,* 2017). Furthermore, foraging preference is influenced by flower resources, flower structure, and other adaptive relationships (Dotter *et al.,* 2005; Knudsen *et al.*, 2006).

Furthermore, the factors mentioned above, the distance of foraging, flower availability, flower cover, and types of hosst plants are also important determinants of the plant-bumblebee foraging relationship. The intricate nature of morphological characteristics, including corolla length and proboscis length, in numerous non-native flower species may significantly influence the rate of flower visitation and utilization by native bumblebee species in this region. Furthermore, the spatial foraging behavior of *Bombus* species depends on their behavioral flexibility and nutritional requirements. Therefore, bumblebees may prefer flowers from host plants with symmetrical structures and higher nectar availability for foraging. The occurrence of more feeding generalist *Bombus* species may also be linked to their foraging strength in native flora. Additionally, the regular flowering of perennial wildflowers and seasonal cultivated flowers provides bumblebees with additional floral resources, which may result in foraging biases in local habitats with both natural and invesive floras. However, establishment of a few invesive species of plant in the early stages may affect the foraging patterns of bumblebee species in this area. The change in plant species composition from native to non-native in specific habitats can also impact the foraging patterns of bumblebees in this human-dominated heterogeneous landscape. Conservation efforts often include the use of pollinator-friendly plantings to enhance habitats for bumblebees. It is important to do more research that compares pollinator-friendly native and non-native plants at various sizes through experiments. Therefore, it is recommended to consider the visitation of bumblebees to specific native and non-native flora in coming studies to confirm exact understandings of foraging preferences by different bumblebee species. This information can be applied for conservation purposes in this landscape. In conclusion, the findings indicate a higher foraging frequency of *Bombus* species within native flora based on our sampling efforts. We also observed that certain *Bombus* species exclusively foraged on specific flowers within both the native and non-native categories, highlighting their specific floral preferences. However, future alterations in land use patterns and changing in climate should be taken into account as they may have an influence on the floral composition, especially for native species. Such changes could pose a survival risk for many native *Bombus* species in this region. Further detailed studies investigating the interaction between plants and bumblebees are required to afford a more inclusive knowledge of these dynamics in future.

4.5 Conclusions

In conclusion, the study highlights the significance of plant families and floral color as significant factors influencing the foraging behavior, abundance, and diversity of bumblebees. The choices of food in bumblebees are greatly affected by the shapes of flowers, what various plant families provide, and the specific colors of the flowers. These factors collectively revel a fundamental part for guiding the choices and interactions of bumblebees with various plant species in their environment. Furthermore, habitat structures, including vegetation dynamics, microclimatic variations, topographic factors, and anthropogenic disturbances, contribute to the assembly of bumblebee communities in the CHAL region, particularly along altitudinal gradients. The study also emphasizes the emerging risk factors that bumblebees face in the region, which are expected to worsen with increasing human pressure and environmental changes. The identification of priority plant families that are frequently foraged by *Bombus* species can inform future conservation efforts in the CHAL. It is recommended to develop altitude-specific strategies to address the emerging risk factors in each altitudinal zone, forming an effective conservation action plan for bumblebees in the region.

CHAPTER 5

5. VARIATION IN NETWORK PROPERTIES BETWEEN BUMBLEBEES AND PLANT CATEGORIES (NATIVE VS NON-NATIVE) ALONG ELEVATIONAL GRADIENTS

Abstract

Plant-pollinator networks play a major role in shaping ecological information, with between and multiple level interactions between plants and their pollinators being vital for ecosystem functioning. The altitudinal gradients present in this region provide distinct opportunities for investigating the properties of pollination networks, given the diverse habitats and temperature ranges. This study aims to examine the variations in ecological network properties between bumblebees and different plant categories (native vs. nonnative) along elevational gradients within the Chitwan Annapurna Landscape (CHAL) of Nepal.We performed bipartite network analysis to examine interactions between 16 *Bombus* species and four categories of plants (native, nonnative cultivated, nonnative naturalized, and nonnative invasive) at high and low altitudinal ranges within three river basins. Our results reveal distinct network structures for high and low altitude regions. Overall, our study provides valuable insights into the relationships between bumblebees with native plant versus nonnative flora. In this study, most of the bumblebee species attracted towards the native flora rather than nonnative flower categories. The plant-bumblebee interactions network analysis attributed the plant specific foraging behavior of certain bumblebee species (*B. asiaticus, B. novus*) where as some species are more generalist (*B. festivus, B. tunicatus, B. haemorrhoidalis*). This study highlights the flower specificity for specific bumblebee and rarity of specific flower for pollination by specific *Bombus* species.

5.1 Introduction

Research on plant-pollinator interactions at the level of whole ecological ecosystems (Moldenke, 1975) has been conducted since long time. The study of mutualistic networks has come to be a necessary means for addressing ecological inquiries, both in theory and in practical applications. It plays a crucial role in comprehending patterns and processes, including how pollinators choose specific flowers in their foraging behavior. The examination of pollinator interactions at the community level has produced valuable insights into numerous ecological relationships (Bascompte & Jordano, 2007). While most pollinators and plants exhibit generalist tendencies, the investigation of pollination networks serves as a means to assess plant/pollinator interactions within the community (Waser *et al.*, 1996). Species involved in interactions with one another, creating complex networks that play a decisive role in the ecosystems (Tylianakis *et al.*, 2008). the loss of specific species interactions can have serious consequences for ecosystem functioning, even if the interacting partners are still present (Aizen *et al.*, 2012). Pollinator communities have received more attention during the past ten years (Waser & Ollerton, 2006), which highlights their importance in ecological research and conservation initiatives. This awareness that these communities might be seen as useful complex networks has been a major factor in attracting this attention (Proulx *et al.*, 2005). The interactions between individual plants and pollinator species in a community environment may be fully understood through the use of plant-pollinator networks. Moreover, recent studies have shown how human-induced changes can impact the structure of interaction networks, even when species richness remains unaffected (Albrecht *et al.*, 2007; Lopezaraiza-Mikel *et al.*, 2007; Memmott *et al.*, 2007; Aizen *et al.*, 2008). These findings reinforce previous recommendations for conserving network structure as part of conservation efforts (McCann, 2007).

Altitudinal gradients offer distinct chances to explore characterstics of pollination network at different elevations, give the wide range of habitats and temperature they involve. The valuable approach to calculating the potential effects of local climate change on pollinator distribution by comparing the networks of insects and plants acting as pollinators along these gradients. The decline in bee diversity is usually attributed to factors such as reduced floral diversity, habitat loss, pesticide usage, and pathogens. (Cameron *et al.*, 2016; Goulson *et al.*, 2015). However, it's important to note that certain species of bumblebees have experienced more significant losses than others (Cameron & Sadd, 2020). However, it is crucial to highlight that specific bumblebee species have encountered more pronounced declines than others (Cameron & Sadd, 2020).

Plants and pollinators form a specialized mutualistic network that is inherently bipartite. Within this network, plants and animals function as nodes, and the connections between them represent the mutualistic nature of the plant-pollinator relationship. Conservationists ignored the structure of networks rather they have instead focused on the threat status of individual species. Conversely, ineffective management strategies may arise when they neglect the ecological network within which a threatened species is trapped.

Recent research focused on communities of plants and their pollinators has employed the characterization of network properties. They have employed null models that use species abundances to characterize aspects such as species link distributions (how species are connected in the network) and the distribution of specialists and generalists (species with specific versus broad ecological interactions). Invasive plant species usually significantly change the richness and abundance of native plants, that support a different flora condition thereby reversing the successional cycle (Meiners *et al.,* 2002; Fenesi *et al.,* 2015). According to (Traveset & Richardson 2014, Bezemer *et al.,* 2014; van Hengstum *et al.,* 2014), Changes in floral communities can impact associated animal groups, including pollinator insects and their pollination services (Traveset & Richardson, 2014; Bezemer *et al*., 2014; van Hengstum *et al.*, 2014). Plant invasions can impact pollinators by affecting foraging opportunities, as invasive flowering plant species may provide more food options (Lopezaraiza-Mikel *et al.,* 2007; Bartomeus *et al.,* 2008; Russo *et al.,* 2016). Such changes can result in decreased insect movements or widespread pollen transfer, consequently reducing the diversity and reproductive success of native plants (Chittka & Schürkens, 2001; Carvalheiro *et al.,* 2014; Charlebois & Sargent, 2017). Meanwhile, invasive plant species may experience increased reproductive success due to enhanced pollination (Stout & Tiedeken, 2017). However, these invasions often reduce the amount of pollen and its sap available to native flora (Hanula & Horn, 2016; Fenesi *et al.*, 2015). Species interactions form complex networks that are crucial for the equilibrium and working of ecosystems (Tylianakis *et al*., 2008). Removal of exotic plants can alter the number of pollinators consuming both native and alien plants, potentially leading to reduced pollination of rare native species (Carvalheiro *et al.,* 2008).

In mutualistic networks, specialist species often interact with generalist partners, leading to nested structures (Bascompte & Jordano, 2007). These networks demonstrate robust stability, where disruptions that eliminate specialists may not necessarily outcome widespread losses within a community (Memmott *et al.*, 2007; Kaiser-Bunbury *et al.*, 2010). Through a network analysis, critical species may be identified, whose loss may trigger cascade extinctions in the community, revealing important information about the interactions' structure, like connectivity and nestedness (Memmott *et al.,* 2007; Kaiser-Bunbury *et al.,* 2011).

Conducting a network analysis on native and non-native flora and bumblebees along elevation gradients in three river basins (Kaligandaki, Marsyngdi, and Budigandaki) in mid-Himalya, Nepal can help evaluate variations in network structure.

The mutualistic associations of plants and pollinators lay the foundation for pollination networks, establishing crucial connections within them. Using a network approach, the inter-connections between plants and their pollinating animals can be visualized, capturing all interactions within the group. However, our understanding of plant-bumblebee visitation network properties at high altitudes remains limited, including whether these properties are dependent on altitude and how they change along the gradient in the CHAL regions of Nepal. Our research stands out for its unique emphasis on exploring patterns of bumblebee diversity and their contributions to plant-pollinator networks whole elevational gradients in three river basins in CHAL regions of the central Himalaya, Nepal. This comprehensive approach allows to find out in depth understanding of interactions between bumblebees and plants across various elevations, providing valuable insights into the ecological dynamics of this region.

5.2 Materials and Methods

5.2.1 Data collection

This study was conducted across elevation gradients of three river basins within the Chitwan Annapurna landscape (see Figure 1), which were categorized into upper and lower altitudinal gradients. The upper point was established at the 2800 m where most flowering plants were clustered. Similarly, lowere point was selected on the basis of highest clustered of flowering plants. The lowere point was established at 1700 m asl.

Two plots (40m x 40m) were established at each point along the same walking transect. The bumblebees were observed and captured all possible foraging workers within 30 minutes by three people. During the collection, the number of bumblebee visitation in each flower was noted. The plot observation was carried out 12 times during ascending and descending the trastsects in sampling months of the collection years. The census of visitation of each species of bumblebees in flowers was carried out for 30 minutes (between 9 am to 5 pm). During the collection, if bumblebee touched the reproductive parts of flower, then that visit was considered as valid for collection.

5.2.2 Species collection and identification

An insect net was used to collect bumblebees and ethyl acetabe was used to kill them immediately. Later, they were kept in vials having few layers of tissue, and added few drops of ethyl alcohol to prevent mold-growth during transportation. For identification of collected specimens, a stereoscopic microscope was used along with published identification keys (Williams, 1991; Williams *et al.,* 2009; Williams et al., 2009; An *et al.,* 2014; Saini *et al.,* 2015), and the Himalayan Bumblebees identification guide (William, 2022). Finally, insect pins were used to label the specimens and stored as voucher specimens at Central Department of Zoology, Tribhuvan University, Kathmandu.

Similarly, the unknown flowering plants were also collected from the sampling plots and prepared herbarium which were identified from National Herbarium, Godawari, Lalitpur.

5.2.3 Network analysis

The visitation interaction between bumblebees (worker) and flowering plants in these plots was analysed using bipartite network (Dormann *et al.,* 2020) in R software. Higher level was represented by 16 *Bombus* spp. and lower level was represented by four categories of plants in this network.

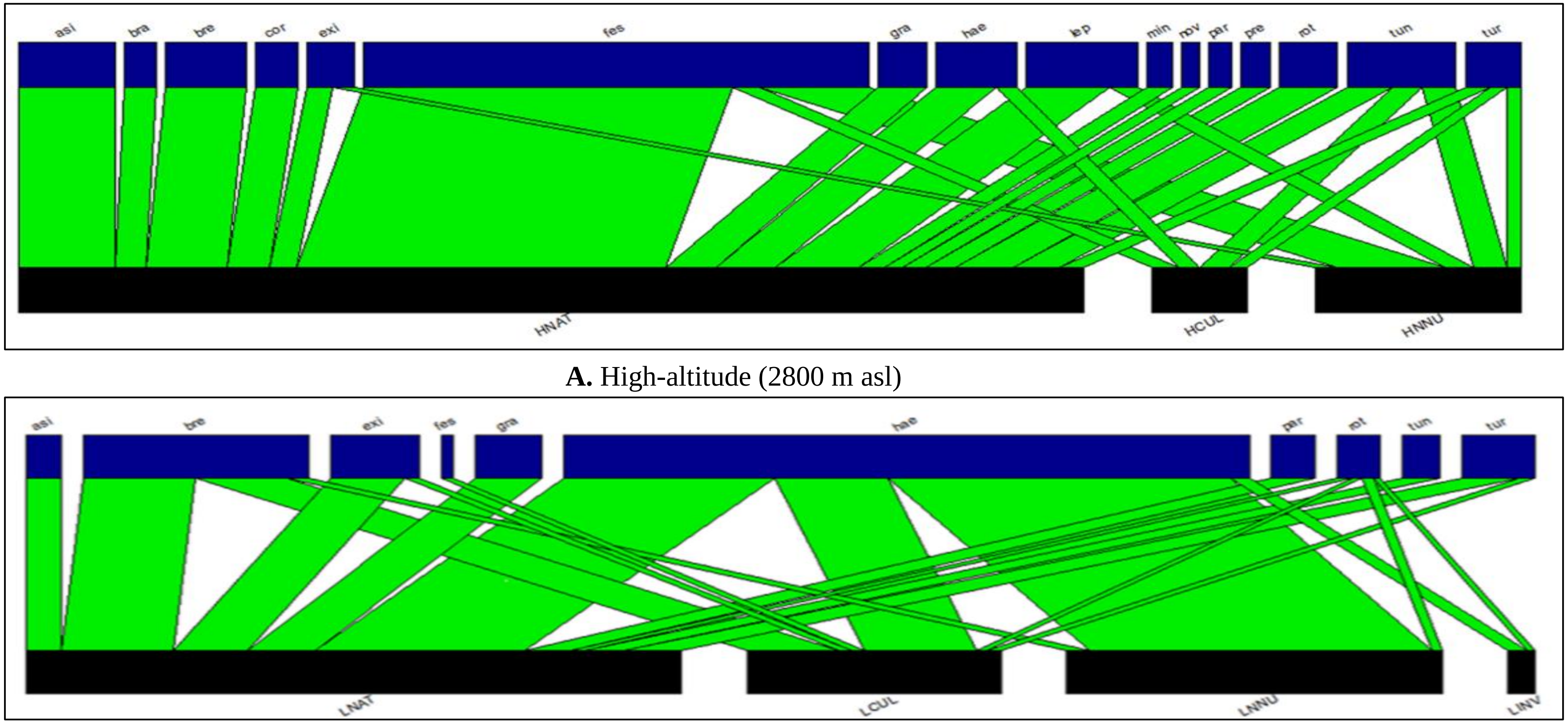
The following network properties were calculated to define the interaction of bumblebees and foraging plants.

Network Properties	
Connectance	Comparison of proportion of possible link and actual link
Linkage density	Number of interaction and their strength
Generality	Average diversity of interaction in the network for each species
H_2 specialization	The deviation of observed and expected interactions based on marginal total per species
Weighted NODF	Measurement of the interaction of specialists and generalists
Vulnerability	Dependence of plants on insects pollinators in network
Web asymmetry	When specialist plant is pollinated by generalist and generalist plant pollinated by both generalist and specialist pollinators then interaction become asymmetric
Interaction Evenness	Closeness of species and interaction to each other
Robustness	Tolerance of haphazard removal of a species in network
Partner Diversity	Number of different interaction partners
Nestedness	Few linked species of interaction are the subsets of large interaction
Link per Species	Average number of link formed by each species with their partner species in network

5.3 Results

5.3.1 Bipartite network analysis of cetagories of plants and *Bombus* spp. in high- and low-altitudes

During study, 2401 encounters of bumblebees were recorded with different categories of plants, and the resulting network comprised of 45 interactions between 4 categories of plants and 16 *Bombus* spp.



A. High-altitude (2800 m asl)

B. Low-altitude (1700 m asl)

Figure 16: Bipartite network of *Bombus* spp. and categories of plants in high- and low altitudinal region (Pollinators are represented in the upper level, and categories of plants in the lower level. Box size is proportional to the total number of visits and thickness of link is proportional to the frequency of the particular link) **A**) High Network Category: *Bombus asiaticus* (asi), *B. branickii* (bra), *B. breviceps* (bre), *B. cornutus* (cor), *B. eximius* (exi), *B. festivus* (fes), *B. grahami* (gra), *B. haemorrhoidalis* (hae), *B. lepidus* (lep), *B. miniatus* (min), *B. novus* (nov), *B. parthenius* (par), *B. pressus* (pre), *B. rotundiceps* (rot), *B. tunicatus* (tun), *B. turneri* (tur); high altitude native (HNAT), high altitude nonnative cultivated (HCUL), high altitude nonnative naturalized (HNNU) and high altitude nonnative invasive (HINV) **B)** Low Network Category (species and plant categories): *Bombus asiaticus* (asi), *B. breviceps* (bre), *B. eximius* (exi), *B. festivus* (fes), *B. grahami* (gra), *B. haemorrhoidalis* (hae), *B. parthenius* (par), *B. rotundiceps* (rot), *B. tunicatus* (tun), *B. turneri* (tur); low altitude native Category (LNAT), low altitude nonnative cultivated (LCUL), low altitude nonnative naturalized (LNNU) and low altitude nonnative invasive type (LINV).

The high altitude-based and low-altitude based networks are shown in Figure 17. In both cases, majority of interactions were found as weak while only few interactions were found as very strong. Similarly, the highest interaction frequency was observed in natural category (10 species) and lowest interaction frequency was obaserved in invasive category (2 species) in low-altitude based network. But in high-altitude, invasive category was absent and the highest interaction was observed in native cagerogy (16 species) and the lowest interaction was observed in cultivated category (4 species).

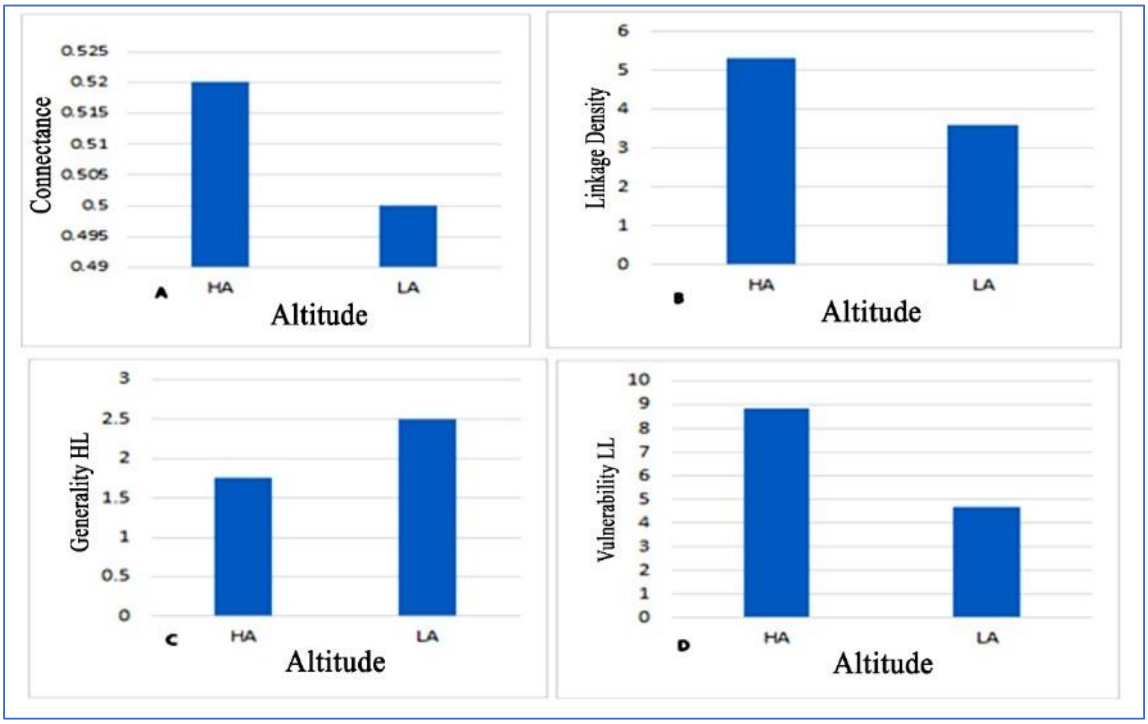
B. festivus, B. tunicatus, and *B. turneri*, were linked with all three categories of plants: natural, non-native cultivated, and non-native naturalized, but species like *B. asiaticus, B. branickii, B. breviceps, B. cornutus, B. grahami, B. miniatus, B. novus, B. parthenius, B. pressus,* and *B. rotundiceps* linked with only one category of plant in the high-elevational region.

In the lower elevation, *B. haemorrhoidalis* and *B. rotundiceps* were linked to all four categories of plants (Fig. 17). This suggests that these species have interactions with a wider range of plants. While species like *B. asiaticus*, *B. grahami,* and *B. tunicatus* were linked with only one category of plant.

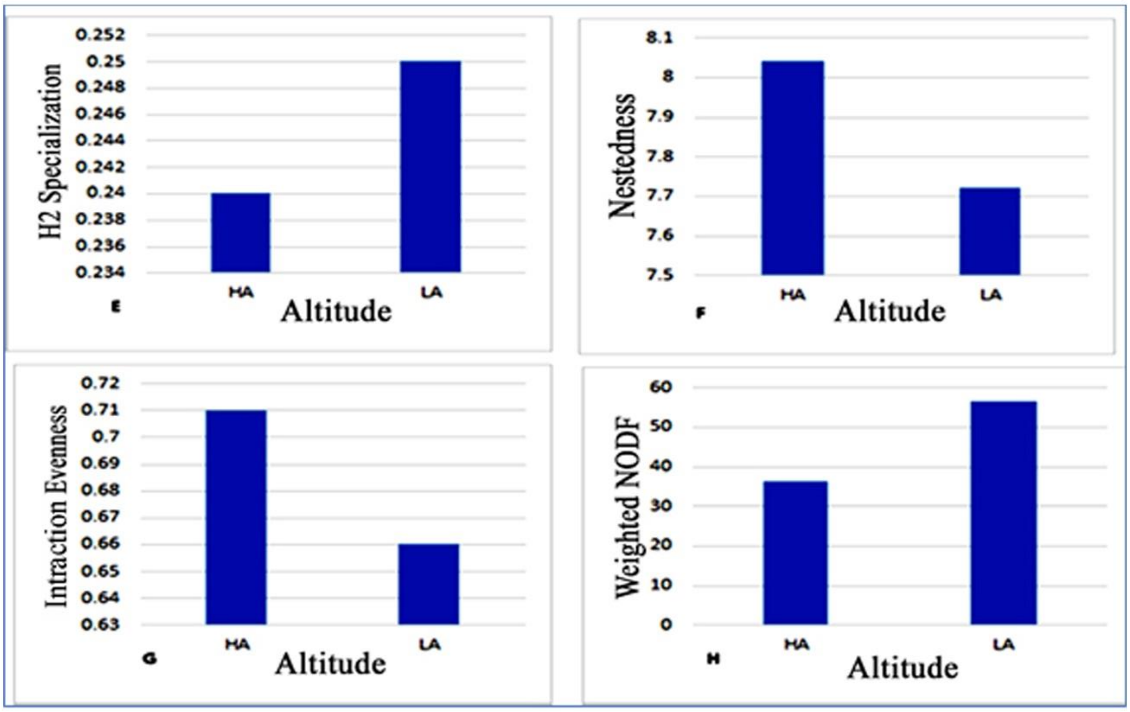
5.3.2 Network properties analysis category of plants and Bumblebee species in high and low altitude level

Different network properties were studied for determining the actual situation of bumblebees in the three river basins of CHAL region of Central Himalaya Nepal. The values of network properties were shown in Figure 18.

The highest values of connectance, linkage density, vulnerability, nestedness, interaction evenness, web asymmetry, robustness and partner diversity were found in high altitudinal range. But in low altitudinal range, the highest values of generality HL, H_2 specialization, weighted NODF, liner per species, robustness, and partner diversity HL were found.



Connectance
0.525
0.52
0.515
0.51
0.505
0.5
0.495
0.49
A
HA
LA
Altitude
Linkage Density
6
5
4
3
2
1
0
B
HA
LA
Altitude
Generality HL
3
2.5
2
1.5
1
0.5
0
C
HA
LA
Altitude
Vulnerability LL
10
9
8
7
6
5
4
3
2
1
0
D
HA
LA
Altitude



H2 Specialization
0.252
0.25
0.248
0.246
0.244
0.242
0.24
0.238
0.236
0.234
E
HA
LA
Altitude
Nestedness
8.1
8
7.9
7.8
7.7
7.6
7.5
F
HA
LA
Altitude
Intraction Evenness
0.72
0.71
0.7
0.69
0.68
0.67
0.66
0.65
0.64
0.63
G
HA
LA
Altitude
Weighted NODF
60
50
40
30
20
10
0
H
HA
LA
Altitude

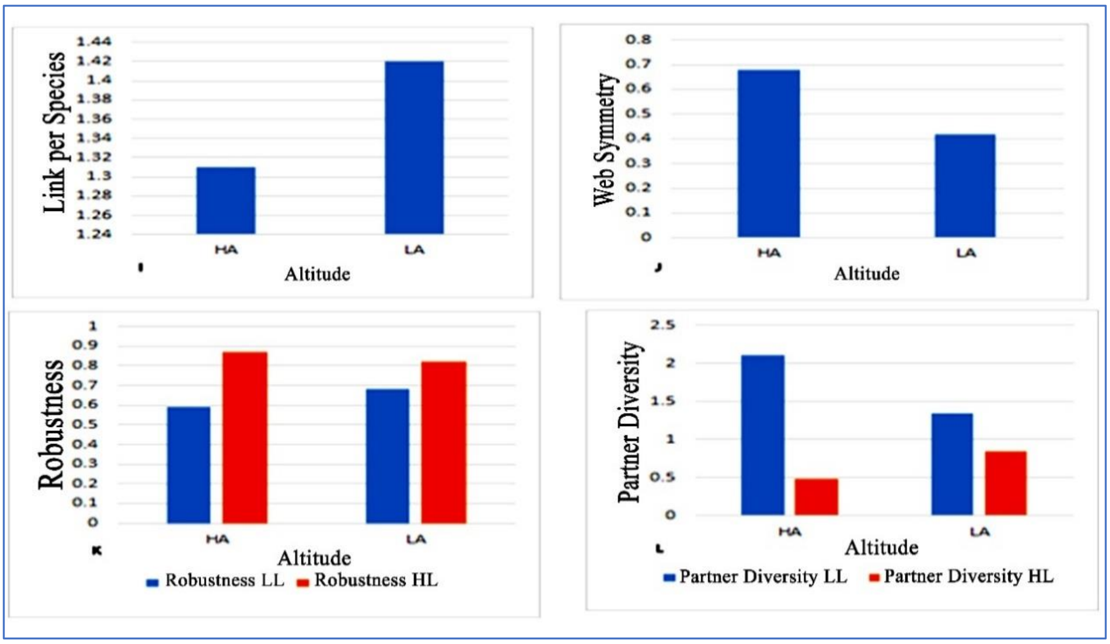


Figure 17: Network properties: HA= High Altitude, LA= Low Altitude, A) Connectance, B) Linkage density, C) Generality, D) Vulnerability LL, E) H_2 Specialization, F) Nestedness, G) Interaction evenness, H) Weighted NODF, I) Link per species, J) Web asymmetry, K) Robustness HL/LL and L) Partner diversity HL/ LL

5.4 Discussion

In the high altitudinal range, the Connectance and Linkage density were high, indicating a more interconnected network in this altitudinal range. When a plant-pollinator network is highly interconnected in a certain place, it generally indicates a more diverse and stable ecosystem. A high level of inter-connectivity means many species of plants and pollinators are interacting forming a complex web of relationships. In such a network, the presence of multiple plant species provides a variety of resources for pollinators (Viekere *et al.*, 2021). When a species vulnerability LL value is high, it means that it depends heavily on a certain partner species for interactions in the high altitudinal range. It's important to note that species vulnerability can vary across different kinds of ecological networks including food webs, mutualistic networks that can be affected by various variables such as species traits, habitat availability, and interactions with other species. Understanding and addressing species vulnerability in ecological networks is crucial for conservation and ecosystem sustainability (Couet, *et al.*, 2022). High nestedness is observed, signifying a non-random arrangement where species with fewer interactions are nested within the interactions of generalists (Cantor *et al.,* 2017). (Saavedra *et al.,* 2011), (Bascompte *et al.,* 2007) and (Almeida-Neto *et al.,* 2008) provide insights into the concept of nestedness, its measurement, and its implications for ecological systems. Nestedness is just one of the many patterns that can be observed in ecological networks, and its study helps to understand the structure and dynamics of complex ecological interactions. Interaction evenness is high, indicating a relatively balanced distribution of interactions among species (Vázquez *et al.,* 2007). A highly even network with multiple interactions among species can lead to redundancy in ecological functions. If multiple species are involved in similar interactions, the network can still function properly even if some species are lost or their interactions are disrupted. (Almeida-Neto *et al.,* 2008). Web asymmetry is high, suggesting that interactions are asymmetrical, with certain species playing more important roles as pollinators or resources compared to others (Vázquez *et al.,* 2007). High web asymmetry can affect the overall stability of the mutualistic network. Asymmetry might lead to decreased resilience to disturbances and environmental changes, making the network more susceptible to collapse or regime shifts (Valdovinos *et al.,* 2010). Robustness HL is high, representing that the network is relatively robust to the loss of highly connected *Bombus* species (Liu, 2021). Robustness in mutualistic networks indicates the capability of the network to balance its stability and functionality in the face of perturbations, such as species extinctions or environmental changes. A robust mutualistic network can withstand disturbances and continue to support mutualistic interactions between species, contributing to ecosystem health and stability (Saavedra, *et al.,* 2011). Partner diversity LL is high, suggesting that certain categories of plants in the high altitudinal range cooperate with diverse partners (Wani *et al.,* 2023).

At lower altitudes, there is a high level of Generality (HL) and H2 (Specialization), suggesting that *Bombus* species in these areas typically engage with a restricted group of partner species, demonstrating increased specialization. Conversely, the network at these altitudes features generalist species that engage with numerous partners and exhibit low level of specialization (Almeida-Neto *et al.*, 2008). Weighted NODF is high, suggesting a higher nestedness in the network as in high altitudinal range (Cantor *et al*., 2017). Weighted NODF is a metric applied to measure nestedness in ecological networks, including mutualistic networks. More Weighted NODF shows a strong nested pattern in network, where specialists interact with appropriate partners of generalist species. This nestedness tendency can have significant ecological implications for the stability, persistence, and functioning of the mutualistic network (Pires *et al*., 2018). Link per species is high, indicating that each species in the low altitudinal range has a relatively higher number of interactions (Galiana *et al.*, 2022). This high link density can have several ecological implications for the mutualistic partners and the overall network structure and functioning. Robustness LL is high, suggesting that the network is relatively robust indicating the possible loss of low-connected species of plants in low altitudinal range (Liu *et al.*, 2021). Partner diversity HL is high, suggesting that certain *Bombus* species in the low altitudinal range interact with a diverse set of partners (Wani *et al.,* 2023).

Lower values of all connectance, linkage density, generality, and vulnerability, along with higher values of H2' and modularity, suggest higher specialization within ecological networks (Morris *et al.,* 2014). These metrics help us understand organization and alteration of species interactions, highlighting the extent to which species are specialized or generalist in their ecological roles. In ecological networks, high modularity and H2' values indicate a high amount of network-level specialization. This means that species within the network tend to interact more exclusively within specific groups or modules, indicating a tightly structured and specialized system (Classen *et al.,* 2020). Regarding the threats to pollinators and pollination, several factors can pose global risks to these important ecological processes. Pollinators and their pollination processes are widely recognized to be at risk from the issues outlined, which include changes in land use, intensification of conventional agriculture, pollution, diseases, and invasive alien species. (Vanbergen & the Insect Pollinators Initiative 2013; IPBES 2016). Mutualistic networks having more connectance, modularity, or nestedness are more likely being structurally or dynamically robust (Bascompte *et al.,* 2003; Olesen *et al.,* 2007; Thebault & Fontaine 2010; Saavedra & Bascompte 2014), mutualistic networks with high levels of connectance, modularity, or nestedness are more likely to be dynamically or structurally stable. In their study, (Thebault & Fontaine 2010) found evidence supporting the negative correlation between network size and connectance, which was previously established. The networks observed in the sdisturbed habitat exhibited greater size and species richness, but at the same time, they displayed lower connectance, indicating fewer realized interspecific contacts. In addition, once network size was standardized. (Thebault &

Fontaine, 2010) found that while disrupted networks were more specialized, they were also less nested, vulnerable, and had fewer insect visits per plant species. High values of nestedness were found in high altitudinal regions, while high levels of connectance were found at low altitudinal levels. Levels of specializations and network robustness are more sensitive, while nestedness is resilient to sampling intensity (Nielsen *et al.,* 2007) (Rivera-Hutinel *et al.,* 2012). Research on Mt. Olympus in Greece (Minachilis *et al.*, 2020) discovered that high elevation communities' bumblebee-plant networks had more nestedness. The existence or lack of particular categories and variability in the number of bumblebees interacting with each category may represent differences in the ecological circumstances and plant communities throughout altitudinal ranges. Jordano (2016) anticipates an elevation gradient to lead to an increase in nestedness. Furthermore, a high species richness typically correlates with lower connectance, whereas connectance is frequently linked to ecosystem stability. Consequently, according to Tylianakis *et al.*, (2010), as specialization decreases along the elevation gradient, one can anticipate an increase in connectance (and consequently, stability) and a rise in generalization. Abiotic factor like temperature, and biotic factor like presence of foods, frequently affect in limiting species richness and influencing interaction networks, as discussed by Jordano, (2016).

Regarding interaction frequencies within each category, the Natural category exhibited the highest frequency of interactions in both high and low altitudinal ranges. This suggests that bumblebees showed more frequent interactions with plants categorized as Natural as opposed to those in other categories. These results underscore the variations in interaction patterns among different plant categories and across altitudinal ranges (Traveset *et al*., 2015). The Natural category stands out as particularly significant in terms of both interaction frequency and connectivity with bumblebee species, evident in both high and low altitudinal ranges. It's worth emphasizing that the predominant method for examining changes in plant-pollinator interactions in invaded environments involves analyzing the structure of flower-visitor interaction networks. Nevertheless, it is crucial to recognize that, despite the integration of non native plants into interactive networks (Lopezaraiza-Mikel *et al*., 2007; Traveset *et al*., 2015), network structure metrics may remain unaffected (Vila *et al*., 2009). The occurrence of invasive species may profoundly influence on the structure of interaction networks, leading to changes in the balance of interactions and the density of connections (Kaiser-Bunbury *et al.*, 2011). Additionally, invaders might contribute to the formation of larger, more interconnected modules within the network. These alterations can trigger ripple effects in the ecosystem, leading to shifts in community composition and changes in ecosystem function (Albrecht *et al*., 2014). The expected influence on network structure is projected to be impacted by relative number of invasive species within the network (Stout *et al*., 2014). Taking into account the various influences on network structure, the doubt of functional outcomes of structural characteristics, and the ability to reshape networks by the invaders (Campos-Navarrete *et al*., 2013). Native animals may potentially utilize invasive plants as an alternative food source, provided they can effectively access these plants. Accessibility found on the compatibility between the visitors of the invasive species and their flowers, as highlighted by Jesse *et al.* (2006). Nevertheless, the viability of this food source relies on factors such as its nutritional value and its implications for the health and fitness of the native animals consuming it.

5.5 Conclusion

The bipartite network analysis carried out in high-and low-altitudinal ranges exhibited variation of interaction between *Bombus* spp. with different categories of plants. In case of high-altitude, all 16 species showed interaction with three categories of plants including native, cultivated and naturalized. But in low-altitude, only 10 species involved in interaction, but with all categories of plants including invasive plants. In both cases, native plants showed interaction with all *Bombus* spp. involved respectively. Therefore, native plants are very important for conservation of bumblebees. While complex and resilient ecosystem was observed in the high-altitude environment, even more complex and highly structured network with a mix of generalist and specialist species was observed in low-altitude environment.

CHAPTER 6

6. SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

Bumblebee (*Bombus* spp.) are important insects responsible for the pollination of high altitude plants. The Chitwan Annapurna region is under rapid habitat loss due to current climate change and huge anthropogenic pressure. The present study focused on taxonomic diversity, foraging association of *Bombus* species and their mutualistic interaction along the altitudinal gradient of Chitwan Annapurna Landscape. The field survey was carried out in the most flowering seasons of (April to November) of 2018 and 2019. To gather bumblebees, we conducted three walkable transects (ranging from 600 to 3500 m asl) along the Kaligandaki river (Mustang site), Marshyandi river (Manang site), and Budhigandaki river basins (Gorkha site). The transects passed across different habitats (agricultural areas, forests, grasslands, and home gardens). The *Bombus* specimens (foraging workers) were collected by sweeping net and some individuals were also collected by hand picking method that were observed inside the flower. Three researchers were walked along the transect, following 20 meters right and left of the transect. The collected specimens were carefully transferred to killing jar having cotton bulb with ethyl acetate. The entire walking process (three times towards up and three times down) was done. The total field collection was performed for 108 days (April-May, July-August, October –November). Whenever a bumblebee was encountered, we stopped at that specific points and spent 30 minutes for extensive searching around the point along the transect. All the possible foraging worker *Bombus* species were trapped before continuing the walking along the transects. Some specimens were also captured opportunistically along the transects between two specific points. A total of 16 *Bombus* spp. were collected during the whole study period, they were: *B. asiaticus, B. branickii, B. breviceps, B. cornutus, B. eximius, B. festivus, B. grahami, B. haemorrhoidalis, B. Lepidus, B. miniatus, B. novus, B. parthenius, B. pressus, B. rotundiceps, B. tunicatus, Bombus turneri*. The relative abundance of *Bombus* species vary with altitude, habitat, transect sites and foraging plant types. The relative abundance of *B. haemorrhoidalis* was highest (20.29%), followed by *B. festivus* (19.57%) and *B. eximius* (18.84%). However, *B. branickii*, *B. miniatus*, *B. novus*, and *B. pressus* exhibited the lowest abundance, each accounting for 1% relative abundance. The impact of elevation on bumblebee richness was determined to be significant, with the peak richness observed at mid-elevation (2000-3000 m asl.). This result revealed that the elevation as a significant factor for species richness showing the highest species at the mid-elevation range (2000-3000 m asl.) probably due to increased availability of host plants. The Gorkha site has highest species richness than the mustang site. This is probably associated with the microclimatic factors anf habitat heterogeneity and more vegetation dynamics towards the eastern sites of Himalaya and more specifically in this study area. In the habitat-based analysis, the forest habitat of the Gorkha site recorded the highest species richness (Shannon index H′= 2.18), followed by the grassland habitat of the Mustang site (Shannon index H′= 2.10). Overall, the species diversity was relatively greater in the habitats of the Gorkha transect site compared to those of the Manang and Mustang sites. The altitudnnal gradients of the study areas have lead to diverse microclimatic conditions and vegetation dynamics in different habitats having a significant effect on bumblebee abundance, number of species, and diversity. The mid-elevation ranges in CHAL exhibited the highest species richness, likely due to the intensified availability of pollinator-dependent flowering plants within this particular range. The relative frequency of *Bombus* spp. exhibited notable variation concerning the categories of flowering plants (such as native, non-native cultivated, non-native naturalized, and invasive) and the flower color along the sampling routes. Linear mixed-effects models, cluster analysis, and corresponding analyses (CA) were employed to investigate the foraging relationships between *Bombus* species and plant families, flower color, and plant categories. This study investigated that the plant families and colour of flower were important biotic factors for the forging choice of particular *Bombus* species in this landscape. Furthermore, a substantial change in the foraging magnitude of identified *Bombus* species was observed between native and exotic plant categories. For plant-bumblebee network anlysis, six plots (plot size: 40m x 40m) were established along the three transects (2 plots in each transect). The upper point was established at the 2800 m where most flowering plants were highly clustered during the survey. Similarly, lowere point was selected on the basis of highest clustered of flowering plants. The lowere point was established at 1700 m asl. The bumblebees were observed and captured all possible foraging workers within 60 minutes by three people. During the collection, the number of bumblebee visitation in each flower was noted. The plot observation was carried out 12 times during ascending and descending the trastsects in sampling months of the collection years. If bumblebees were in contact with the reproductive structure of a particular flower, it was considered as valid individual for the data collection. The bipartite network analysis carried out in high-and low-altitudinal ranges exhibited variation of interaction between *Bombus* spp. with different categories of plants. In case of high-altitude, all 16 species showed interaction with three categories of plants including native, cultivated and naturalized. But in low-altitude, only 10 species involved in interaction, but with all categories of plants including invasive plants. In both cases, native plants showed interaction with all *Bombus* spp. involved respectively. Therefore, native plants are very important for conservation of bumblebees. While complex and resilient ecosystem was observed in the high-altitude environment, even more complex and highly structured network with a mix of generalist and specialist species was observed in low-altitude environment. The plant-bumblebee interactions network analysis attributed the plant specific foraging behavior of certain bumblebee species (eg: *B. asiaticus, B. novus*) where as some species are more generalist (e.g: *B. festivus*, *B. tunicatus*, *B. haemorrhoidalis*). In overall, this study provided valuable insights about the complex relationships between *Bombu*s spp. and plant categories along elevational gradients in CHAL. The upcoming shift in elevation of suitable flowering plants is expected to pose a significant limitation on species diversity and community composition in this region. Within both altitudinal ranges, the highest foraging interaction was observed with native plants. As this study indicated native plants as the most preferred plants by *Bombus* spp.

6.2 Conclusion

The research identified a mid-domain effect, wherein geographic factors of the landscape, along with favorable environmental conditions such as high habitat heterogeneity, likely contributed to the creation of a diverse array of food resources for bee communities.This study identified a mid-domain effect, where the geographic factors of the landscape, combined with favorable environmental conditions such as high habitat heterogeneity, likely played a role in creating a diverse range of food resources for bee communities. These factors likely contributed to the observed pattern of species distribution and abundance. The higher diversity profiles observed in natural habitats (forests and grasslands), in comparison to agricultural and human settlement habitas, may be attributed to anthropogenic disturbances such as intensive application of pesticides and cattle grazing. However, the complex series of environmental, social and ecological factors such as rapid changes in habitat utilization, agricultural intensification, microclimatic variation, topographic factors and shifts in cropping system possibly affecting in community composition and abundance of bumblebees in the CHAL. In other hand, plant families and the flower color were significant explanatory factors for foraging preference of bumblebees in this study. In both altitude ranges, native plants exhibited the highest interaction frequency, suggesting a robust foraging association of bumblebees with native rather than exotic categories of plant species in the CHAL area. Therefore, native plants are very important for conservation of bumblebees.

In overall, this study provided valuable insights about the complex relationships between *Bombu*s spp. and plant categories along elevational gradients. As this study indicated native plants as the most preferred plants by *Bombus* spp., these findings can contribute for proper conservation steps of those plant species in CHAL region.

6.3 Recommendation

i. Many bumblebee species exhibit colour patters and cryptic behavior therefore detail study covering all altitudinal gradinets would be recommended in CHAL.

ii. To identify specific host plant for the pollination of *Bombus* species, it is highly recommendate to explore the pollen load in the body of these bumblebee so that their pollination contribution can be known for specific flowering plants in cultivated as well as wild ecosystem. This can be important information to identify the plant specific pollination behavior of *Bombus* species.

iii. In recent day, Chitwan Annapurna Landscape is under the threat of rapidely growing invasive plants, so that the impact on native flora is still poorly documented. Therefore, the study on further consequence on pollinator communities including bumblebees is highly needed.

iv. The detail study on plant-bumblebee interaction covering large scale altitudinal gradients is highly recommended. This is important to identify specific *Bombus* spp. for reproductive success of specific wild and cultivated flowering plants so that this may be conservation priority of *Bombus* species in CHAL.

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APPENDICES

Appendix 1. Participated in national and international conferences


Central Department of Zoology, TU

this Certificate in recognition for your

Oral Presentation in the

First National Conference on Zoology: Biodiversity in a Changing World

organized by

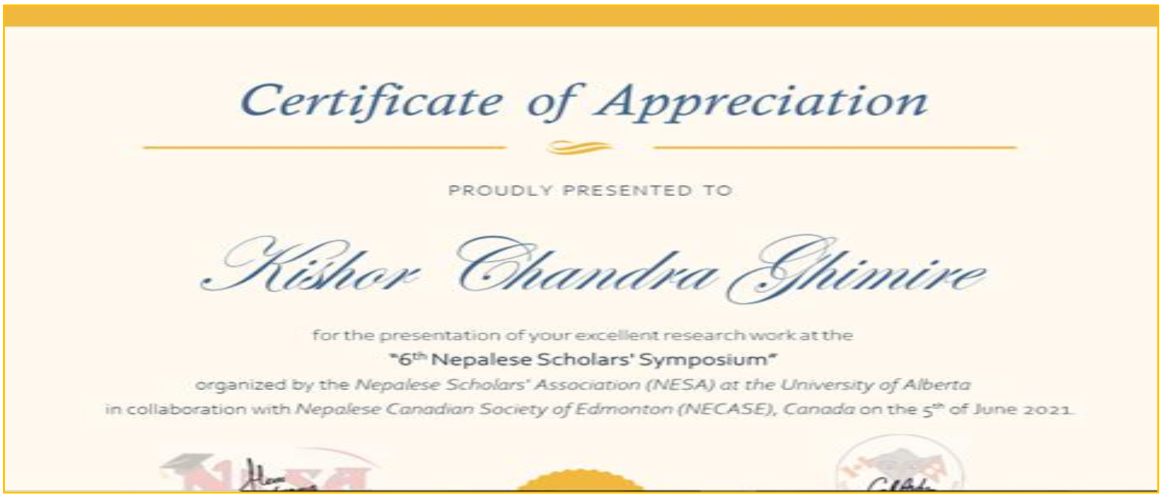
Central Department of Zoology & Alumni Association of CDZ, Tribhuvan University

during 28 – 30 November 2020.

Prof Dr Tej Bahadur Thapa, Conference Chair and Head, Central Department of Zoology

Prof Dr Ram Prasad Khatiwada, Dean, Institute of Science & Technology, Tribhuvan University

Prof Dr Dharma Kanta Baskota, Vice Chancellor, Tribhuvan University


Certificate of Appreciation

PROUDLY PRESENTED TO

Kishor Chandra Ghimire

for the presentation of your excellent research work at the

"6th Nepalese Scholars' Symposium"

organized by the *Nepalese Scholars' Association (NESA) at the University of Alberta*

in collaboration with *Nepalese Canadian Society of Edmonton (NECASE), Canada* on the 5th of June 2021


HIMALAYAN KNOWLEDGE CONCLAVE

Sixth Graduate Conference on Environment and Sustainable Development

SUSTAINABILITY THROUGH KNOWLEDGE AND INNOVATION

CERTIFICATE OF APPRECIATION

This certificate is awarded to

Mr KISHOR CHANDRA GHIMIRE

in recognition as an ORAL PRESENTER in the Sixth Graduate Conference held on August 5-6, 2020.

Appendix 2. Published papper

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Community dynamics of bumblebee across elevation gradients and habitat mosaics in Chitwan Annapurna Landscape, Nepal

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A R T I C L E I N F O

Keywords:
Bombus spp.
Gorkha

A B S T R A C T

The species composition of bumblebees (*Bombus* species) across the elevation gradients in Chitwan-Annapurna Landscape (CHAL) was studied from April to November 2019. We performed opportunistic surveys to collect the bumblebee specimens. The walking transects were followed in

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Species Composition and Conservation Risk Factors for Bumblebees (*Bombus* spp.) Across the Chitwan Annapurna Landscape, Nepal

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Foraging Discrepancy of *Bombus* Species with Native Versus Non–native Flora in Chitwan Annapurna Landscape, Nepal

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***Author for correspondence**

ABSTRACT
We compared the foraging frequency of bumblebee (*Bombus* spp) with native versus non-native flowring plants
... Landscape (CHAL). Specimens were collected using a sweeping net along the altitudinal

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Research Article

FORAGING VARIATION OF *Bombus* SPECIES WITH PLANT FAMILIES AND FLORAL COLORS IN CHITWAN ANNAPURNA LANDSCAPE, NEPAL

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***(Received:** September 27, 2021; **Revised:** February 10, 2022; **Accepted:** March 01, 2022)*

Abstract

Little is known about the effect of the type of vegetation and floral color on the foraging behavior of *Bombus* species. In this work, we have investigated the differential foraging association of bumblebees (*Bombus* spp.) with the specific flower color and the plant families in Chitwan-Annapurna Landscape (CHAL). The specimens were collected between April to October 2019 and field visits were carried out by following accessible walking transects between 600 to 3500 m asl covering different habitats of the study area. The bumblebees were collected by opportunistic methods using a sweeping net. We found that the relative frequency of *Bombus* spp. varied significantly with the families of local flowering plants and the particular colors of flowers. Some of the bumblebees visited at the specific plant family for nectar and pollen indicating the specific association with particular flower morphology and color. This study, therefore, gives an insight into the differential foraging preference of *Bombus* spp. to certain plant families with selected specific colors in CHAL.

Keywords: *Bombus* spp., CHAL, foraging, floral color, plant families, pollinators

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Distribution of Bombus haemorrhoidalis Smith and its Interrelationship with Host Plants in Chitwan Annapurna Landscape of Central Nepal

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Abstract

Occurrence and variety of flowering host plant of native Bombus pollinators are viewed as basic alternatives than the imported species. The use of native bumblebee species for pollination was considered more significant than imported bumblebees to reduce environmental impact and pest problems. B. haemorrhoidalis is the most dominant species of Chitwan Annapurna Landscape in agricultural and wild flora during April to September 2019. We followed assessable walking trails and used insect net for sample collection. The effect of different environmental variables on the floral host plant resources of this native bumblebee was examined. With eight locations ranging from 1407 to 2506 meters above sea level, twenty-seven species of seventeen plant families were identified as pollen and nectar foraging host plants. B haemorrhidalis distribution frequency is correlated with relative humidity (0.07438968) and altitude (0.495657857). The most visited plant family was

K.C. Ghimire and D.R. Bhusal/BMCJoSR, 5, 85-96 (Dec. 2022) 85

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Diversity of Bumblebees (Bombini, Apidae: Hymenoptera) in Chitwan Annapurna Landscape (CHAL) of Central Himalaya, Nepal

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Abstract

Bumblebees (Hymenoptera: Apidae: genus Bombus Latreille, 1802) are the effective group of pollinators for various crops and wild plants in the north high-hills of Nepal. Over the past few decades, it has been expected that the population of these insects has declined since there are

Appendix 3. Photo plates of identified 16 *Bombus* Species

Photo Plate 1

FV- Frontal view, LV- Lateral view DV- Dorsal view

Phot Plate 2

B. festivus : F.V. L.V. D.V.

B. miniatus : F.V. L.V. D.V.

B. haemorrhoidalis : F.V. L.V. D.V.

B. branickii : F.V. L.V. D.V.

FV- Frontal view; LV- Lateral view; DV- Dorsal view

Photo Plate 3

B. cornutus : F.V. L.V. D.V.

B. novus : F.V. L.V. D.V.

B. turneri : F.V. L.V. D.V.

B. lepidus : F.V. L.V. D.V.

FV- Frontal view; LV- Lateral view; DV- Dorsal view

Photo Plate 4



B. parthenius : F.V. L.V. D.V.



B. pressus : F.V. L.V. D.V.



B. rotundiceps : F.V. L.V. D.V.



B. asiaticus : F.V. L.V. D.V.

FV- Frontal view; LV- Lateral view; DV- Dorsal view

Appendix. 4. Tables

Table 1- Network properties

Network Properties	High altitude	Low altitude
Connectance	0.52	0.50
Linkage density	5.31	3.59
GeneralityHL	1.76	2.49
VulnerabilityLL	8.86	4.69
H2(Specialisation)	0.24	0.25
Nestedeness	8.04	7.72
Interaction eveness	0.71	0.66
Weighted NODF	36.17	56.53
Link per species	1.31	1.42
Web assymetry	0.68	0.42
RobustnessLL	0.59	0.68
RobustnessHL	0.87	0.82
Partner diversityLL	2.11	1.34
Partner diversityHL	0.49	0.84

Table 2. Presence - absence status of *Bombus* species with their foraging plant family. *Bombus* species: *Bombus asiaticus(*asi), *Bombus branickii* (bra*), Bombus breviceps(*bre*), Bombus cornutus*(cor*), Bombus eximius* (exi*), Bombus festivus(*fes*), ombus. graham(*gra*), Bombus haemorrhoidalis(*hae*), Bombus Lepidus(*lep*), Bombu sminiatus(*min*), Bombus novus(*nov*), Bombus parthenius(*par*), Bombus pressus(*pre*), B. rotundiceps(*rot*), Bombus tunicatus*(tun*), Bombus turneri*(tur*)*

Families	asi	bra	bre	cor	exi	fes	Gra	hae	lep	min	nov	par	pre	rot	tun	tur
Fabaceae	+	–	+	–	+	+	–	+	+	–	–	–	–	+	–	+
Acanthaceae	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–
Apocynaceae	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
Asteraceae	+	–	+	–	+	+	–	+	–	–	–	–	–	+	+	–
Balsaminaceae	+	–	+	–	+	–	–	+	–	–	–	+	–	+	–	–
Cactaceae	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–
cannabaceae	–	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–
Cannaceae	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Compositae	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
Convulvulaceae	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–
Cucurbitaceae	+	–	+	–	+	–	–	+	–	–	–	–	–	–	–	–
Ericaceae	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–
Gesneriaceae	–	–	–	+	+	–	+	+	–	–	–	–	–	–	–	–
Iridaceae	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
Lamiaceae	–	–	+	–	+	–	+	+	–	–	–	+	–	–	+	–
Lauraceae	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Liliaceae	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
Lythraceae	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–
Malvaceae	–	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–
Oleaceae	+	–	+	+	+	+	–	+	+	–	–	+	–	+	+	–
Ophiopogon	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–
Papaveraceae	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
Polygonaceae	–	–	–	–	–	+	–	+	–	+	–	–	–	–	–	–
Ranunculaceae	+	–	–	+	+	+	+	–	+	–	+	–	–	–	+	+
Rosaceae	+	+	+	–	+	+	–	+	+	–	–	–	+	+	–	+
Solanaceae	+	–	–	–	–	+	–	+	–	–	–	–	–	+	+	+
Verbenceae	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
Veronicaceae	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–
Viburnaceae	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–

Appendix 5. Field photos

Field photograph

Trifolium repens

Collected specimens

Opuntia dillei

Opuntia dillei

Bumblebee foraging on the *Opuntia dillei*

Landscape of study area

Host plant *(Solanum tuberosum)*

Researcher in the study area

Host Plants

Solanum truberosum

Rosa brunonii

Dahlia pinnata

Dahlia pinnata

Chrysojasminum humile *Solanum melongena*

Rosa brunonii

Rosa brunonii *Chrysojasminum humile*

Appendix 6. Consent Letter from the Authority

नेपाल सरकार
वन तथा वातावरण मन्त्रालय
राष्ट्रिय निकुन्ज तथा वन्यजन्तु संरक्षण विभाग
इकोलोजी शाखा

फोन नं. ४२२०८५० / ४२२०९१२ / ४२२७०२६
फ्याक्स नं.: ४२२७६७५

पत्र संख्या: ०७४/७५ इको
चलानी नम्बर: ३२४८

पो.ब.न.-८६०
बबरमहल,काठमाण्डौ
Email: info@dnpwc.gov.np
http//:www.dnpwc.gov.np

मिति:- २०७५।३।११

विषय:- अध्ययन अनुमति सम्वन्धमा।

श्री रारा राष्ट्रिय निकुन्ज कार्यालय,हुटु, मुगु / श्री सगरमाथा राष्ट्रिय निकुन्ज कार्यालय, नाम्चेबजार, सोलुखुम्बु
शे-फोक्सुन्डो राष्ट्रिय निकुन्ज कार्यालय, सुलिगाड, डोल्पा
श्री अन्नपूर्ण संरक्षण क्षेत्र कार्यालय, पोखरा, कास्की

प्रस्तुत विषयमा त्यस संरक्षित क्षेत्रमा निम्नानुसार अध्ययन अनुसन्धान अनुमति प्रदान गरिएको व्यहोरा आदेशानुसार अनुरोध छ।

अनुसन्धानकर्ताको नाम	पहिलो	बीचको	थर
	किशोर	चन्द्र	घिमिरे
अनुसन्धानकर्ताको ठेगाना	स्थायी: हर्मी-६, गोरखा	ई-मेल:	फोन नं.:
	अस्थायी: किर्तिपुर	kishor.ghimire@gmail.com	9845054181
सम्बद्ध संस्था	संस्थाको नाम:	ठेगाना:	
	त्रिभुवन विश्वविध्यालय, प्राणी शास्त्र केन्द्रीय बिभाग	कीर्तिपुर	
पद	विद्यार्थी		
अनुसन्धानको तह	विद्यावारिधि	संस्थागत:	
अनुसन्धानको शिर्षक	Species Composition and Floral Association of Bunblebees (Bombus spp.) in Chitwan Annapurna Landscape, Nepal.		
अनुसन्धानको विधि	Extensive field survey, specimen sample collection	नमुना संकलन: गर्ने	नमुनाको परिक्षण: नेपालमा
अनुसन्धानको समयावधि	१ भाद्र, २०७५ देखि ३० श्रावन २०७६ सम्म		
अनुसन्धानका शर्तहरु	१. अनुसन्धानकर्ताले राष्ट्रिय निकुन्ज तथा वन्यजन्तु संरक्षण ऐन २०२९ र नियमावली २०३० तथा यस मातहतका सबै नियमावलीहरुको पूर्ण पालना गर्नु पर्ने छ। २. अनुसन्धानकर्ताले विभाग र सम्बन्धित संरक्षित क्षेत्र कार्यालय संग समन्वय गरि कार्य गर्नु पर्ने छ। ३. अनुसन्धानकर्ताले आपनो अनुसन्धानको प्रस्ताव सम्बन्धित संरक्षित क्षेत्र कार्यालयमा समेत पेश गर्नु पर्नेछ। ४. अनुसन्धानकर्ताले अनुसन्धान समाप्त भएपछि एक प्रति कागजी प्रतिवेदन र एक प्रति विधुतीय प्रतिवेदन विभाग र सम्बन्धित संरक्षित क्षेत्र कार्यालयमा बुझाउनुपर्ने छ। ५. अनुसन्धानकर्ताले नतिजाहरु प्रकाशित गर्दा अनुसन्धानमा संलग्न कर्मचारीको योगदानको आधारमा सह लेखकको रुपमा समावेश गराउनु पर्नेछ। ६ सकलित नमुना विदेश लैजान नपाइने।		

ऋषि रानाभाट
सहायक इकोलोजि

बोधार्थ:
श्री किशोर चन्द्र घिमिरे : सम्बन्धित संरक्षित क्षेत्र कार्यालयसंग समन्वय गरी अध्ययन अनुसन्धान हुन।
श्री त्रिभुवन विश्वविध्यालय,प्राणी शास्त्र केन्द्रीय बिभाग, किर्तिपुर : उपरोक्त विद्यार्थीले सोधकार्य सम्पन्न गरेपछि १ प्रति सोधपत्र अनिवार्यरुपमा यस विभागमा पठाउने व्यवस्था हुन।

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Community dynamics of bumblebee across elevation gradients and habitat mosaics in Chitwan Annapurna Landscape, Nepal

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ABSTRACT

The species composition of bumblebees (*Bombus* species) across the elevation gradients in Chitwan-Annapurna Landscape (CHAL) was studied from April to November 2019. We performed opportunistic surveys to collect the bumblebee specimens. The walking transects were followed in the accessible places along the Kaligandaki, Marshyandi, and Budhigandaki river basins in different habitats (e.g., agricultural, forest, grassland and home garden). We identified 16 *Bombus* species from the sampling areas. The highest relative abundance was of *B. haemorrhoidalis* (20%), followed by *B. festivus* (20%) and *B. eximius* (19%). The least abundant species were *B. branickii*, *B. miniatus*, *B. novus*, and *B. pressus* with 1% relative abundance of each. We examined the effects of elevation on bumblebee richness and found a significant relationship. The Highest species richness was detected in the mid-elevation. Likewise, the highest species richness and diversity were found in the forest habitat in Gorkha site (n = 12, Shannon index H' = 2.18) followed by the grassland habitat of the Mustang site (n = 11, Shannon index H' = 2.10). Whereas, comparatively, species diversity was higher in habitats of the Gorkha site comparing Manang and Mustang. The elevation gradients create immense variations in microclimatic conditions and vegetation dynamics, which influence bumblebee abundance, species richness and diversities in different habitats in the study area. The mid-elevation range (2000–3000 m asl) of CHAL exhibited the highest species richness probably due to the higher availability of pollinator-dependent flowering plants in this range. The landcover composition and anthropogenic activities along the elevation gradient is the governing factor for the species composition, distribution and diversity of bumblebees in CHAL. We recommend to decision-makers for formulating their conservation strategies under a socio-ecological framework.

1. Introduction

The Mountain landscape is sensitive to change in climate and land cover [1,2]. Previous studies [1] have indicated that the sub-alpine and alpine regions are experiencing the impact of climate change in different parts of the world and including Asian bumblebees [1,2]. The high degree of spatial complexities and abiotic stresses in the mountain landscapes suggests that the mountain ecosystems should be very challenging environments. In mountains, increases in the mean temperature affect the diversity, abundance and

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distribution of insects and are expected to vary along an elevational gradient [2,3]. In general, the temporal and spatial distribution and diversity of bee communities in different habitats along elevation gradients are difficult to predict because of the differences in microhabitat and host plant types [4]. Among the bee communities, bumblebees are higher-elevation insects that play an important role in the pollination of flowering plants [5–10]. These are well adapted to cold and temperate habitats at higher latitudes and elevations [11].

The altitudinal distributions of bumblebees have been documented across different parts of the world, though the factors underlying this variation remain poorly understood [12,13], especially along elevation gradients under complex landscape structures

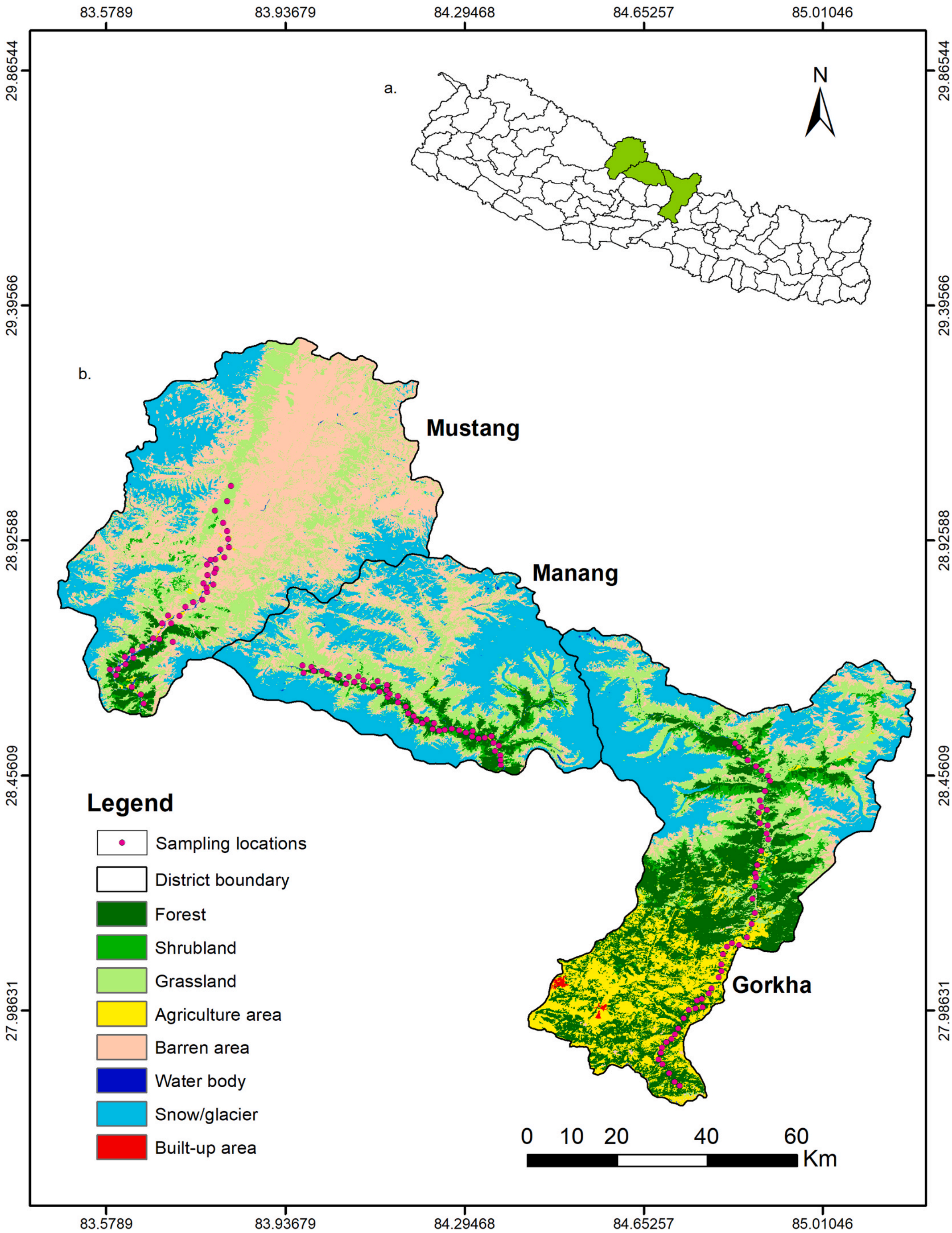

Fig. 1. Map of Chitwan Annapurna Landscape (CHAL). a. Location of three districts of Nepal, b. Detailed map of study districts, Gorkha, Manang and Mustang with Sampling sites: Kaligandaki River valley (A), Marshyandi River valley (B) and Budhigandaki River valley (C).

[13–16]. They are greatly affected by habitat types, land-use change, vegetation composition and availability of suitable nesting habitats [17–20]. Besides the environmental factors, the thermal tolerance limit [21–24] of the bumblebees is also one of the major limiting factors to determining the range distribution along the elevation gradients [25]. The effects of habitat type along elevation gradients that typically impact, host plant variation may alter the diversity and abundance of bumblebees and this has been reported in many parts of the world [26–28]. In more general, habitat quality determines the density and dispersibility of bumblebees, particularly in a natural versus agricultural habitat [21,29]. Therefore, it is difficult to predict how the abundance and diversity of bumblebees interact with different habitat types, elevation gradients, and other landscape features [30–34]. Previous studies suggest the effect of habitat types on abundance and diversity of bumblebees. The destruction of natural habitats effects the species composition of bumblebees typically *Bombus* species [24,33,35–39]. There is much global evidence regarding the loss of bumblebee, in general, are associated with increased food stresses, reduced floral diversity, continuing habitat loss, increased pesticide use, and disease [40,41]; however, this is species-specific where some suffered greater losses than others [42].

Our understanding of the response of bumblebees to different habitat types in the Himalayan Mountains is still rather limited. Recently, the increasing temperature and anthropogenic pressures, such as agricultural intensification, pesticides use and habitat loss caused extensive modifications of the Himalayan landscape [43,44]. In the context of bumblebees studied in the Himalayan region, the species composition of these faunas has been intensively studied in the western [45,46], specifically in Indian parts. However, the taxonomic status and species composition in the central and some parts of the eastern Himalayas still need to be explored [10,46,47]. In the central Himalaya, the study of bumblebee particularly on the taxonomic richness and vulnerability to climate change, the effect of habitat types and other anthropogenic pressures remain poorly understood and need to be explored extensively [39,47]. The bumblebee abundance, species richness and distribution patterns have been largely understudied in focusing on the altitudinal gradients of this region.

The Chitwan Annapurna Landscape (CHAL) is important of the mid-Himalayas with a unique flora with flowering plants and habitat types. In recent days, this landscape is expected to be greatly threatened by climate change and habitat loss by many invasive and alien plant species (IAPS), infrastructures development, landslide, erosion and use of pesticides in agriculture [39,48–54]. Bumblebees are important pollinators and play an important role in conserving the germplasm of higher-elevation flora [39]. However, the pattern of distribution, diversity and abundance of these faunas along with elevation and habitat gradients in this landscape has not been well studied. Therefore, this study aimed to explore species richness, diversity, and composition of bumblebees along elevation and habitat gradients in CHAL focusing on Kaligandaki, Marshyandi, and Budhigandaki River basins.

2. Materials and methods

2.1. Site description and field design

This study was carried out along an elevation gradient (from 600 to 3500 m asl) in CHAL focusing on major three river basins [Fig. 1 (a)]: Kaligandaki (Mustang site 28˚87′ 65.15″ N - 83˚79′ 47 65″ E), Marshyandi (Manang site, 28˚57′ 52. 62″ N - 84˚18′ 66. 28″ E), and Budhigandaki (Gorkha site, 28˚18′ 36.44″ N - 84˚85′15.79″ E). These river basins are hotspots of biodiversity with diverse habitat types, including agriculture, forest, grassland, and human settlements [Fig. 1(b)]. These areas have immense microclimatic variation and differed from each other by ecological settings typically microclimatic types and vegetation structures that create suitable habitats for many species of bee communities. Furthermore, these river valleys are important transit routes for migratory birds, as well as supporting populations of various endangered species including the snow leopard (*Panthera uncia*), red panda (*Ailurus fulgens*) and the Himalayan black bear (*Ursus thibetanus*) [55,56] and include one of the important protected areas (Annapurna Conservation Area) of Nepal. The study sites comprise a wide range of subtropical monsoon climate with very high rainfall in the south (below 1000 m) to a temperate climate in the mid-hills (1000–4000 m) and an alpine/arctic climate with very low rainfall above 4000 m [57]. CHAL has a rich cultural heritage, with over four million people who have a high dependency on forest resources and ecosystem services for their livelihoods and well–being. In recent days, this area is heavily threatened by increasing human activities, rapid human migration and settlement, ecosystem degradation by IAPS and the impact of climate change which threating the heavily loss of biodiversity from this area.

2.2. Bumblebee surveying and identification

For the bumblebee survey, we followed three walking transects along the three river valleys (Kaligandaki - Mustang site, Marshyandi - Manang site and Budhigandaki - Gorkha site). We followed the opportunistic survey along the transect (20 m right and left) and passed different habitats. In this method, two field researchers were involved at a slow pace along the transect. The sweeping nets (entomological nets) were used to collect the bumblebees. The specimens were immediately killed using ethyl acetate. Field surveys were conducted in the flowering season between April to November 2019. We spent 45 days and repeated these walking transects twice (The first visit - between April–June (25 days), the second visit-between August–November (20 days)) in the field. Whenever a bumblebee was encountered, we stopped and spent 30 min at the point for searching and collecting all possible foraging specimens (workers) before continuing walking along the transect. Meanwhile, we noted geographic coordinates and elevation using Global Positioning System (GPS) (Garmin eTrex 10) and habitat types. The number of individuals of bumblebee collected per 30 min in specific sampling points ($n = 138$) along the transect were used as abundance. Specimens were stored in airtight containers with a few layers of tissue and the addition of a few drops of ethyl alcohol to prevent the growth of mold during transport to the laboratory. We identified the species and calculated species richness along the specific elevational gradient and habitat types (e.g., Agricultural, Grassland, Home Garden and Forest) from the collected specimens. The agricultural habitat was considered as the farmland area, grassland as open space with flowering herbs scattered inside the forest. Similarly, a home garden was considered if the sampling route was present inside the human settlement area such as the front yard and backyard, and a forest habitat was considered, if the habitat is covered with trees along with bushes and herbs. In all three sites, bumblebees were captured from 138 sampling points along the elevation gradients. From where species richness was calculated from 22 specific elevation bands creating 100 m intervals along the elevation gradients (1350–3550 m asl).

The survey was carried out between 9 a.m. and 6 p.m. during the absence of rain and high wind speed. The collected specimens were subsequently dry–mounted using standard insect pins and deposited in the Entomological Museum of the Central Department of Zoology, Tribhuvan University, Kathmandu, Nepal. The collected specimens were observed under a stereoscopic microscope and identified using published identification keys [e.g., 45–47].

2.3. Data analysis

The data were analysed in R version 4.0.3 (https://www.r-project.org/). We analysed the mean elevation of 16 identified species from our collection and compared the mean elevation within the whole sampling area (WSA) and per site. To find the effect of elevational gradients with bumblebee individuals recorded along the study transects, we performed generalized linear model (GLM) for the abundance of the species and used quasi-poisson error structure since the simple Poisson model showed over dispersion. We analysed the study points that are above 1300 m asl for the species richness.

For the diversity and corresponding analysis (CA), the relative abundance of 16 bumblebee species in the collection was calculated by performing cross-tabulation (two-way table) between bumblebee species versus sites and habitats. After that, we applied diversity analysis using the R package vegan [58]. We calculated Shannon's diversity index (H′) and inverse Simpson's (In λ). Similarly, correspondence analysis (CA) was used to visualize the association of bumblebee abundance with habitat types the R package FactoMineR [59].

3. Results

3.1. Species composition

We collected a total of 656 individuals and 16 species of bumblebee (*Bombus* spp.) were identified. The relative abundance of *Bombus* species in the whole sampling area and their proportion within three 3 sites were analysed (Table 1). We obtained; the highest relative abundance of *B. haemorrhoidalis* (20%), *B. festivus* (20%) and *B. eximius (*19%). The least abundant species were *B. branickii (1%), B. miniatus (1%), B. novus (1%)*, and *B. pressus (1%)*.

The maximum number of species was represented from the Gorkha site (i.e. 15 species) and the relatively higher dominant species from this site were *B. asiaticus*, *B. haemorrhoidalis*, and *B. branickii*, and 10 species were reported from the Manang site (MAN). Whereas, 11 species were reported from Mustang (MUS) site, similarly, *B. turneri* and *B. parthenius* were reported as dominant species in the Manang site (MAN).

In the Mustang sites, *B. miniatus*, *B. lepidus*, and *B. grahami* were major dominant species [Fig. 2 (a)]. Furthermore, we compared, the relative composition of *Bombus* species in our collection by habitats per site [Fig. 2 (b,c,d)]. In the agricultural habitat, *B. asiaticus* was a majorly dominant species followed by *B. cornutus*. Some of the species such as *B. branickii* found only in agricultural habitats. Similarly, species, like *B. rotundiceps, B. parthenius*, *B. tuncatus*, *B. haemorrhoidalis* and *B. festivus* were most dominant in the forest. Whereas, *B. minniatus* and *B. novus* were specific in this habitat. In the grassland habitat, *B. turneri, B. rotundiceps, B. lepidus* and

Table 1
Relative abundance (%) of Bombus species in whole sampling area (WSA) and proportion (%) of each species occurrence among 3 sites (GOR: Gorkha site; MAN: Manang site; MUS: Mustang site). The number in parenthesis indicates the number of individuals.

Sites	WSA (%)	GOR (%)	MAN (%)	MUS (%)
Bombus asiaticus	4.35 (29)	100 (29)	–	–
B. branickii	0.72 (5)	100 (5)	–	–
B. breviceps	5.07 (34)	71.4 (24)	14.3 (5)	14.3 (5)
B. cornutus	2.17 (15)	100 (15)	–	–
B. eximius	18.84 (124)	65.4 (81)	3.8 (5)	30.8 (38)
B. festivus	19.57 (129)	48.1 (62)	14.8 (19)	37 (48)
B. grahami	3.62 (24)	40 (10)	20 (4)	40 (10)
B. haemorrhoidalis	20.29 (134)	78.6 (105)	14.3 (19)	7.1 (10)
B. lepidus	3.62 (24)	40 (10)	20 (4)	40 (10)
B. miniatus	0.72 (5)	–	–	100 (5)
B. novus	0.72 (5)	100 (5)	–	–
B. parthenius	2.17 (15)	33.3 (5)	33.3 (5)	33.3 (5)
B. pressus	0.72 (5)	100 (5)	–	–
B. rotundiceps	5.8 (39)	62.5 (24)	25 (10)	12.5 (5)
B. tunicatus	5.07 (34)	71.4 (24)	14.3 (5)	14.3 (5)
B. turneri	5.07 (34)	28.6 (10)	42.9 (15)	28.6 (9)

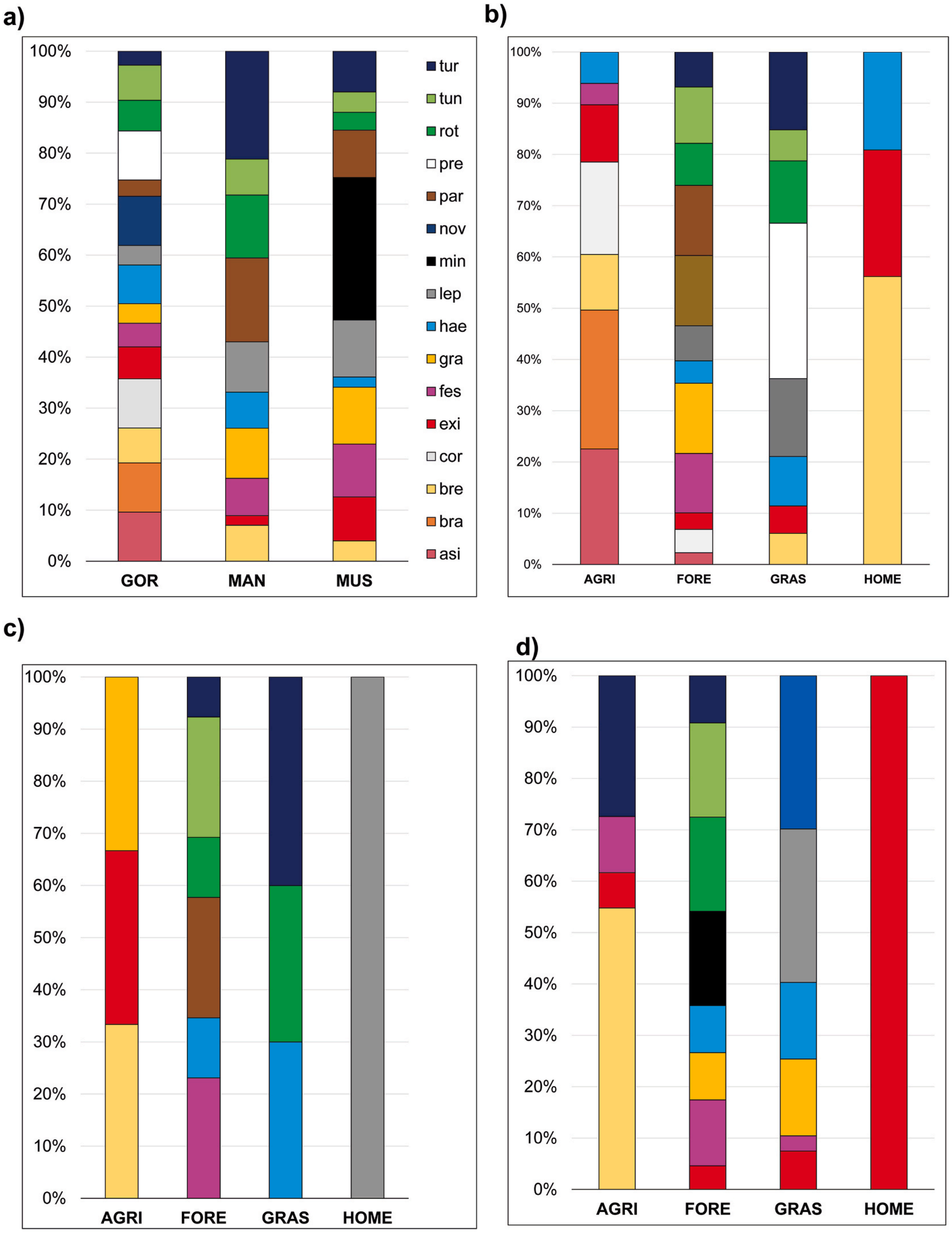

Fig. 2. Species composition of bumblebee; a. Relative abundance by sites; b. Relative abundance of bumblebee in GOR: Gorkha site c. Relative abundance of bumblebee in MAN: Manang site d. Relative abundance of bumblebee in MUS: Mustang site.

B. haemorrhoidalis were the dominantly recorded but *B. pressus* was specifically observed from this habitat. The species *B. breviceps, B. eximus* and *B. haemorrhoidalis* were predominantly recorded from home garden habitat.

We also compared the site-based composition of the *Bombus* species. In the Gorkha site, *B. asiaticus* was the most dominat in agricultural habitat followed by *B. cornutus*. Similarly, *B. festivus*, *B. grahami, B. novus*, *B. parthenius* and *B. tunicatus* were predominantly recorded from the forest habitat of this site. Whereas, *B. pressus* was represented in the grassland of this site followed by *B. turneri*. Likewise, *B. breviceps* was mainly represented from the home garden habitat in this site.

In the case of the Manang site, the most abundant species in agricultural land were *B. breviceps, B. eximus, B. grahami.* In the forest area, the most dominant species were *B. festivus*, *B. parthenius* followed by *B. tunicatus*. Similarly, *B. lepidus* was specific to the home garden habitat of this site. Whereas, in Mustang site, *B. breviceps* and *B. turneri* were most abundant species in agricultural land, but *B. miniatus*, *B. rotundiceps*, *B. tunicatus* were dominant in the forest habitat. Similarly, *B. lepidus* and *B. parthenius* were most abundant in the grassland habitat, and *B. eximus* was specifically recorded from the home garden habitat.

3.2. Elevational records of Bombus species

Within the whole study area, the highest mean elevation record was observed (Table 2) in *B. pressus* followed by *B. cornutus* and *B. lepidus.* The species recorded from the lowest mean elevation were *B. haemorrhoidalis* (2189 m asl), *B. rotundiceps* (2121 m asl), and *B. eximus* (2257 m asl). In the case of site-based analysis, we found variation in their mean elevation records. In the Gorkha site, some species such as *B. pressus* (3000 m asl), *B. grahami* (2734 m asl) and *B. cornutus* (2700 m asl) were in higher mean elevation records. In the Manang site, some species such as *B. breviceps* (3500 m asl), *B. rotundiceps* (2799 m asl) and *B. lepidus* (2669 m asl) were recorded from the highest mean elevation, whereas, Mustang site, *B. lepidus* was recorded at the highest mean elevation (2706 m asl), followed by *B. festivus* (2597 m asl) and *B. tunicatus* (2270 m asl).

3.3. Effect of elevation and landscape factors

We examined the change in bumblebee abundance, richness and species diversity with the landscape factors (elevation, sites and habitats) and found a significant effect of elevation on the abundance and richness of bumblebees (Table 3). In our model, we used elevation gradients and habitat types as fixed factors and species names as random factors along the elevation gradients. We detected, the species richness changed from lower to higher elevation and exhibited a mid–domain effect. The highest species richness was recorded in the mid-elevation zone specifically between 2000 and 3000 m asl (Fig. 3). Similarly, we assessed the relation of habitat types with the abundance of bumblebees.

In our corresponding analysis (CA), among three axes, the first axis contributed the highest variance (48%) followed by second axis (43%) resulting in a cumulative variance of 91% (Table 4). The variance in an abundance of bumblebees along axis-1 was mostly contributed by agriculture and forest followed by home garden and the least being contributed by grassland. Whereas, along axis-2, grassland contributed most of the variance (64%) and with least contribution from the forest habitat (3%) (Table 5). The species were distinctly ordinated and the majority of species were particularly associated with forest habitat followed by agricultural land (Fig. 4). The key species such as *B. festivus, B. grahami,* and *B. tunicatus* were dominant. Whereas, from the agricultural land were *Bombus branickii, Bombus asiaticus* and *B. cornutus.* In the case of grassland, the most abundant species were *B. haemorrhoidalis, B. lepidus, B. turneri.* Likewise, *B. haemorrhoidalis, B. eximius, B. lepidus* were majorly abundant species in home garden habitat.

We compared the species diversity profiles such as species number, Shannon-Weiner index and Inverse Simpson were calculated. The diversity indices changed across the sampling habitats by sites and exhibited a specific pattern i.e., forest habitat showed the highest diversity profiles followed by grassland, agriculture and home garden [Fig. 5 (a)]. We found the highest species richness and diversity in forest habitat of Gorkha site (S = 12, Shannon index H' = 2.18) followed by grassland habitat of Mustang site (S = 11, Shannon index H' = 2.10) [Fig. 5 (a)]. Similarly, Inverse Simpson (In λ) was higher in the forest habitats of Gorkha site (In $\lambda = 7.71$) followed by a grassland of Mustang sites (In $\lambda = 6.92$) and forest habitats of Manang sites (In $\lambda = 5.26$) [Fig. 5 (b)].

4. Discussion

In the present study, the landscape characteristics such as elevation and habitat composition are the important factors to shape the

Table 2
Mean elevation of *Bombus* species recorded in the study area. WSA (Whole study area), GOR (Budhigandaki River valley), MAN (Marshyandhi River valley), MUS (Kaligandaki River valley).

Species	WSA	GOR	MAN	MUS
Bombus asiaticus	2556 ± 307	2556 ± 307	–	–
B. branickii	2660	2660	–	–
B. breviceps	2399 ± 677	1988 ± 388	3500	1709
B. cornutus	2700 ± 267	2700 ± 267	–	–
B. eximius	2257 ± 191	2245 ± 220	2202	2324 ± 116
B. festivus	2443 ± 531	2337 ± 476	2395 ± 593	2597 ± 593
B. grahami	2452 ± 327	2734 ± 376	2191	2432 ± 270
B. haemorrhoidalis	2189 ± 418	1880 ± 406	2351 ± 277	2337 ± 16
B. Lepidus	2673 ± 34	2644 ± 15	2669	2706 ± 21
B. miniatus	2592	–	–	2592
B. novus	2536	2536	–	–
B. parthenius	2353 ± 184	2554	2191	2315
B. pressus	3000	3000	–	–
B. rotundiceps	2121 ± 714	1833 ± 531	2799 ± 991	1731
B. tunicatus	2309 ± 164	2527 ± 11	2132	2270
B. turneri	2353 ± 215	2550	2271 ± 277	2240 ± 41

Table 3
Table: Summary of model showing relationship of bumblebee individual number with elevation.

Coefficient	Estimate	Std. Error	t-value	p-value
Intercept	−4.22	2.01	−2.1	0.037
Elevation (m)	0.002	0.0008	3.45	0.0007

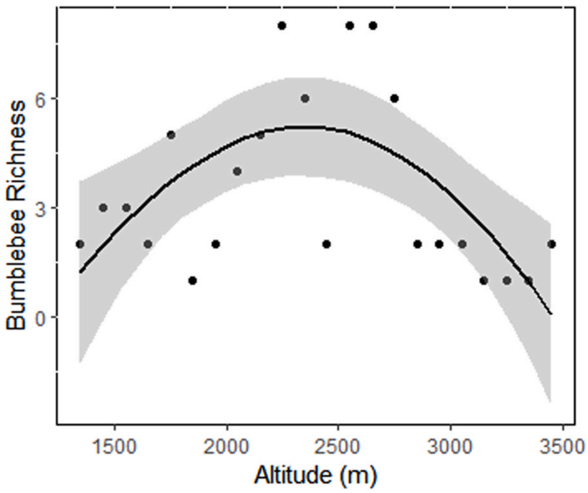

Fig. 3. Patterns of species richness and elevation gradients.

Table 4
Eigenvalue from the correspondence analysis (CA) for the three axes with variance and cumulative variances.

Eigenvalue	Axis-1	Axis-2	Axis-3
	0.441	0.398	0.088
% Of Variance	47.593	42.9	9.507
Cumulative % of Variance	47.593	90.493	100

Table 5
Contribution (%) of habitat types in abundance of bumblebees in two corresponding axes.

Habitats	$Axis_1$	$Axis_2$
Agriculture	42.223	26.219
Forest	40.157	2.692
Grassland	2.323	63.573
Home garden	15.297	7.516

abundance and diversity of bumblebees. We detected; elevation is one of the most important factors for the distribution of bumblebees and landscape characteristics such as habitat type also influence the species composition of bumblebees. The landscape features and vegetation dynamics present within three river basins affect the composition and diversity of bumblebees in this study area.

In our study, the proximate landscape factors along the elevation gradients are important filtrating factors because of distinct vegetation dynamics in different elevation [6,15,34,60,62], landscape dynamics and particularly climate microclimatic factors types [63].

In the case of the mid-domain effect detected in our study, the geographic factors of this landscape along with suitable environmental parameters such as the presence of high habitat heterogeneity [64] that finally created suitable and diverse food resources for bee communities and have contributed to this pattern [65,66]. The site-based habitat-wise variation of species composition along the elevation gradient of this study is in line with the conclusion of some previous studies from the other parts of the Himalayan landscape [10,19]. This might be attributed to the variation of vegetation dynamics and microclimatic changes within habitat types and microclimatic variation within the river valleys of three sites [62].

The higher diversity profiles in the natural habitats such as forest and grassland compared to agriculture and human settlement sites are attributed to anthropogenic disturbance [67] that might be the major factor for the bumblebee distribution in the study area.

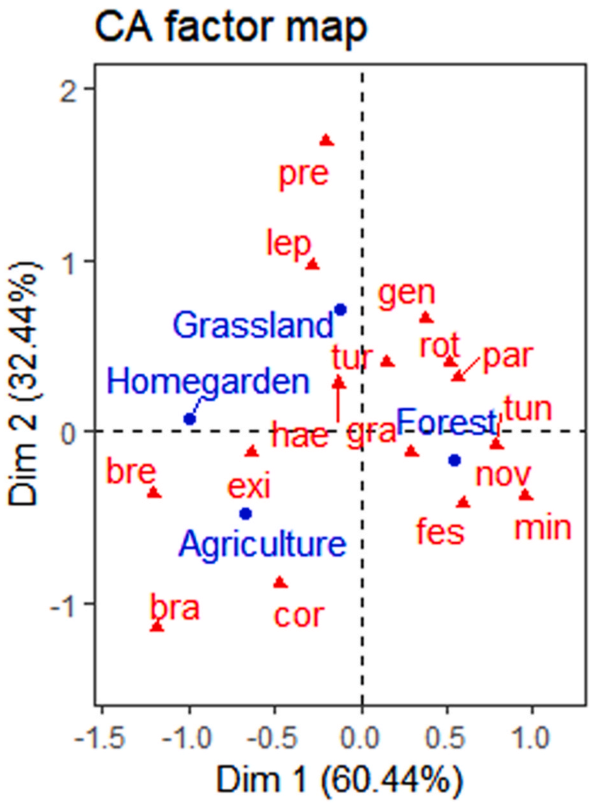

Fig. 4. Correspondence analysis of *Bombus* species with habitat types; AGRI: Agriculture, FORE: Forest-FORE, GRAS: Grassland, and HOME: Home Garden; asi: *Bombus asiaticus*, bra: *Bombus branickii*, bre: *Bombus breviceps*, cor: *Bombus cornutus*, exi: *Bombus eximius*, fes: *Bombus festivus,* gra: *Bombus graham,* hae: *Bombus haemorrhoidalis,* lep: *Bombus lepidus,* min: *Bombus miniatus*, nov: *Bombus novus*, par*: Bombus parthenius,* pre: *Bombus pressus,* rot: *Bombus rotundiceps,* tun: *Bombus tunicatus,* tur: *Bombus turneri.*

On the other hand, rapid habitat loss and increasing use of pesticides in farming land may be impacting the abundance and distribution of specific bumblebee species [18,20,68]. However, socio-ecological systems with a range of social, ecological and environmental factors [69] such as rapidly changing habitat utilization, pesticide application [35] and change in the farming patterns are the influencing factors on the abundance and diversity of bumblebee in CHAL. The general trends of the Himalayan landscape (e.g., CHAL) is characterized by the higher richness of vegetation typically flowering plants and lower mean temperature in eastern river valley parts compared to western parts [10,35,36,45,70–72].

In this study, some of the species such as *B. asiaticus, B. eximius, B. festivus, B. haemorrhoidalis, B. rotundiceps* and *B. turneri* exhibited a particularly large elevation variation in different area as well as within the same area. In this study, the most abundant three species *B. rotundiceps, B. haemorrhoidalis, B. eximius* were observed at relatively low mean elevations as similarly reported from parts of Himalaya by Streinzer et al. and Decourtye et al. [10,36]. The distribution of particular, *Bombus* species along elevation gradients might be related to the critical thermal limits of the particular *Bombus* species along elevation gradients [12,13,73,74]. Some of the species such as *B. breviceps* exhibited vast variation in their mean elevation range comparing the three sites of this study and also with previous studies [10,36,75]. Some of the species were confined to a relatively narrow elevation zone, or from specific sites, demonstrating their high specificity in this study area. For example, *B. asiaticus, B. branickii, B. cornutus, B. pressus, B. novus* were only present at the eastern side of our area whilst *B. miniatus* was only present at the western side. These species may be particularly adapted to unique microhabitats as they are restricted to a very limited elevation range in our study sites.

The body physiology such as the thermal limits of these species and more generalist food plants are likely to be important environmental factors influencing this distribution [39,40,45,76]. The recent impacts of climate change in the Himalayan region, such as species range shifts [77], might be one of the important factors influencing the elevation range of many bumblebee species in this region. Recent evidence further suggests CHAL facing drastic landcover change that finally influences the vegetation dynamic of this area [54] that possibly impact the composition and species diversity of bee communities including bumblebees. Additionally, the availability of species–specific food plants within a particular habitat and the presence of suitable nesting sites might also be important for the distribution of bumblebee species in our study area. In these particular river basins, the higher species richness observed at mid-elevation (2000–3000 m) regions indicates high food availability (i.e., suitable host plants). The availability of some dominant pollinator-dependent flowering plants in the mid-elevation region might link to the highest species richness of bumblebees. We identified, some of the dominant bumblebee attracting flora from this region such as *Cucurbita pepo, Solanum melongena, Vigna*

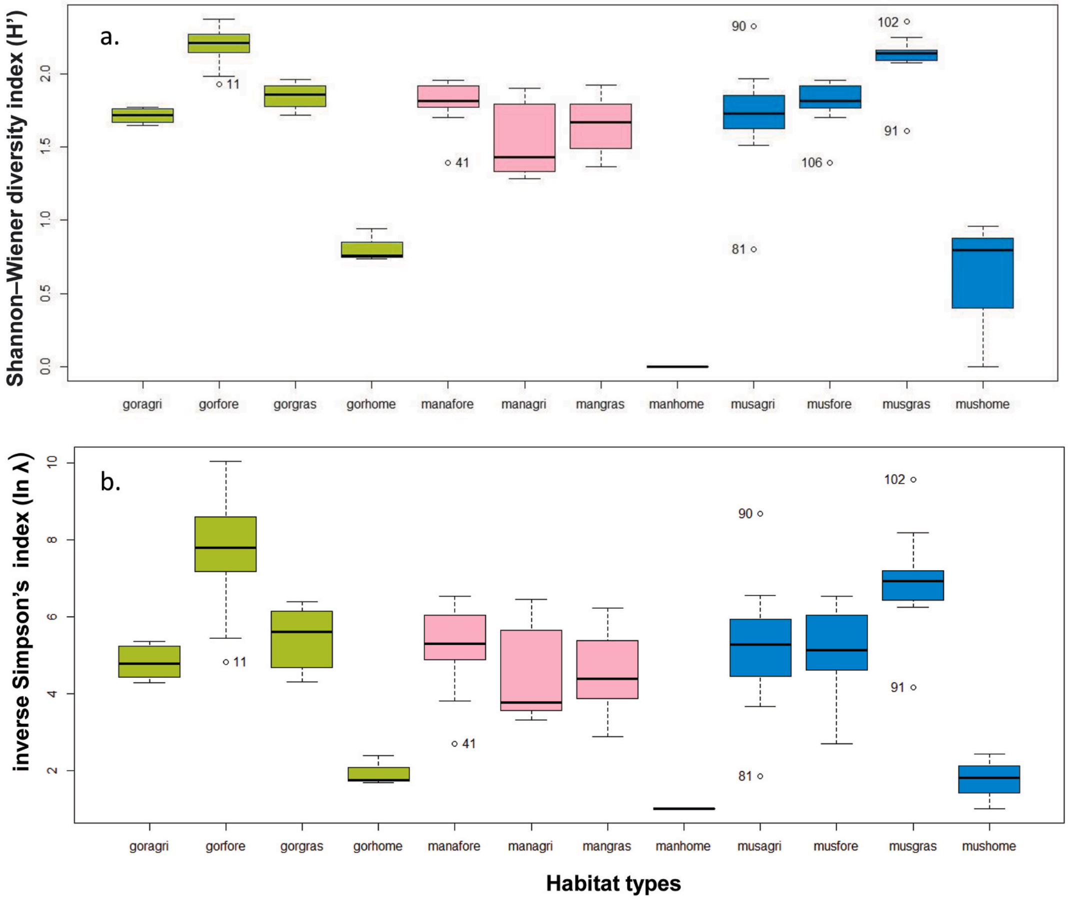

Fig. 5. Species diversity profile of bumblebee: a. Shannon–Wiener diversity index (H′) in four different habitats of three study sites; b. inverse Simpson's index (In λ). Here, goragri: Gorkha-agricultural habitat, gorfor: Gorkha-forest, gorgras: Gorkha Grassland, gorhome: Gorkha-homeland, managri: Manang-agricultural habitat, manfor: Manang forest, mangras: Manang Grassland, manhome: Manang homeland, musagri: Mustang agricultural habitat, musfor: Mustang forest, musgras: Mustang Grassland, mushome: Mustang homeland.

unguiculata, Berberis asiatica, Reinwardtia indica, Rubus ellipticus, Vigno mungo, Diploknema butyracea, Osbeckia nepalensis, Biden pilosa, Cirsium wallichii, Impatiens scabrida, Rosa brunonii and *Anaphalis busua.*

Our study highlights the strong impact of environmental changes on bumblebee distributions, and we suggest strategies for the conservation of threatened species that include protecting the areas of high species richness. Overall results indicate that the variation in proximate landscape factors along elevation gradients, combined with other landscape factors such as habitat characteristics particularly, the availability of suitable host plants in the particular habitat is important for the assemblage of bumblebees. Moreover, the habitat features, typically the presence of suitable flowering plants were important in influencing the composition of *Bombus* species [28,75]. In this context, the landscape complexity along the elevation gradient is an important determining factor for the species composition, distribution and diversity of bumblebees in CHAL.

5. Conclusions

The habitat heterogeneity along elevation gradients in different river basins appeared to act as important environmental filtering for the community assembly of bumblebees in this region. Higher species richness was observed at a mid-elevation of study areas, likely driven by microhabitat structure with the availability of suitable food plants. It can be suggested that emerging risk factors such as rapid biological invasion and climate change are likely to alter the habitat of bumblebee communities along gradients. In the future, elevation shifting of suitable flowering plants will be the important limiting factor for shaping the species diversity and community composition of bumblebee species in this region. Our results are an important step toward a future assessment of species distribution and composition in the mid-Himalayas of Nepal. In this study, the sampling was restricted to major river basins along elevation gradients. Therefore, additional species are expected to be found in the other areas of CHAL. We further explain the effects of socio-ecological factors influence on assemblages of bumblebees at the local and regional scales in this area and we provide the suggestion for decision-makers to support their conservation strategies.

Author contribution statement

Kishor Chandra Ghimire: Conceived and designed the experiments; Performed the experiments; Contributed reagents, Analysed and interpreted the data, Wrote the paper, Anjeela Pandey: Performed the experiments, Ichha Roka: Performed the experiments, Jagan Nath Adhikari: Analysed and interpreted the data, Daya Ram Bhusal: Conceived and designed the experiments; Analysed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Species Composition and Conservation Risk Factors for Bumblebees (*Bombus* spp.) Across the Chitwan Annapurna Landscape, Nepal

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***Abstract*:** The study was conducted in Chitwan Annapurna Landscape(CHAL) to explore the species composition of bumblebee (*Bombus* spp.) and their conservation risk factors along elevation gradients. The field surveys were conducted in a range of different habitats along an altitudinal gradient (500 to 3500 m asl) in the Kaligandaki, Marsyangdi, and Budhigandaki river basins of study area. A total of 656 *Bombus* specimens were identified comprising 16 different species with eight new records (*Bombus grahami, B. pressus, B. branickii, B. cornutus, B. novus, B. turneri, B. lepidus, and B. asiaticus*) from this region. The highest relative abundance was of *B. haemorrhoidalis* followed by *B. festivus*. The major survival risks factors for bumblebee were habitat loss, ecosystem alternation by invasive plants, pesticide application and nesting sites destruction by many human activities. The severity of conservation risk factors varies along elevation gradient that determine on species filtering of bumblebee along the CHAL.

***Keywords*:** Bumblebee, Altitudinal gradient, Species distribution, Conservation implication

Bumblebees (Hymenoptera: Apidae) are an important group of pollinators in the alpine and subalpine regions of the world (Bingham and Ranker 2000, Yu et al 2012, Streinzer et al 2019). Although, the understanding of their dispersal limitation along altitudinal gradients is vastly lacking from many parts of the world. In mountainous regions, it is difficult to predict how bumblebees interact with local and landscape feature (Fourcade et al 2019). It is known that the diversity and distribution of bumblebee is strongly influenced by elevation gradients and the geographical location of the mountain, as well as the specific ecological adaptations of each species and their thermal tolerance (Burkle and Alarcón 2011). Beside this is also influenced by the human disturbance and other limiting ecological factors such as habitat alternation. The Himalayan range is a hotspot of bumblebee (Williams et al 2009, Bhusal 2020). However, taxonomic richness, vulnerability to climate change and other anthropogenic pressures remain poorly known (Williams 2009, Williams et al 2010, Saini et al 2015, Streinzer et al 2019), particularly, in heterogeneous landscapes. The declines of bumblebees were some have also shown the influence of landscape composition on bumblebee populations (Vray et al 2019). Similarly, the conservation risks of bumblebees are thought to be driven by a range of interacting human-induced threats, including habitat loss (and the associated loss of food and nesting resources), pesticide use, the introduction of new pathogens and non-native species, and the increasing threat posed by the climate change (Biesmeijer et al 2006, Goulson et al 2015, Potts et al 2016). Though the threats facing bumblebees and the impacts of their decline have been relatively well characterized in some parts of the world, they remain largely understudied in many parts of the world where the threats are often rapidly increasing and the impacts of insect pollinators decline are expected to be more severe (Timberlake and Morgan 2018). Within the Himalayan region, bumblebees have been intensively studied along altitudinal gradients, particularly in the West Himalaya (Saini et al 2015), but such studies are poorly documented in the Central and Himalaya (Williams et al 2010, Saini et al 2011). In the recent days, this region is facing the increasing threat of climate change and growing human pressure that directly and indirectly affects the biodiversity of this region, particularly, the high altitude ecosystem of this region are now threatened by intensive grazing, expansion of agricultural land and other rapid land use change (Telwala et al 2013, Sharma 2016). Furthermore, this region has high conservation risks of bumblebees that to be driven by a range of other human-induced threats, including loss of food and nesting resources, increasing pesticide use, the introduction of new pathogens and non-native species, and climate change (William et al 2010, Streinzer et al 2019, Bhusal 2020). However, the conservation issues of Bumblebee have been poorly reported from central Himalaya Nepal (Bhusal 2020). The present study was conducted in the Chitwan Annapurna Landscape (CHAL) in the central Nepal where altitudinal and climatic gradients are apparent, giving rise to a range of distinct ecological zones, each with their own unique assemblage of flowering plant species.

MATERIAL AND METHODS

Study area: This study was carried out along an altitudinal gradient (from 500 to 3500 m asl.) in three river valleys of the Chitwan Annapurna Landscape (CHAL): Kaligandaki (Mustang site 28◦87' 65.15" N - 83◦79' 47 65" E), Marshyandi (Manang site, 28◦57' 52. 62" N - 84◦18' 66. 28" E), and Budhigandaki (Gorkha site, 28◦18' 36.44" N - 84◦85'15.79" E) (Fig. 1). The CHAL region contains ranges from a subtropical monsoon climate with very high rainfall in the south (below 1000 m) to a temperate climate in the mid-hills (1000-4000 m) and an alpine/arctic climate with very low rainfall above 4000 m (Chhetri et al 2017). The area hosts diverse habitat types, including agriculture, forested, grassland, and human settlements. The study area is rich in biodiversity and includes the Annapurna conservation area which is an important transit route for migratory birds, as well as

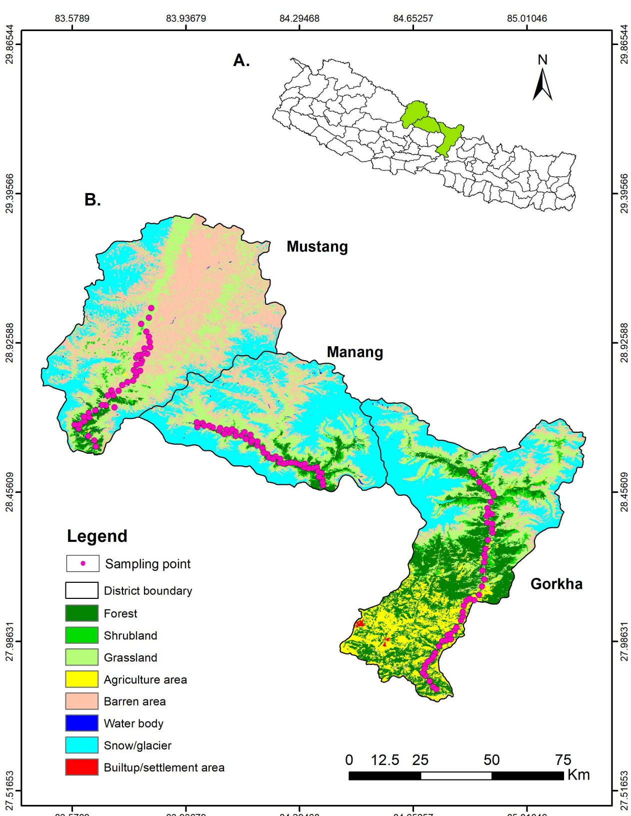


Fig. 1. Study area showing sampling points for bumblebee with in Kaligandaki, Marsyangdi and Budhigandaki River basins

supporting populations of various endangered species including the snow leopard, red panda, and the Himalayan black bear (Adhikari et al 2019, Chetri et al 2019). The landscape has a rich cultural heritage, with over four million people who have a high dependency on forest resources and ecosystem services for their livelihoods and well-being.

Bumblebee surveying and identification: Field surveys were conducted throughout the entire flowering season between April and November 2019 and followed three accessible walking routes (transects) along the river valleys of the Kaligandaki, Marshyandi, and Budhigandaki Rivers (Fig. 1). Opportunistic surveys were conducted along the three transects from 500 to 3500 m (Goulson et al 2005). Whenever a bumblebee was detected at a particular point along the route, we stopped and observed this point for up to one hour, or until the observer was satisfied that all possible species on the site were completely collected at a point for thirty minutes. Those individuals only collected which area foraged only in the floral parts. The survey was carried out between 9 am and 6 pm when rain was absent and wind speeds were low. *Bombus* species were captured using an entomological net and immediately killed using ethyl acetate. During the survey, habitat characteristics, host plant species, species frequency, and altitude and GPS location were recorded. Specimens were stored in airtight containers with a few layers of tissue and the addition of a few drops of ethyl alcohol to prevent the growth of mold during transport. Specimens were subsequently dry-mounted using standard insect pins and deposited in the Entomological Museum of the Central Department of Zoology, Tribhuvan University, and Kathmandu. The collected specimens were observed under stereoscopic microscope and identified using published identification keys for adjacent regions, e.g., Kashmir (Williams 1991), Nepal (2009), Sichuan (Williams et al 2009), North China (An et al 2014) and India (Saini et al 2015).

Conservation risk assessment for the bumblebee: Two approaches were applied to identify risk factors for the conservation of bumblebee in this area: (i) performed direct observation of habitat characteristics and noted presence and absence of particular threats in the local sampling sites across the altitudinal gradients and ii) conducted a household survey in the sampling sites which assessed local residents' experience-based perception of changes in local habitat characteristics, trends and patterns, perceived risk to pollinators, and attitudes towards pollinators. The data based semi structured questions about the history, severity and impact of these threats was collected. The survey was conducted on a total of 540 people, with 180 people from each site equally spread across the altitudinal range of our study area. The survey included respondents above 25 years of age and prioritized local farmers, teachers, social workers, shepherds and community forest user groups.

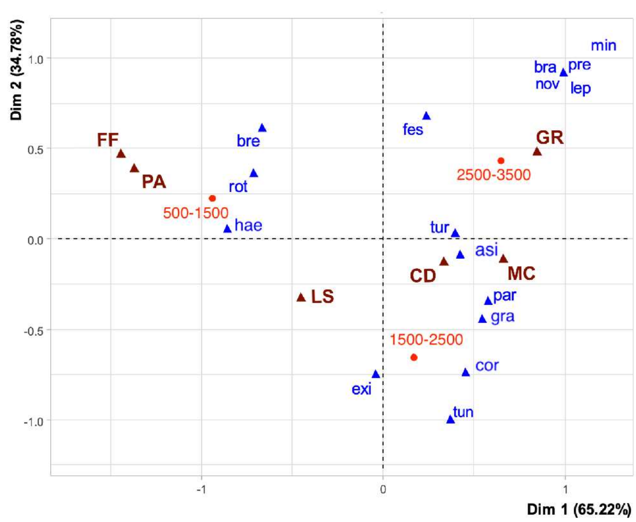
Data analysis: The relative composition of 16 Bombus species was determined. The non-parametric data from the habitat assessment were quantified using the cross tabulation (two-way contingency) to determine the frequency distribution of each threat within the three elevation gradients. The relative number of risk factors was produced and the thus produced data was used to performed for correspondence analysis (CA) among *Bombus* species, severity of threat, and three elevation gradients. All data were analyzed using R program (R core Team 2022).

RESULTS AND DISCUSSION

Species composition in study area: A total of 656 *Bombus* specimens were collected comprising 16 different species. In this study, eight species (*Bombus grahami, B. pressus, B. branickii, B. cornutus, B. novus, B. turneri, B. lepidus, and B. asiaticus*) were new records for the CHAL. In study area, the highest mean abundance (Levene's test: n = 136, df = 15, F = 3.40, P = 0.001) was observed *in B. Pressus*, *B. cormutus* and followed by *B. lepidus* and *B. branickii* (Table 1). Similarly, a lower mean abundance was observed in *B. tunicatus, B. eximius* and followed by the lowest mean abundance of *B. haemorrhoidalis.* Some species such *as B. festivus*, *B. grahami*, *B. asiaticus* and *B. lepidus* had a higher frequency variation within the study area (Fig. 2). The correspondence analysis (CA)was performed to determine the pattern of species, altitudinal gradients and associated risk factors. The species were well ordinated along the altitudinal gradients with specific associated risk factors present thereby. The highest species richness was in high altitude (16 species) followed by in mid altitude region (10 species), whereas, only 7 species recorded from the lower altitudinal gradients. Five species including *B. branickii*, *B. lepidus*, *B. miniatus*, *B. novus* and *B. pressus* were limited to the high elevation of the study area. Similarly, four other species (*B. cornutus*, *B. ghrami*, *B. parthenius* and *B. tunicatus*) were limited to mid and high elevations, with *B. tunicatus* most dominant at the mid elevation of the study area. Seven species *B. asiaticus*, *B. eximius*, *B. festivus, B. haemorrhoidalis, B. rotundiceps*, *B. turneri* were observed from all elevational gradients. The major survival risks for bumblebee were habitat loss by emerging invasive plants (IV), increasing trends of pesticide application (PA), colony destruction by local people (CD), increasing monocultures (MC), habitat loss landslides (LS), forest fire (FF), and intensive grazing (GR). However, the relative severity of each risk factors differed across the elevation gradient. The intensity (Friedman chi-square -X^2 = 33.807, P = 0.053) of these threats at each elevation was determined. The forest fire, pesticide application and invasive species (in particular *Ageratina adenophora* and *Parthenium hysterophorus*) were the most substantial threats at lower elevations (500- 1500 m) level of this landscape. Similarly, colony destruction by

Table 1. Relative abundance (%) of *Bombus* species and their mean elevation in sampling area and individual collection

Sites	WSA (%)	Mean elevation (m. above sea level) recorded
Bombus asiaticus	4.35 (29)	2556
B. branickii	0.72 (5)	2660
B. breviceps	5.07 (34)	2399
B. cornutus	2.17 (15)	2700
B. eximius	18.84 (124)	2257
B. festivus	19.57 (129)	2443
B. grahami	3.62 (24)	2452
B. haemorrhoidalis	20.29 (134)	2189
B. lepidus	3.62 (24)	2673
B. miniatus	0.72 (5)	2592
B. novus	0.72 (5)	2536
B. parthenius	2.17 (15)	2353
B. pressus	0.72 (5)	3000
B. rotundiceps	5.8 (39)	2121
B. tunicatus	5.07 (34)	2309
B. turneri	5.07 (34)	2353



Elevation level (m asl): (500-1500, 1500-2500, 2500-3500), The Risk factors are codded as: PA- pesticide application, IV- invasive plants, PA-pesticide application, CD- colony and nesting site destruction, MC- monocultures, LS-landslides, FF- forest fire, GR- intensive grazing

Fig. 2. Corresponding analysis (CA) for *Bombus* species abundance, specific elevation range and associated risk factors

local shepherds, emerging monocultures tendencies and pesticides application in commercial vegetables farms were the major risk factor at the mid elevation (1500-2500 m). Beside this, wider range of landslides occurrence was also recorded at the mid elevation region whilst at high elevations (2500- 3500 m) over-grazing and intense habitat loss by some human activities during herbal collection are notable risk factors for the important host plant for bumblebee. These factors impacting on species composition of bumblebee (Hoiss et al 2012, Sydenham et al 2015, Miller-Struttmann and Galen 2014). Furthermore, this might be linked with the critical thermal limits (Martinet et al 2015, Oyen et al 2016) of bumblebees which determine their altitudinal distribution (Dudley et al 2017). In this study, some of the species such as *B. asiaticus, B. eximius, B. festivus, B. haemorrhoidalis, B. rotundiceps, B. turneri* exhibited a particularly wider range i.e. from lower to higher altitudinal range in this study. Furthermore, this altitudinal variation in the distribution of *Bombus* species might be link with the critical thermal limits of these species driven by environmental temperatures (Oyen et al 2016), and habitat selection process along altitudinal gradients (Carvel 2002, Saini et al 2012, Diaz-Forero 2013, Goulson et al 2015). Similarly, the climate and land-cover change in this landscape probably alter the bumblebee species richness and community composition in CHAL (Fourcade et al 2019). The most abundant three species, *B. rotundiceps, B. haemorrhoidalis, B.eximius,* were observed at relatively low mean elevations as also reported by Williams et al (2009) and Streinzer et al (2019) in other parts of Himalaya. In case of *Bombus breviceps,* its mean altitudinal distribution in eastern sites was found to be at lower elevations, these species were recorded up to a similar altitudinal level in the western Himalaya (Saini et al 2015). Some of the species were confined to a relatively narrow altitudinal zone, or from specific sites, demonstrating their high specificity in this study area. For example, *B. asiaticus, B. branickii, B. cornutus, B. pressus, B. novus.* these species may be particularly adapted to unique microhabitats and vegetation types as they are restricted to a very limited altitudinal range in our study sites. In summary, altitude appears to act as an important environmental filter for the community assembly of bumblebees in area. In addition, vegetation dynamics, micro climatic variation, topographic factors and anthropogenic disturbance are also likely to be influencing bumblebee communities in this landscape.

Some of the species such as *B. breviceps, B. rotundiceps,* and *B. haemorrhoidalis,* appear mostly with lower altitudes having threats like FF, PA. At mid-altitudes, species *B. asiaticus, B. cornutus, B. parthenius, B. grahmi, B. tunicatus* are most prevalent, corresponding with CD and MC. At high altitudes, species *B, festivus, B. branicki, B. novus, B. pressus, B. Lepidus* and *B. miniatus* are most common, and the threats most likely to be encountered was GR. Many authors suggested, the habitat destruction by infrastructure development and the resulting changes in landscape configuration and permeability is likely to reduce the availability of food resources, hibernation and nesting sites for bumblebees (Kells and Goulson 2003, Otterstatter and Thomson 2008, Osborne et al 2008, Wermuth and Dupont 2010). At mid-altitudes, the colony destruction evidence, and extensive monocultures, were the important threats to bumblebee observed in recent years from CHAL region (Bhusal 2020). Periodic forest fires are a natural phenomenon in this region their increasing frequency and intensity as a result of human activities is a cause for concern, given their potential to destroy bumblebee nests and foraging habitat particularly at lower elevations. In the mid altitude region, shepherds frequently destroy bumblebee colonies using fire, to obtain protein sources from their larvae. Ultimately, this will have a negative impact on the habitats and food resources for bumblebees.

An additional threat identified from the lower to mid elevation gradients of our study area is the spread of rapidly-growing invasive plants species, such as *Ageratina adenophora and Parthenium hysterophorus*. In the last 10 years, these species have proliferated across the CHAL, particularly at lower elevations (below 1500 m), with negative effects on the local biodiversity (Sheathe et al 2019, Maharjan et al 2019) including native flora including for host plants of bumblebees. Some of the study from other parts of the world have listed invasive plants as an important potential driver of bee declines bee communities (Fiedler et al 2012, Morales et al 2013); however, the exact nature of their impacts upon bee populations and the mechanisms by which this occurs, remains unclear. Further studies are required to clarify the extent of the threat posed by invasive plants in this region and identify the likely effects on bumblebees. At high altitudes, species *B. festivus, B. branicki, B. novus, B. pressus, B. lepidus,* and *B. miniatus* are most common even in the high livestock grazing sites. The intense livestock grazing in the mid to high altitude regions of the study area were found to be degrading the important grassland ecosystem, likely reducing the availability of floral resources for bumblebees). Indeed, a recent study in the in the Western Himalaya showed that livestock grazing patterns can shift the abundance and community composition of grassland flora, with likely knock-on effects for insect pollinators (Hatfield and LeBuhn 2007, Hatfield 2007). The collection of the medicinal herb such as caterpillar fungus (*Ophiocordyceps sinensis*) is also being a high risk factor for the natural habitat of bumblebee bee of that region. While collecting this *O. sinensis,* thousands of people are deployed in the flowering seasons and search this herb digging on ground that may destroy suitable and specific foraging flowering plant for bumblebee and other bee communities leading to crisis on food plants.

CONCLUSION

It attributes the microclimatic and food resources change along the elevation gradient that majorly limit the shaping of distribution and diversity of Bombus species in study area. The severity and type of risk factors for the survival of bumblebees in this region vary along altitudinal level that affecting in species composition and abundance. The further identification species specific risk factors along elevation level and suitable mitigation approaches for the sustainable conservation of bumblebee and pollinator communities from CHAL is recommended.

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Research Article

FORAGING VARIATION OF *Bombus* SPECIES WITH PLANT FAMILIES AND FLORAL COLORS IN CHITWAN ANNAPURNA LANDSCAPE, NEPAL

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Abstract

Little is known about the effect of the type of vegetation and floral color on the foraging behavior of *Bombus* species. In this work, we have investigated the differential foraging association of bumblebees (*Bombus* spp.) with the specific flower color and the plant families in Chitwan-Annapurna Landscape (CHAL). The specimens were collected between April to October 2019 and field visits were carried out by following accessible walking transects between 600 to 3500 m asl covering different habitats of the study area. The bumblebees were collected by opportunistic methods using a sweeping net. We found that the relative frequency of *Bombus* spp. varied significantly with the families of local flowering plants and the particular colors of flowers. Some of the bumblebees visited at the specific plant family for nectar and pollen indicating the specific association with particular flower morphology and color. This study, therefore, gives an insight into the differential foraging preference of *Bombus* spp. to certain plant families with selected specific colors in CHAL.

Keywords: *Bombus* spp., CHAL, foraging, floral color, plant families, pollinators

INTRODUCTION

The impacts of multilevel spatial structure on pollination services have been addressed globally. And the global decline in bumblebee species is often linked with the loss or alteration of vegetation composition (Williams & Osborne, 2009). However, the foraging association of bumblebees with vegetation types, floral color, and structures within the specific plant family has been poorly explored. It is usually suggested that the flower choice behavior of bee communities for floral colors in particular plant families is influenced by their innate preferences and life experiences (Gumbert, 2000). However, there is a long debate on how *Bombus* species select a specific floral color and how they preferentially visit plants with selected floral traits (Lunau & Maier, 1995; Gumbert, 2000; Junker *et al*., 2013). They possess innate preferences for the recognized patterns of flowers, such as shape (Zeil, 1997) and flower symmetry (Giurfa *et al*., 1996). Bumblebees have trichromatic color vision with photoreceptors, maximally sensitive at about 350, 440, and 540 nm (Backhaus, 1991; Peitsch *et al*., 1992), and spontaneously use floral guides of natural flowers (Lunau, 1992; Lunau *et al*., 2006) of a specific color.

The floral structure and the floral scent are also described as important factors for the foraging attraction of many species of bumblebees (Odell *et al*., 1999). The bumblebees are also attracted to inflorescences, a plant–level attractant, based on the number of pollen-producing flowers (Thairu & Brunet, 2015). Bumblebees have varying tongue lengths depending on the species, which amongst other factors, determine their preferences for certain specific plants (Fussell & Corbet, 1991). Hence, tongue length is believed to be an important factor in niche separation in bumblebee communities (Hoelzel *et al*., 1989). The species with a long tongue can easily reach the stigma of the flower with a long corolla, while the short tongue bites the corolla and makes its way to stigma (Goulson & Williams, 2001).

Recently, it has been suggested that the color preferences by the key bee pollinators are one of the vital factors that tend to exhibit weak phylogenetic signals for facilitating the coexistence of related species, especially on a tropical–subtropical island (Tai *et al*., 2020) in the plant-pollinator interaction (Ibanez *et al*., 2016). Many authors have claimed the main color preference of bumblebees is a generalization and not absolute (Raine & Chittka, 2008; Brunet *et al*., 2015), although, bumblebee strongly prefers flowers with blue-purple colors (Raine *et al*., 2006; Rausher, 2008; Dyer *et al*., 2012). In other cases, many Bumblebee species prefer pink flowers of *Impatiens sulcate* (Balsaminaceae) over yellow-colored flowers of *Impatiens scabrida* (Balsaminaceae) when both of these plants are growing side by side (Saini *et al*., 2011). Several studies have also confirmed that some specific plants, for example, the legumes (Fabaceae), are in particular immensely selected by long-tongued bumblebees (Goulson *et al*., 2015; Hülsmann *et al*., 2015) whereas the most bumblebees visiting plant families in this other parts of the urban ecosystem of Nepal Himalaya were: Cucurbitaceae, Fabaceae, and Verbenaceae (Bhusal *et al*., 2019). In general, perennial plants are the major food resources for bumblebees (Fussell & Corbet, 1992; Dramstad & Fry, 1995; Leonhardt & Bluthgen, 2012); and bumblebees forage those plants that have noticed higher protein content (Leonhardt & Bluthgen, 2012; Somme *et al*., 2014; Vaudo *et al*., 2016) than the least visited plants species (Goulson *et al*., 2015). In the conservation issue of bumblebees under climate change scenario, it is urgent to identify the priority host plant for the foraging resources of Himalayan bumblebees is highly needed. Further, it is important to identify, how the preferred plant species diversity and composition are influential factors for determining the bumblebee abundance and diversity in different habitats in the Himalayan landscapes. This study aims to establish whether there exists any foraging preference between the flower color and particular plant families visited by the bumblebees in the Chitwan Annapurna landscape.

MATERIALS AND METHODS

Study area

This study (Fig. 1) was carried out along an altitudinal gradient (from 500 to 3500 m asl) in three river valleys of the Chitwan-Annapurna Landscape (CHAL), following Kaligandaki (west site), Marsyangdi (mid site), and Budhigandaki (east site) in central Nepal. These sites host diverse habitat types (agriculture, forest, grassland, and human settlements). The study area is rich in biodiversity and includes the Annapurna conservation area which is an important transit route for bird migration, as well as for many endangered species including the snow leopard, red panda, and the Himalayan black bear (Oli *et al*., 1994; Chetri *et al*., 2019). This area is particularly facing many threats under current climate change, and experiencing the rapid invasion of some notorious invasive plants (Bhusal, 2020; Sharma *et al*., 2020; Shrestha & Shrestha, 2021) that might be affecting the pollination process and native floral composition.

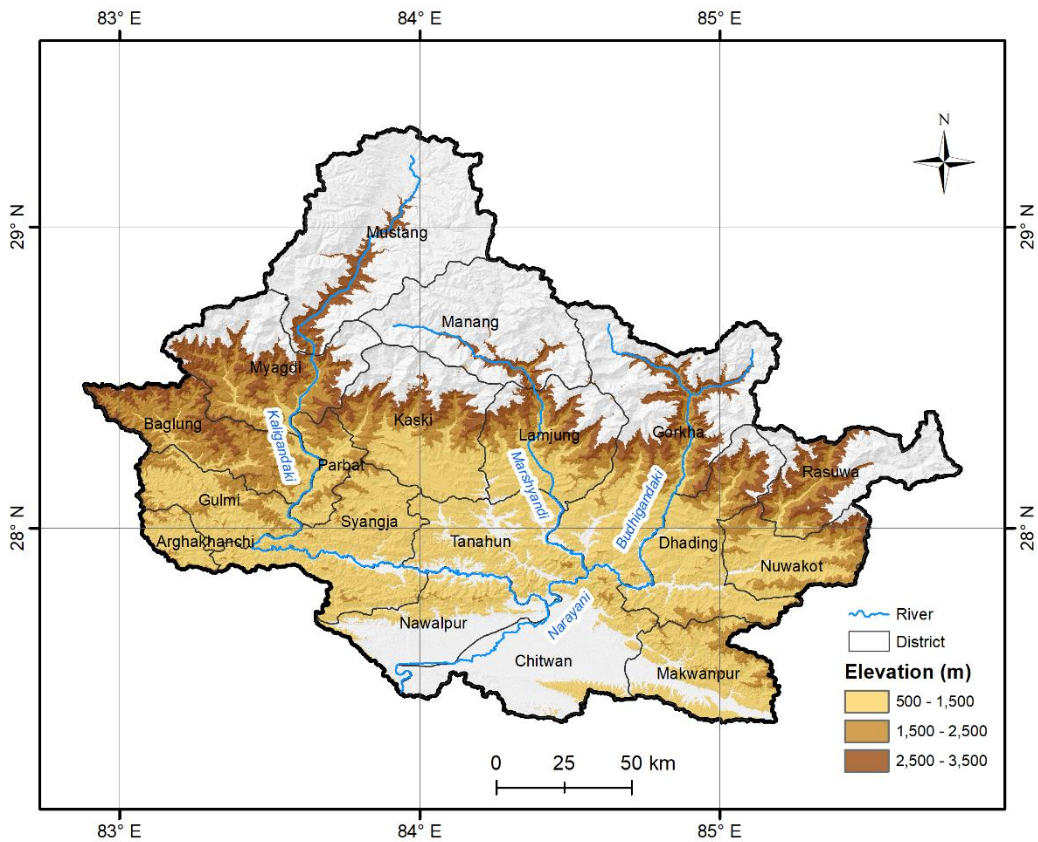

Figure 1. Map of study area showing walking transects(Kaligandaki, Mashyandi and Budhigandaki region)

Bumblebee surveying and identification

Field surveys were conducted throughout the entire flowering season between April and November 2019. We followed three accessible walking routes (transects) along with the river valley sites (Kaligandaki, Marsyangdi, and Budhigandaki) of the study area. Extensive surveys were conducted along the three walking transects from 500 to 3500 m asl. Whenever a foraging bumblebee was detected at a particular point along the walking route, we observed around the flowering plants present there and the bumblebees on the site were collected and recorded their number. The survey was carried out between 9 am and 6 pm (especially morning and afternoon time) when rain was absent and the wind speed was low. *Bombus* spp. were captured using an entomological net, and were immediately killed by using ethyl acetate. During the survey, the habitat type (Agriculture, human settlement, grassland, and forest), altitude, and GPS locations of the collection points were recorded. Specimens were stored in airtight containers with a few layers of tissue paper and the addition of a few drops of ethyl alcohol to prevent the growth of mold during transportation. Specimens were subsequently dry–mounted using standard insect pins and deposited in the Entomological Museum of the Central Department of Zoology, Tribhuvan University, Kathmandu. We collected 600 individual workers, and the collected specimens were observed under a stereoscopic microscope and identified using published identification keys for adjacent regions (Hanley *et al*., 2008; Williams *et al.,* 2010; An *et al*., 2014; Saini *et al*., 2015).

Data Analysis

We performed the cross-tabulation (two–way contingency) to find out the relative frequency of bumblebees with foraging plant families and corresponding floral colors. The Chi-square (χ^2) level of significance (<0.05) was observed for each contingency table. Similarly, we analyzed the effect of bumblebee number with families of foraging plants by performing a linear mix effective model (lmm) in **lme4** package in R. The cluster analysis (CA) and corresponding analysis (CA) were also performed to explore how closely the *Bombus* species were associated the particular plant families and the color of their flowers.

RESULTS

Foraging association of bumblebees and plant families

The relative frequency of bumblebees and their foraging plant families was cross-tabulated from the samples we got (χ^2 = 579.68, df = 448, p-value < 0.001). Some *Bombus* species were recorded only from the single plant families (Table 1). The bumblebee species, *Bombus branickii* was recorded from the Rosaceae family's plants. The *Bombus miniatus* and *B. haemorrhoidalis* were recorded only from the single plant family Polygonaceae. Likewise, *B. novus* was recorded only in the family Ranunculaceae whereas, *B. pressus* were found to be associated mainly with plants Rosaceae. In our study sites. Moreover, *B. flavescens* was recorded from only two plant families, viz. Asteraceae and Lamiaceae. In the same way, *B. cornutus* was recorded from three plant families such as Ranunculaceae, Oleaceae, and Gesneriaceae. Likewise, *B. parthenius* was recorded from Balsaminaceae, Lamiaceae, and Oleaceae; *B. lepidus* were noted from four plant families (Oleaceae, Ranunculaceae, Fabaceae, and Rosaceae); and *B. turneri* from four plant families (Ranunculaceae, Solanaceae, Fabaceae, and Rosaceae). *Bombus grahami* was recorded wider plant species representing from five plant families as Gesneriaceae, Ericaceae, Papaveraceae, Lamiaceae, and Ranunculaceae. Similarly, the *B. tunicatus* was recorded from six plant families: Asteraceae, Oleaceae, Ranunculaceae, Solanaceae, Lamiaceae, and Verbenaceae. *Bombus asiaticus* and *B. rotundiceps* were recorded from a large number of plant families such as Ophiopogon, Cactaceae, Solanaceae, Oleaceae, Fabaceae, Balsaminaceae, Rosaceae, and Asteraceae. On the other hand, the *B. asiaticus* has commonly exhibited foraging association with Ranunculaceae, Solanaceae, Fabaceae, Rosaceae, Oleaceae, Asteraceae, Cucurbitaceae, and Balsaminaceae. *Bombus breviceps* was recorded from nine plant families: Convolvulaceae, Lythraceae, Oleaceae, Fabaceae, Balsaminaceae, Rosaceae, Asteraceae, Lamiaceae, and Cucurbitaceae. In the same way, *B. festivus* was found most commonly representing plant species from eleven families such as Cannabaceae, Ericaceae, Malvaceae, Liliaceae, Polygonaceae, Ranunculaceae, Solanaceae, Oleaceae, Fabaceae, Rosaceae, and Asteraceae. *Bombus eximus* was recorded from 14 plant families: Acanthaceae, Gesneriaceae, Laureceae, Veronicaceae, Viburnaceae, Lamiaceae, Ranunculaceae, Oleaceae, Fabaceae, Balsaminaceae, Cucurbitaceae, Rosaceae, and Asteraceae. However, *B. haemorrhoidalis* was recorded from nineteen plants families of Acanthaceae, Apocynaceae, Cannabaceae, Compositae, Genenculeceae, Iridaceae, Malvaceae, Polygonaceae, Verbenaceae, Convolvulaceae, Lythraceae, Lamiaceae, Solanaceae, Oleaceae, Fabaceae, Balsaminaceae, Cucurbitaceae, Rosaceae, and Asteraceae. In our study, most foraging families by *Bombus* species were Rosaceae, Oleaceae, Ranunculaceae, Fabaceae, and Asteraceae; and the least foraging plant families were Apocynaceae, Cactaceae, Cannabaceae, Iridaceae, Lauraceae, Liliaceae, Ophiopogon, Papaveraceae, Verbenaceae, and Viburnaceae.

The plant families were classified in cluster analysis (Fig. 2) based on the relative abundance of *Bombus* species (χ^2= 579.68, df=448, p < 0.001). A total of 29 families of foraging plants were clustered.

Relative foraging association of bumblebees with plant families and colors

In our cluster analysis, some plant families and bumblebees exhibited relatively lower foraging associations. These plant families were Polygonaceae, Cannaceae, Liliaceae, Gesneriaceae, Ericaceae, Papaveraceae, Iridaceae, Apocynaceae, Compositae, Cactaceae Ophiopogon, Viburnaceae, Veronicaceae, Acanthaceae, Cannabaceae, Lauraceae. Similarly, twelve plant families in the sampling sites were recorded with a higher number of foraging associations with the identified bumblebee species. These were Asteraceae, Cucurbitaceae, Solanaceae, Balsaminaceae, Rosaceae, Fabaceae, Oleaceae, Ranunculaceae, Malvaceae, Verbeceae, Lythraceae, and Convulvulaceae.

Table 1. Presence - absence status of *Bombu*s species with their foraging plant family. *Bombus* species: *Bombus asiaticus(asi), Bombus branickii (bra), Bombus breviceps(bre), Bombus cornutus(cor), Bombus eximius(exi),, Bombus festivus(fes), Bombus graham(gra), Bombus haemorrhoidalis(hae), Bombus Lepidus(lep), Bombus miniatus(min), Bombus novus(nov), Bombus parthenius(par), Bombus pressus(pre), Bombus rotundiceps(rot), Bombus tunicatus(tun), Bombus turneri(tur)*

Families	asi	bra	bre	cor	exi	fes	fla	gra	hae	lep	min	nov	par	pre	rot	tun	tur
Fabaceae	+	–	+	–	+	+	–	–	+	+	–	–	–	–	+	–	+
Acanthaceae	–	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–
Apocynaceae	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
Asteraceae	+	–	+	–	+	+	+	–	+	–	–	–	–	–	+	+	–
Balsaminaceae	+	–	+	–	+	–	–	–	+	–	–	–	+	–	+	–	–
Cactaceae	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–
Cannabaceae	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–
Cannaceae	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Compositae	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
Convolvulaceae	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–
Cucurbitaceae	+	–	+	–	+	–	–	–	+	–	–	–	–	–	–	–	–
Ericaceae	–	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–	–
Gesneriaceae	–	–	–	+	+	–	–	+	+	–	–	–	–	–	–	–	–
Iridaceae	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
Lamiaceae	–	–	+	–	+	–	+	+	+	–	–	–	+	–	–	+	–
Lauraceae	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Liliaceae	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
Lythraceae	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–
Malvaceae	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–
Oleaceae	+	–	+	+	+	+	–	–	+	+	–	–	+	–	+	+	–
Ophiopogon	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–
Papaveraceae	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
Polygonaceae	–	–	–	–	–	+	–	–	+	–	+	–	–	–	–	–	–
Ranunculaceae	+	–	–	+	+	+	–	+	–	+	–	+	–	–	–	+	+
Rosaceae	+	+	+	–	+	+	–	–	+	+	–	–	–	+	+	–	+
Solanaceae	+	–	–	–	–	+	–	–	+	–	–	–	–	–	+	+	+
Verbenaceae	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
Veronicaceae	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–
Viburnaceae	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–

Relative foraging preference of bumblebees with floral colors

We recorded the particular color where bumblebees mostly prefer to visit. In the cluster diagram, we found that most of the bumblebee species preferred white, yellow, and purple color flowers (Fig. 3). Whereas, orange, red, blue, and pink colors were relatively less visited by the *Bombus* species in our field records. Furthermore, we performed a corresponding analysis (Fig. 4) to find out the relationship between the *Bombus* species and the floral color preference. The relative frequency of *Bombus* species was cross-tabulated (χ^2 = 97.24112, $p<0.05$), between the floral colors and the relative visiting frequency of *Bombus* species. There indicated, 12 species of bumblebees mostly prefer to visit white-colored flowers. However, *Bombus cornutus*, *B. eximus*, and *B. haemorrhoidalis* were preferring the yellow flowers. While, *B. grahami* preferred mostly the red–colored flowers, *B. breviceps* foraged highly on the purple flowers. Similarly, blue and orange were seen as less-visited flowers by many species of identified bumblebees.

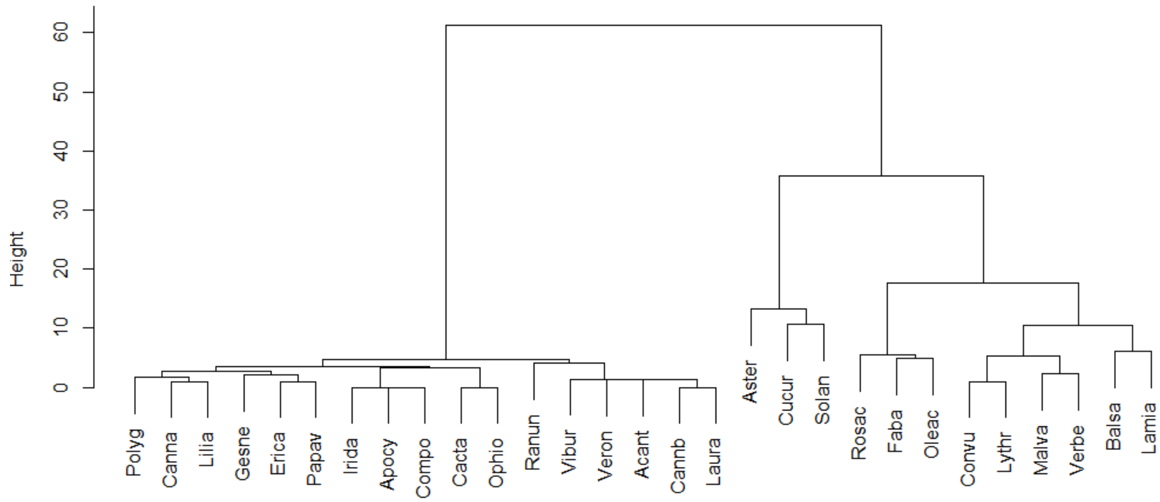

Figure 2. Cluster analysis (method= ward, distance = Euclidean) of plant families based on the relative abundance of *Bombus* species. Plant families are: *Fabaceae (Faba), Acanthaceae (Acant), Apocynaceae (Apocy), Asteraceae (Aster), Balsaminaceae, Cactaceae (Cacta), Cannabaceae (Cannb),, Cannaceae (Canna), Compositae (Compo), Convulvulaceae (Convu), Cucurbitaceae (Cucur), Ericaceae (Erica), Gesneriaceae (Gesne), Iridaceae (irida),, Lamiaceae (Lamia), Lauraceae (Laura), Liliaceae (Lilla), Lythraceae (Lythr), Malvaceae (Malva), Oleaceae (Oleac), Ophiopogon (Ophio), Papaveraceae (Papav), Polygonaceae (polyg), Ranunculaceae (Ranun), Rosaceae (Rosac), Solanaceae (Solan), Verbenceae (Verbe), Veronicaceae (Veron), Viburnaceae (Vibur).*

Table 2. Foraging relation (linear mixed model) of *Bombus* species and with colours and plant families

Fixed factors (Colour and Plant families)	Estimate	Std.Error	t value
(Intercept)	10.1192	1.72251	5.875
Orange colour	–2.97649	4.09755	–0.726
Pink colour	–4.21245	2.39768	–1.757
Purple colour	0.01049	1.7703	0.006
Red colour	–2.22082	2.22836	–0.997
White colour	–1.43949	1.66404	–0.865
Yellow yolour	–1.03265	1.76356	–0.586
Acanthaceae	–8.48302	3.34983	–2.532
Apocynaceae	–5.6876	4.41702	–1.288
Asteraceae	–4.39638	1.28474	–3.422
Balsaminaceae	–4.44065	1.68987	–2.628
Cactaceae	–3.6876	4.41702	–0.835
cannabaceae	–4.0067	3.58663	–1.117
Cannaceae	–7.78532	4.32607	–1.8
Compositae	–7.28077	4.32833	–1.682
Convulvulaceae	–4.43046	1.95781	–2.263
Cucurbitaceae	–3.11777	1.68974	–1.845
Ericaceae	–3.03304	3.14858	–0.963
Gesneriaceae	–7.36038	2.54733	–2.889
Iridaceae	–6.69487	4.43195	–1.511
Lamiaceae	–4.93624	1.83437	–2.691
Lauraceae	–7.77157	4.42314	–1.757
Liliaceae	–6.81471	4.48232	–1.52
Lythraceae	0.32098	2.43447	0.132

Malvaceae	–4.75103	2.55619	–1.859
Oleaceae	–2.04918	1.67733	–1.222
Ophiopogon	–7.28077	4.32833	–1.682
Papaveraceae	–0.19215	4.42324	–0.043
Polygonaceae	–0.81138	2.36456	–0.343
Ranunculaceae	–3.79444	1.77825	–2.134
Rosaceae	–2.58771	1.50101	–1.724
Solanaceae	–2.3222	1.36507	–1.701
Verbenceae	1.20654	2.21716	0.544
Veronicaceae	–7.78803	3.18993	–2.441
Viburnaceae	–7.53304	3.14858	–2.393

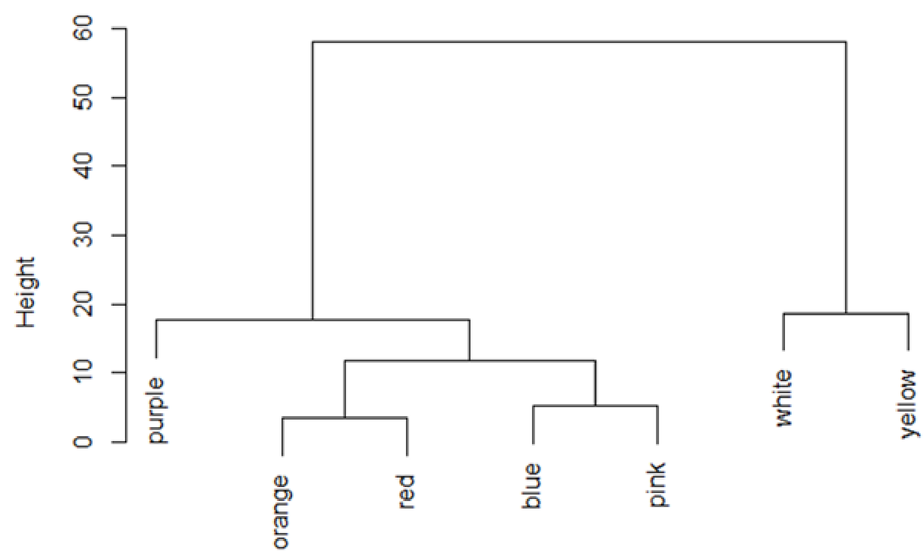

Figure 3. Clustering of flower colors based on the relative frequency of bumblebees

DISCUSSION

Our results indicated that the flower morphology and color of a particular plant family is an important factor to determine the foraging association of Bombus species. Some previous studies have also suggested that family-level variation was distinct in many *Bombus* species especially, family Fabaceae and Verbenaceae which were important plants family visited by a wider number of Bombus species (Westphal *et al*., 2003; Hanley *et al*., 2008). In our study, the most foraging plant families were Rosaceae, Oleaceae, Ranunculaceae, Fabaceae, and Asteraceae whereas, the least foraging plant families were Apocynaceae, Cactaceae, Cannabaceae, Iridaceae, Lauraceae, Liliaceae, Ophiopogon, Papaveraceae, Verbenaceae, and Viburnaceae. Among these, the family Rosacea was highly visited by bumblebee species in many parts of the world (Somme *et al*., 2014). The differential visiting preference by bumblebees is generally conceded with the flower scent, which is an important long-distance signal for native bees (Heinrich *et al*., 1977; Dötter *et al*., 2005). Probably, the floral traits and resources are the determining factors that alter the species foraging selection and community dynamics of bumblebees (Potts *et al*., 2010). Many authors have suggested the effect of morphological characters of flowers and bees, such as corolla length and proboscis on the rate of flower visitation by bumblebees, where short-tongued bees foraged faster on short–corolla flowers than the long-tongued bees. Many studies have suggested the availability of floral resources for frequent visitation of the bumblebee to some plant families such as Oleaceae that alters the foraging preference of bee communities (Bäckman & Tiainea, 2002; Pywell *et al*., 2005). As suggested, it is probably related to the flower structures and the resources rewarded. The floral structures, such as symmetrical and asymmetrical flowers are also important for the nectar production that affect the foraging selection by many insect pollinators (Møller, 1995; Goulson *et al*., 2015; Reininghaus, 2017). A recent study has suggested that the temperature differences across the floral surface, which may be due to floral architecture constraints within the plant families, might be the important factor for the foraging preference of bumblebee species (Rands & Harrap, 2021). On the other hand, the phonological period of many plant species and colonial peaks are majorly important (Reininghaus, 2017) for the foraging association of bumblebees and plants types. Seasonal and spatial variation in the composition of floral resources may pose challenges for bumblebees' foraging selection in this mosaic landscape. Plant species within habitats at different times lead to temporal turnover in the floral assemblage, which itself may vary spatially that might affect foraging discrimination in plant and bumblebees (Simanonok & Burkle, 2014). However, there is a long debate regarding the native and non-native plant and bumblebees foraging association in different parts of the world (Giurfa *et al*., 1996; Raine & Chittka, 2007; Schiestl & Johnson, 2013). Probably the bee individuals can exhibit the higher and more general visiting behavior in native plant families rather than the non-native plant species.

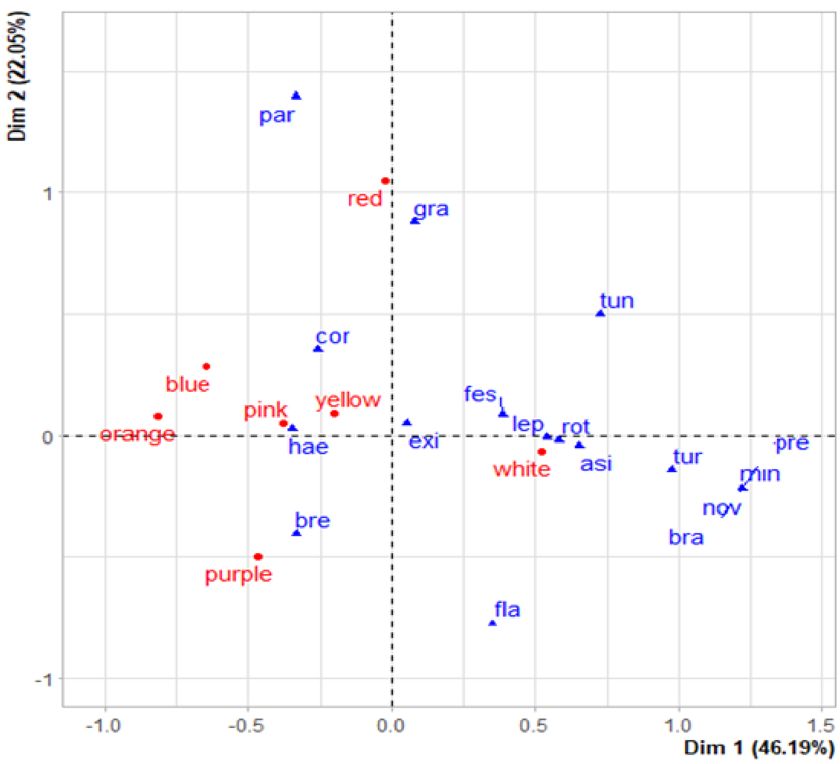

Figure 4. Corresponding Analysis based on the relative frequency of *Bombus* species and flower color. The Bombus species are: *Bombus asiaticus(asi), Bombus branickii(bra), Bombus breviceps(bre), Bombus cornutus(cor), Bombus eximius(exi),, Bombus festivus(fes), Bombus graham(gra), Bombus haemorrhoidalis(hae), Bombus Lepidus(lep), Bombus miniatus(min), Bombus novus(nov), Bombus parthenius(par), Bombus pressus(pre), Bombus rotundiceps(rot), Bombus tunicatus(tun), Bombus turneri(tur)*

On the other hand, flower color is often one of the main traits that majorly determine the foraging association of bee communities including the bumblebees (Raine & Chittka, 2007; Schiestl & Johnson, 2013). Colors appear to be particularly important to bumblebees for flower recognition (Menzel & Shmida, 1993; Spaethe *et al*., 2001; Dyer & Chittka, 2004). In this study, the higher foraging intensity was towards the white and yellow-colored flowers by the bumblebees, indicating that they prefer more saturated colors over the less saturated ones (Rohde *et al*., 2013), and those of high purity (Lunau *et al*., 1996). Additionally, the bees' communities have a trichromatic color vision in the UV light (Spaethe *et al*., 2001; Dyer & Chittka, 2004; Hempel *et al*., 2014) that might be responsive to white and yellow flowers because these flowers are melittophilous and regularly absorb UV light (Kevan *et al*., 1996).

CONCLUSIONS

The effects of plant families and the color of flowers are important explanatory factors for the foraging association of bumblebees with particular plant families in the study area. The structures of flowers and resources rewarded by different plant families and associated specific colors of the flowers are vital in determining the foraging preference by *Bombus* species. This study indicated the differential foraging association by *Bombus* species to the plant families across the landscape scale. This is important to identify the priority-based plant families in local and across landscape scales for the foraging preference by bumblebee species and can be implemented in conservation programs. In the future, the experimental approach is needed along the altitudinal gradients to extract a more conclusive idea regarding the foraging choice of *Bombus* species to the particular plant families species.

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AUTHOR CONTRIBUTIONS
KCG contributed for data collection, taxonomic work, data analysis and manuscript writing. AP contributed to the fieldwork and taxonomic work for bumblebees. GDJ contributed for the plant identification and data analysis. DRB performed data analysis and manuscript writing and visualization.

CONFLICT OF INTEREST
There is no conflict of interest between the authors in this publication.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Foraging Discrepancy of *Bombus* Species with Native Versus Non–native Flora in Chitwan Annapurna Landscape, Nepal

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ABSTRACT

We compared the foraging frequency of bumblebee (*Bombus* spp) with native versus non-native flowring plants in Chitwan Annapurna Landscape (CHAL). Specimens were collected using a sweeping net along the altitudinal gradients following an accessible walking trail between 600 to 3500 m asl. The random sampling was carried in different habitat types (agriculture, forest, grassland and home garden) along the transects. The relative frequency of *Bombus* species was significantly vary with the flowering plant (native types and non native cultivated, non native naturalized and invasive) in sampling rout along the altitudinal gradients. Some *Bombus* species were found to be foraged only to the specific plants within the study sites indicating that the specific flowering preference. The overall results indicated the differential foraging choice of *Bombus* species with native and non-native plant communities in the study area.

Key words: pollinators, foraging, host plants, *Bombus* spp, CHAL

INTRODUCTION

Many studies documented declines in insect pollinators, including bumblebees Bombus spp., globally (Goulson et al. 2015, Potts et al. 2016). Many factors are known to be driving this decline: land use change, habitat loss, pesticide use, biological invasion, pathogens; and possibly climate change (Goulson et al. 2005, 2015). Insect pollinators exhibit flower-visiting behaviors that influence on pollen dispersal and reproductive success of many plants. The spatial distribution, diversity and abundance of floral resources may also be important for maintaining community composition of insect pollinators including bumblebees (Osborne et al. 1999, Walther-Hellwig and Frankl 2000, Bhusal et al. 2019). Many ecological factors and plant features have been explained regarding the relationship between bee communities and the foraging host plants that crucial factors to alter the species richness, species diversity and abundance of the bumblebees (Potts et al. 2010) in the different habitats. The impacts of exotic plants on the pollination and reproductive success of natives have been widely reported; however, in spite of its importance for the invasive process, the role of native plants in the pollination and reproduction of exotic plants has been explored in different by different researchrs. However, knowledge is limited how the choice of flowers, foraging time, and the number of flowers visited by pollinators is determined to the success of pollination. In the current global change scenario, it is critical to understand how insect pollinators are affected by plant invasions (Carvallo et al. 2013) and vice versa.

Bumblebees forage in a very complex and and highly dynamics environment, where they obtain nectar and pollen vary widely among plant species. These resources are accompanied by myriad floral cues, including odors, color of flower, morphology, and spatiotemporal availability of host plants that may influence on foraging decisions(Brunet et al. 2015, Vaudo et al. 2015). It is known that the nonnative plants have the potential to cause direct and indirect impacts on those species, as well as the functioning of ecosystems (Mack et al. 2000). Some previous studies suggested that invasive plant species generally reduce the abundance and diversity of local and native plant species, which may affect alterations of pollinator communities (Biesmeijer et al 2006, Geib and Galen 2012, Geib et al. 2015). Bumblebees (*Bombus* spp) are widespread, efficient pollinators that are important for many native plant species and the reproductive success of many local crop species. The foraging relation of bumblebees is often difficult to investigate since it might be affected by range shifts of native flora, change of floral composition, and phenology under the current climate change scenario (Ogilvie 2017, Woodard 2017, Suzuki-Ohno et al. 2020). However, the mechanism of these interactions is not fully understood. The morphology, colour of the flower, and the foraging Bombus species are also important for the determination of the foraging behavior and the flower choice by bumblebees (Inouye 1980, Simonds and Plowright 2004, Raine and Chittka 2005). For example: the presence of pollen baskets on the hind legs, mandibles and a long tongue used to remove pollen grain from anther and to moisten the pollen grains with regurgitated droplet of nectar are some specific adaptive features of true pollinators community for the pollination success. Foraging behavior and preference by different bumblebee to a specific plants species with the particular evolutionary features such as shape and size of corolla and plant functional features also play a key feature in predicting reproductive success (Gomez et al. 2008, Ordano et al. 2008, Córdoba and Cocucci 2011). Many other ecological factors, the diversity and abundance of flora locally available to bumblebees, the distance between the nesting sites and foraging plants (Dramstad 1996, Walther-Hellwig and Frankl 2000, Goulson and Stout 2001) are also the notable factors for the foraging choice of many Bombus species. The foraging patterns of bumblebees especially focusing on native versus non-native flowering plants across altitudinal gradients is poorly studied theme from Hindu Kush Himalaya (Bhusal 2020) region including central Himalaya, Nepal. In this context, the exploration regarding the foraging choice of bumblebees is crucial especially with limited historical and current occurrence data from this region.

The floral abundance and density also affect the species-specific foraging patterns especially in alpine bumblebees (Shibata and Kudo 2020). There might be a functional relationship between flower characters and colour and particular bumblebee species. The Bombus species were significantly affected by the families of the host plants in both (open and closed) type of floral structures under high pressure of urbanization and land use change (Hulsmann et al. 2015, Bhusal et al. 2019). There is a growing debate on the benefit of native and non-native plants in landscapes levels especially for the pollinator's management concerns It is generally suggested that native plants support greater faunal diversity and biomass than non-native planting (Burghardt et al. 2008). It is known that non native species have many advantages over native species, such as faster growth and reproduction rates, higher ecological tolerance, or more effective dispersal mechanisms (Sladonja et al. 2018). The correlations between floral traits and pollen reward affected pollinator preferences and may facilitate selection of floral traits by specific bumblebee (Brunet et al. 2015). The outward preference of native fauna for native flora is often explained based on the long history of the association, the two having coevolved over millions of years While plants have evolved a variety of physical and chemical means to attract pollinators, those insects that have co-evolved with them are believed to be much more likely to have the specialized behavioral and physiological adaptations (Tallamy 2004). The co-evolutionary explanation may again partly support the apparent differences in pollinator preference between native and non-native plant species. The foraging response of Bombus species depends upon the colour, morphology and adaptation of flower-and bumblebee. With respect to the qualitative and quantitative changes in floral resources, bumblebees extensively forage multiple plant species. In general, studies of impacts of nonnative plant species on bumblebees' communities are much harder to perform It is suggested that non-native plants are a novel resource for the resident pollinator community, but may not be able to successfully attract pollinators if they have a specialized floral morphology (Bode et al. 2020). Some non-native provides benefits (including the economic and environmental benefits of increased pollination services) but others have negative impacts on native pollinators (Stout and Morales 2009). Impacts of invasive alien species on specialist and generalist bee taxa may differ and probably vary according to landscape context. Pollinator limitation may limit invasion success, but a plant able to attract many pollinators in different conditions will be a successful invasive species.

In this context, we hypothesized in this study that the foraging preference of Bumblebees (*Bombus* species) vary with native versus non-native plant species in the human-dominated heterogeneous Chitwan annapurna landscape (CHAL) Nepal.

MATERIALS AND METHODS

Study area

This study (Fig. 1) was carried out along an altitudinal gradient (from 500 to 3500 m asl) in three river valleys of the Chitwan Annapurna Landscape (CHAL) following Kaligandaki (west site), Marsyangdi (mid site), and Budhigandaki (east site) in central Nepal. These sites host diverse habitat types (agriculture, forest, grassland, and human settlements). The study area is rich in biodiversity and includes the Annapurna conservation area which is an important transit route for bird's migration, as well as for many endangered species including the snow leopard, red panda, and the Himalayan black bear (Oli et al. 1994, Adhikari et al 2019, Chetri et al. 2019). The landscape has a rich cultural heritage that harbors millions of people who have a high dependency on forest resources and ecosystem services especially on agriculture production and destruction of natural habitat by infrastructure development and land-use change. This area is particularly facing many threats under current climate change and facing the rapid invasion of some notorious invasive plants (Pandey et al. 2020, Bhusal et al. 2020) that might be affecting on native floral composition in term of reproductive success and competition for natural resources affecting on physiological and morphological abnormality that ultimately distressing on pollination process. In the long run, this competition adversely affecting on the maintenance of plant-pollinators interaction and ecosystem service of this landscape.

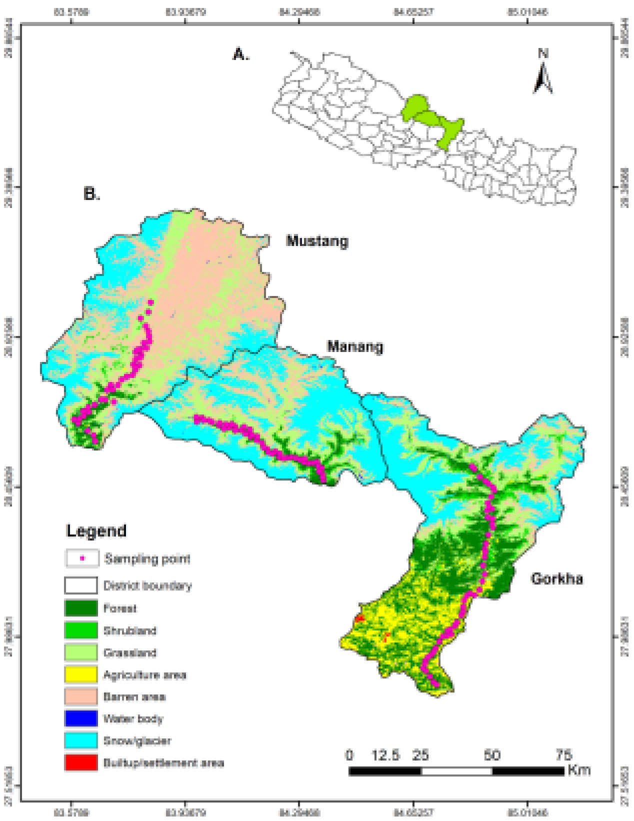

Figure 1. Study area showing GPS points of bumblebee collection

Bumblebee surveying and identification

Field surveys were conducted throughout the entire flowering season between April and November 2019. We followed three accessible walking routes (transects) along with the river valley sites (Kaligandaki, Marsyangdi, and Budhigandaki) of the study area. Extensive surveys were conducted along the three walking transects from 500 to 3500 m asl. Whenever a foraging bumblebee was detected at a particular point along the walking route, we observed around the flowering plants present there and the bumblebees on the site were collected and recorded. The survey was carried out between 9 hrs and 18 hrs (especially morning and afternoon time) when rain was absent and wind speeds were low. *Bombus* species were captured using an entomological net and immediately killed using ethyl acetate. During the survey, we also noted habitat types (agriculture, human settlement, and forest) altitude, and GPS location of the collection points were recorded. Specimens were stored in airtight containers with a few layers of tissue and the addition of a few drops of ethyl alcohol to prevent the growth of mold during transport. Specimens were subsequently dry-mounted using standard insect pins and deposited in the Entomological Museum of the Central Department of Zoology, Tribhuvan University, and Kathmandu (www.cdztu.edu.np). We collected 600 individuals workers and collected specimens were observed under a stereoscopic microscope and identified using published identification keys for adjacent regions (Williams 1991, Williams et al. 2009, 2010, An et al. 2014), and India (Saini et al.

2015)

Data analyses

The relative frequency composition of identified *Bombus* species was analyssed. We also, identificed plant species and were cataegorised: native type (NAT) and non native type. The non native species were further categoried into non native cultivated (CUL), non native invasive (INV) and non native naturalized (NNU). Similarly, the habitat types (forest, home garden, agriculture and grassland) were classified. We further divided the nature of the flower into open and close types of flowers. The number of foraging observation (response variables) of particular *Bombus* species with their host plants across the walking transit was analysised by performing linear mix effective model (lmm) in lme4 package in R. The category, habitat and nature of flower were applied and explanatory variables. A crosstab (two-way contingency table) was prepared between *Bombus* species (at the row) and category of plants and habitat types (at the Column). Meanwhile, the Chi-square (χ^2) level of significance (<005) was observed for each contingency table. Thus prepared data were subjected for cluster analysis and corresponding analysis (CA) to explore how closely *Bombus* species related to the particular habitat types and categories of foraging plants in the study area. Similarly, we calculated relative foraging presence of *Bombus* species in different flowering plant. If *Bombus* species were recorded more than 20 times in our sample categoried as very common (VC), if it is 10 to 20 categoried as common (C), if it is between 5 to 10 that it is categoried as rare (R), and if it is less than 5 categoried as very rare (VR) (Supplementary table 1).

RESULTS

We catagoried all the identified plants native and non native (INV, CUL, NNU) types of plants. The sum of the relative frequency conservation within non

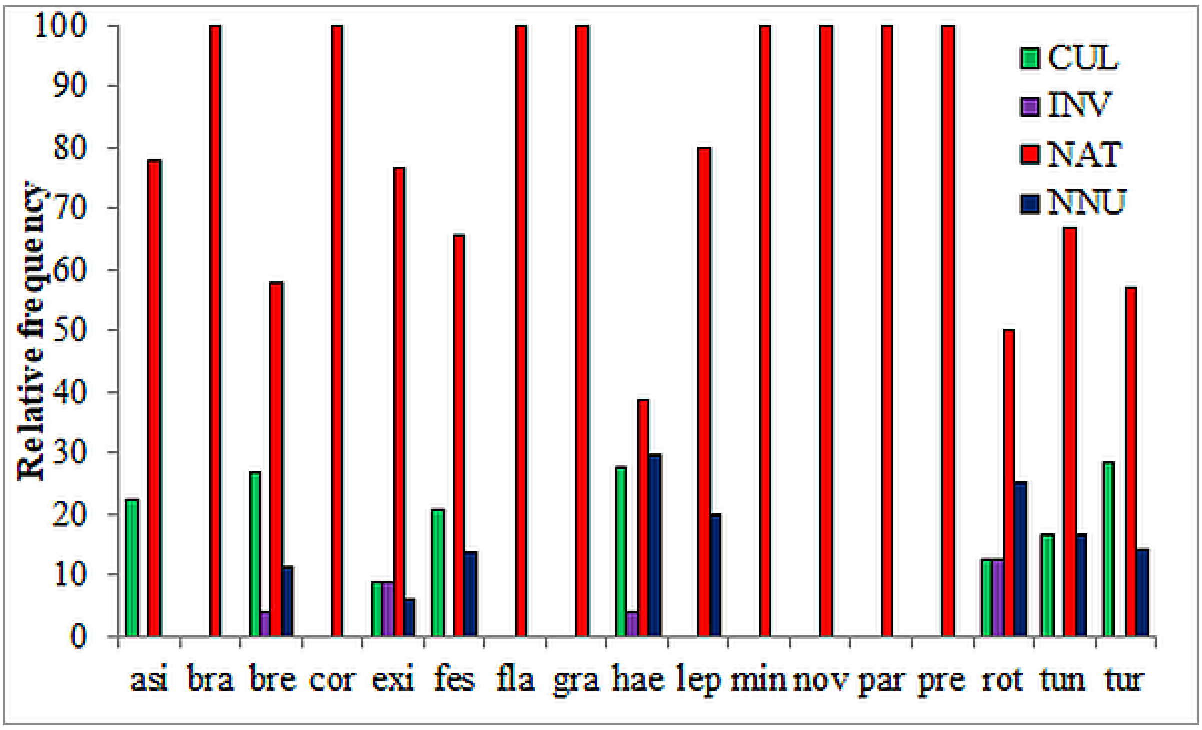

Figure 2. Relative abundance of *Bombus* species in different categories of plants: Native type (NAT), nonnative cultivated type (CUL), nonnative invasive type (INV) and nonnative naturalized (NNU); *Bombus asiaticus* – asi, *B. branickii*- bra, *B. breviceps* - bre, *B. cornutus*- cor, *B. eximius*- exi, *B. festivus*- fes, *B. grahami* - gra, *B. haemorrhoidalis*- hae, *B. lepidus*- lep, *B. miniatus*-min, *B. novus*- nov, *B. parthenius* - par, *B. pressus*-pre, *B. rotundiceps*- rot, *B. tunicatus*- tun, *B. turneri*- tur

native floral versus native flora was determioned. We resulted the frequency observation of identified bumblebees (χ^2 = 40.383, df = 16, p-value = 0.0006844) were significantly vary with native (NAT) and sum of the non native (CUL, INV, NNU) flowering plants (Fig. 2). In our study most of the species were found to be foraged in native type of flora. Whereas, only one species for example: *B. haemorrhoidalis* was found to be foraged relatively higher in non native group. Similarly, *B. rotudinceps* was found to be reported almost equally in both (native and non native) types of flora. Meanwhile, we carried out the frequency observation (Chi square value = 50.141, df = 16, p-value = 0.00002177) within the catagories of native and non native plants (Fig. 3). Similarly, we performed cluster analysis of the catagories of plants based on the relative foraging frequency of identified Bombus species, The corresponding analysis (CA) between foraging records of Bombus species versus categories of plants showed the well ordination (Fig. 4). We found most of the Bombus species were ordinate towards the native types of plants rather than non native types.

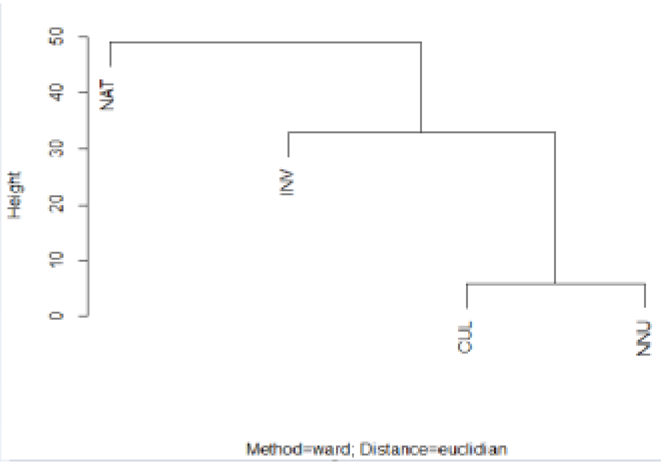

Figure 3. Cluster analysis plant categories of the basis of bumblebee foraging records

Effect of category of host plants, habitat types and nature of plants on foraging frequency of *Bombus* species

To find the effect of catagories, habitat types (Forest, seminatural, agricultural, home garden) and nature of flowers (open and close types of floweres) we performed linear mixed model (lmm) fitted by maximum likehood ['lmerMod']. We fitted the

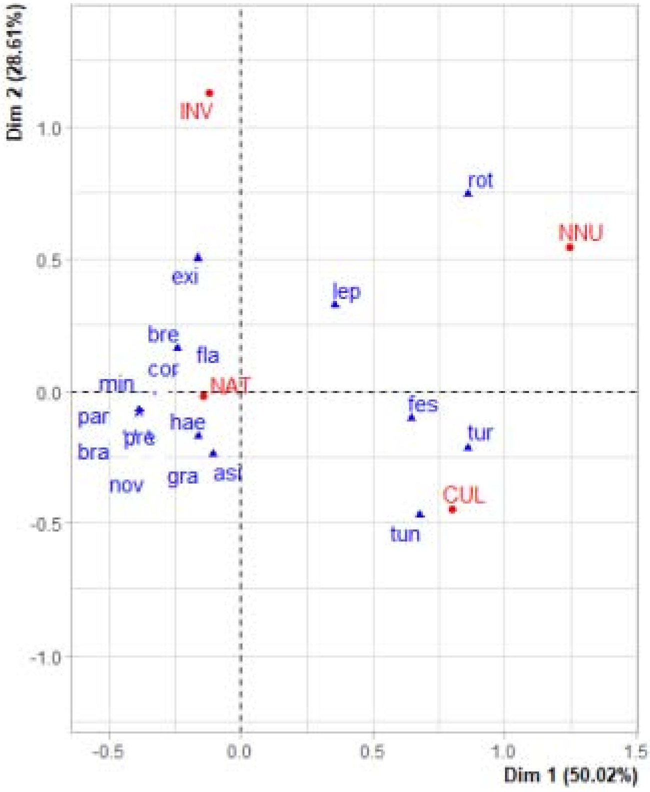

Figure 4. Corresponding analysis (CA) between plant categories and foraging records of *Bombus* species

frequency (n= 248) of *Bombus* species observed along the transet with nature of flower as random factor whereas, category and habitate types were as fix explanatory factors in this model (Table 1). There was significant variation of category of foraged plants whereas no significant variation was observed within habitat types.

Table 1. Summary of Linear mixed model between number of bunblebee caught, plant categories and habitat fit by maximum likelihood ['lmerMod']

Fixed factors	Estimate	Std. Error	t value
(Intercept)	4.8261	0.7077	6.819
INV	1.9391	1.7319	1.12
NAT	2.0392	0.993	2.054
NNU	4.277	1.2085	3.539
Forest	-2.0791	1.0561	-1.969
Grassland	-1.8566	1.1028	-1.684
Home garden	-0.4548	1.0337	-0.44

Relative foraging frequency of *Bombus* species with flowering plant

We analysed the foraging preference based on our foraging records of *Bombus* species in different flowering plant in our samples. On the basis of relative foraging records of *Bombus* species in different flowering plant during the study we analysed as more very common (VC), Common (C), Rare(R) and very rare (VR). we found that certain *Bumbus* species were highly specific for the specific plant species and some were commonly found to be foreged in many types of host plants. The highest relative frequency of bumblebees has been found foraging the host plants were *Anemone elongate, Jasminum humile, Rosa brunonii, Solanum tuberosum* and *Trifolium repens*. In this study, *Bombus asiaticus* was generally foraged on wide range of plant species such as *Anemone elongata, Solanum tuberosum, Rosa brunonii, Jasminum humile, Impatiens stenantha, Glycine max, Cucurbita* sp., *Cirsium arvense, Cirsium falconeri*. Similarly, *B. branickii* was seen to be foraged only on *Rosa brunonii* in our study areas. *B. brevicep* was like to forage on *Clinopodium* sp., *Cucurbita pepo, Cirsium falconeri*. Other species such as *B. cornutus* was seen foraging on *Anemone elongata, Chirita bifolia, Jasminum humile*. In this study *B. eximus* was observed to be foraging mostly on *Jasminum humile* and *Bidens pilosa*. *B. festivus* was also seen to be foraging specially on *Jasminum* and *Trifolium* species. *B. flevesens* recorded on *Clinopodium* sp., *Cirsium arvense* and *Cirsium falconeri*. *B. graham* was mostly foraged on *Anemone elongata, Chirita bifolia, Colquhounia coccinea, Corydalis casimiriana, Gaultheria fragrantissima* during our field study. *B. haemorrhoidalis* was most commonly foraged on *Solanum viarum, Cuphea procumbens, Ipomoea purpurea*. *B. lepidus* foraged on *Jasminum humile, Anemone elongate* and *Cotoneaster frigidus*. We observed *B. miniatus* was only foraging on *Aconogonum molle*. *B. novus* was found to be mostly foraging on *Anemone elongata* in our field areas while *B. parthenius* was seen on the host plant of *Jasminum humile, Colquhounia coccinea* and *Imatiens scabrida*. Similarly, we found, *Rosa brunonii* was specific foraging host plant for *B. pressus*. *Bombus rotundiceps* was commonly found to be foraging on *Trifolium repens*, *Jasminum humile, Ophiopogon* sp., *Rosa brunonii*, *Solanum tuberosum*. *B. tunicatus* visited on *Anemone elongate* and *Anaphalis contorta*. Similarly *B. turneri* frequently foraged on *Rosa brunonii,* and *Solanum tuberosum*.

DISCUSSION

Overall foraging response of bumblebees community differed significantly between native and non native flora. Some Bombus species were found to be foraged only to the specific flowers within the study sites indicating that the specific flowering preference of certain bumblebees in the study area. In this study, most of the *Bombus* species were recorded to be foraged at native plants, while relatively few species were observed in non natives types. This result indicated that the native plants can benefit native pollinators rather that non native types of plant species. It is suggested, the flowers of non native plants are foraged on by native bees (Williams et al. 2011, Drossart et al. 2017), but whether they prefer native or non native plants is still unclear. In our study, the most foraging plant under the families were Rosaceae, Oleaceae, Ranunculaceae, Fabaceae and Asteraceae whereas, least foraging like Apocynaceae, Cactaceae, Cannabaceae, Iridaceae, Lauraceae, Liliaceae, Ophiopogon, Papaveraceae, Verbenceae and Viburnaceae. However, some previous studies (Westphal et al. 2003, Hanley et al. 2008) suggested that the plants under the certain selective families such as Fabaceae and Verbenaceae were the most attractive plants to visit the bumblebee workers. There is long debate about the spatial foraging preference of different insect pollinators including bumblebees with the local flora. Many researchers suggested, the foraging preference of *Bombus* species between native and non native type of flowers is probably assocuiated with the many ecological and evolutionary relation. Some The floral characters such as morphology, color and scent are probably associated with the attraction of the bumblebee (Stone et al. 2003, Cnaani et al. 2006, Fornoff et al. 2017) with their foraging plants. Moreover, it is linked with flower resources, structure of flower, and other adaptational relationship (Dotterl et al. 2005, Dobson 2006, Knudsen et al. 2006).

Beside this, the foraging distance (Elliott 2009), flower availability, flower cover and types of host plants (Williams and Osborne 2009) are the major determining factors for the plant -bumblebees (Goulson et al. 2008, Krishna and Keasar 2019) foraging relation. The effect of complexity of morphological characters of many non native flower species such as corolla length and of length of proboscis (Inouye 1980, Smithson and Macnair 1996, Dhzono et al. 2011) might be important for the rate of flower vistitation and flower use by native bumblebees species (Inoue and Yokoyama 2006) of this region. Moreover, the spatial foraging relation of the Bombus species depending on their behavioral plasticity and nutritional requirements (Delaplane and Mayer 2000, Shafir et al. 2003, Drossart et al. 2020). So the flowers of the host plants of these families being symmetrical with higher availability of nectar for foraging might be preferred more by bumblebee than the flowers from (Kells and Goulson 2003, Sepp et al. 2004, Bhusal et al. 2019). In the other hand, the occurrence of more feeding generalist *Bombus* species might be linked with foreging strength in native types of flora. Another reason can be more specialized interactions of generalist species with the native types of flora in our study sites. Therfore, their foraging patterns can vary with the range of plants available (Seitz et al. 2020). In the other hand, the regular flowering of (perennial) wildflower and the seasonal cultivated flowers and therefore bumblebees rely on additional floral resources that may have the some foraging biases in the local habitats with native and non native flora. Similarly, In case of non native flowers, had a few plant species in the early establishment phase might affect on the foraging patterns of bumblebee species in this area. The change in plant species composition from native to non native in the particular habitats may impacted on foraging patterns of bumblebees in this human dominated heterogenous landscape. The pollinator friendly plantings are often used to enrich habitats in bumblebee conservation efforts. More research that experimentally compares native versus non native pollinator friendly plants in this landscape, at different scales is essential. Thus, we recommend the bumblebees visitation to the particular native and non native flora be considered in future studies to ensure precise and accurate interpretations of foraging preference by different bumblebee species that can be implemented for the conservation point of view in this landscape.

In sum up, our findings suggest that the higher foraging frequency of *Bombus* species within native flora in our sampling effort. some *Bombus* species were found to be foraged only to the specific flowers within the native and non native categories indicating that the specific flowering preference of certain bumblebees. The future changes in land use pattern and climate change might affect on the future floral composition especially on native flora that will be the survival risk for many native species of Bombus from this region. For the more conclusive thought, detail study especially plant-bumblebees interaction will be necessary in furure.

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Supplementary table 1. Relative frequency of *Bombus* species in sampling sites with foraging in flowering plant. Frequency on more than 20 = very common (VC), 10 to 20 = common (C), 5 to 10 = rare (R), Less than 5= very rare (VR)

Plant species

Allium sativum (CUL), B. fes $^{(VR)}$
Abelmoschus esculentus (CUL), B. hae $^{(VR)}$
Anaphalis busua (NAT), B. exi $^{(VR)}$, B. fes $^{(R)}$
Anaphalis contorta (NAT) B. tun $^{(C)}$
Anaphalis griffithii (NAT) B. hae $^{(VR)}$
Anemone elongate (NAT), B. asi $^{(C)}$, B. cor $^{(VC)}$, B. exi$^{(VR)}$, B. fes$^{(VR)}$, B. gra $^{(C)}$, B. lep $^{(C)}$, B. nov $^{(VC)}$, B. tun $^{(C)}$, B. tur $^{(C)}$
Antirrhinum majus (NNU) B. exi$^{(VR)}$ n=1, B.tun$^{(C)}$
Bidens pilosa (INV), B. bre$^{(VR)}$, B. exi$^{(VR)}$, B. hae$^{(VR)}$, B. rot$^{(C)}$
Canna hybrid (CUL), hae$^{(VR)}$
Canna indica (CUL), fes$^{(VR)}$
Cannabis sativa (CUL), exi$^{(VR)}$
Cascabela thevetia (CUL), B. hae$^{(VR)}$
Cirsium verutum (NAT), B. bre$^{(VR)}$, B. exi$^{(VR)}$
Chrysojasminum humile (NAT), B. asi$^{(C)}$, B. bre$^{(VR)}$, B. cor$^{(VC)}$, B. exi$^{(C)}$, B. fes$^{(VC)}$, B. hae$^{(VR),}$ B. lep$^{(VC)}$, B. par$^{(VC)}$, B. rot$^{(C)}$, B. tun$^{(C)}$
Cirsium arvense (NAT), B. asi$^{(C)}$, B. exi$^{(R)}$, B. fla$^{(VC)}$, B. hae$^{(R)}$
Cirsium falconeri (NAT), B. asi$^{(C)}$, B.bre$^{(C)}$, B. exi$^{(VR)}$, B. fla$^{(VC)}$, B. hae$^{(VR)}$
Clinopodium umbrosum (NAT) B. bre$^{(VC)}$, B. fla$^{(VC)}$, B. hae$^{(VR)}$
Colquhounia coccinea (NAT), B. exi$^{(VR)}$, B. gra$^{(VC)}$
Corydalis casimiriana (NAT), B. gra$^{(VR)}$
Cotoneaster frigidus (NAT), B. hae$^{(VR)}$, B. lep$^{(VC)}$
Cucumis melo (NAT), B. hae$^{(VR)}$
Cucumis sativus (NAT), B. hae$^{(VR)}$
Cucurbita pepo (CUL), B. asi$^{(C)}$, B. bre$^{(VR)}$, B. exi$^{(R)}$, B. hae$^{(R)}$
Cuphea procumbens (NNU), B. bre$^{(VR)}$), B. hae$^{(R)}$
Dahlia pinnata (CUL), B. fes$^{(VR)}$, B. hae$^{(VR)}$
Duranta erecta (NNU), B. hae$^{(VR)}$
Elsholtzia fruticosa (NAT), B. hae$^{(VR)}$
Fagopyrum cymosum (NAT), B. fes$^{(VR)}$, B. hae$^{(VR)}$
Gaultheria fragrantissima (NAT) B. fes$^{(VR)}$, B. gra$^{(VC)}$
Glycine max (NAT), B. asi$^{(C)}$
Helianthus annuus (CUL), B. hae$^{(VR)}$
Henckelia bifolia (NAT), B. cor$^{(VC)}$, B. exi$^{(VR)}$, B. gra$^{(VR)}$
Hibiscus rosa-sinensis (NAT), B. hae$^{(VR)}$
Impatiens glandulifera (NAT), B. rot$^{(C)}$
Impatiens scabrida (NAT), B. bre$^{(R)}$, B. exi$^{(R)}$, B. hae$^{(R)}$, B. par$^{(VC)}$
Impatiens stenantha (NAT), B. asi$^{(C)}$, B.exi$^{(VR)}$
Ipomoea nil (NNU), B. hae$^{(VR)}$
Ipomoea purpurea (NNU), B. bre$^{(VR)}$, B. hae$^{(R)}$
Iris domestica (NAT), B. hae$^{(VR)}$
Koenigia mollis (NAT) B. min$^{(VC)}$
Lantana camara (INV), B. hae$^{(VR)}$
Lindera pulcherrima (NAT), B. exi$^{(VR)}$
Malva cachemiriana (NAT), B. fes$^{(R)}$, B. hae$^{(VR)}$
Neillia rubriflora (NAT), B. exi$^{(VR)}$
Ophiopogon parviflorus (NAT), B. rot$^{(C)}$
Opuntia orbiculata (NNU), B. ror$^{(C)}$
Parochetus communis (NAT), B. fes$^{(C)}$
Persicaria nepalensis (NAT), B. fes$^{(R)}$
Phaseolus vulgaris (NNU), B. fes$^{(R)}$, B. hae$^{(VR)}$
Prinsepia utilis (NAT), B. exi$^{(VR)}$, B. tur$^{(C)}$
Rosa brunonii (NAT), B. asi$^{(C)}$, B. bra$^{(VC)}$, B. bre$^{(VR)}$, B. fes$^{(VR)}$, B. pre$^{(VC)}$, B. rot$^{(C)}$, B. tur$^{(VC)}$
Rubus nepalensis (NAT), B. exi$^{(VR)}$, B. fes$^{(VR)}$, B. hae$^{(VR)}$
Scutellaria discolor (NAT) B. hae$^{(VR)}$
Solanum betaceum (CUL) B. hae$^{(VR)}$
Solanum lycopersicum (CUL), B. hae$^{(R)}$
Solanum melongena (CUL), B. hae$^{(VR)}$
Solanum tuberosum (CUL), B. asi$^{(C)}$, B. fes$^{(R)}$, B. hae$^{(VR)}$, B. rot$^{(C)}$, B. tun$^{(C)}$, B. tur$^{(VC)}$
Solanum viarum (NNU), B. hae$^{(R)}$
Strobilanthes attenuate (NAT), B. exi$^{(VR)}$, B. hae$^{(VR)}$
Trifolium repens (NNU), B. bre$^{(VR)}$, B. exi$^{(VR)}$, B. fes$^{(C)}$, B. lep$^{(VR)}$, B. rot$^{(C)}$, B. tur$^{(C)}$
Viburnum erubescens (NAT), B. exi$^{(R)}$, B. hae$^{(VR)}$
Vigna unguiculata (CUL), B. bre$^{(VR)}$, B. hae$^{(VR)}$

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Distribution of Bombus haemorrhoidalis Smith and its Interrelationship with Host Plants in Chitwan Annapurna Landscape of Central Nepal

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Abstract

Occurrence and variety of flowering host plant of native Bombus pollinators are viewed as basic alternatives than the imported species. The use of native bumblebee species for pollination was considered more significant than imported bumblebees to reduce environmental impact and pest problems. B. haemorrhoidalis is the most dominant species of Chitwan Annapurna Landscape in agricultural and wild flora during April to September 2019. We followed assessable walking trails and used insect net for sample collection. The effect of different environmental variables on the floral host plant resources of this native bumblebee was examined. With eight locations ranging from 1407 to 2506 meters above sea level, twenty-seven species of seventeen plant families were identified as pollen and nectar foraging host plants. B haemorrhidalis distribution frequency is correlated with relative humidity (0.07438968) and altitude (0.495657857). The most visited plant family was Balsaminaceae and plant was Imatian scrabida. This study gives the knowledge of abundance of host plants, ecological and biological relationship of the B. haemorrhoidalis in Nepal.

Keywords: *bumblebees, foraging, host, pollinator, pant families, species*

Introduction

Bumblebees (Hymenoptera, Apidae) are pollinators of many wild as well as agricultural plants. They have high thermoregulatory behavior. Rapid pollination, burst the pollen sac by vibrating the wings and ability to forage at low ambient temperature (Miller-Struttmann et al., 2014) and light makes them the most consistent and efficient pollinators (Heinrich, 1979; Abrol, 2012). So, they behave as significant pollinators, mainly in an alpine surroundings (Yu et al., 2012), gradually, accelerated world human population stresses the usage of such crop pollinators specifically for commercial plants grown under extensive (Griffiths and Robberts, 1996). Pollination by these important actors' aids in fruit production, weight, size, and other chemical characteristics to achieve cost-effective production (Aizen et al., 2008; Klein et al., 2007).

Different species bumblebees like *Bombus terrestris, B. impatiens, B. occidentalis* and a number of other are used for commercial pollination of various crops within the world (Kwon and Saeed, 2003; Velthuis and van Doorn, 2006). These species are expensive to import (Velthuis and van Doorn, 2006), and they compete for nesting locations, food,

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and other resources with native pollinator species, particularly Bombus (Couvillon et al., 2010).

34 species of bumblebees have been recorded from Nepal (William et al., 2010). Among them*, B. haemorrhoidalis* is a dominant pollinator of wild as well as agricultural flowers in the lower to mid altitude region of mid himalya (William 2010 and Streinzer M., et al., 2019) and it is the major pollinator in commercial farm of cardamom (Sinu et al 2007). Fourteen CHAL species of plants have been classified in the CITES Annexes, and their extinction is due to a variety of factors, one of whom is reproductive success. Plant reproductive success has diminished due to the decline of suitable pollinators. However, no research has been done on the interaction between pollinators and hostplants, that results in a decline in plant reproductive rate due to a shortage of adequate pollinators. This study fills a knowledge gap in CHAL on the abundance of *B haemorrhoidalis* and their forage plants at various elevation levels.

Materials and Methods

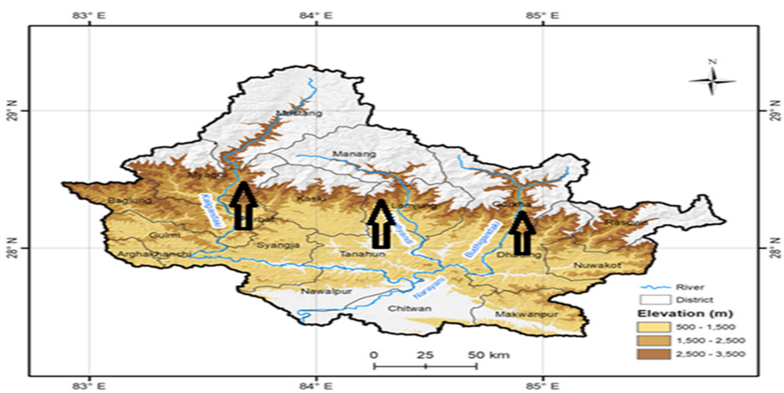

Fig: 1- study area

Surveys were conducted along an altitudinal gradient (from 500 to 3500 m asl) in three river valleys of the Chitwan Annapurna Landscape (CHAL) Kaligandaki (west site), Marsyangdi (mid site), and Budhigandaki (east site) in central Nepal (Figure 1) throughout flowering season between April and November 2019 at 9am to 6 pm. We observed around the flowering plants present at any location along the walking path when a foraging bumblebee was noticed, and the bumblebees on the site were collected and recorded. An entomological net was used to capture Bombus species, which were then killed with ethyl acetate. We noted habitat types, altitude, and the GPS position of the collecting points during the survey. To prevent mold growth during shipment, specimens were kept in airtight containers with a few sheets of tissue paper and a drops of ethyl alcohol and were subsequently dry-mounted using standard insect pins. The collected specimens were deposited in the Entomological Museum of the Central Department of Zoology, Tribhuvan University, Kirtipur (www.cdztu.edu.np). The Collected specimens were observed under a stereoscopic microscope and identified using published keys for adjoining regions, eg. Nepal (William 2010), North China (An et al 2014), India (Saini et al 2015), Kashmir (Williams 1991) and Sichuan (Williams et al 2009).

Results

Collected 103 specimens of *B. haemorrhoidalis* showed wide range of variations providing the successful adaptation in the nature. It was found in agricultural and natural mountainous landscape between 1407 and 2505 meters in altitude. Grassland, home gardens, dense forest trees, and cultivated crops were among their preferred forage plants. The distribution frequency of *B. haemorrhoidalis* was highest in lower altitudes and low in high altitudes (Fig 3). This species was not recorded highest altitude of the 2505 masl in our study areas. During our field, 40% specimens were recorded from agricultural land, 31 % from forest, 25% from grass land and 4% from home garden (fig. 2)

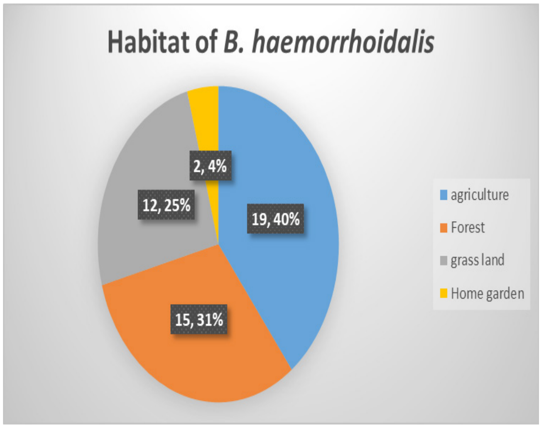

Fig: 2 Habitat distribution

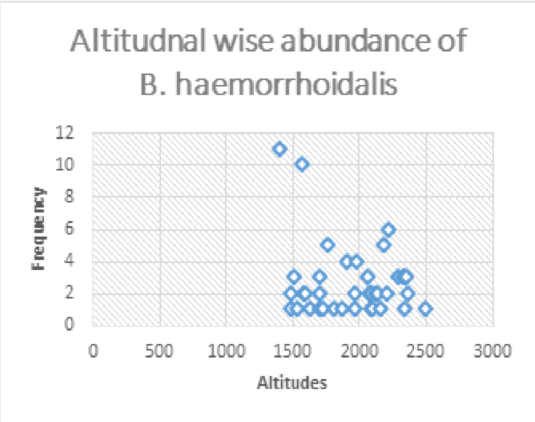

Fig: 3 Altitudinal distribution

The correlation between frequency of B. haemorrhoidalis with environmental variables (temperature and relative humidity) and altitude was calculated, we obtained (Relative humidity = 0.07438968), (Temperature = -0.193060815) and altitude (Altitude = 0.495657857). Then, we concluded that frequency of B haemorrhoidalis was negatively correlated with relative temperature but positively correlated with altitude and humidity. Twenty-six species belonging to seventeen plant families were observed as host plants in eight new habitat ranging from 1407-2505 m altitude (Fig 4 and 5).

The families of plants visited for nectar and pollens belonged to Asteraceae, Balsaminaceae, Brascicaceae, Caprifoliaceae, Cucurbitaceace, Fabaceae, Hypericaceae, Lamiaceae, Liliaceae, Malvaceae, Melastomataceae, Oleaceae, Papaveraceae, Polygonaceae, Rosaceae, Solanaceae and Verbanaceae. The most commonly visited *Imatiens scabrida, Solanum tuberosum* and *Cucurbita* sp, belonging to the families Balsiminaceae, Solanaceae and Cucurbitaceae respectively (Fig 4 and fig 5). The hills of Tatopani and Dharapani in Mustang and Manang Districts (fig. 6) had the least number of bumblebees, which could be attributed to a lack of flowering host plants for these bees to forage on. The highest frequency of B haemorrhoidalis was recorded to be the foremost common wild *Imatiens scabrida* flowering host plants on Lower kerauja and Lapu with common visitation of the determined humblebee species. Sunflower, cucumber, and potato were among the cultivated plant species visited by bumblebees for nectar and pollen, while *Jasminum hummile* (yellow Jasmine) was a common wild herb with a medium level of bumblebee visitation.

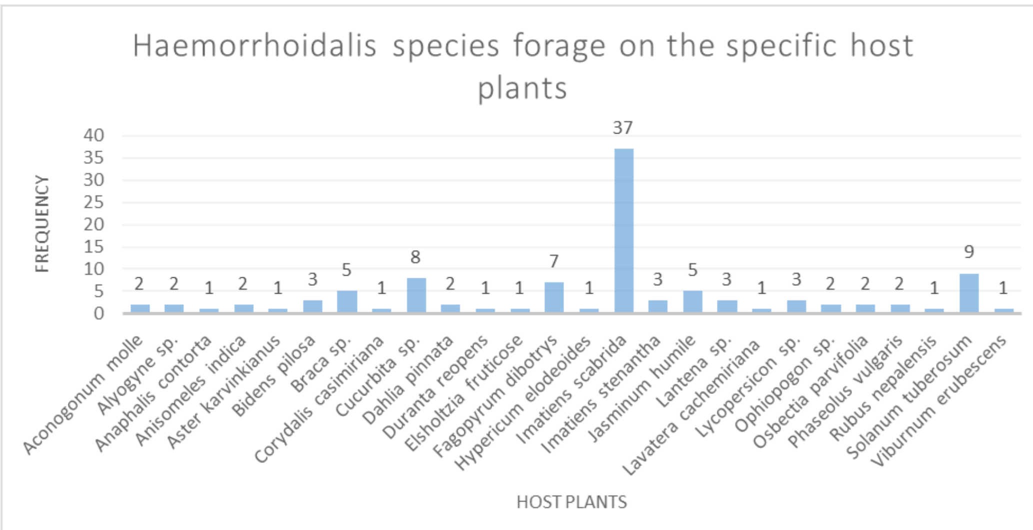

Fig: 4 Foraging frequency of B haemorrhoidalis on specific host plants

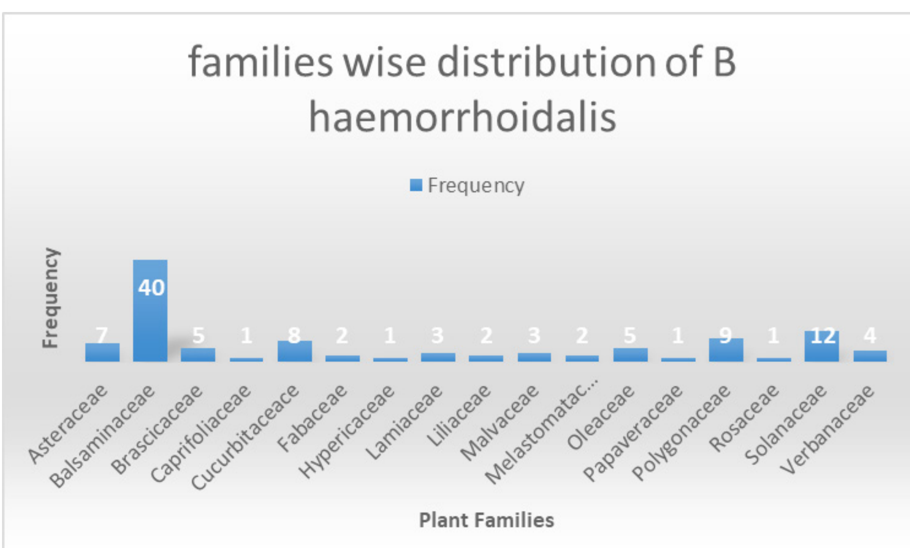

Fig: 5- Foraging of *B haemorrhoidalis* on families of host plants

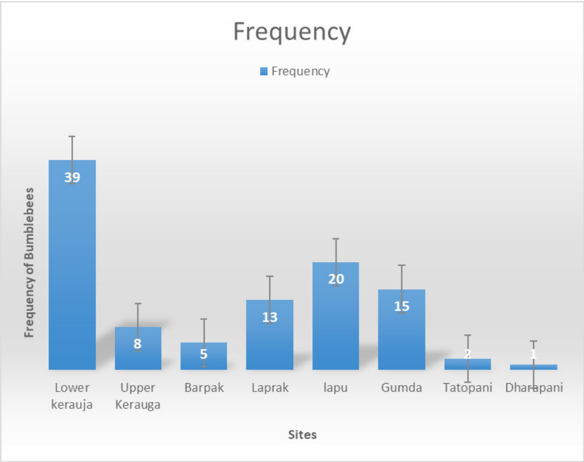

Fig: 6 Site-wise abundance of *B. haemorrhoidalis* in study areas

The thoracic pubescence of *B. haemorrhoidalis* is black, and it has a huge body (20±4.4mm) with long and large wings (20±3.2mm); the forewing is three times longer than the width. Terga1-2 had a bright yellow color, whereas Terga3-5 was an orange-red color. The length of antennal segment 3 was 1.5 times that of antennal segment 4.

Discussion

In nature, a variety of bee species play an important role in pollination and the survival of plant species. A few bee species have been used for agricultural pollination and expansion in order to meet people's food needs and the economic value of the yields in specific areas. The *B. haemorrhoidalis* was important pollinator of wild as well as agricultural plants of wide range of altitude (1407-2505 m asl) and the abundance frequency of this species was also higher lower altitudinal region of mid Himalaya region Nepal. (William et al., 2008) recorded *B. haemorrhoidalis* at 1000m asl in Kasmir India whereas (William et al 2010) recorded this species at the altitude of 850 m asl, in Nepal and (Saini et al, 2015) recorded at the altitude of (1000-2700 m asl) in India. The abundance frequency of *B. haemorrhoidalis* was correlated to temperature. Foraging of *B. haemorrhoidlis* was found positively correlated with altitude and humidity while negatively correlated with temperature. The study correlates with (Peat and Goulson, 2005) who stated that foraging rates of *B. haemorrhoidalis* was positively correlated with humidity while temperature did not significantly influence foraging rate. Positive correlation between foraging of bumblebee and humidity is because nectar secretion rates were higher at high humidity (Peat and Goulson, 2005). They also indicated that bumblebees avoid collecting pollen when the foliage is covered in dew or rain-water droplets, which would make grooming pollen into the corbiculae harder. Also, bumblebee's frequency was found positively correlated with altitude which also correlates with (Williams et al., 2010; Streinzer et al., 2019) who has decided species diversity of bees increases with altitude. At contrast with (Hoiss et al., 2012) who concluded that species richness and abundance of bees showed a linear decline with increasing altitude because the diversity of species decline linearly with decreasing temperatures. But bumblebees are able to forage at extremely low temperatures (Allen-Wardell et al., 1997; Saini et al., 2012) so their frequency was found negatively correlated with temperature and positively correlated with altitude in the study area. *B. haemorrhoidalis* had found frequently visiting host plant of family Balsaminaceae, Solanaceae and Polygonaceae. Bumblebee prefers to visit flowers that are larger and more symmetrical and hence enhances pollen transport Moller (1995). So the flowers of the host plants of these families being symmetrical with higher availability of nectar for foraging might be preferred more by bumblebee than the flowers from other rest families. Flowers of balsaminaceae are attractive to pollinators when they are open, since they provide either pollen or nectar, which provides sugar to pollinators (Delaplane and Mayer, 2000). This could be the reason for the greater visitation of bumblebee towards the host plants of balsaminaceae. We also observed that the open flowers of Solanaceae were highly visited by bumblebee, it correlates with the study on *Comarum palustre* (Rosaceae), in which bumblebees were the main visitors (Somme et al., 2014). Flower aroma is thought to be a crucial long-distance signal for native bees looking for their first floral meal, and it can impact bee behavior, including flower attractiveness (Heinrich et al., 1977; Dotterl et al., 2005). The flowers of Rutaceae are symmetrical and are often sweet-scented which might be the reason that the bumblebees were found to be attracted towards the flowers of the host plants of this family. According to, the bumblebee's species composition is influenced by the landscape context and habitat quality (Carvell et al., 2011).

The agricultural field, forest and grassland was rich in *B haemorrhoidalis* than the home garden. The diversity and abundance of the bumblebee in the specific habitat is affected by distance between nest and floral resources (Hines and Hendrix, 2005) so it may be the reason for higher diversity and abundance of bumblebee in agricultural land of study area. Also Ockinger and Smith (2007) recorded higher species richness of the bumblebees in the field boundaries within the 100 m of a semi natural habitat. The reason for high diversity is due to the resources provided by the agricultural land and the presence of the perennials and herbaceous food plants in the in agricultural land. Goulson (2010) revealed the reasons for the highest abundance of bumblebee in the specific habitat is due to the inflow of the foraging bumblebee in the specific habitat from the other areas so the abundance of the bumblebee in agricultural land was higher than the grass land and the human settlement in the study area. The landscape context and the habitat quality influence the species composition of bumblebee as stated by (Carvell et al., 2011).

B. haemorrhoidalis had found repeatedly visiting host plants in the balsaminaceae, solanaceae and cucurbitaceae families. Bumblebees prefer to visit open, more symmetrical blooms, which improves pollen transmission (Moller 1995). Therefore, the flowers of these host plants are symmetrical and have a higher availability of nectar for foraging, bumblebees may prefer them over flowers from other rest families. Pollinators are attracted to *Imatiens* blooms as they open, especially as sources of pollen or nectar, both of which provide a rich source of sugar to pollinators (Delaplane and Mayer, 2000). This could also explain why bumblebees are foraging to *Solanum tuberosum* host plants in higher numbers. The funnel shaped flowers of cucurbita sps. were also heavily visited by bumblebees, which is consistent with the findings of a previous study on *cucurbita* sps. Of host plants, in which bumblebees were the primary visitors (Somme et al., 2014). Eugenol and geraniol are frequent floral scent molecules present in bee-pollinated plants (Dobson 2006; Knudsen et al. 2006), and hence could be employed to influence bumblebee-plant interactions.The flowers of the Balsaminaceae family are symmetrical and frequently sweet-scented, which could explain why bumblebees were drawn to the blooms of the plant.

Conclusion

B. haemorrhoidalis Smith was mostly observed in the altitudinal range of 1407- 2505 m asl on agricultural field. As floral resources, this species was found on twenty-seven plants from seventeen different plant families, those floral plants were the main source for provision of nectar and pollens found in wild and agricultural land. The range of the simplest determined species, *B. haemorrhoidalis* became quite numerous and the maximum typically visited plants belonged to circle of relatives Balsaminaceae but, flowers with longer to be had floral resources have been visited greater than quick season flowering.

Acknowledgement

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Diversity of Bumblebees (Bombini, Apidae: Hymenoptera) in Chitwan Annapurna Landscape (CHAL) of Central Himalaya, Nepal

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Abstract

Bumblebees (Hymenoptera: Apidae: genus Bombus Latreille, 1802) are the effective group of pollinators for various crops and wild plants in the north high-hills of Nepal. Over the past few decades, it has been expected that the population of these insects has declined since there are still unexplored areas and rich floral and faunal diversity. However, there are very few scientific publications as well as little researches have been carried out focusing on bumble bees in high-hills of Nepal. Therefore, this research was focused to explore the bumblebees in Chitwan Annapurna Landscape (CHAL) in Manang, Mustang and Gorkha districts with altitudinal gradients from 500 -2700 m asl from June to September 2019. The data were collected following accessible walking trails by opportunistic survey method and specimens were captured using sweeping net. Total 7 subgenera with 8 species of genus Bombus were identified. Bombus festivus (subgenus: Festivobombus) was the most dominant species. The diversity of bumblebee species was more in Gorkha site compared to the Manang and Mustang sites. Bombus haemorrhoidalis, B. eximus, and B. rotundiceps were the dominant species in Gorkha site, whereas B. festivus, B. lepidus, and B. tunicatus were dominant in Manang districts. Bombus festivus, B. tunicatus, and B. turneri were the most encountered species in Mustang. This study indicates that each district has the unique diversity of bumblebees particularly a site-specific variation of the Bombus species, which are likely to the variations in floral resources, microclimate and habitat types.

Keywords habitat types, pollinator, subgenus, species dominant

1. Introduction

Bumblebees (Hymenoptera, Apidae, Latreille, 1802) are important social insects that pollinate both wild and agricultural plants. It has been suggested that they originated in the central Asian mountains where temperature variations occur (Williams, 1985). Since they can show endothermic behaviour, bumblebees are better adapted than most other bees to their activity in cool climates (Williams, 2007). The bumblebee's foraging actions are related to nectar gathering (Goulson, 2010), whilst the choice of food plants are based on the pollen's characteristics (Roger *et al*., 2016). Typically, an old bumblebees have high experiences of collecting pollen (Raine and Chittka, 2007). Long- tongue species are more specialized in their choice of food plants and are the pollinators of deep nectar flowers based on the length of their tongues (Kawakita *et al*

2004; Goulson, *et al*. 2006). They avoid feeding the bloom that has already been visited by other bees since their feet smell and leave a scent after they have fed (Bumblebee Conservation Trust, 2014). According to Chacoff *et al*. (2010) and Bommarco *et al*. (2012), bumblebees provide buzz pollination for seed production or in seed breeding process, making them significant pollinators of agricultural production and natural ecosystems (Sabir *et al*, 2011).

According to the recent taxonomy, all bumblebees are classified under the genus *Bombus*, which contains 250 species worldwide and 34 species from Nepal (Cameron *et al*., 2007; Williams, *et al*., 2010). The size of species is greater in cooler climates than in warmer ones (Goulson *et al*. 2005; Peat *et al*. 2005). In the Himalaya, they can be found in between 1000 and 56000 meters above sea level (Williams, 1985). Higher species richness has been noted in the mountains of central Asia and the mountains to the east of Tibet (Williams, 1994). Although a typical species is found in the lowland tropics of South East Asia, Central America, and South America, they are primarily restricted to the Northern Hemisphere (Williams, 2007; Goulson, 2010). In the Himalayas, they are typically located between 1,000 and 5,600 meters above sea level.

The environmental variables affect the floral and faunal diversity (Williams *et al*. 2010; Rawat, 2017). West Himalaya has temperate broad leaf forests, parched alpine meadows, and pastures at high elevations because of the region's comparatively low annual rainfall (Rawat, 2017). As a result of the formation of moist alpine meadows at higher elevations and subtropical broadleaf forests at the eastern part, which experiences high annual precipitation of up to 5,000 mm (Dhar and Nandargi, 2006), the East Himalaya is found to have particularly rich biodiversity and is regarded as a global hotspot of biodiversity (Myers *et al*. 2000). Even though the Central and the West Himalya (Williams, 1991; Saini *et al*., 2015) have both conducted extensive research on bumble bee composition (Williams *et al*. 2010).

The Central Himalaya, specifically from Nepal and the Indian state of Sikkim, has the highest abundance of bumblebees (Williams, *et al*. 2010, Saini, *et al*. 2015). In Nepal, both the eastern and western species reach the limits of their ranges, and the overlap of these two faunal zones may be a factor in the region's high bumble bee diversity (Williams, *et al*. 2010). Even though the range and variety of the bumblebee fauna have been well studied in regions like North America, the Eastern, and Western United States, it is still essential to catalog the species of bumblebees from other continents like South-East Asia and Africa.

There is still a deficit of knowledge on the bumblebee's relationship with its host plant, feeding habits, and ecology. Therefore, in order to conserve, protect, and preserve Nepal's natural ecosystem, a complete species inventory is still required. Hence, this study has proposed to collect the species diversity, floral and faunal relationships, bumblebee ecology etc. The scientists may conduct a thorough investigation into the species inventory of the bumblebee. The interaction between bumblebees and their host plants, as well as the presence of an appropriate climatic pattern, must also be clarified

2. Materials and methods

2.1 Study areas

The study was conducted in Chitwan Annapurna Landscape (CHAL) specially focusing Gorkha, Manang and Mustang districts represents high Himalayan ecosystem within the range of 500- 2700-meter altitude. It possesses diverse micro-climatic variation that creates suitable habitats for many species of pollinating insects including bumblebees. This landscape has high biodiversity value and contains seven major sub-river basins: Trishuli, Marsyangdi, Seti, Kaligandaki, Budigandaki, Rapti and Narayani. Kaligandaki river basin is the most important river basin in Nepal. It is originated from the southern edge of Tibetan Plateau. It covers around 11,7770 sq. km area and has longitudinal range of 82°53' - 84°26'E and latitudinal range of 27°43' - 29°19'N and covers the altitudinal range from 188m- 8,143m asl. Diverse climatic condition is found in this river basin so the temperature, precipitation and vegetation vary in these areas. **(Fig 1**)

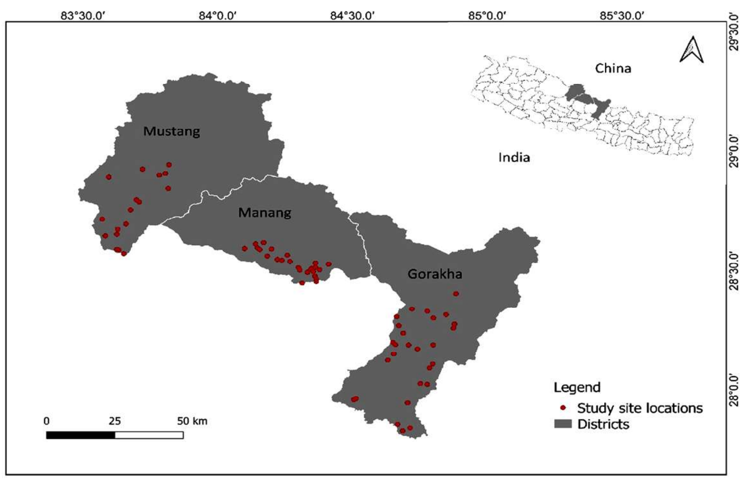

Fig. 1. Map of Chitwan Annapurna Landscape (CHAL). Sampling sites: Gorkha site, Manang site and Mustang site

2.2. **Methods**

An accessible walking trails was followed in Manang, Mustang and Gorkha districts with in the range of 500- 2700-meter altitude. The species were collected by opportunistic method (Goulson 2010). Whenever bumblebee observed along the route, searched them by making a circle of fifty meter radius from the standing point of observer and survey was carried until 40 minutes. The field sampling was conducted in suitable timing for Bumblebees i.e. April to August. Only unidentified specimens were collected by using standard insect collection protocol using a sweeping net. In the field frequency of bumblebees, host plant, temperature and humidity were recorded. Species were identified by using identification key developed by Williams *et al.* (2010), An *et al*. (2014) and Saini *et al*. (2015). The collected specimen was preserved in Central Department of Zoology, Tribhuvan University as a voucher specimen. Data obtained from the field were managed and analysed using Microsoft Excel 2010.

3. Results

3.1. List of identified species

Among 387 individual 8 species of 7 subgenera were recorded. Species were identified using identification key developed by Williams *et al.* (2010), An *et al*. (2014) and Saini *et al.* (2015). The recorded sub genera, their species and diagnostic characteristics of these species are described, likewise, location, altitude, habitat and host plants are mention below.

1. Sub genus -Alpigenobombus

Bombus breviceps, Smith, 1852, (**Photo 2a**)

Diagnostic features

Females: The first two abdominal tergites are black, creamy white, and the third to fifth abdominal tergites are brick red in color. The queen's length is 18 to 21 millimeters, while the worker's is 10 to 16 millimeters. The head is covered in thick pubescence, with the exception of the molar space, the clypeus, the labrum, the area laterals to and in front of the ocelli, and the narrow stripes on the inner and post orbits. heavily infuscated wings. Between prominent lateral tubracles, the labrum has a deep median furrow and a basal transverse depression. The distal half of the metabasitarsus' posterior margin is concave, and the proximal margin's thick pubescence (the auricle) extends to the projection's outer surface as a few sparse hairs.

Distribution

Gumda, alt- 1709, female 2, hab- agricultural field, hp- *Imatiens scabrida*, Gumda, alt- 1702, female 1, hab- agricultural field, hp- *Cucurbita sp* , Barpak, alt- 2780, female 3, hab-grassland, hp- *Parochetus communis*, Gumda, alt- 2082, female 2, hab- grassland, hp- *Strobilanthes attenuta*, Gumda, alt-2040, female 3 , hab- grassland, hp- *Strobilanthes attenuta*, alt- 2017, female 1 , hab- grassland, hp- *Imatiens scabrida*, Gumda, alt- 2570 female 3, hab- grassland, hp- *Anaphalis busua* , Gumda, alt- 1656, female 4, hab- agricultural field, hp- *Aster karvinkianus*

2. Sub genus - Melanobombus

Bombus (Melanobombus) *eximus*, Smith, 1852 (**photo 2b**)

Diagnostic features

Female - Pubescence of thorax is black, hair of the mid and hind tibiae and the basitarsi orange, metatasomal tergum 2 black. It is very large species, queen length is 28-29 mm and worker's length is 14-19 mm. Wings light orange brown with the mid basitarsus having the distal posterior corner forming nearly a right angle and not sharply pointed. Hind basitarsus with the posterior margin nearly straight. Oculo-molar distance approximately equal to the proxomal breadth of the mendible. Labrum with the lamella irregular but nearly straight and about half of the labrum. Ocello-ocular area along the inner eye margin with scattered large puncture but with few small punctures.

Distribution

Lapu, alt 1760, female 2, hab- agricultural field, hp- *Cucurbita sp*, Lapu, alt 2353, female 2, hab – forest, hp - *Jasminum humile*, Lapu, alt 2371, female 6, hab – forest, hp - Jasminum humile, Laprak alt 2400, female 2, hab – forest, hp - *Jasminum humile*, , Laprak alt 2413, female 3, hab – forest, hp - *Jasminum humile,* Lapu alt 2423, female 2, hab-forest, hp - *Trifolium repens* , Laprak alt 2351, female 3,hab- forest, hp -*Cercium verutum*, , Laprak alt 2279, female 1, hab-agricultural field, hp- *Lindera pulcherrila*, Lapu ,alt 2256, female 1, hab – agriculture, hp - *Solanum tuberosum*, , Laprak alt 2092, female 2, hab – forest, hp - *Prinsepia uitilis*, , Laprak alt 2202, female 3, hab - forest, hp - *Viburnum erubescens*, Lapu, alt 2284, female 1, hab – forest, hp - *Jasminum humile*, , Laprak alt 2263, female 1, hab – forest, hp - *Opuntia dillei*, Barpak, alt 2237, female 5, hab- agriculture, hp - *Cucurbita sp*., Laprak, alt 2066 female 1, hab – grassland, hp – *Anemone elongate*,Lapu, alt 2043, female 1, hab – grassland, hp – *Anemone elongate*, alt 2039 female 1, hab – grassland, hp – *Anemone elongate*, alt 2044, female 2, hab – forest, hp - *Colquhounia coccinea*, Barpak, alt 2258, female 1, hab – grassland, hp - *Vibumum erubescens*, Lapu, alt 2351 female 1, hab-forest, hp - *Rosa brunonii*, , Laprak alt 2422, female 1, hab - agricultural field, hp - *Solanum tuberosum*, Barpak, alt 2544, female 3, hab – agricultural field, hp - *Cucurbita sp*. , Laprak alt 2351, female 2, hab – forest, hp - *Colquhounia coccinea*, , Laprak alt 2592, female 2, hab – agricultural field, hp - *Gaultheria fragrantissima*, , Laprak alt 2423, female 3, hab - forest, hp -*Lavatera cachemiriana*, , Laprak alt 2424, female 2, hab - forest, hp - *Lavatera cachemiriana*, Barpak,alt 2003, female 2, hab - forest, hp - *Lavatera cachemiriana*, Barpak,alt 2013, female 3, hab – forest, hp - *Opuntia dillei*.

3. Sub genus - Festivobombus

Bombus (festivobombus) *festivus* Smith, 1861 (**Photo 2c**)

Queen length is 22–25 mm, worker length is 12–17 mm. The pubescence is black in the thorax and white in the two abdominal tergite, except for the labrum, clypeus, and molar spaces, the head is covered in thick pubescence. Thick pubescence is evenly present on the visible thorax and abdomen. Strongly infuscated wings. a labrum with a deep median furrow and a basal transverse depression. The lateral and basal margins of the clypeus are extremely protuberant, and they curve back to join the gena and supraclapeal region. The mesobasi tarsus' distoposterior is entirely spherical. The meta tibia's outer corbicular surface is shiny, smooth, and devoid of any long, stout hairs that protrude from the surface.

Distribution

Ghasa,, alt – 1472, Female 3, hab – agricultural field, hp - *Allium sativum*, Ghasa, alt – 1506, Female – 6, hab – agricultural field, hp - *Phaseolus vulgaris*, Ghasa, alt – 1506, female 1 (dead), hab – home garden, hp - *Canna indica*, Lower Kerunja alt – 1690, female 1, hab - home garden, hp – *Lavatera cachemiriana*, Lower Kerunja, alt – 1732, female 1, hab – agricultural field, hp - *Solanum tuberosum*, Lower Kerunja, Lete, alt-1948, femmale 10, hab- forest, hp - *Jasminum humile* , Lete, alt- 1948, female 1, hab- home garden, hp - *Dahalia pinnata* , Lete, alt – 2492, female 10, hab – forest, hp- *Cotoneaster frigidus*, Lete, alt – 2559, Female 20, hab - forest, hp - *Rosa brunonii*, Lower Kerunja alt-2595, female 10, hab – forest, hp - *Rosa brunonii*, Lower Kerunja, alt- 2632, Female 10, hab- Forest, hp - *Rosa brunonii*, Ghasa, alt-2633,female 2, hab-forest, hp- *Trifolium repens*, Ghasa, alt-2638, female 2, hab – grass land, hp - *Anemone elongate*, Ghasa, alt- 2660, Queen 10, hab – forest, hp - *Cotoneaster frigidus*, Thumi, alt- 2691, Female 2, hab- grassland, hp- *Trifolium repens*, Thumi, alt- 2693, female 4, hab- forest, hp- *Jasminum humile*, Thumi, alt- 2724, female 2, hab- forest, hp - *Rosa brunonii*, Thumi, alt-2724, Female 1, hab – forest, hp- *Jasminum humile*, Thumi, alt- 2724, Female 1, hab- forest hp- *Jasminum humile*, Thumi, alt- 2724, Female 2, hab-forest, hp- *Jasminum humile*, Thumi,,alt- 2724, female 3, hab forest, hp - *Jasminum humile*, Thumi, alt- 2774, Female 2, hab- forest, hp- *Jasminum humile*,Ghasa, Kabra, alt-2774, female 1, hab-forest, hp - *Jasminum humile*, Kabre, alt- 2774, Female 1, hab agriculuural field, hp- *Solanum tuberosum*

4. Sub genus - Orientalibombus

Bombus haemorrhoidalis Smith, 1852, (**Photo 2d)**

They are a huge species with totally black queen pubescence on the head and thoracic dorsum, white abdominal tergites 1 and 2, black abdominal tergum 3, and brick red tergites 4-5; worker with head, thorax, and abdominal tergum 3 totally black, yellow; abdominal tergites 1 and 2; abdominal tergites 4 and 5 are brick red; wings are firmly infuscated; pubescence is short and extremely even. Except for the malar space, the clypeus, a region lateral to and in front of the ocelli, and narrow stripes on the inner and post orbits, the head is completely covered in pubescence. Labrum having lateral tubercles that are prominent, a lateral lamella that is broad and takes up more than half of the labrum's basal width, and a basal transverse depression that extends apically as a deep median furrow between them.

Distribution

Yeya, alt-1407, female 6, hab- agricultural field, hp -*Lantena sp.*, Yeya, alt- 1518, female 1, hab - grassland, hp- *Elsholtzia fruticose*, Yeya, alt- 1539, female 5, hab- agricultural field, hp - *Lantena sp.*, Yeya, Yeya, alt- 1571, female 1, hab- agricultural field, hp- *Aster karvinkianus*, Yeya, alt- 1709, female 2, hab- agricultural field, hp- *Anisomeles indica* , Dharapani, alt-1816 , female 5, hab-agricultural field, hp- *Fagopyrum dibotrys*, Dharapani, alt-1870, female 2,hab-agricultural field, hp- *Fagopyrum dibotrys*, Yeya, Lower kerunja, alt- 1906, female 1, hab-garden, hp -*Lavatera cachemiriana*, Lower kerunja, alt- 1973, female 1, hab-garden, hp- *Lavatera cachemiriana*, Lower kerunja alt-1594, queen 2, hab-agricultural field , hp- *Cucurbita* sp., Lower kerunja, alt-1760, queen 2 , hab-agricultural field, hp- *Cucurbita* sp., Thumi, alt-1700, queen 1, hab- agricultural field, hp- *Duranta reopens*, Barpak, alt-2349, queen 1 , hab- forest, hp- *Viburnum erubescens*, Barpak, alt-2326, queen 1, hab –forest, hp- *Jasminum humile*, Barpak, alt-2090, queen 2, hab-agricultural field, hp- *Solanum tuberosum*, Barpak, alt-2097, queen 1, hab- agricultural field, hp- *Solanum tuberosum* , Barpak, alt-2126, queen 1, hab -forest, hp- *Solanum tuberosum*, Barpak, alt-597, female 2, hab-agricultural field , hp- *Cucurbita sp*.,Barpak**,** alt-1906, female 2 , hab-garden, hp-

Dahlia pinnata, Barpak, alt-2067, female 6, hab- forest, hp- *Opuntia dillei*, Barpak, alt-2083 , female 2 , hab- forest, hp- *Imatiens scabrida*, Barpak, alt-2093, female 1, hab - forest, hp- *Imatiens scabrida*, Barpak, alt- 2366, female 1 , hab- grassland, hp- *Impatiens stenantha*, Laprak, alt- 2505, female 2, hab- agricultural field , hp- *Anaphalis contorta*, Laprak, alt-2592, female 2, hab- grassland, hp- *Imatiens scabrida*, Laprak, alt- 2590, female 2, hab- grassland, hp- *Aconogonum molle*, Laprak, alt-2614, female 1, hab- grassland, hp- *Hypericum elodeoides*, Barpak, alt-2284, queen 1, hab- grassland, hp- *Vibumum erubescens*, Laprak, alt-2443, female 2, hab- forest, hp- *Rosa brunonii*, Laprak, alt-2739, queen 2, hab- forest, hp- *Jasmium humile*, Laprak, alt-2360, queen 1, hab- agricultural field, hp- *Cucurbita sp*., Barpak, alt-2020, queen 1, hab- garden, hp- *Cercium verutum*, Barpak, alt-2060, queen 1, hab- forest, hp- *Impatiens stenantha*, Barpak, alt 2081, queen 1, hab- agricultural field, hp- *Impatiens stenantha*, Dharapani, alt – 1904, Female 1, hab- home garden, hp- *Lavatera cachemiriana*, Bokekhola, alt-1386, Female 2, hab- agricultural field, hp- *Phaseolus vulgaris*,

5. Sub genus - Pyrobombus

a. *Bombus lepidus* Skorikov, 1972, (**Photo 2e**)

Female – Head, pronotum, metanotum, abdominal tergites 1 and 2 is yellow, mesonotum, malar space, and tergum 3 is black, and abdominal tergites 4-5 is brick red. Truncate, bean-shaped lateral tubercles on the anterior labrum's edge are divided in the middle by a shallow median depression that is the same length as the tubercle. The rest of the labrum is macro perforated, with the exception of the raised section of the lateral tubercle. Unpunctured area adjacent to the lateral ocellus in the ocello-ocular region that is the same size as the lateral ocellus. One-fourth of the space between the lateral ocellus and the eye margin is covered by a band of punctures along the eye margin.

Distribution

Thanchock, alt- 2633, female 3, hab – grassland, hp - *Trifolium repens*, Larjung, alt-2655, female 1, hab- grassland, hp- *Anemone elongate*, Larjung, alt – 2669, female 10, hab- forest, hp- *Jasminum humile*, Thanchock, alt – 2691, female 6, hab- grassland, hp - *Trifolium repens*, Thanchock, alt-2722, female 3, hab – forest, hp - *Trifolium repens*.

b. *Bombus rotundiceps* (Friese, 1916), **Photo 2f)**

Female- The first three abdominal tergites pubescence are black and dingy yellow. Brick red describes the final 3. Except for the malar space, clypeus, the region lateral to and in front of the ocelli, and the short stripes on the inner and post orbits, the head is covered in heavy pubescence. Thick pubescence is evenly seen on the thorax and abdomen. Labrum with pronounced lateral tubercles that are slightly bluntly elevated and a deep median furrow between them that displaces the ridge between them to form a lamella that overhangs the apical border. The lateral and basal margins of the clypeus are substantially protuberant, and they curve back to connect the gena and the supraclypeal area, respectively.

Distribution

Danaque, alt- 2780, female 1, hab- forest, hp - *Jasmium humile*, Danaque, alt – 2098, queen 1, hab – grassland, hp - *Bidens pilosa*, Gumda, alt – 2090, queen 1, hab – agricultural field, hp – *Solanum tuberosum*, Gumda, alt – 1822, female 1, hab – forest, hp – *Osbectis parvifolia*, Gumda, alt – 1852, female 1, hab – agricultural field, hp – *Osbectia parvifolia*, Thumi, alt – 1731, female 4, hab - forest, hp – *Trifolium repens*, Thumi, alt – 1656, female 5, hab – agicultural field, hp – *Biden pilosa*, Thumi alt – 1660, female 3, hab - agricultural field, hp – *Stribilianthes tomentosa*,Thumi, alt – 1470, Female – 2, hab – agricultural field, hp – *Impatiens glandulifera*, Gumda, alt – 1320, Female – 5, hab – forest, hp - *Opuntia dillei,* Gumda, alt – 2632, female 1, hab – forest, hp - *Jasminum humile*

6. Sub genus - Bombus

Bombus tunicatus (Smith, 1852), (**Photo 2g**)

Female- Mesonotum and abdominal tergum 3 are black, pronotum, metanotum, and abdominal tergum 1 are white, abdominal tergites 4 and 5 are brick red, and there is a well-developed black stripe between the wings in the queen. Worker with a black head, mesonotum, and third abdominal tergite; white pronotum and metanotum; and brick-red abdomen tergite 4 and 5. The visible portions of the thorax and abdomen are uniformly covered in pubescence. With prominent lateral tubercles and a basal transverse depression that extends apically as a deep median furrow between them, the labrum has a lamella that overhangs the apical border. Labrum's anterior edge is complete; the abdominal tergum is crimson; the lateral tubercles are bean-shaped and do not connect in the middle. The lateral and basal margins of the clypeus are substantially protuberant, and they curve back to connect the gena and the supraclypeal area, respectively. presence of numerous, big punctures all over the central, flattened portion of the clypeus.

Distribution

Boksekhola alt – 2132, female 1, hab- forest, hp- *Rosa brunonii*, Danque, alt – 2270, female 10, hab- forest, hp - *Rosa brunonii*, Khokhethati, alt – 2515, female 10, hab – 10, hp - *Anemone elongate*, Danque, alt – 2515, female 8, hab – grassland, hp - *Anemone elongate*, Lete, alt – 2536, female 2, hab – grassland, hp - *Anemone elongate*, Lete, alt – 2536, female 2, hab- home garden, hp - *Antirrhinum Majus*.

7. Sub genus - Psithyrus

Bombus turneri (Richards, 1929), (**Photo 2h**)

Female- Strongly protruding as a broad, rounded triangle from the labrum is the labral lamella. The pubescence of the thoracic dorsum is black with sparsely interspersed yellow hairs anteriorly and posteriorly.

Distribution

Gumda, alt – 2270, female 2, hab – forest, hp - *Jasmium humile*, Boksekhola, alt – 2592, female 3, hab- grassland, hp - *Anaphalis busua*, Thumi, alt – 2096, female 1, hab

– forest, hp - *Imatiens scabrida*,Thumi, alt – 2127, female 1, hab – forest, hp - *Imatiens scabrida*, Lowerkerunja, alt – 2211, female 1, hab-forest, hp- *Prinsepia uitilis*, Barpak, alt – 2191, female 2, hab – forest, hp - *Chirita bifolia*, Upperkerunja, alt 2550, Female 10, hab - forest, hp - *Rosa brunonii*, Boksekhola, alt – 2550, Female 7, hab – forest, hp - *Jasminum humile*.
(alt= altitude, hab= Habitat, hp= Host plant)

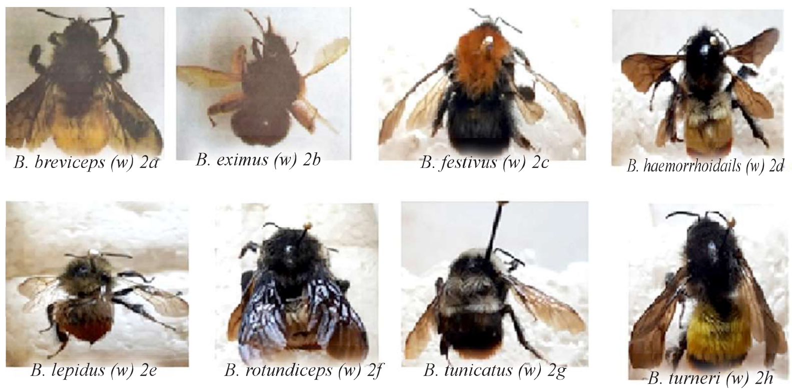

Fig. 2: photos of different identified species of bumblebees in CHAL region of Nepal

Discussion

Species variation in three sites are related to the local microhabitats, floral composition and land use change. Due to the different microhabitat such as grassland, agricultural land, forest and home garden of three sample sites, the distribution pattern of bumblebees' species are different. Due to their limited foraging ranges, bumblebees require close proximity between landscape elements that provide nesting sites, foraging habitats, and undisturbed structures for hibernation (Diekötter *et al* 2006)). The diversity and abundance of flowering plant species, as well as vegetation shape and height, were habitat variables that influenced the overall abundance, species richness, and foraging activity of bumblebees. According to their unique needs for foraging and nesting, many Bombus species may respond to certain ecological factors (Carvell *et al*. 2002). The size, color, and shape of the flowers, the amount and kind of pollen, the season, and the location are some of the variables that affect the floral composition of host plants (Tai *et al* 2020). The bumblebees prefer flower grown on the open slope of mountain instead of closed deep and dense forest. For these insects, the shape of the flower is crucial when selecting a bloom. Flowers have funnel-shaped that facilitate direct movement and make it easier to collect pollen and nectar. Bumblebees have also been observed to favor symmetrical flower over asymmetrical ones (Moler, 1995; Vaudo, 2016). Instead of color and categories, host plant selection was heavily influenced by host plant families and flower types (Bhusal *et al*. 2019). Land use change in Europe's subalpine regions is a main driver in biodiversity changes, and it also has a serious influence on the presence of European bumblebees (Fourcade, 2019, Marsal, 2018).

During several field survey in the Gorkha, Manang and Mustang of three river basins of

Kaligandaki, Marsyngdi and Budigandaki of Chitwan Annapurna Landscape, over 387 bumble bee specimens were collected, belonging to 8 species. As a biodiversity hotspot and an important area for conservation priority, the central Himalayan range is a site to the first systematic study of bumble bee diversity (Myers *et al*., 2000).Out of eight subgenera of *Bombus*, *B. festivus* was most abundant as the species of this subgenus inhabit in high alpine grassland, open grassland, semi-desert and tropical hill forest and have short, medium or may have long tongue-length visiting shallow to deep flowers while subgenus Pressibombus was least abundant as the species of this subgenus inhabit in higher alpine grassland and semi-desert (Williams *et al*. 2008). *Bombus festivus* was found more abundant representing 25.91% of total species recorded. *B. haemorrhoidalis* was low in abundance in Manang and Mustang but high in Gorkha as it was recorded from low elevation and warmest altitude as a species of western Oriental region (Williams, 1991; Streinzer *et al* 2018). Similarly, *B. asiaticus* was recorded as it a Himalayan species (Williams, 1991). The least abundance species were *B. asiaticus, B. cornotus* and *B. pressus*. All the recorded species were recognized as Himalaya species. So, no species have yet been assessed for red list status. Of the total, only *B. tunicatus* is categorized as endemic species from Himalayan (Williams and Jepsen, 2017). Species richness (11) of study area is higher than the species richness (eight) of Iowa as reported by Hines and Hendrix (2005) and from Japan (six) recorded by Teruyoshi *et al* (2006) and from Scotland (six) recorded by Brodie (1996). The reason for higher diversity of bumblebee in central Himalaya (especially Nepal) is because of overlap of both eastern and western bumblebee species (Williams *et al*., 2010).

5. Conclusion

The Chiwan Annapurna Landscape CHAL's Gorkha, Manang, and Mustang Districts provide a variety of climatic settings for bumblebees. A total of 387 bumble bee specimens were collected, and 8 species from 7 subgenera were identified. The *Bombus festivus* (subgenus: Festivobombus) was the most common species in the research area. When compared to the Gorkha, Manang and Mustang sites, the Gorkha site had the highest species richness. The main species at the Gorkha site were *B. haemorrhoidalis, B. eximus*, and *B. rotundiceps*, whilst *B. festivus, B. lepidus*, and *B. tunicatus* were main species recorded in Manang, and *B. festivus, B. tunicatus*, and *B. turneri* were major species in Mustang during our field study.

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