Indian Journal of Ecology (2022) 49(6): 2223-2228
DOI: https://doi.org/10.55362/IJE/2022/3813

Manuscript Number: 3813
NAAS Rating: 5.79

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 (*Bombu*s 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 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.

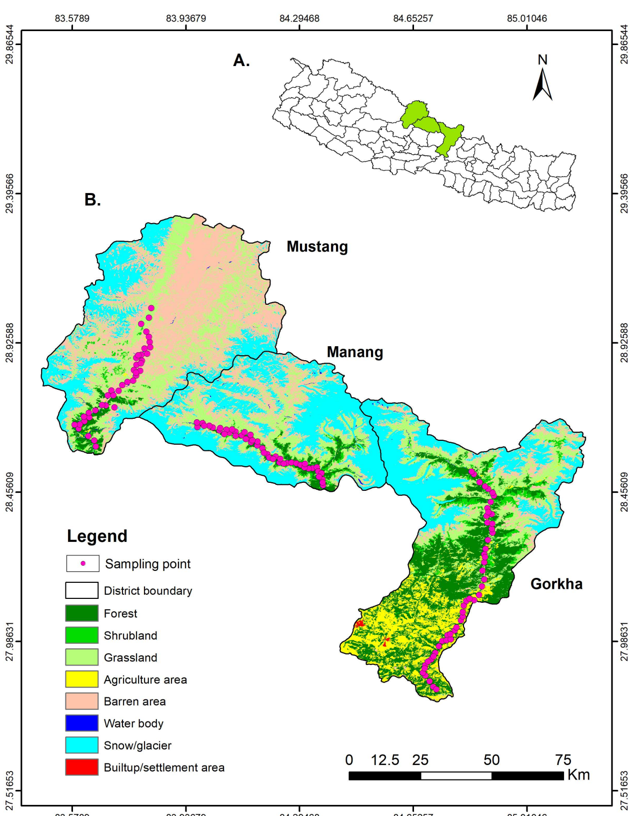


Fig. 1. Study area showing sampling points for bumblebee with in Kaligandaki, Marsyangdi and Budhigandaki River basins

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.

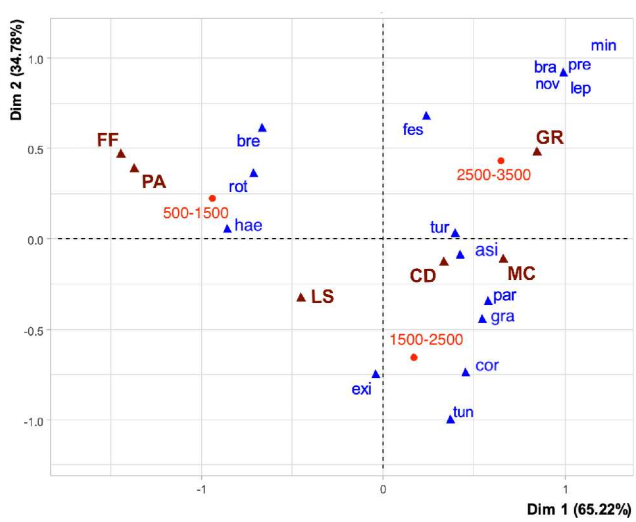
Data analysis: The relative composition of 16 Bombus species was determined. The non-parametric data from the habitat assessment were quantified using the cross tabulation (two-way contingency) to determine the frequency distribution of each threat within the three elevation gradients. The relative number 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.

ACKNOWLEDGMENT

Authors are thankful to the Nagao Natural Environment Foundation, Japan, Mohamed Bin Zayed Species Conservation Fund, and University grant commission Nepal for supporting in field expenses.

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Received 22 August, 2022; Accepted 27 November, 2022