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Exploring of bumble bee fauna and its biogeographical affiliation in district Swat, Khyber Pakhtunkhwa, Pakistan

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Abstract
To explore the diversity of Bumble bee fauna of District Swat surveys were carried out from April to September 2015. To find out a complete profile of a Bumble bee fauna of the study area sampling was carried out at seven different locations including Madyan, Matta, Qalagai, Kalam, Utror, Charbagh and Mankyal. During the surveys a total of 314 specimens of bumble bees were collected belonging to three different species namely, *Bombus rufofasciatus*, *Bombus asciaticus* and *Bombus haemorrhoidalis*. *Bombus rufofasciatus* was the most abundant species reported and represented 37 % of the total collection, while *Bombus asciaticus* presented 35.70 % and *Bombus haemorrhoidalis* 27.30 %. It was the very first study of its kind in the study area, and further exploratory studies are recommended to increase the understanding of the ecology of the bumble bee fauna.

Keywords: *Bombus*, bumble bee, diversity, Simpson's index, Shannon's index, swat

1. Introduction
Insects belong to the class Insecta of the Phylum Arthropoda, which is the prime class of Arthropoda. All insects belong to the major category of animal Phylum Arthropoda. Insects occur everywhere and until now plentiful of insect species i.e. approximately hundred thousand, have been described (Triplihorn and Johnson, 2005) [29]. Generally, bumble bee generation reproduces once a year. Queens emerge in the spring from the hibernation period (Kearns and Thomson, 2001) [16]. In search of pollen and nectar most species of bumble bee can travel several miles away from their colonies (McFrederick and LeBuhn, 2006) [19].
These bees are the primary pollinators and are the main service providers with greater pollinator diversity and play very important role in ecosystems by providing key role of Pollination, which is essential for the essential for all living organisms (Potts *et al*., 2010) [24]. Some bees such as Apis species provide honey and present a well evolved social behavior while majority of them are solitary and commonly known as non-apis bees, also called wild bees or pollen bees (O, Toole, and Raw, 1991) [22].
All apis bees, bumble bees and carpenter bees belong to family Apidae, which is further divided into three subfamilies; Apinae, Nomadinae and Xylocopinae. There are two main types of bumble bees, the nest-making species which live with queens and workers, belong to genus *Bombus*, while the other type of bumble bees are parasitic species which have no worker cast and belong to genus Psithyrus (Chandler, 1950) [9].
There are about 250 species of bumble bees divided into 15 subgenera under a single genus Bombus (Williams *et al*., 2008; Michener, 2007) [35, 20]. Bumble bees are the most fascinating, easily recognized bees after the honey bees, but larger in size. However, both belong to family Apidae. They have many structural features in common. Bumble bees are basically eusocial i.e. each colony has a solitary and sub-social phase (O, Toole, and Raw, 1991) [22].
Like other wild bees, bumble bees also provide a vital ecological service of collecting nectar and pollen from flowering plants (Bohart, 1972) [5] and take great part in pollination activities (Yang and Owen, 1989; Jennertsen *et al*., 1991; Bergman *et al*., 1996; Wang *et al*., 2009) [42, 14, 2, 32]. However, many species of bumble bees are considered generalists which are closely related with specific environment (Goulson, 2004; Goulson *et al*., 2006) [10, 11].
As compared to other bees, bumble bees have larger bodies. Like other bees they are energetic and bright in color (Koch, *et al*., 2011) [17]. They have a wide range of distribution, as can be

found in the areas as low as at the sea level to the peaks of the Himalaya (Williams, 1985) [36]. They can be found in the areas of temperate climate, and commonly occur throughout Eurasia and Northern America, some species exist in more extreme environment such as cool slopes of the Himalayas, while other live in tropical forests. They are absent from Africa south of Sahara. They were deliberately introduced into New Zealand in the late 1800s to early 1900s (O, Toole, and Raw, 1991) [22].

Bumble bees prove diel patterns of movement while foraging (Yang and Owen 1989; Bergman *et al*., 1996) [42, 2]. Which affect pollination (Wang *et al*., 2009) [32] and is different among different species (Teras, 1985; Yang and Owen, 1989) [31, 42]. Bowers (1986) [6] reported that in early summer the diversity of queens in early time of June. Bumble bees shows their life activities e.g. foraging and nesting activities in Whereas in moderate type of weather bumble bees show their life cycle completed from the spring to autumn (Bowers 1986, Charman *et al*., 2010) [6, 8]. Bumble bees are involved mostly in the foraging activities but their foraging activity is greatly affected by seasonal variation like rain fall, snowfall, declining of temperature in cold areas and low quantity of flowers in which affect foraging activities (Wardell *et al*., 1998) [33].

Due to the destructions and changes occurring in global and regional climate (Williams, 1998) [39] the population of bumble bees is declining all over the world and the population decline have been recognized in recent year due to the use of increase in land such as conversion of wild land into agricultural and urban land, food modification, habitat destruction by disturbing natural ecosystems and excessive use of pesticides (Osborne *et al*., 1991; Partap and Partap, 1997; Kearns *et al*., 1998; Winfree *et al*., 2009) [21, 23, 15, 41].

Pakistan occupies an important geographic position by having four important mountain ranges i.e. the Himalaya, the Karakoram, the Hindu Kush and the Suleiman range. Among these ranges, the Himalaya, the Karakoram, the Hindu Kush are northern in location. These mountain series are main bio-geographic boundaries between tropical and subtropical flora and fauna (Rafi *et al*., 2010) [25]. Due to distinct habitats and ecological zones, understandings about bio geographical affiliation of bumble bee fauna from Pakistan is very below the adequate level, beside the fact that Pakistan is the main intersection of Palearctic, Oriental and the Afrotropical regions.

However, few studies on bumble bees have been conducted in Pakistan (Williams, 1991) [37]. From Himalayan Kashmir, while from northern Pakistan (Suhail *et al*., 2009) [27]. A list of bumble bees is published described species of bees from Pakistan, in which they reported species of bumble bees from Pakistan without their distribution details (Ascher and Rasmussen, 2010) [1]. Recently, Irshad *et al*., (2014) [13] reported 28 species of bumble bees from different areas Pakistan; however, there is not a single record of bumble species from District Swat. Awareness regarding bumble bee fauna and their distribution in Swat district is not studied before.

Therefore, keeping in view their importance this study was conducted to explore, assess distribution and geo-graphical affiliation of the bumble fauna in District Swat, Pakistan.

2. Materials and methods

2.1 Study Area

The present study was carried out in District swat which is situated in the northern of 35° North Latitude while 72° East Longitude (Figure 3.1). The District Swat lies in the part of Mountainous chain, which is the branches of Hindu Kush, so the greater area of swat is restricted with high foothills and mountains, the peaks of which are buried by endless snow in winter.

Though these massive series of mountains run unevenly, some to the East while others to the West, but the overall direction is North-South. The valley is surrounded by the sky-high peaks (Man, 2008). Margins of the valley are above 600 meters in the southern and increase towards the North rapidly. There are numerous mountain crests which are ranging about 6000 meters above the sea level, usually covered with uninterrupted snow. Swat is topographically a mountainous region, placed amongst the slopes hills of the Hindu Kush Mountain series. Swat District lies in the moderate region.

2.2 Collection sites

Surveys were carried out for collection of the bumble bee from seven predetermined collections of District Swat namely Madyan, Mankyal, Matta, Qalagai, Kalam, Charbagh, Utror (Figure 3.1).

2.3 Survey, Collection and Preservation

Bumble bees specimens were collected during April 2015 to Sep 2015 after 15 days interval. During day hours' time from variety of habitats such gardens, fruits orchards, vegetables fields, grasses and other plants from 10: 00 AM to 4: 00 PM during the sunny days. The collection was made with the help of aerial net. (Gullan and Cranston, 2005) [12].

After collection, bumble bees were killed with the help of Ethyl Acetate and fixed on setting board with entomological pins. When the specimens became dried, they were separated from starching sheets, labeled and transferred into the wooden collection boxes, comprising Naphthalene bolls in the boxes to protect the collected specimens from ants and other insect attacks. Whole field records were noted e.g., collection date, time, collector name, area, remarks on a predesigned preformed (Appendix-I). These samples were brought to the laboratory of (National Insect Museum (NIM) National Agriculture Research Centre), Islamabad for identification.

2.4 Identification

Identification of the bumble bee up to species level was done with the help of museum housed bumble bee species up to the species level and by using keys of Bombus species (Bingham *et al*., 1897; Wililams, 1991; and Mischner, 2007) [4, 37, 20], under compound microscope. It is important to note that its values decrease with the increase in size of sample because this index is dependent upon sample size. Values of this index range mostly from 0 to 1 and higher diversity is considered in selected locality if the values tend towards zero. Shannon (H) was used to measures the richness and abundance of the calculated species in the selected localities and Evenness (e^H/S) for calculation of evenness.

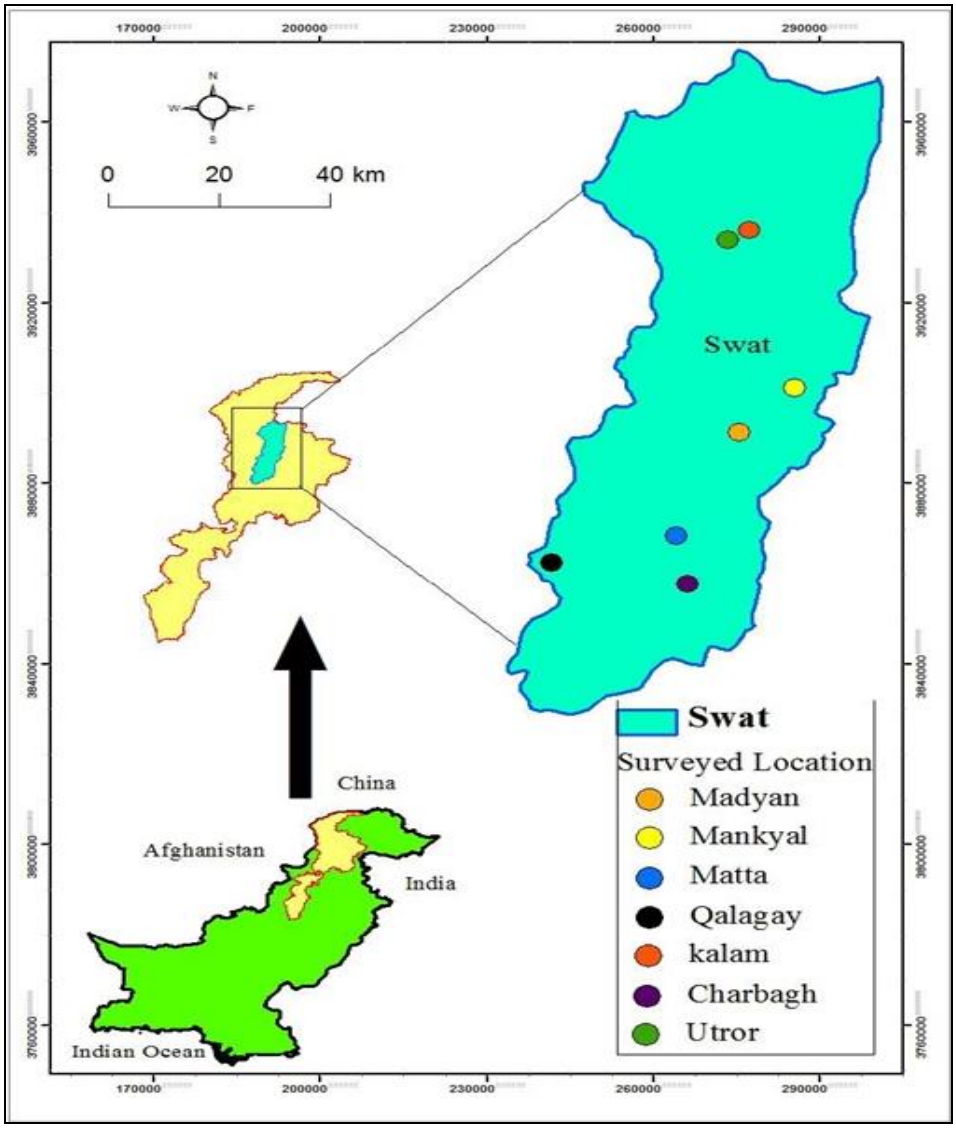

Fig 1: Map of the Study Area and Collection Sites (Prepared in Geographical Information System, GIS)

3. Results and discussion

During the survey a total of 314 specimens were collected from seven selected localities of Swat. Out of 314 collected specimens three species under genus Bombus were identified. Among 314 specimens 116 individuals were belonged to *Bombus rufofasciatus.* While 112 specimens belong to *Bombus asciaticus* followed by *Bombus haemorrhoidalis* with 86 specimens.

During the present study, total abundance of specimens of Bombus species collected from seven selected localities of district Swat are given in Table 4.2. Validation of the species were made with their synonyms, diagnostic characters i.e. body length, colors, hairs mouth parts, legs, antennae and wings characters etc. Photographs of all species are provided. Taxonomic sources were also provided for the identified bumble species. The relative percentage abundance of each species collected from each collection site of district swat is given in Table 4.3.

1. *Bombus rufofasciatus* Smith, 1852 (Figure. 2)

Synonym*: B. prshewalskyi* Morawitz, 1880; *B. rufocinctus* Morawitz, 1880; *B. chinensis* Dallatorre, 1890*; B. przewalskyi* Morawitz, 1890

This species was collected from four sites including Madyan, Qalagay, Kalam and Utror.

Diagnostic Characters: Body length 14-29 mm; head black pubescent; prominent eye; antennae are elongated; ocello-ocular region sparsely or thoroughly punctured thorax: pale white or grayish pubescence with a dark band between wing bases; dark fuscous wings coloration; pubescence prominently blackish, while slightly rufescent on torsi, bright white pubescence on the basal two abdominal segments, bright rufi-fulvous on the apical four paler fulvous red; pubescence on abdominal segments of males.

Remarks: This is the first record of *Bombus rufofasciatus* from Swat. During the study a total of 116 specimens were collected from collection sites. Earlier this species was reported from Pir Panjal (Williams, 1991) [37] while from Gilgit Baltistan: Darkot, Doarian, Hunza, Rama forest and Samgam (Irshad *et al*., 2014) [13].

Distribution: Widely distributed species in Pakistan, Nepal, Bhutan, Myanmar, Tibet, and China (Williams, 1998) [39].

Bio-geographical affiliation: Palearctic

2. *Bombus asciaticus Morawitz*, 1875. (Figure. 3)

Synonym*: Bombu shortorum* Linnaeus, 1761*; Bombus longiceps* Smith, 1878; *Bombus regelli* Moravitz, 1880; *Bombus regelli* Vogt, 1909; *Bombus heicens* Wang, 1982; *Bombus huangcens* Wang, 1982*; Bombus flavicollis* Wang,

1985; *Bombus asiaticus* Wang, 1985.
This species was collected from three sites including Madyan, Mankyal and Utror.

Diagnostic Characters: Body length: 17-31 mm, black pubescent; eyes poorly prominent; antennae are long; lightly or narrowly punctured ocello-ocular-malar neighborhood. Grey or pale thorax with whitish pubescence, wing bases are band and black in color. First abdominal segment and second segment of anterior part is white in color while other abdominal segments are orange.

Remarks: New record for Swat. Earlier reported from Gilgit-Baltistan, By Suhail *et al*. (2009) [27] and Maryam (2012) [18].

Distribution: Afghanistan, Pakistan, Nepal, Mongolia, China, Kazakhstan, India, Kyrgyzstan, Tajikistan Saini and Ghattor, 2007 [26], Gupta, 2010; Saini and Ghattor, 2007 [26]; Suhail *et al*., 2009 [27].

Bio geographical affiliation: Paleo-oriental species.

3. *Bombus haemorrhoidalis* Smith, 1852. (Figure 4)

Synonym: *Bombus orientalis* Smith, 1854; *Bombus buccinatoris* Smith, 1879; *Bombus assamensis* Bingham, 1897 [4]*; Bombus montivolans* Richards, 1929*; Bombus orientalis* Richards, 1929.
This species was collected from two site including Mankyal and Charbagh.

Diagnostic Characters: Body length about 16-21 mm; wings pattern dark fuscous; pubescence conspicuously blackish, while a little pubescent on tarsi, bright creamy pubescence on the basal two abdominal fragments, bright rufi-fulvous on the apical four; pubescence on abdominal segments of males whitish fulvous red than in the female.

Remarks: New record for Swat. Earlier Irshad *et al*., (2014) [13] reported this species from Abbottabad, Haripur, Laberkot, Peshawar, Timerggarh (Khyber Pakhtunkhwa); while Williams (1991) [37] from Murree, Pir Panjal. During study 86 specimens of *Bombus haemorrhoidalis* collected from three different localities, during collection the weather was arid and hot, as a result of hot summertime many vital forage plants tends to welt leading, which decrease together the total number of species and also individuals of insect pollinators.

Distribution: Widely distributed species in Pakistan, Kashmir, Himichal Pradesh, Uttaranchal, Nepal, Bengal, Sikkim, Thailand, Vietnam, Tibet, Myanmar, Bhutan (Brunetti, 1923).

Bio geographical affiliation: Palearctic

Adult

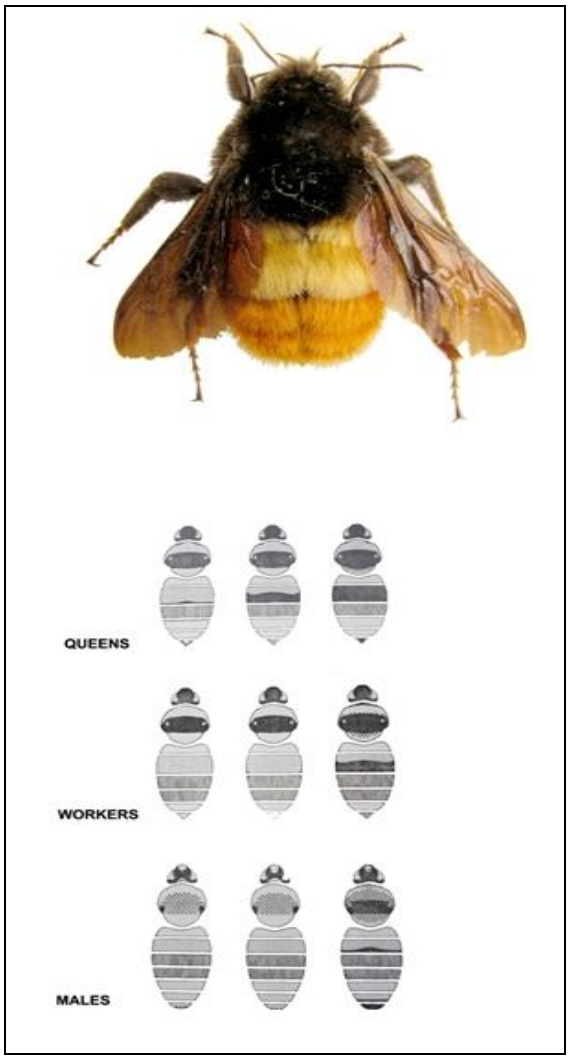

Fig 2: *Bombus rufofasciatus* By Willium (1991)

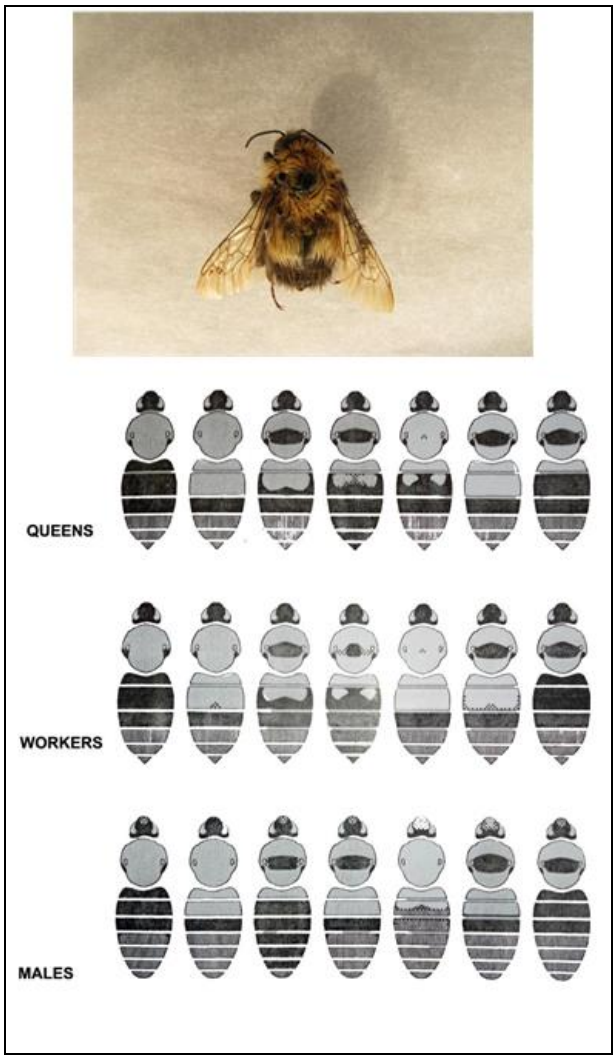

Fig 3: *Bombus asiaticus* By Willium (1991)

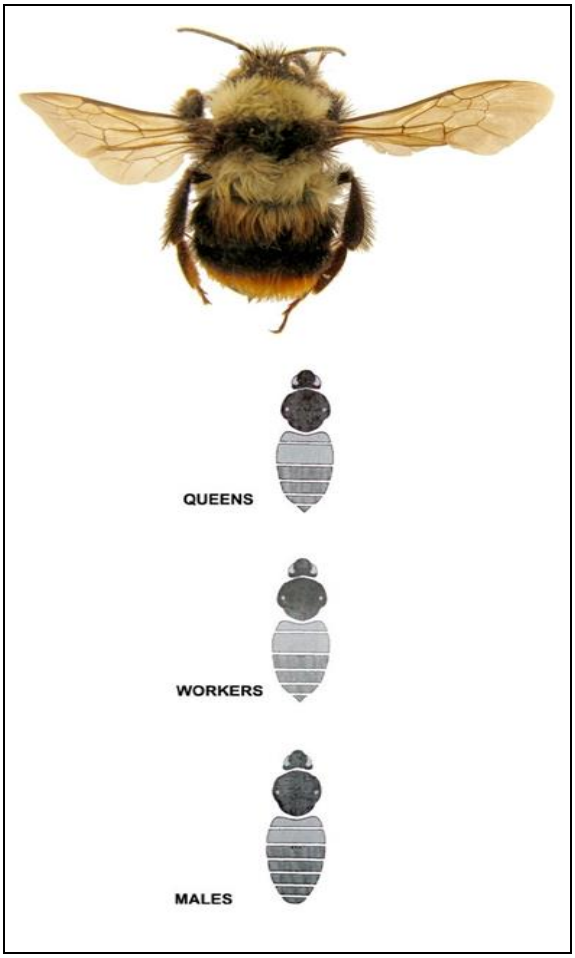

Fig 4: *Bombus haemorrhoidalis* By Willium (1991)

Table 1: Composition of Bumble bee species (Hymenoptera) at selected localities in District Swat

S. No.	Localities	Species	Latitude	Longitude	Altitude	
					Meter	Feet
1.	Madyan	*B. asiaticus* *B. rufofaciatus*	35º14	72º53	1362	4470
2.	Mankyal	*B. haemorrhoidalis* *B. asiaticus*	35º32	72º61	2044	6709
3.	Matta	*B. rufofaciatus*	34º93	72º41	343	1128
4.	Qalagai	*B. rufofaciatus*	34º56	72º19	1284	4213
5.	Kalam	*B. haemorrhoidalis* *B. rufofaciatis* *B. asiaticus*	35º47	72º59	2300	7550
6.	Charbagh	*B. haemorrhoidalis* *B. asiaticus*	34º83	72º44	1030	3372
7.	Utror	*B. asiaticus* *B. rufofaciatus*	35º49	72º46	2300	7500

Table 2: The collective rank list of species collected from different localities in District Swat.

Dominance rank	Name of species	Abundance	Madyan	Mankyal	Matta	Qalagai	Kalam	Charbagh	Utror
1.	*B. rufofasciatus*	116	22	00	13	30	31	00	20
2.	*B. asiaticus*	112	37	33	00	00	00	00	42
3.	*B. haemorrhoidalis*	86	00	19	00	00	35	32	00
Number of individuals		ΣN=314	N=59	N=52	N=13	N=30	N=66	N=32	N=62
Number of species			02	02	01	01	02	02	02

As in the above table shows that all the localities had a great diversity of species, whereas *Bombus rufofasciatus* (116) was at top of the collective rank list of species with large number of specimens, while 112 of *Bombus asciaticus* and 86 of *Bombus haemorrhoidalis*.

The relative percentage abundance of identified species was calculated. The highest relative percentage abundance accounting 37.00 percent of the total was observed for *Bombus rufofaciatus* that was followed by *Bombus asciaticus* with relative abundance of (35.70%). The lowest relative

percentage abundance 27.30 percent was observed for *Bombus haemorrhoidalis*.

Table 3: Relative percentage abundance of each species collected from district Swat.

S. No.	Name of species	Abundance of individual per species	Relative percentage abundance
1.	*B. rufofasciatus*	116	37.00
2.	*B. asiaticus*	112	35.70
3.	*B. haemorrhoidalis*	86	27.30
Total		314	100

Diversity Indices

PAST software (Simpson, 1949) was used to study several indices such as the Simpson's index (1-D) is generally used to measure the most abundant species in the sample while for measuring species richness it is considered less sensitive

The species diversity index, evenness and richness of Bombus species was

calculated for the collected species (Table 3). The table shows that all the three species found in each localities whereas Shannon's index of diversity was showed that maximum richness and abundance of species was found in Kalam (0.6874), followed By Madyan (0.6778), Utror (0.6693) and Mankyal (0.6565). While Matta, Qalagai and Charbagh yielded showed very low Shannon's index of diversity value that was almost equal to zero (00). Similarly, evenness found was highest in Kalam (0.9943) than Madyan (0.9848) and Utror (0.9765), while a comparatively low value of evenness was observed in Mankyal (0.964) Madyan (0.8573) Higher Simpson's index.

Table 4: The collective rank list of taxa of Species collected from different Localities of district Swat

Selected localities	Madyan	Mankyal	Matta	Qalagai	Kalam	Charbagh	Utror
Taxa_S	02	02	01	01	02	01	02
Shannon_H	0.6778	0.6565	00	00	0.6874	00	0.6693
Evenness_ e^H/S	0.9848	0.964	01	01	0.9943	01	0.9765
Simpson_ 1-D	0.4848	0.4638	00	00	0.4943	00	0.4764

(1_D) values was observed for the Kalam, that was 0.4943 and lowest was observed from Mankyal (0.4638).

This study was conducted to explore the diversity and bio-geographical affiliation of the bumble bee fauna of District Swat and measured to be the first study of its kind in the area. Over-all 314 specimens of Bumble bees were collected during the current study from the early spring season April 2015 to early autumn September 2015 from seven different locations, which belonged to family Apidae Order Hymenoptera and genus *Bombus*. Specimens were identified at species level yield three different species including *Bombus rufofasciatus*, *Bombus asciaticus* and *Bombus haemorrhoidalis*.

Bombus rufofasciatus is a Palearctic species. From Pakistan, this species was reported from Pir Panjal (Williams, 1991) [37] while from Gilgit Baltistan: Darkot, Doarian, Hunza, Rama forest and Samgam (Irshad *et al*., 2014) [13]. During this study the species were collected from various localities of study area namely Madyan, Matta, Qalagai, Kalam and Utror. During survey specimens of Bombus asciaticus were reported from three localities of study area i.e. Madyan, Mankyal and Utror. Earlier this species was reported from Gilgit-Baltistan, by Suhail *et al*. (2009) [27].

During study *Bombus haemorrhoidalis* was the second most abundant species observed during the survey time and was represented by 86 specimens that were collected from Mankyal, Matta, Kalam and Charbagh. High abundance of the species was observed at Charbagh having flowering plants in abundance, providing good foraging ground for the species. Earlier Irshad *et al.,* (2014) [13] reported this species from Abbottabad, Haripur, Laberkot, Peshawar, Timergara (Khyber Pakhtunkhwa); while Williams (1991) [37] from Murree, Pir Panjal.

A high relative percentage abundance was observed for *Bombus rufofasciatus* that was 37.00 percent, followed with 35.70 percent by *Bombus asciaticus*. The lowest relative percentage abundance 27.30 percent due to some factors e.g. temperature, pollinated plants, modification in natural environment, intensification of agriculture increased use of pesticides which have bad for pollinators (Thompson 2001 and Biesmeijer *et al*.2006) [30, 3] was observed in *Bombus haemorrhoidalis*. All the collected species from these areas were compared with the previously recorded species from other different areas of Pakistan which were housed in National Insect Museum at National Agricultural Research Center, Islamabad for reconfirmation.

Oder hymenoptera, family Apidae of genus Bombus was represented by three species i.e. *Bombus rufofasciatus, B. asiaticus* and *B. haemorrhoidalis* (Suhail *et al.,* 2009) [27] reported species of genus Bombus from northern area of Pakistan *Bombus rufofasciatus*, *B. asiaticus* and *B. haemorrhoidalis* while Saini and Ghattor (2007) [26] reported two species *B. rufofasciatus* and *B. asciaticus* from different areas of India. Mariyam (2012) [18] reported *Bombus asciaticus* from apricot orchids of Gilgit- Baltistan Pakistan. In a similar study conducted by Saini and Ghattor (2007) [26] reported *Bombus asciaticus* from Lahuaul-Spiti valley of Himachal Pradesh India. Recently Irshad *et al*., (2014) [13] reported 28 different species of genus Bombus from northern areas of Pakistan areas including *B. rufofasciatus, B. asciaticus, and B. haemorrhoidalis.* In Present study, this species was collected from some collection sites of District Swat.

During the present survey diversity of Bombus species was shows that maximum richness and abundance was found in Kalam, Madyan, Utror, while minimum value were observed in Mankyal, Matta, Qalagay and Charbagh respectively. Similarly, evenness is found to be highest in Kalam than Madyan and Utror while comparatively low value of evenness is found in Mankyal and Madyan which have small difference in values due to less amount of pollinated plants, crops, low temperature, absence of host flowers and rainfall (Ricketts *et al.,* 2004). High simpson's index values were observed from Kalam which were abundant of pollinated plants, crops and the most suitable temperature was recorded during surveys (Cane and Wcislo 1996) [34].

The lowest diversity was observed from Mankyal because some pollinators cannot show their foraging activities and reduced due to low temperature and snowfalls Torchio (1991) Pakistan fauna has geo-graphical affiliations with the Palearctic and Oriental zones (O' Toole 1993) but we don't

have an adequate scientifically documented information about the geographical affinities of the bumble bee's fauna known to Pakistan.

Eventually this study proved to be very helpful to describe some features of bumble bee diversity of District Swat. Furthermore, more research studies are recommended to evaluate different aspects of ecology of the Bumble bee fauna of District Swat.

4. Conclusion

The Swat District of Khyber Pakhtunkhwa display varied climatic environments for bumble bee fauna such as Palearctic distribution. Total 314 specimens of bumble bees were collected. From the above results, it is concluded that maximum number of specimens of *B. rufofasciatus* were observed from four localities while specimens of *B. asciaticus and B. haemorrhoidalis* were collected in minimum number. Total identified specimens were belonging to three species of one genus namely *B. rufofasciatus*, *B. asciaticus* and *B. haemorrhoidalis.* Among reported species two species i.e. *B. rufofasciatus and B. haemorrhoidalis* are Palearctic. However, one species *B. asciaticus* is Palearctic and Oriental.

In the current study, it is concluded that different species of bumble bee fauna have been collected from seven different localities. According to Banaszak (1995) some important factors that leads to reduce pollinators like destruction of habitats, absence of pollinated flowers, and destruction of nesting due to urbanization and extraordinary use of insecticides are important factors affecting bumble bee fauna.

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