DIVERSITY OF GROUND DWELLING ANTS (HYMENOPETRA: FORMICIDAE) IN RAJBIRAJ, SAPTARI, NEPAL

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A Thesis submitted in Partial fulfillment of the requirement for the award of degree of the Master of Science in Zoology with special paper Entomology

Submitted to:

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April 2022

DECLARATION

I hereby declare that the work presented in the thesis entitled **"DIVERSITY OF GROUND DWELLING ANTS (HYMENOPTERA: FORMICIDAE) IN RAJBIRAJ, SAPTARI, NEPAL"** has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

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This is to recommend that the thesis entitled **"DIVERSITY OF GROUND DWELLING ANTS (HYMENOPTERA: FORMICIDAE), IN RAJBIRAJ, SAPTARI, NEPAL"** has been carried out by Kopila Yadav for partial fulfillment of Master Degree of science in Zoology with special paper Entomology. This is her original work and has carried out under my supervision. To the best of my knowledge, this work has not been submitted for any other degree in any institutions.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Kopila Yadav entitled **"DIVERSITY OF GROUND DWELLING ANTS (HYMENOPTERA: FORMICIDAE), IN RAJBIRAJ, SAPTARI, NEPAL"** has been accepted as a partial fulfillment for the requirements of Master's Degree of Science in Zoology with special paper Entomology.

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LIST OF ABBREVIATIONS

Abbreviated form	Detailed of Abbreviations
asl	above sea level
B	Bait traps
cm	centimeter
GPS	Global Positioning System
H	Hand collection
Km	Kilometer
P	Pitfall traps
Sp	Species
Spp	More species of same genus

ABSTRACT

Ants are important in terms of biodiversity as they are the most diverse, abundant and ecologically significant organisms on earth. Ants were collected using pitfall traps, bait traps and manual collection in three different habitats viz. forest, cultivated land and grass land in spring and winter seasons. This study documented the ant's genera and assess species richness and their diversity in different habitats and seasons using different methods. Altogether 1350 ants were collected representing four subfamilies, 17 genera and 36 morphspecies. Formicinae was the most dominant sub-family (62.59%), followed by Myrmicinae (22.59%), Ponerinae (13.92%), Pseudomyrmicinae (0.89%). *Camponotous* was the most abundant genus as well as the most adapted genus which was most specious genus (12 morphospecies). Among the three sites, the similarity index (0.85) was found highest between forest and grassland. Species richness (10), Shannon diversity index (0.97) and abundance (448) were higher in spring in comparison to winter season. Similarly species richness (17), Shannon diversity index (1.03) and evenness (0.36) were found highest in forest, while species abundance was least in cultivate land. The One-way ANOVA concluded that relationships between habitats and ant diversity as well as with seasons were statitistically insignificant as the $p<0.05$. Pitfall trap was most effective method for ants collection as the ants collected through this method was maximum (946) as compared to bait trap (404) and manual hand collection in all habitats and seasons.

1. INTRODUCTION

1.1. Background

Ants are one of the most successful groups of organisms, present in all the terrestrial ecosystems of the earth (Holdobler and Wilson, 1990). They are eusocial organism, characterized by brood care overlapping generation of workers within the colony and a highly developed cast system (Agosti et al. 2000). Ground dwelling ant species have small body size, small stationary nests and fairly restricted foraging range (Holldobler and Wilson 1990). These attributes guarantee a tight habitat connection for the ant and make them sensitive to environmental changes (Agosti et al. 2000). Ants are important functionally at many different tropical levels and play critical ecological roles in soil turn over, nutrient cycling, plant protection, seed dispersal and seed production (Agosti et al. 2000). Ants are responsible for a wide range of scientific research including studies in behavior, ecology and evolutionary biology (Andersen et al. 2003) Ants perform several significant funct ional roles, as predators of other arthropods whereas sometimes behaving as destructors in nature of being serious herbivores (Holldobler and Wilson 1990). Ground dwelling ants exert a strong influence on the arthropod community in tropical rain forests (Holldobler and Wilson, 1998). Ants can be sampled rapidly, and the diversity of ants in a community is a good indicator for the diversity of other invertebrate species (Alonso, 2000). They have been used as a powerful tool in several ecological studies (Folgrait, 1998; Lach et al. 2010).

Ants are the social insects evolving since cretaceous period. There are 17 extant subfamilies, 338 genera and 13,911 species (Bolton 2021). Over 80% of the ants, species described fall under four subfamilies viz: Myrmicinae, Formicinae, Ponerinae and Dolichoderinae (Guenard, 2013).Different collecting methods have been used to sample ant fauna and these vary in their efficacy and selectivity in capturing ant species (Bestelmeyer et al .2000). A large percentage of the ground dwelling fauna was captured using a combination of different trapping system (Bestelmeyer et al. 2000; Fisher, 1999; Olson, 1991). Pitfall traps are cost- effective techniques and are probably most widely and frequently used method for ground -dwelling ants. It provides a reasonably good estimation of species richness and relative abundance. These traps are easy to use and can be operated continuously during day and night over extended periods of time with little attention required but may not be effective fo r species associated with soil, deep litter and vegetation (Majer,1997). Nepalese myrmeco fauna represent includes 8 known sub-families; Amblyoponinae Dolichoderinae, Dorylinae, Formicinae, Leptanillinae, Myrmicinae, Ponerinae and Pseudomyrmicinae with 48 genera and 128 species with 33% of total species (Subedi, et al. 2020).Among eight subfamilies, Myrmicinae is the largest subfamily which comprises of 41.37% ant species of genera *Myrmica*, *Strumigenys, Meranoplus* and *Pheidole* having more species followed by sub-family Formicinae which comprises of 20.68% ant species with genera *Formica, Polyrhachis* , *Camponotus and Prenolepis* having more species (Bharti and Subedi 2020). The data on Nepalese ants are also available in different web -based resources such AntWeb, 2020 and AntWiki, 2020. Ants in Nepal occupy a variety of habitats such as leaf litter, trees, soil and dead logs while tramp species prefer human modified habitats (Bharti and Sharma 2009).

1.2 OBJECTIVES

1.2.1. General Objective

To explore the diversity of ground-dwelling ants in Rajbiraj, Saptari district, Nepal.

1.2.2. Specific Objectives

To evaluate diversity of ants in different habitats.
To compare diversity of ants in winter and spring season. To access the efficacy of collection methods.

1.3 Rationale of the study

Ants are important components of ecosystem not only because they constitute a great part of the animal biomass but also because they act as ecosystem engineers. Despite in great importance of ant ecology, the diversity and distrib ution of ants in Nepal is not well known. Few researches have been done in diversity and distribution of ants in Nepal (Collingwood, 1970, Elmes and Radchenko, 2009, Adhikari 2017,Neupane 2018, Adhikari, 2020, Bharti, Subedi and Alonso, 2020) were done in Nepal. However, no study in ants was seen from Rajbiraj, Saptari. Study was conducted to determine ant diversity, species richness and evenness index according to various habitats, seasons and methods. The research will create a preliminary base for the study of the ground dwelling ants in the future for the other researchers. Identifying ants in genus level can provide useful information on environmental monitoring, conservation, evaluation and ecological research.

2. LITERATURE REVIEW

2.1. In the context of Nepal

Forel (1906) first reported Nepalese ants; *Aphaenogaster pachei* and Myrmica *pachei*.

Collingwood (1970) published the first list of 34 species of ants of Nepal from the collections ofthe1954 BritishMuseumexpeditiontotheKhumbuHimalRegion. Hecollected34species of ants from different parts of Nepal from the altitudes ranging 850m to 4500m asl. Out of which, 12 were generally distributed over the whole Himalayan region, 12 were eastern Himalayan, eight were western Himalayan and two were endemic to Nepal.

Elmes and Radchenko (2009) recorded the two different species of *Myrmica: M. webri and M. alperti* from Makalu-Barun National Park, Nepal.

Adhikari et al. (2016) documented 30 genera and 70 morphospecies in three habitats and two seasonsinLahachowk,Kaski.

Sixteen genera using bait traps, pitfall traps and leaf litter method in Shivapuri Nagargun National Park, Nepal were recorded and reported new genera *; Pachycondyla* and *Echinopola* to Nepal (Neupane and Subedi 2018)..

Subedi et al. (2020) updated checklist of Nepalese ants that included 128 named species in 48 genera and 8 sub-families (Myrmicinae, Formicinae, Ponerinae, Dolichoderinae, Dorylinae, Pseudomyrmicinae, Leptanillinae and Amblyoponinae) with 33% of total species where Myrmicinae was the largest with 53% of total species followed by Formicinae.

Adhikari et al. (2020) recorded 12 genera using pitfall traps, using bait traps and opportunistic hand collection in attitudinally and seasonally in Phulchowk hill, Lalitpur, Nepal.

Subedi et al. (2021) recorded six sub-families and 36 genera from 70 ant species along the north and south belt transects in eastern, central and western Nepal by using vegetation beating, sweeping and hand collection methods in selected forest types. Forest an t species richness was high in western forests of Nepal.

2.2. In the global context

Andrade & Del- Claro (2007) investigated the variety of ant diversity on the ground of an ecological reserve in three different areas of Cerrado forest. Found 77 ant spe cies distributed in 22 genera and 6 subfamilies. *Camponotous* and *Pheidole* were the most common genera. Similarly, Chavhan and Pawar (2011) explored ants in forest, grassland and human habitats located around Amrawati city. They found 34 species, 20 genera by collecting samples in three habitats where *Crematogaster, Pheidole* and *Camponotus* were the most dominant species. Raja (2017) studied ants of Medinipur West Bengal, India and applied all search out method where 34 species and 20 genera of ant species were found in which *Crematogaster* was most dominant one.

Ryder et al. (2010) surveyed species diversity and distribution patterns of the ants in a lowland primary rainforest in Western Amazonian Ecuador, using canopy, fogging, pitfall traps, baits, hand collecting, mini -Winkler devices and subterranean probes to sample ants. A total of 489 ant species compromising 64 genera in nine subfamilies were identified.

Mahalakshmi and Channaveerappa (2016) explored the diversity of ants in the Campus of Maharani's Science college, Karnataka. Captured a total of 978 individuals representing 20 morphospecies belonging to 12 genera and four subfamilies. Myrmicinae was the most dominant subfamily followed by Formicinae, Ponerinae and Pseudomyrmicinae. Manikandan, Anusuyadevi and Sevarkodiyone (2018) studied diversity of ants in three different sites of Thiruthangal, Sivaksi taluk, Virudhunagar, India. Noted totally eleven species of ants belonging to four subfamilies where seven species found in residential sites, five ant species were found in industrial sites and eleven species found in agricultural sites. Myrmicinae was a dominant subfamily in three study sites and Dolichoderinae had a low relative abundance during the study period. Khan (2018) surveyed the diversity of outdoor ants in a college campus of Kajrat city, Ahmednagar district, Maharashtra, India. Nine species were recorded belonging to subfamily Myrmicinae, Formicinae and Dolichoderinae.

Hazra (2018) surveyed ant species diversity at Contac Municipality, Purbi, Medinipur West Bengal, India and recorded 15 species belonging to five subfamilies of ants. Out of the five subfamilies, Formicinae was the most dominant family in terms of species richness followed by Myrmicinae, Psudomyrmicinae, Dolichoderinae and Ponerinae.

Fisher and Robertson (2002) studied species richness and composition of ant's assembling in adjacent mountain forest and secondary grassland habitats in the central plateau of Madagascar was evaluated .They used five quantitative methods and compared methods within and across habitats. They collected 26 species in grassland and 31 species in forest. Deblauwe and Dekoninck (2007) was studied diversity and distribution of ground-dwelling ants in lowland rainforest in Southeast Cameroon where they studied the effect of vegetation type on ant species density, activity and composition and observed 145 ant species with the help of pitfall trap. Differences in species density, activity and composition between vegetation types were explained by developmental stage of vegetation type.

Graham et al. (2004) studied habitat disturbance, species richness, equitability and abundance of ants in the Fall -Line Sandhills, at Fort Benning, Georgia. They collected 48 species of ants belonging to 23 genera over four years of sampling and noted that highly disturbed areas had fewer species and greater number of ants than did moderately or lightly disturbed areas. Leal et al. (2012) surveyed the relative effects of habitat fragmentation and habitat structure on ant species and functional composition in the Atlantic forests of northeast Brazil were examined and found fragment size and tree density were the most important variables predicting species richness and functional diversity. Chavan and Patkar (2014) compared grou nd-dwelling ants in undisturbed and disturbed habitat of Great Indian Bustard Wildlife Sanctuary in Maharashtra state, India. They used pitfall trap, scented trap and visual searching to collect ants and found more in undisturbed site.

Forys and Allen (2005) explored the relationship between sprawl and biodiversity using a data set of ant species collected from 46 habitat patches located in the increasingly suburbanized Florida Keys, USA. They identified 24 native and 18 nonnative species of ants using bai t transects and found that neither the overall number of native species nor the number of rare native species were significantly affected by the amount of development or proximity to roads and the number of non-native species was significantly correlated with the amount of development.

Bruhl and Eltz (2010) studied the community of ground -dwelling ants in different plantations in Sabah, Malayesia, over 2 years using tuna baiting. Nine of the 23 ant species baited in the plantations were never recorded inside forest and most common species was *Anoplolepis* *gracilipes* , an invasive species present at 70% of all bait sites and known to cause ecological meltdown in other situations.

Santoandre et al.(2019) studied ant taxonomic and functional diversity showing di fferential response to plantation age in two contrasting biomes in Argentina. A total of 12,435 ants collected during samplings in both biomes and found opposite environmental similarity gradient between natural habitats and plantation ages.

Levings (1983) examined patterns of species distributions in ground ants on Barro Colardo island (BCI), Republic of Panama, using baited transect samples and Berlesc extraction of litter arthopods. More species and more individuals were collected in wet than dry seasons using either methods. Evenness of species abundance at baits also increased during the wet seasons. Overall seasons, fewer species and fewer individuals were collected at drier,

3. MATERIALS AND METHODS

3.1. Study Area

The study was carried out in Rajbiraj, Saptari (26º32'60"N and 86º45'0"E.) Nepal. The study was conducted in three habitats viz forest, cultivated land and grass land in winter (January - February) 2020 and spring (April-May) 2020. The average temperature of spring was 32ºC (maximum 38º c and minimum 26ºC), winter was 21º c(maximum 27º c and minimum 14º c).This study area has tropical climate. Different types of vegetation were found in different habitats. In forest, vegetation like *Carica papaya, Cocus nucifera, Dalbergia sissoo, Ficus racemosa, Litchi chinensis, Mangifera indica, Melian azedarach* . In grassland *Cynodon dactylon, Imperata cylindrical, Oplimenus* sp. of grass were available. *Zea mays, Brassica species, Triticum astivum, Eleusine corocana, Oryza sativa, Glysine max* were crops species found in cultivated land. Forest contains sandy soil with stone but cultivated, and grassland had alluvial soil. In forest, anthropogenic activities were relatively low in comparison to cultivated land and grassland. Forest and cultivated land were protected against grazing but grassland was open type.

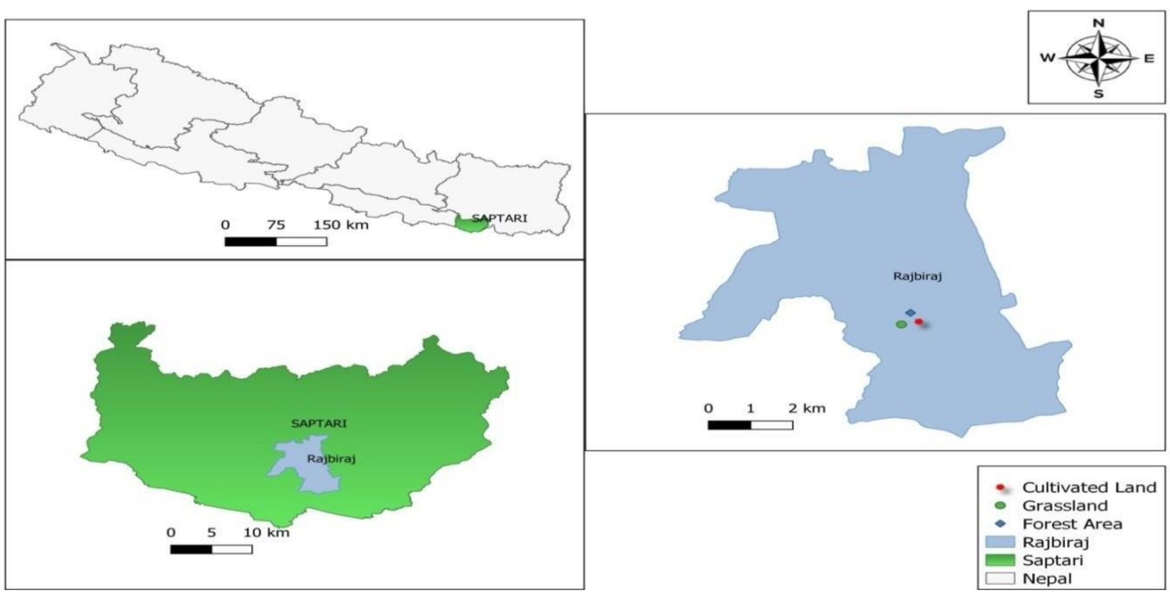

Figure1: Map of study area

3.2 Materials

Pitfall traps, Bait trap, Camera ,Digger, Ethyl Alcohol ,Cotton ,GPS ,Vials, Measuring tape, Feather weight forceps ,Stereo microscope.

3.3. Methods

3.3.1. Sampling Methods

Samples were collected three times in two season: winter season (January -February) 2020 and spring season (April -May) 2020. Ants were collected at three sites: forests, cultivat ed land and grass land using pitfall traps (Santondare et al. 2019), bait traps (Adhikari 2020) and hand collection method (Subedi et al. 2021). For removal of sampling errors, three different ant collection methods to collect maximum number of ant species from study area. The trapped ants of all this methods were preserved in vials containing 70% ethanol.

1.*Pitfall Trap*

Pitfall trap consists of a plastic cup with opening of 12 cm in height and eight cm in diameter, buried at ground level. Ten pitfall tra ps were placed in 100 m area in 10 m distance at each site. Each cup carried 25 ml of soapy water. Samples were collected after 48 hrs.

2.*Bait Trap*

Bait traps consists of paper (10*10) cm. Ten Bait traps were placed in 100 m area with the distance of 10 m apart. 0.25 gm of sugar and butter were added in each trap and samples were collected after 30 minute of its placement with the help of feather -weight forceps and collected specimens were preserved in 70% ethanol.

3.*Opportunistic Manual Collection*

Hand collection of ants from each sampling plot was carried out to collect representative individuals of all species found in the each site (under stones, under logs, under moss) after using the baits.

3.3.2. Identification of Ants

Ants were photographed by using Samsung digital camera and identified genus level with the help of stereo-zoom trinocular microscope, based with the help of taxonomic keys (Bolton 1994, Holdobler and Wilson, 1990) . Collected specimens were deposited to the Zoology Department of Amrit Campus.

3.4 Data processing and statistical analyses

The data were analyzed using Microsoft Office Excel, 2007. The results were used to indicate the ant diversity in habitats, season and method. Species diversity was simply calculated by counting the number of species in different habitats and seasons. Shannon -Weinner index was used to calculate the species diversity indices. Evenness index was used to know the closeness of species of ant in type of habitats, method and seasons. The diversity index of each sampling plot was first calculated with the presence data of species richness and the frequency of each species by using

Shannon-Weinner Diversity index $(H') = -\sum(Pi)*(\ln Pi)$

Pielou's Evenness Index $(J)' = H'/Hmax$

To measure the similarity between two community samples, coefficient of Sorensen was used as the following equation.

$QC = 2a/(2a+b+c)$

One- way ANOVA was used to calculate the relation between the habitats heterogeneity and species richness as well as to find out the association between the seasons and species richness.

4. RESULTS

In total 1350 ant specimens were collected which represented four subfamilies (Formicinae, Myrmicinae, Ponerinae and Pseudomyrmicinae), 17 genera (*Camponotus, Polyrachis, Catalacus, Oecophylla, Par atrechina, Nylanderia, Aphaenogaster, Crematogaster, Pheidole, Monomorium, Lophomyrmex, Tetramorium, Leptogeny, Brachyponera, Odontoponera, Odontomachus, and Tetraponera*) and 36 morphospecies through three different methods (Pitfall traps, Bait traps and opportunistic manual collection)(Table 1). Out of four subfamilies, Formicinae represented the most abundant subfamily where as Pseudomyrmicinae the least (Figure 2)

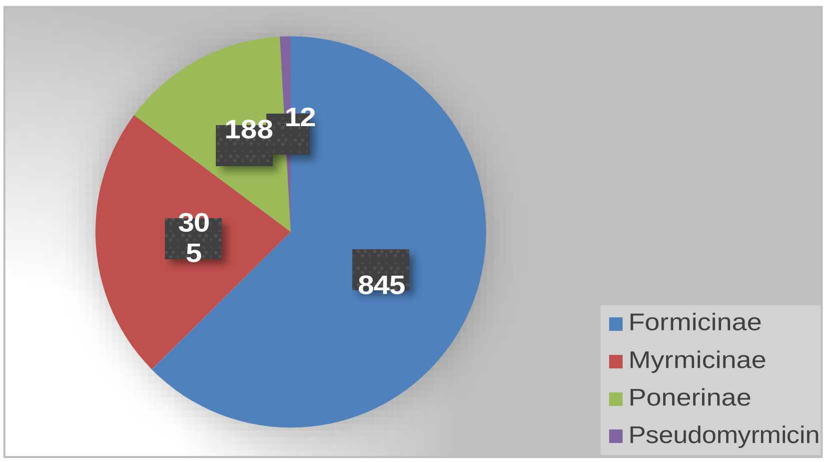

Figure 2:Subfamily wise ant abundance

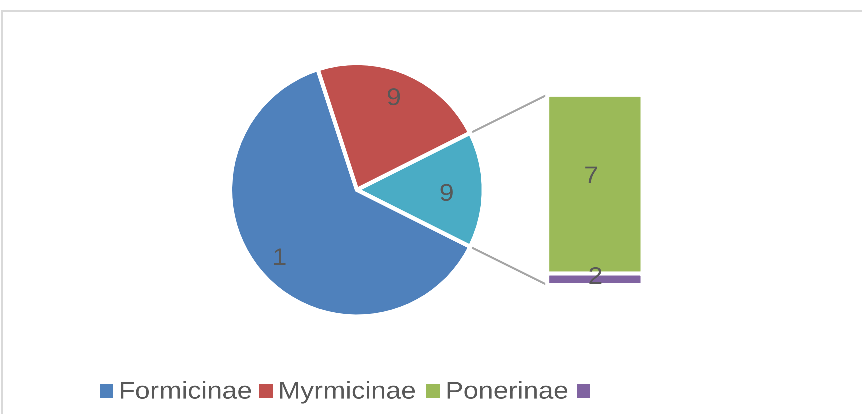

Figure 3:Subfamily wise ant morphospecies

4.1. Species richness

Four subfamilies (Myrmicinae, Formicinae, Ponerinae and Pseudomyrmicinae),17 genera *(Camponotus sp, Polyrhachis sp, Oecophylla sp, Paratrechina sp, Nylanderia sp, Aphaenogaster sp, Crematogaster sp, Pheidole sp, Monomorium sp, Catalacus sp, Lophomyrmex sp, Tetramorium sp, Leptogeny sp, Brachyponera sp, Odontoponera sp, Odontomachus sp, and Tetraponera sp*) are reported. Formicinae bear five genera with 18 morphospecies followed with Myrmicinae with seven genera and nine morphospecies, Ponerinae with four genera and seven morphospecies and Pseudomyrmicinae with single genera and two morphospecies (Table 1).

Table 1: Ant genera reported from the study area

S.N	Sub-Family	Genus	Morphospecies
1.	Formicinae	*Camponotus* Mayer,1861	12
		Polyrachis Smith,1857	2
		Nylandria Forel,1894	1
		Oecophylia Smith*,1866*	1
		Paratrechina Fisher, 2014	2
2.	Myrmicinae	*Pheidole* Westwood,1839	2
		Aphenogaster Mayar,1853	1
		Crematogaster Lund,1831	2
		Tetramorium Mayr,1855	1
		Lophomyrmex Emery,1892	1
		Catalacus Smith,1853	1
		Monomorium Mayr,1855	1
3.	Poneriane	*Brachyponera* Emery, 1900	2
		Leptogeny Roger, 1861	2
		Odontoponera Mayr,1862	2
		Odontomachus Latreille,1804	1
4	Pseudomyrmicinae	*Tetraponera* Smith,1852	2
	Total	17	36

4.2. Diversity of ants in different habitats

The study was conducted in three sites: forest, cultivated land and grassland. In forests, species richness (S), Shannon's index of species diversity (H') and evenness index (J) were calculated as: S= 17,H'= 1.00and J= 0.36. The maximum number of ant individuals collected at this site was *Camponotous sp1, Aphaenogaster sp, Odontoponera sp1, Odontomacus sp* and *Crematogaster sp*(Appendix III).

In cultivated land, species richness (S), Shannon's index of species diversity (H') and evenness index (J) were calculated as S=15, H'= 0.97, and J= 0.35. The number of ant individuals collected at this site was *Polyrachis sp1, Camponotous sp.12* were most abundant species at this site (Appendix III).

In grass land, species richness (S), Shannon's index of species diversity (H') and evenness index (J) were calculated as S=16 ,H' =0.96 and J= 0.34 .The number of ant individuals collected at this site was *Catalacus sp1, Tetraponera sp1* were most abundant species at this site.(Appendix III).

In one- way ANOVA it is proved that significant difference between ant species composition in various habitat at $p<0.05$ level of significance (p - value=0.002), F- value (16.9)

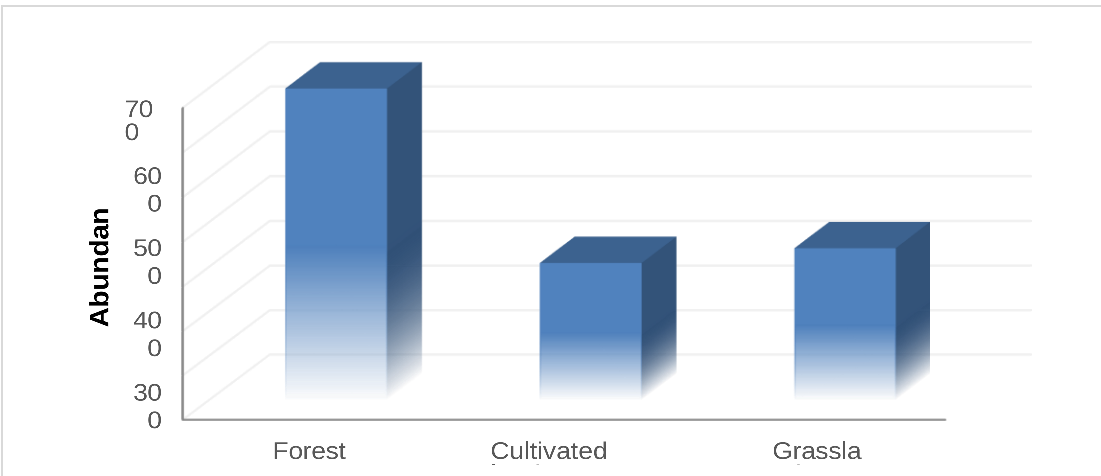

Figure 3: Ants abundance reported in different habitats

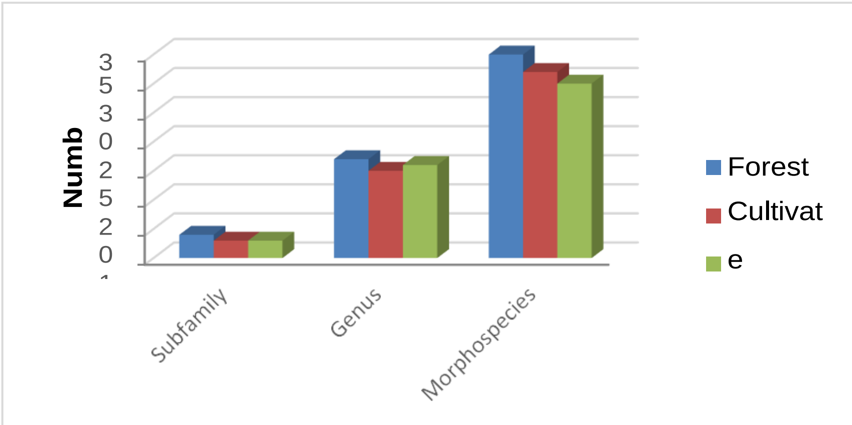

Figure 4: Subfamilies,genus, morphospecies of ants in forests, cultivated land and grassland.

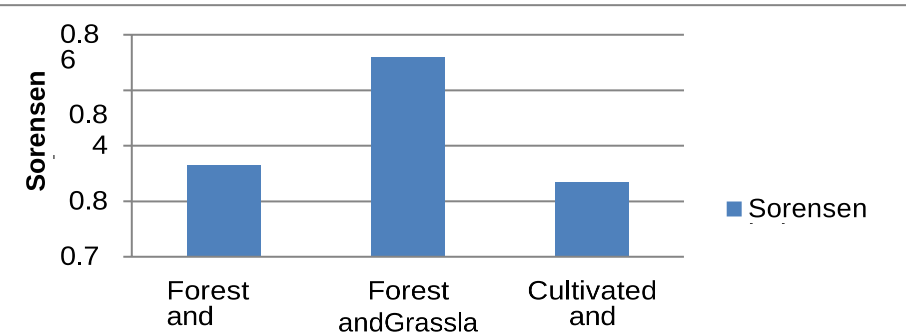

Figure 5: Sorensen index between habitats

4.3. Variation of ants in winter and spring season

This research was done in winter and spring season four sub -families, 17 genera and 36 morphospecies. Four subfamilies 16 genera, 32 morphospecies were collected in spring season and three subfamilies, 12 genera, 20 morphospecies were collected in winter seasons. Maximum species richness (20) was recorded in spring as compared to winter (16). Similarly, the Shannon index of species richness (H') was recorded as the highest during spring season as H'=1.06 and winter H'=0.93 as a greater number of individuals was collected in spring (902), and winter (448). Higher species richness (16) in forests was recorded in spring as compared to winter (15)(Table 2).The ANOVA comparisons of ant species richness across the two seasons detected significant difference between the seasons at p< 0.005 level of significance (p-value=0.015), F-value (16.9).

Table 2: Ant diversity reported in two seasons with habitats

Seasons	Habitats	Species richness(S)	Shannon diversity Index (H')	Evenness index (J)	No. of ants collected
winter	Forests	15	0.59	0.22	226
	Cultivated land	16	0.55	0.20	98
	Grassland	15	0.56	0.20	124
spring	Forests	16	1.00	0.36	473
	Cultivated land	17	0.59	0.21	211
	Grassland	16	0.59	0.22	218

4.4. Variation of ants collected through different methods

Pitfall method collected maximum ant ind ividuals (946) followed by Bait method (347) and Opportunistic hand collection (57)(Figure 5). Majority of ants collected by pitfall method included *Camponotus sp1, Camponotous sp2, Camponotous sp3, Camponotous sp4, Camponotus sp5, Camponotous sp8, Camponotus sp10, Aphaenogaster sp , Polyrachis sp, Leptogeny sp, Brachyponera sp, Pheidole sp2, Odontomachus sp* and *Odontoponera sp. Camponotous sp6, Camponotus sp7, Camponotous sp9, Camponotus sp12, Polyrachis sp ,Nylandria sp, Paratrechina sp, Aphenogaster sp, Crematogaster sp1, Tetramorium sp, Lophomyrmex sp, Monomorium sp, Leptogeny sp, Brachyponera sp* were collected through bait traps and *Catalacus sp, Oecophylla sp* and *Tetraponera sp* were collected through hand collection only.

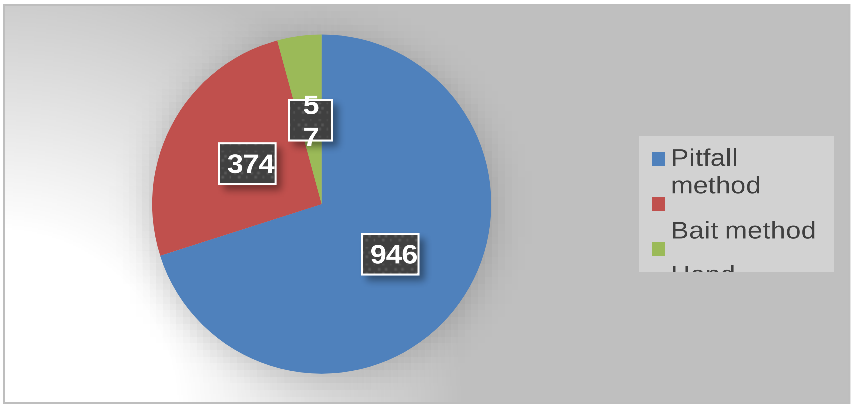

Figure 6: Abundance of ants collected in different sampling methods.

5. DISCUSSIONS

5.1 Species richness

The present study recorded 1350 ant specimens representing four subfamilies (Formicinae, Myrmicinae, Ponerinae and Pseudomyrmicinae), 17 genera and 36 morphospecies in Rajbiraj(Table 2) . Out of 17 genera most speciose genus was *Camponotus* (12 morphospecies) followed by *Crematogaster* , *Pheidole* *,Leptogeny, Odontoponera* and *Brachyponera* with two morphospecies were more diverse genera and with single specimen. This result somehow agree with the study that recorded *phedole,crematogaster* and *Camponotus* are the most prevalent genera globally (Wilson, 1976; Ryder Wilkey et al . 2010).In this study,*camponotus ,aphenogaster,crematogaster* and *Leptogeny*were the most abundant genera,occurring almost 89%of samples out of the five subfamilies formicinae was the most dominant family in term of species richness Hazara(2018).Similar result was documented from Cerrado forest by Andrade and Del-Claro (2007) they documented *Camponotous and Pheidole* were the most common genera. *Camponotous* was the most frequent occurring species visual everywhere. These are called carpenter ants because of their nesting behaviours (Chavan and Pawar, 2011). Abundance of Myrmicinae is more due to availability of food and nesting sites and they have high potential to adopt varying environmental conditions. They are found in different habitats, *Pheidole* nests in soil while *Crematogaster* nests on dead wood of trees (Anderson, 2000). Only genus *Tetraponera* represents Pseudomyrmicinae has been recorded. These are solitary forages and make them in dead woods and rotten logs (Chavan, 2014).

5.2 Ant diversity comparison among habitats

Study show that considerable variation of the an t diversity in habitat wise i.e forest was slightly richer in ant species (35morphospecies) than the cultivated land (32morphospecies) and the grassland (30morphospecies)(Figure 5). This finding was agreed with the Fischer and Robertson (2002), recorded 19 species from grassland and 31 species from forest in Plateau of Madagascar. Species recorded by Fischer and Robertson (2002) was highly maximum in compared to the verdict because they used five methods of data collection. Species composition in grassland and cultivated land was different from forest due to the absent of higher vegetation line (Fischer and Robertson, 2002). Calcattera et al. (2010) recorded higher species 39 in forest and 29 in grassland of Argentina that was parallel with Rajbiraj outcome. Similarly, in Amravati City of India, Chavan and Pawar (2011) recorded 30 species of ants in forest, 22 species of ants in human settlement and 15 species in grassland that was agree with this finding. Lower species richness and evenness of ants in differ ent habitats is due to disturbance (Bruhl et al. 2003). Similar result was documented from Georgia by Graham et al. (2004). They documented that highly disturbed areas had fewer species and greater number of ants than moderately or lightly disturbed areas . Leal et al. (2012) found fragment size and tree density were the most important variables predicting species richness and functional diversity. Study area, anthropogenic disturbance in cultivated land mostly occurred during tilling and harvesting period. Similarly in grassland livestock disturbance occurred. Due to this reason in grassland, least number of morphospecies was collected in comparison to forest and cultivated land. Grazing is also a cause to reduce and affected the faunal composition, includin g ant species in grassland Hays and Holl (2003) that is similar to result of study. Deblauwe and Dekoninck (2007) reported the ant species richness generally increases with increase in vegetation. By the similarity measurement, forest and grass land (0.86) showed the most similar ant species diversity. It is possible that the places of forest may coexist of similar microhabitat types occurring in grassland. Similar result with the study of ants in Lahachowk, Kaski, Nepal (Adhikari et al. 2020).

5.3 Seasonal variation

The study was carried in winter and spring season. Maximum species richness (20 morphospecies) was recorded in spring compared to winter. Study shown considerable variation of the ant community due to seasonal variation. This conclusion was mostly compared to the conclusion described by some of the researchers as for seasonal pattern of ants was studied in five seasons in Punjabshivalik range of North -West Himalaya which conclude the only 5 species were recorded during winter season and 40 species during summer season (Bharti et al. 2009). Similarly ,composting and activity patterns of ants was observed by various methods in three different habitats in summer and winter in the semi -arid Karoo, south Africa which showed the result that ant abundance was greater during summer than winter (Lindsey and skinner,2001). Species richness varied in different seasons temperature and moisture availability (Adhikari 2016). (Rico - Gray et al. 1998). They alter and gradually halt their activities and process to hibernation due to cold as temperature and moisture availability decreases in winter and gradually increased from spring. Thus species richness is low in winter in comparison to spring season correlated with outcome result. Anusuyadevi and Sevarkodiyone (2018). found that species richness was high in dry season than wet season fewer species and fewer individuals were collected at drier, sunnier sites. He found that moisture availability is an important contributes to these patterns of among site an d among season variation as well as moisture availability may affect the distribution of suitable nest sites. As the weather warmed the activity increased in different rates in different habitats, depending upon the temperature and moisture availability (Le vings, 1983).

5.4 Effectiveness of ant collection methods

The study has shown variation in the total individuals of the ants collected Pitfall method was the most successful method of ant collection (946) followed by bait method (374) and then hand collection method (57)(Figure 7). This study was covalent to the study done upon the ant by using different techniques in eight different localities in the Venezuelan Llanos, Savannas Romero and Jaffe (1989) . The best result of ant collection was obtained by a combination of hand collecting and pitfall traps. It was concluded the pitfall method was the most successful method with 28 genus and 91 species Gadagkar et al.(1993). Majer and Delabie (1994) compared leaf litter and soil and fauna which had shown that a combination of pitfalls, litter shifting, baiting and hand sorting increase the efficiency and of specimens captures in comparison to any single method by itself. Martelli et al (2004). Litter sifting yielded more individuals, more species and more occurrences of most species than pitfall traps, but neither method captured all species.Barech et al. (2016) sampled ants in the saline Dry Lake Chott El Hodna in Alergia, a Ramsaar Conservation Wetland. More species of ants were collected by pitfall trapping (20) than hand sampling (15). This result was correlated with the study. In the same way, composition and activity patterns of ants was observed by various methods (pitfall method, quadrant method and dig sampling method) in three different habitats in summe r and winter in the semi-arid Karoo, South Africa which concluded that pitfall method recorded the most species as compared to dig sampling method or quadrant sampling (Lindsey and Skinner,2001). So, pitfall traps was the easiest and most effective method as these traps were fully opened for whole day and night and highest number of ant species were collected

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The present study has been carried out to explore the species richness, species diversity, evenness and abundance of ants in Rajbiraj. Altogether four subfamilies, 17 genera and 36 morphospecies were recorded. This study concludes that subfamily Myrmicinae and Formicinae were the dominant among the other recorded sub-families. This study showed that the most preferred habitat was forest area inhabiting large number of morphospecies followed by Cultivated land and grass land. In case of Season, spring season was the most diverse (20 morphospecies) in comparison to winter season (16 morphospecies) .Likewise, Shannon diversity index was highest in spring season. Pitfall traps was the most effective technique for ant collection over bait traps and manual collection in all habitats and seasons. This result indicated ,spring season and forest were the best time and habitat for ant fauna respectively.

6.2 Recommendations

Based on the results following are the recommendations.

The research was carried out for two seasons so; in depth, research should be designed to cover more season within a year and in between year.

Moreover, continuous monitoring of the ant fauna is necessary so that any changes in the environment that may occur in future can be identified and appropriate measures can be taken to counterthem.

In this research three methods were used for data collection, for more exploration of ants' other effective method leaf litter shifting could be used.

The taxonomic work of present study was done up to genus, further the taxonomic study could be extended up to species level.

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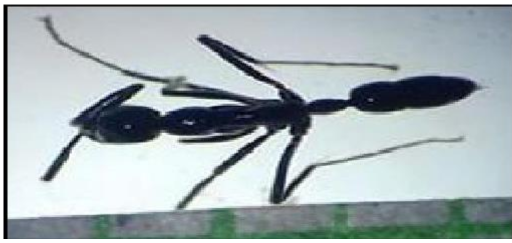
8. APPENDICES

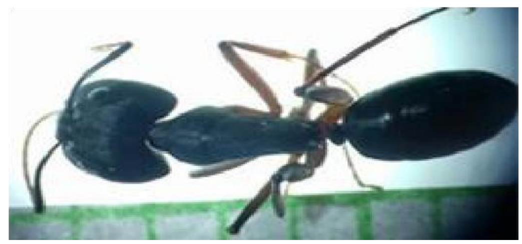
APPENDIX I: Photo plate of representative genus recorded in study area



Leptogeny(Ponerinae)

Tetraponera(Pseudomyrmicinae)

Camponotous A(Formicinae)



Camponotous B(Formicinae)

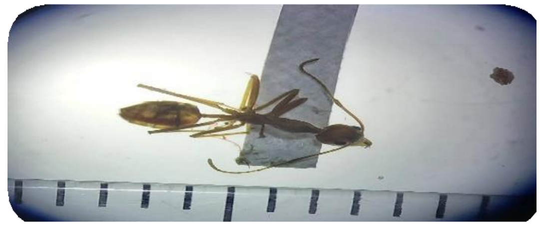
Crematogaster(Myrmicinae)

Brachyponera(Ponera)

Odontomachus(Ponera)

Paratrechina(Formicinae)

Polyrachis(Formici



Oecophylia(Formicinae)

Catalacus(Myrmicinae)

Odontoponera(Ponera)

Appendix II: Ant Genera with Shannon index reported in the Research

S.N	Sub-family	Genus	No of ants	Shannon diversity index
1.		*Camponotous sp1*	105	0.19864
2.		*Camponotous sp2*	90	0.36524
3.		*Camponotous sp3*	75	0.36709
4.	Formicinae	*Camponotous sp4*	37	0.30102
5.		*Camponotous sp5*	16	0.1917
.		*Camponotous sp6*	30	0.27293
7.		*Camponotous sp7*	28	0.2636
8.		*Camponotous sp8*	45	0.3257
9.		*Camponotous sp9*	60	0.35509
10.		*Camponotous sp10*	35	0.29367
11.		*Camponotous sp11*	16	0.1917
12.		*Camponotous sp12*	8	0.12129
13.		*Polyrachis sp1*	58	0.35227
14.		*Polyrachis sp2*	37	0.30102
15.		*Nylandria sp1*	73	0.36635
16.		*Oecophylia sp1*	20	0.21915
17.		*Paratrachina sp1*	48	0.3332
18.		*Paratrachina sp2*	64	0.35981
19.		*Pheidole sp1*	39	0.30787
20.		*Pheidole sp2*	34	0.2898
21.		*Aphenogaster sp1*	35	0.29367
22.		*Crematogaster sp1*	32	0.28165
23.		*Crematogaster sp2*	8	0.12129
24.		*Tetramorium sp1*	60	0.35509
25.	Myrmicinae	*Lophomyrimex sp1*	45	0.3257
26.		*Catalacus sp1*	2	0.04304
27.		*Monomorium sp1*	50	0.33772
28.		*Brachyponera sp1*	26	0.25361
29.		*Brachyponera sp2*	19	0.21267
30.	Ponerinae	*Odontoponera sp1*	31	0.27737
31.		*Odontoponera sp2*	24	0.24291
32.		*Odontomachus sp1*	33	0.2858
33.		*Leptogeny sp1*	28	0.2636
34.		*Leptogeny sp2*	27	0.25869
35.		*Tetraponera sp1*	9	0.13158
36.	Pseudomyrmicinae	*Tetraponera sp2*	3	0.05898

Appendix III: Ants reported in different habitats

S.N.	Species	Forests	Shannon index	Cultivated land	Shannon index	Grassland	Shannon index
1.	*Camponotous sp1*	44	0.17408	30	0.22642	31	0.21762
2.	*Camponotous sp2*	49	0.18631	17	0.15955	24	0.18644
3.	*Camponotous sp3*	23	0.11234	27	0.21299	25	0.19122
4.	*Camponotous sp4*	23	0.11234	8	0.0946	6	0.07093
5.	*Camponotous sp5*	9	0.05604	4	0.05627	3	0.04155
6.	*Camponotous sp6*	19	0.098	6	0.07654	5	0.06177
7.	*Camponotous sp7*	14	0.07832	5	0.06673	9	0.09573
8.	*Camponotous sp8*	20	0.10169	18	0.16561	7	0.0796
9.	*Camponotous sp9*	36	0.15276	12	0.12615	12	0.11754
10.	*Camponotous sp10*	17	0.09039	7	0.0858	11	0.11054
11.	*Camponotous sp11*	8	0.05116	3	0.045	5	0.06177
12.	*Camponotous sp12*	4	0.02955	4	0.05627	0	-
13.	*Polyrachis sp1*	26	0.12243	15	0.14686	17	0.1492
14.	*Polyrachis sp2*	25	0.11913	5	0.06673	7	0.0796
15.	*Nylandria sp*	39	0.16103	16	0.15331	18	0.15497
16.	*Oecophylia sp*	11	0.06534	5	0.06673	4	0.05203
17.	*Paratrachinasp1*	21	0.1053	14	0.14019	13	0.12429
18.	*Paratrachina sp2*	31	0.13818	13	0.1333	20	0.16603
19.	*Pheidole sp1*	23	0.11234	7	0.0858	9	0.09573
20.	*Pheidole sp2*	22	0.10885	5	0.06673	7	0.0796
21.	*Aphenogaster sp*	22	0.10885	8	0.0946	5	0.06177

22.	*Crematogaster sp1*	17	0.09039	8	0.0946	7	0.0796
234.	*Crematogaster sp2*	6	0.04084	2	0.03262	0	-
24.	*Tetramorium sp*	29	0.13203	16	0.15331	15	0.13714
25.	*Lophomyrimex sp*	27	0.12568	5	0.06673	13	0.12429
26.	*Catalacus sp*	0	-	2	0.03262	0	-
27.	*Monomorium sp*	27	0.12568	12	0.12615	11	0.11054
28.	*Brachyponera sp1*	15	0.08244	4	0.05627	7	0.0796
29.	*Brachyponera sp2*	9	0.05604	2	0.03262	8	0.08784
30.	*Odontoponera sp*	22	0.10885	4	0.05627	5	0.06177
31.	*Odontoponera sp2*	14	0.07832	3	0.045	7	0.0796
32.	*Odontomachus sp1*	12	0.06978	8	0.0946	13	0.12429
33.	*Leptogeny sp*	14	0.07832	7	0.0858	7	0.0796
34.	*Leptogeny sp2*	14	0.07832	5	0.06673	8	0.08784
35.	*Tetraponera sp*	5	0.03534	2	0.03262	2	0.03007
36.	*Tetraponera sp2*	2	0.01676	0	-	1	0.01706

Appendix IV: Data analysis of two season

Diversity index/ Seasons	Spring	Winter
Species richness (S)	20	16
Shannon Diversity (H)	0.9350	1.0694
Evenness Index (J)	0.3372	0.4644
Number of ants collected	902	448

Appendix V: Ant collected from specific collection methods

S.N.	Season → / Site / Genus ↓	Winter			Spring		
		Forests	Cultivate land	Grass land	Forests	Cultivate land	Grass land
1	*Componotous sp1*	P,B,H	P,B	B	P,B	P,B	P,B,H
2	*Componotous sp2*	P,B	B	H	P,B,H	B	P,B,H
3	*Componotous sp3*	P	B,H	P,B	B	H	P,B
4	*Componotous sp4*		B	H	PB	P,B,H	H
5	*Componotous sp5*		P,B,H	P,B	B	H	
6	*Componotous sp6*	P,B,H	H	P,B	B,H	P,B	
7	*Componotous sp7*		P,B	H	P,B,H	B,H	H
8	*Componotous sp8*	P	P,B		P,B	H	H
9	*Componotous sp9*				P,B	B,H	H
10	*Componotous sp10*	P,B,H		P	B	P,B	B
11	*Componotous sp11*				H,B		
12	*Componotous sp12*	P,B		H		P,B	P,B
13	*Polyrachis sp1*	P,B,H	P,B,H		P,B	B,H	P,B,H
14	*Polyrachis sp2*	P	P,B		B,H	P,B,H	H
15	*Nylandria sp1*				P,B,H	P,B,H	B,H
16	*Oecophylia sp1*		H			P,H	
17	*Paratrechina sp1*	P,H	P,B,H			P,B,H	P,B
18	*Paratrechina sp2*		P,B			H	
19	*Pheidole sp1*	P,B	P,H	H		P,B,H	P,B

20	*Pheidole sp2*	P,B,H	P,B		P,B,H	P,B	
21	*Aphenogaster sp1*		P,B	P,B	P,B,H	P,B	B,H
22	*Crematogaster sp1*	P,B,H			P,B,H	P,B,H	B,H
23	*Crematogaster sp2*	P	B		P,B,H	B	P,B
24	*Tetramorium sp1*			P,B,H	B	B,H	B
25	*Lophomyrmex sp1*	P,B,H	P,B	P,B		P,B,H	B,H
26	*Catalacus sp1*		H	H		H	H
27	*Monomorium sp1*	B,H	P,B		P,B,H	B,H	P,B,H
28	*Brachyponera sp1*	P,B	P,B	B,H		P,B,H	P,B,H
29	*Brachyponera sp2*		B		P,B,H	B,H	P,B,H
30	*Leptogeny sp1*			P,B	P,B,H	H	
31	*Leptogeny sp2*		P,B		B,H	P,B,H	P,B
32	*Odontoponera sp1*		P,B,H		P,B,H	P,B,H	B,P
33	*Odontoponera sp2*				P,B,H	B	P
34	*Odontomachus sp1*	P,B,H	B	B,H	P,B,H	H	P,B,H
35	*Tetraponera sp1*		H		H	H	
36	*Tetraponera sp2*		H			H	