Species Composition of Ants (Hymenoptera: Formicidae) and Thermal Tolerance along Environmental Gradient in the Bale Mountains National Park (BMNP), Ethiopia

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GRADUATE PROGRAMMES

This is to certify that the Dissertation prepared by Chirotaw Ayele Gizaw, entitled: Species composition of Ants (Hymenoptera: Formicidae) and Thermal Tolerance along environmental gradient in the Bale Mountains National Park (BMNP), Ethiopia and submitted in fulfillment of the Requirements for the Degree of Doctor of Philosophy (Biology: Zoological Sciences) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

This study was conducted to investigate the species composition of ants (Hymenoptera: Formicidea) and thermal tolerance of *Camponotus negus* and *Camponotus acvapimensis* in the Bale Mountains National Park (BMNP), Ethiopia. Ants were collected from different altitudes and habitats using manual search, baits and pitfall traps from December 14, 2013 to October 08, 2015. Heat and cold tolerance of ant colonies (*C. negus*) from different altitudes of the BMNP (Gaysay and Sanetti) and from Dilla (*C. acvapimensis*) was determined. The time taken (minutes) by ant colonies to lose mobility at a stressfully high temperature (knock-down resistance) of colonies were measured from each site as an index of heat tolerance. For heat tolerance experiment 10 to 12 individuals from each colony (for a minimum of 24 individuals per colony) were placed into two Petri dishes in identical water baths (Stuart digital water bath, RE300DB, Keison International Ltd, UK). Initially the temperature in both baths was set and maintained at 45 °C for 90 minutes. After 90 minutes, the temperature was raised by 5 °C, and continued to rise by 5 °C every 30 minutes thereafter until all ants had succumbed to heat stress. Time was recorded starting from the time the first ant completely lost mobility till the last ant stopped moving. The time required for an individual to recover from exposure to extremely low temperatures (chill-coma recovery) was used to assess cold tolerance of ants. For cold tolerance experiments, 24 to 27 ants from each colony were placed in a Petri dish covered in ice for 20 minutes. Then the time for each individual to recover from chill-comma was recorded. From a total of 162 sample collections made, 16 species under 8 genera and 4 subfamilies namely; Dorylinae, Formicinae, Myrmicinae and Ponerinae were identified. Subfamily Myrmicinae was the most diverse with nine species

(56.25%) and the genus *Tetramorium* was the most speciose containing five species. *C. negus* was the most abundant species of 54.12% of the samples collected. The highest ant species diversity was observed in the altitude range of 2000– 2500 m followed by 3000–3500 m and the lowest was in the altitude range of 2500– 3000 m and 3500–4000 m. Thus there was no clear pattern of species diversity with elevation. The highest ant species diversity was recorded from the rainforest. *C. negus* from Sanetti (3970 m) had high heat tolerance (143.52 ± 1.80 minutes) than from Gaysay (3040 m) (98.30 ± 1.82) minutes, but cold tolerance did not differ between the two sites. Colonies from Sanetti on average tolerated heat for additional 45 minutes compared to Gaysay. *C. acvapimensis* had similar heat tolerance as *C. negus* from Sanetti but it had very low cold tolerance which took 549.79 ± 18.77 seconds to recover from chill-comma compared to *C. negus* from both Gaysay and Sanetti which were 113.94 ± 19.40 and 84.04 ± 18.77 seconds respectively. *C. acvapimensis* needed nearly 5 times more time to recover from chill-coma compared to colonies of *C. negus* from Gaysay and 6 times than *C. negus* from Sanetti. Hence *C. negus* from Sanetti had higher heat and cold tolerance and may have higher potential to survive in the changing climate of BMNP. In conclusion, there was no clear pattern of species diversity with elevation and the diversity was the highest in the rainforest. *C. negus* from Sanetti had higher heat and cold tolerance and may has higher chance of survival in the changing climate.

Key words: ants, diversity, climate change, thermal tolerance, *Camponotus negus*, *Camponotus acvapimensis*, Bale Mountains National Park

Dedication

This work is dedicated to my father Ato Ayele Gizaw and my mother Wro. Etayehu Workie whom I lost at the final moment of my PhD study. This is a huge loss in my life, but your words of love and courage make me strong to see forward. Rest in peace.

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List of Abbreviations and Acronyms

BEM: Bioclimatic Envelope Models

BMNP: Bale Mountains National Park

CSD: Commission on Sustainable Development

$CT_{max:}$ Critical Thermal maximum

CT_{min}: Critical Thermal minimum

DTR: Diurnal Temperature Range

EMA: Ethiopian Meteorological Agency

EPA: Environmental Protection Authority

EWCO: Ethiopian Wildlife Conservation Organization

HSPs: Heat Shock Proteins

HSR: Heat Shock Response

IBA: Important Bird Area

IPCC: Intergovernmental Panel on Climate Change

LLT: Lower Lethal Temperature

$LT_{50:}$ Acute Lethal Temperature

MEF: Ministry of Environment and Forest

OARDB: Oromia Agriculture and Rural Development Bureau

$P_{breadth}$: Performance breadth

P_{max}: maximal Performance

RBA: Rapid Bioassessment

SCP: Supercooling Point

TNZ: Thermoneutral Zone

T_{opt}: Thermal optimum

TSD: Temperature-dependent Sex Determination

ULT: Upper Lethal Temperature

UN: United Nations

UNCED: United Nations Conference on Environment and Development

UNEP: United Nations Environmental Program

WHO: World Health Organization

Chapter 1: Introduction

1.1. Background of the study

1.1.1 Climate change in mountains

Climate change is not a new phenomenon. The Earth's temperature and climate have changed considerably over the past millions of years. However, the current changes and those projected for the future differ in that they are largely driven by human activities (IPCC, 2007). The continued increase in the atmospheric concentration of carbon dioxide due to anthropogenic emissions is predicted to cause to considerable changes in climate. Global warming refers to the rising average temperature of Earth's atmosphere and oceans. Scientists are certain that the main cause of this warming is the result of increasing concentrations of greenhouse gases produced by human activities such as deforestation and burning fossil fuels. Over the past 100 years, the average global atmospheric temperature has increased by 0.6 °C but much steeper increases are expected in the coming decades (IPCC, 2014).

Mountain ecosystems have recently received considerable interest based on the understanding that climate change might have particularly serious irreversible impacts on physical and biological systems in these habitats (Loader *et. al.*, 2009). Global warming is more prominent at higher elevations than at lower elevations, especially during winter and spring seasons, and such a tendency may continue in future climate change scenarios (Liu *et. al.*, 2009). Most climate models suggest amplification of global warming in high mountains, but observations are less clear (Pepin and Lundquist, 2008). Mountains make up close to 25% of continental surfaces (Kapos *et. al.*, 2000) and snow-topped mountains provide water for roughly 25% of the global gross domestic product (Barnett *et. al*., 2005). Around 40% of the world's population lives in watersheds of rivers originating in mountains (Beniston, 2006). The responses of the climatic conditions and ecosystems in these regions to global climatic change make the detection of global climate change most observable at its early stage. Therefore, analyzing climatic tendencies in high-elevation regions can be an ideal approach to the study of climate change (Liu *et. al.,* 2009).

1.1.2 Human population at High altitude

The highland areas of the world support considerable populations. According to the World Health Organization (WHO) statistics, around 140 million people live permanently at altitudes higher than 2500 m (WHO, 1996). While this is a small fraction of the world's population, it comprises a high proportion of the population in certain countries or regions. The numbers of high-altitude residents are likely to increase as the result of population growth and active migration in many of these regions (Niermeyer *et. al.,* 2001). In addition the number of lowland dwellers travelling for work (soldiers, miners, construction workers, and astronomers), seeking adventure or recreation (skiing, trekking, and climbing) at high altitudes has greatly increased in recent decades (Karinen and Tikkanen, 2012).

The three most dramatic examples of long-term high-altitude residents are populations living on the Tibetan Plateau, the Andean Altiplano, and the Ethiopian Highlands (Scheinfeldt and Tishkoff, 2010). The Ethiopian highlands are among the main areas of the world above 3000 m. More than half of the total population living at altitude greater than 2500 m in Africa is from Ethiopia. The country is intersected by a number of rift valley systems, establishing a connection between the African rift valley in the south and the Red Sea. The valley systems divide the country into three reasonably well-defined regions: the western highlands, the eastern highlands and the rift valley itself with the lowland area (West *et. al.*, 2007).

The Bale Mountains National Park (BMNP) is part of the eastern highlands of Ethiopia. The park is the largest and most important protected area in Afromontane habitat in the whole of Africa and global biodiversity significance and an area of outstanding natural beauty (Alers *et. al.,* 2007). Poorly regulated use of the natural resources is threatening the sustainability of this ecosystem and the livelihoods of the people who depend upon it. Expansion of agricultural land, deforestation, overgrazing, unplanned settlement and fire are the main factors causing the degradation of the ecosystem (Amente, 2006). Moreover, climate change is undoubtedly causing additional pressure in this habitat which needs immediate study. Increased warming and changes in precipitation are possibly to have considerable consequences for humans and ecosystems within the mountains as well as those living downstream (Beniston, 2003).

1.1.3 Climate change and biodiversity

Predicted future rates of climatic change, especially in temperature will exceed any that has occurred over recent geological time. Climate change is considered as a key threat to biodiversity and to the structure and function of ecosystems that may already be subject to significant anthropogenic stress (Graham and Harrod, 2009). Global warming is predicted to have a dramatic impact on many species (Franco *et. al*., 2006). This has been linked to changes in physiology, phenology, species distributions, interspecific interactions, and disturbance regimes. Projected future climate change will certainly result in even more dramatic shifts in the states of many ecosystems (Lawler, 2009). A sufficient and accurate understanding of how global warming affects organisms requires detailed knowledge of how close species are to their thermal limits in nature, and how much extra capacity they possess to respond to further increases in habitat temperature (Stillman, 2003).

Global warming is likely to bring about a host of changes in organismal and population attributes for the reason that temperature is the single most important environmental factor directly affecting molecular, biochemical and physiological processes, particularly in poikilotherms such as reptiles, amphibians and fish. As at present, only little is known about how global warming affects biochemical and physiological characters in natural populations, it is essential to study on its effect and mitigative measures. Rapid changes in climate may alter physiological performance and result in reduced survival or fitness. This may lead to local extinction if populations are unable to respond to global warming (Thomas *et. al.*, 2001).

There is lack of long-term data of species in their natural settings and recent developments in conservation physiology have demonstrated that the physiological response of animals to changes in their environment may be considerably more complex than previously thought. Animal response to climate change should therefore be a crucial component of future conservation efforts (Pidcock, 2010). An understanding of the physiological adaptability of organisms to respond to global warming is essential to predict future species distributions and population dynamics and to implement successful conservation strategies (Wikelski and Cooke, 2006). This emphasizes on the impacts of recent global warming on animal physiological adaptation including thermal regulation and reproduction. There are many studies examining the responses of organisms to variations in thermal environment (Angilletta *et. al.*, 2002), and studies of responses to spatial variation are common. Examples of differences in thermal tolerance across large scales (i.e. across latitudes or altitudes) seem to be common in the literature. Temperature is one of the most important factors influencing the physiology, ecology and evolution of ectotherms, with clear latitudinal and altitudinal influences on the distribution of species (Williams *et. al.*, 2003). For insects, temperature determines survival, population dynamics, and distribution and hence their responses to climate change (Chown *et. al.,* 2006; Deutsch *et. al.,* 2008).

There are many studies on thermal limits and thermal tolerance of animals worldwide but only few studies from Africa and there is very little from Ethiopia. Thus there is a need of research to know how much is biodiversity affected by global warming. This needs well researched information and knowledge on thermal sensitivity of animals in their natural habitats with the changing climate like in mountains and highlands. One of the focuses of this research is studying the difference in thermal tolerance of ants from the Bale Mountains National Park (BMNP). Characterization of thermal tolerances of ants, which are both abundant and important in most terrestrial ecosystems, is vital since thermal constraints can inform how a species may respond to local climatic change (Oberg *et. al.,* 2011). Because ants are geographically widespread, ecologically diverse, and thermophilic, they are an ideal system for exploring the extent to which physiological tolerance can predict responses to environmental change (Diamond *et. al.,* 2013). In this regard knowledge on thermal tolerance is essential to predict the effects of climate change in an organism (Deutsch *et. al.,* 2008).

Ethiopia is experiencing the effects of climate change. Reports show that climate variability and change are among the critical issues affecting the livelihood of most Ethiopians (IPCC, 2014). Despite the fact that Ethiopia's impact on the atmosphere is insignificant, climate change has adverse impacts on various socio-economic activities, particularly agriculture, water resources, forestry, human health, biodiversity and wildlife (CSD, 2002). The Bale Mountains have a unique endemic flora and fauna, resulting from a combination of large area, isolation from the rest of Ethiopian highlands and climatic history (Hillman, 1988; Williams *et. al.,* 2004). Protection of these mountains of immense national and global importance needs consideration of the effect of the climate change in this ecosystem in general and in its biodiversity in particular.

1.1.4 Ant diversity of Ethiopia

The Ethiopian government 2002 national report submitted to Commission on Sustainable Development (CSD) of the United Nations (UN) about the obstacles and challenges the country confront to implement Agenda 21 adopted at the United Nations Conference on Environment and Development (UNCED) in Rio de Janeiro in 1992 states:

"*A major constraint to implementing biodiversity conservation program is the inadequacy of data for most of the lower plants and animals – particularly invertebrates and fungi for the country. It is thus impossible to produce reliable information on species distribution, abundance, and conservation status in general and genetic diversity in particular."*

Thus the study of invertebrates for example ants among others is binding to implement biodiversity conservation program in the country. Ants are known to be ecologically important invertebrates in many ecosystems (Hölldobler and Wilson, 1990). But there are substantial gaps in our understanding of the African ant fauna (Robertson, 2000). A more complete record of African ants is essential to improve our understanding of their ecology, evolution, and behavior. Further study of the African ant fauna is also needed to benefit from their established value in conservation priority setting, biomonitoring, and biological control (Agosti *et. al.,* 2000).

The knowledge ant diversity of Ethiopia is fragmentary. Ants of Ethiopia have received little attention compared to the study of ant species of neighboring countries like Kenya (Hita Garcia *et. al*., 2012). There are few recent studies on the ant fauna of Ethiopia. Rigato (2006) described two new Ethiopian *Strumigenys* ants namely *S. alessandrae* and *S. bartolozzii.* In another publication, Bolton and Fisher (2011) included *Hypoponera exigua, Hypoponera faex, Hypoponera jocose* and *Hypoponera juxta* as they revised the taxonomy of the ponerine ants of the genus *Hypoponera* for the Afrotropical and West Palaearctic regions.

Apart from the need for studying ants of Ethiopia for their ecological significance, studies on ants should also consider their economic importance as pests. There is considerable economic loss of honey in Ethiopia due to insect pests. Some studies show that ants are among the major honeybee enemies and ant attack is the most serious problem in the beekeeping sector (Edessa, 2005; Desalegn, 2007). Specifically the ant *Dorylus fulvus* is found to be the most troublesome and can cause a decrease of honey yield up to 29 % (Desalegn, 2007). Ants are also used in Ethiopia for pest control. In another study the ant *Crematogaster chiarinii* is reported to be used as a potential biological control agent for protecting honeybee colonies from attack by driver ants, *Dorylus quadrates* (Nuru *et. al.,* 2014). Thus the study of ant diversity of Ethiopia is not only for their ecological significance but also they are economically important.

1.2 Research Questions, Hypothesis and objectives

1.2.1 Research questions

There was no study of ant species in the BMNP. This was the first study Species composition of Ants (Hymenoptera: Formicidae) and Thermal Tolerance along environmental gradient. The following are the research questions that this study attempted to unveil:

- Which species of ants are found in the BMNP?
- How ant species are distributed and are abundant with elevation and habitats?
- Do lower altitude ants have higher heat and lower cold tolerance as compared to those at a higher altitude*?*
- Which group of ants is more vulnerable to future climate warming?

1.2.2 Hypothesis

Ant (Hymenoptera: Formicidea) species composition has a certain pattern with elevation and habitats and ants living in a higher elevation are more vulnerable to climate change as compared to those living in lower altitudes.

1.2.3 Objectives

General objective

To investigate ant species composition (Hymenoptera: Formicidae) and thermal tolerance along environmental gradient in the BMNP, Ethiopia.

Specific objectives

The specific objectives of the study are to:

- determine the species composition and abundance of ants in the BMNP;
- find out ant species composition distribution and abundance with elevation and habitats;
- test the heat and cold tolerance of *Camponotus negus* from different altitudes and *Camponotus acvapimensis* and
- predict which group of ants is more vulnerable to future climate warming scenario.

Chapter 2: Literature review

2.1 Animal distribution pattern and climate change

Climate is a major determinant of the distribution and abundance of species (Jeffree and Jeffree, 1994). Global average surface temperatures have increased by 0.6 ± 0.2 1°C since the late 19th century and are expected to rise from 1.4 to 5.8 °C over the next century (IPCC, 2001). Thus, there is a need to develop models linking species distributions to climate change scenarios in order to anticipate the effects of global warming on plant and animal populations (Ludwig *et. al.*, 2001; Lawler *et. al.*, 2006). Models predicting the distribution of organisms may contribute to our understanding of factors controlling patterns of species distribution, as well as for planning of conservation and management programs (Godown and Peterson, 2000). One class of predictive models that has been applied for a variety of scientific and practical purposes is termed Bioclimatic Envelope Models (BEM). These models generate predictive maps of species distribution using data on the climatic characteristics of the sites where the species were recorded (Nix, 1986).

BEM produce valuable, first-order assessments of potential global warming impacts on biodiversity. However, their limitations, together with the urgent need to provide more robust information to policy-makers and conservation practitioners, demand the development of integrated models which consider biotic environment (potential effects of predation, competition or mutualisms on range dynamics); dispersal capacity of species and physiological capacity of organisms to global warming (Hampe, 2004).

In this regard physiological studies help predict effects of global warming through determining which species currently live closest to their upper thermal tolerance limits, which physiological systems set these limits, and how species differ in acclimatization capacities for modifying their thermal tolerances (Somero, 2009). Rapidly global warming will alter the selective pressures acting on all animals because temperature is one of the most important factors affecting energy and water balance; thus, has the potential for severely affecting the fitness of many species.

2.2 Effect of climate change on thermal regulation

Temperature is a universal and pervasive physical characteristic, which affects the functioning of all organisms. Organisms capable of locomotion avoid extreme of temperatures, although what is ‘extreme’ depends on the species, its evolutionary thermal history and the thermal history of the studied individuals. Genetically transmitted effects and individual physiological experiences will influence the thermal behavior of an organism (Cossins and Bowler, 1987).

Thermoregulatory flexibility may afford multiple pathways for ectotherms to cope with a changing climate. Some endotherms, on the other hand, may be so thermally specialized that their only option is to increase energy expenditure to maintain a constant T_b as T_a increasingly deviate from the animal’s Thermoneutral Zone (TNZ), thereby lessening energy available for other fundamental functions such as growth and reproduction (Scholander *et. al.*, 1950 as cited in Boyles *et. al.*, 2011). Predictions about adaptive thermoregulation in endotherms can be made by expanding the framework commonly used in literature on ectotherms (Angilletta *et. al.*, 2006). They argued that performance in endotherms is related to T_b in a manner similar to performance in ectotherms and can therefore be described using thermal performance curves. A performance curve describes the nonlinear relationship between some physiological traits; in this case T_b, and a measure of performance. A performance curve can be described by the maximal level of performance, the optimal T_b for performance, the performance breadth, and the minimum and maximum critical T_b (Figure 1).

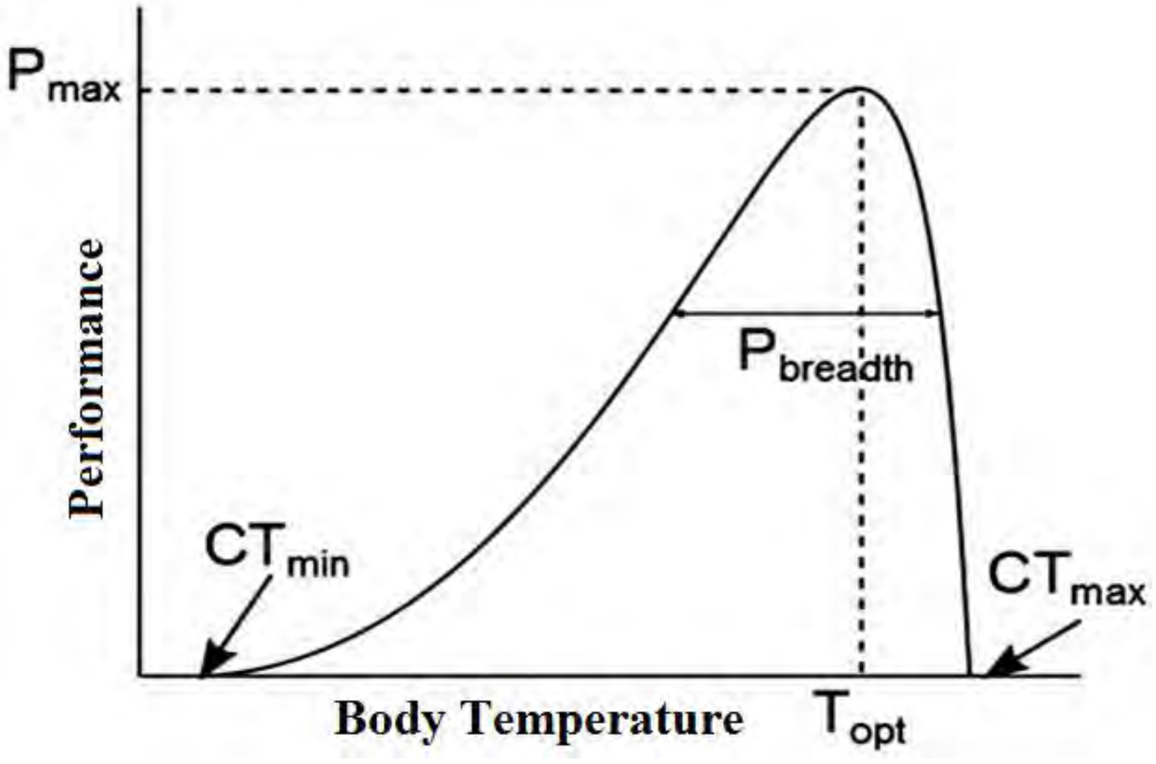

Figure 1: Hypothetical performance curve of ectotherms (Adopted from Boyles *et. al.,* 2011)

Performance curves are usually used in the literature on ectotherms to describe the relationship between body temperature and performance, but they can also apply to endotherms. This hypothetical curve (Figure 1) shows the commonly used descriptors of a performance curve: the thermal optimum (Topt), performance breadth (Pbreadth), critical thermal limits (CTmin and CTmax), and maximal performance (Pmax) (Boyles *et. al.,* 2011).

2.3 Differences in thermal tolerance of organisms

The impact of anthropogenic climate warming on terrestrial organisms is often predicted to increase with latitude, in parallel with the rate of warming. Yet, the biological impact of rising temperatures also depends on the physiological sensitivity of organisms to temperature change. Deutsch *et. al.* (2008) estimated the direct fitness impact of warming on terrestrial insects across latitude. The results showed that warming in the tropics, although relatively small in magnitude, is likely to have the most deleterious consequences because tropical insects are relatively sensitive to temperature change and are currently living very close to their optimal temperature. In contrast, species at higher latitudes have broader thermal tolerance and are living in climates that are currently cooler than their physiological optima, so that warming may even enhance their fitness. Available thermal tolerance data for several vertebrate taxa exhibit similar patterns, suggesting that these results are general for terrestrial ectotherms. These analyses imply that in the absence of ameliorating factors such as migration and adaptation, the greatest extinction risks from global warming may be in the tropics, where biological diversity is also greatest (Burger and Lynch, 1995).

Mammals, as endothermic organisms with complex thermoregulatory adaptation, generally occupy a fundamental niche with broader environmental variability than can ectotherms (Dormann *et. al.,* 2010). However, endotherms, like ectotherms, are also affected by temperature variations (Humphries *et. al.,* 2004), and the survival of species with narrow thermal tolerances will also be influenced by changes in ambient temperatures (Portner and Farrell, 2008).

Thermal tolerance limits differ among species, notably among highly similar congeneric species with different latitudinal or vertical distributions. A powerful approach for examining the thermal tolerance is to compare the physiology of congeneric species that have distribution patterns that reflect thermal gradients (Stillman, 2002). The relationship between environmental temperature and acute thermal tolerance limits found for congeneric porcelain crabs (genus *Petrolisthes*) showed that tropical species are uniformly more heat tolerant than temperate species and, within each latitudinal group, species occurring highest in the intertidal zone have the highest acute lethal temperature LT_{50} values (Stillman and Somero, 2000). But, the most heat-tolerant species are, in general, most threatened by further increases in environment temperature because current maximal environmental temperatures may reach or exceed the LT_{50}. Furthermore, the most warm-adapted congeners of porcelain crabs are further disadvantaged by possessing a relatively small ability to increase LT_{50} during acclimation (Stillman, 2003).

The extent to which phenotypic plasticity might mediate short-term responses to environmental changes is controversial. Nonetheless, theoretical work has made the prediction that plasticity should be common, especially in predictably variable environments by comparison with those that are either stable or unpredictable. Deere *et. al.* (2006) examined these predictions by comparing the phenotypic plasticity of thermal tolerances (supercooling point (SCP), lower lethal temperature (LLT), upper lethal temperature (ULT), following acclimation at either 0, 5, 10 or 15 °C, for seven days, of five closely related ameronothroid mite species. These species occupy marine and terrestrial habitats, which differ in their predictability, on sub-Antarctic Marion Island. All of the species showed some evidence of pre-freeze mortality (SCPs -9 to -23 °C; LLTs -3 to -15 °C), though methodological effects might have contributed to the difference between the SCPs and LLTs, and the species are therefore considered moderately chill tolerant. ULTs varied between 36 °C and 41 °C. Acclimation effects on SCP and LLT were typically stronger in the marine than in the terrestrial species, in keeping with the prediction of strong acclimation responses in species from predictably variable environments, but weaker responses in species from unpredictable environments. The converse was found for ULT. These findings demonstrate that acclimation responses vary among traits in the same species.

2.4 Climate change and animal reproduction

Understanding the mechanisms influencing the timing of reproduction has taken on new urgency as climate change is altering environmental conditions during reproduction, and there is concern that species will not be able to synchronize their reproduction with changing food supplies (Dunn *et. al.*, 2011). One consequence of climate change is an increasing mismatch between timing of food requirements and food availability.

This changes in the timing of events like reproduction and migration for a given animal is called phenology. Many organisms respond to changes in temperature by altering their activity and metabolism. Therefore, anthropogenic induced temperature increases have the potential to affect the phenology of organisms (Hegland *et. al.,* 2009).

Climate change is predicted to be most severe in northern regions and there has been much interest into what extent organisms can cope with these changes through phenotypic plasticity or microevolutionary processes. A red squirrel population in the southwest Yukon, Canada, faced with increasing spring temperatures and food supply has advanced the timing of breeding by 18 days over the last 10 years (6 days per generation). The timing of breeding in this population of squirrels has advanced as a result of both phenotypic changes within generations, and genetic changes among generations in response to a rapidly changing environment (Re´ale *et. al.*, 2003).

Recent global warming has sparked an interest in the timing of biological events, which is a general problem in life-history evolution. Reproduction in many organisms breeding in seasonal environments, e.g. migratory birds, is dependent on the exploitation of a short but rich food supply. If the seasonal timing of the food peak advances owing to global warming, then one would expect the bird to track those changes, hence, initiate migration and breeding earlier. However, when there is competition for territories and a risk of pre-breeding mortality, the optimal response to a shifting food distribution is no longer obvious (Jonzen *et. al.*, 2007).

2.5 Response of animals to climate change

For a species, environmental changes can lead to at least four possible outcomes. Species may (1) become extinct or extirpated, (2) migrate or shift their current distribution range, (3) adapt to the changes through a change in the genetic composition of the population, or (4) employ phenotypic plasticity.

Studies show that terrestrial organisms are shifting their distributions in response to climate change. Changes in distributions are happening at a much faster rate than formerly estimated (Tewksbury *et. al.,* 2011). Using a meta-analysis Chen *et. al.* (2011)

estimated that the distributions of species have recently shifted to higher elevations at a median rate of 11.0 meters per decade, and to higher latitudes at a median rate of 16.9 kilometers per decade. In this way organisms escape the increased temperature caused by climate warming.

Genetic change and phenotypic plasticity are the outcomes that prevent local extinction. Although the rapid rate of climate change will prevent the evolution of adaptive heritable traits in long-lived species (with long generation times), there is evidence that microevolution has occurred in response to climate warming (Bradshaw and Holzapfel, 2006).

2.5.1 Phenotypic plasticity

Phenotypic plasticity is defined as the process through which a genotype gives rise to different phenotypes under different conditions (Garland and Kelly, 2006). It includes phenology (changes in the timing of events), morphology (e.g., color patterns, body shape, and size), physiology, or behavior of a species (Fuller *et. al.*, 2010). This describes the ability of some animals to change a specific phenotype reversibly and repeatedly during their lifetime in response to a change in the environment, far quicker than they could evolve by genetic adaptation. This means many animal species may in fact be able to adapt to changing conditions better than previously thought (Pidcock, 2010).

A major gap in our understanding of how climate change will affect natural populations is how much phenotypic plasticity contributes to the current realized niche of a species, because phenotypic plasticity in the natural setting has been studied in very few species (Fuller *et. al.*, 2010). Lommen *et. al.* (2005) reported on the phenotypic plasticity of the degree of winglessness of ladybird beetle *Adalia bipunctata* (L.). Winglessness in the two-spot ladybird beetle is determined by a single locus with the wingless allele recessive to the winged wild type allele. The expression of the wingless trait is highly variable, with individuals missing a variable part of elytra and flight wings. The degree of winglessness is partly determined genetically. The environmental effect on elytron length relative to maximal elytron length in wingless phenotypes was studied by rearing offspring of single pair crosses of this form at a low (19 °C) or high (29 °C) temperature. Offspring reared at 19 °C showed relatively longer elytra than those reared at 29 °C. This result suggests that temperature determines phenotypic plasticity of elytron length in wingless ladybird beetle.

A recent correlation of rising temperature associated with anthropogenic climate warming involves reductions in the body size of many organisms. This phenomenon is being reported from a growing number of species on multiple continents and appears to apply to both endotherms and ectotherms, in both terrestrial and aquatic environments (Gardner *et. al.*, 2011). Changes in body size have important implications for the thermal biology of endotherms and ectotherms. This is because body size directly affects energy and water requirements for thermoregulation, energy and mass acquisition and utilization rates (Kooijman, 2010) and life-history characteristics (Calder, 1984). Changing body size will, therefore, have implications for resilience in the face of global warming.

2.5.2 Genetic change

Phenotypic plasticity is not the only way organisms adjust to environmental changes. There are studies indicating that at least some populations are able to undergo rapid responses via selective processes (Naya *et. al.*, 2011). Although the rapid rate of climate change will prevent the evolution of adaptive heritable traits in long-lived species (with long generation times), there is evidence that microevolution has occurred in response to climate warming (Bradshaw and Holzapfel, 2006).

The Palaearctic species *Drosophila subobscura* recently invaded the west coast of Chile and North America. This invasion helped to corroborate the adaptive value of the rich chromosomal polymorphism of the species. This species reproduced in the colonized areas in a relatively short period of time the same as those observed in the original Palaearctic area. The rapid response of this polymorphism to environmental conditions makes it a good candidate to measure the effect of global warming on the genetic composition of populations. Indeed, the long-term variation of this polymorphism shows a general increase in the frequency of those inversions typical of low latitudes, with a corresponding decrease of those typical of populations closer to the poles. Although the mechanisms underlying these changes are not well understood, the system remains a valid tool to monitor the genetic impact of global warming on natural populations (Balanya *et. al.,* 2009).

Current empirical evidence suggests that evolution is responsive to climate variation and occurs at rates that make it relevant for consideration of current and projected responses to climate change. For a wide variety of taxa, thermal performance varies within the geographic ranges of species, suggesting both genetic variation in critical traits and localized evolution in response to climate variation (Conover and Schultz, 1995; Gilchrist *et al.,* 2004). Many examples of contemporary evolution in response to climate change exist. In less than 40 years, populations of the frog *Rana sylvatica* have undergone localized evolution in thermal tolerance (Skelly and Freidenburg, 2000), in response to altered temperature in their wetlands. Laboratory studies of insects show that thermal tolerance can change markedly after as few as 10 generations (Good, 1993).

Not all species evolve in synchrony with changing climate? Although not often studied with specific reference to climate change, the factors limiting evolutionary response to a changing environment have been considered closely by evolutionary biologists. There are several potential constraints on evolutionary responses. The three most important and likely to be critical constraints are time lag between change and response, lack of genetic variation, and erosion of genetic variation. Some species will not be able to respond rapidly enough to keep up with climate change (Davis *et. al.,* 2005). Long generation times are the norm in some groups, including important conservation targets (e.g., large mammals). Evidences of changes in climate show that some species can take hundreds or even thousands of years to respond demographically to a new climate regime (Skelly *et. al.,* 2007).

2.5.3 Induction of Heat shock proteins (HSPs)

Organisms cope with extremes in temperature physiologically through induction of heat shock proteins (HSPs), which are up-regulated during periods of cellular stress. Heat shock proteins minimize stress-induced aggregation of damaged proteins and they facilitate removal of irreparable proteins (Lindquist, 1986). Expression of HSPs enhances thermal tolerance and ability to withstand subsequent stress (Krebs and Feder, 1998; Krebs and Bettencourt, 1999). Thus, the heat shock response is a key strategy used by ectotherms to survive elevated temperatures in nature (Feder and Hofmann, 1999). But elevated levels of HSPs may not completely repair proteins denatured by thermal stress; some irreversible protein damage can occur. When a protein is irreversibly damaged, ubiquitin, a low-molecular-mass protein, is bound to the damaged protein, marking it for degradation by cytoplasmic proteases (Hershko and Ciechanover, 1992).

The preferential synthesis of HSPs in response to thermal stress; the heat shock response (HSR) has been shown to vary in species that occupy different thermal environments. A survey of case studies of aquatic (mostly marine) organisms occupying stable thermal environments at all latitudes, from polar to tropical, shows that they do not in general respond to heat stress with an inducible HSR (Berger and Emlet, 2007). Organisms that occupy highly variable thermal environments (variations > 20 °C), like the intertidal zone, induce the HSR frequently and within the range of body temperatures they normally experience, suggesting that the response is part of their biochemical strategy to occupy this thermal niche. The highest temperatures at which these organisms can synthesize HSPs are only a few degrees Celsius higher than the highest body temperatures they experience. Thus, they live close to their thermal limits and any further increase in temperature would push them beyond those limits (Krebs and Bettencourt, 1999). In comparison, organisms occupying moderately variable thermal environments (<10°C), like the subtidal zone, activate the HSR at temperatures above those they normally experience in their habitats. They have a wider temperature range above their body temperature range over which they can synthesize HSRs. Contrary to our expectations, species from highly (in comparison with moderately) variable thermal environments have a limited acclamatory plasticity. Due to this variation in the HSR, species from stable and highly variable environments are likely to be more affected by climate change than species from moderately variable environments (Tomanek, 2010).

2.6 Ants and their importance

Ants are classified in a single family, the Formicidae, within the order Hymenoptera, and are social insects which have been evolving since the Cretaceous. Ants are known to be ecologically important invertebrates in many ecosystems (Hölldobler and Wilson, 1990). They positively affect physical and chemical soil properties, plant and animal distribution, and forest health (Boulton *et. al.,* 2003). Ants are important components of ecosystems not only because they constitute a great part of the animal biomass but also because they act as ecosystem engineers (Ghait and Kale, 2015). Ant biodiversity is incredibly high and these organisms are highly responsive to human impact, which obviously reduces its richness (Folgarait, 1998). Ants provide immense ecosystem services that benefits people i. e. including provisioning, supporting, regulating and cultural services (Daily *et. al.,* 1997; Millennium Ecosystem Assessment, 2005). As per the Millennium Ecosystem Assessment conceptual framework, food and drugs constitute provisioning services provided by ants, while soil modification is a supporting service, and the processes of pollination, seed dispersal and herbivore suppression are regulating services. Since ants have provided artistic, religious and spiritual services to tribal peoples (Ramos-Elorduy, 2009), and have more recently been utilized by scientists for scientific services, they also provide cultural services to mankind (Rastogi, 2011) (Figure 2).

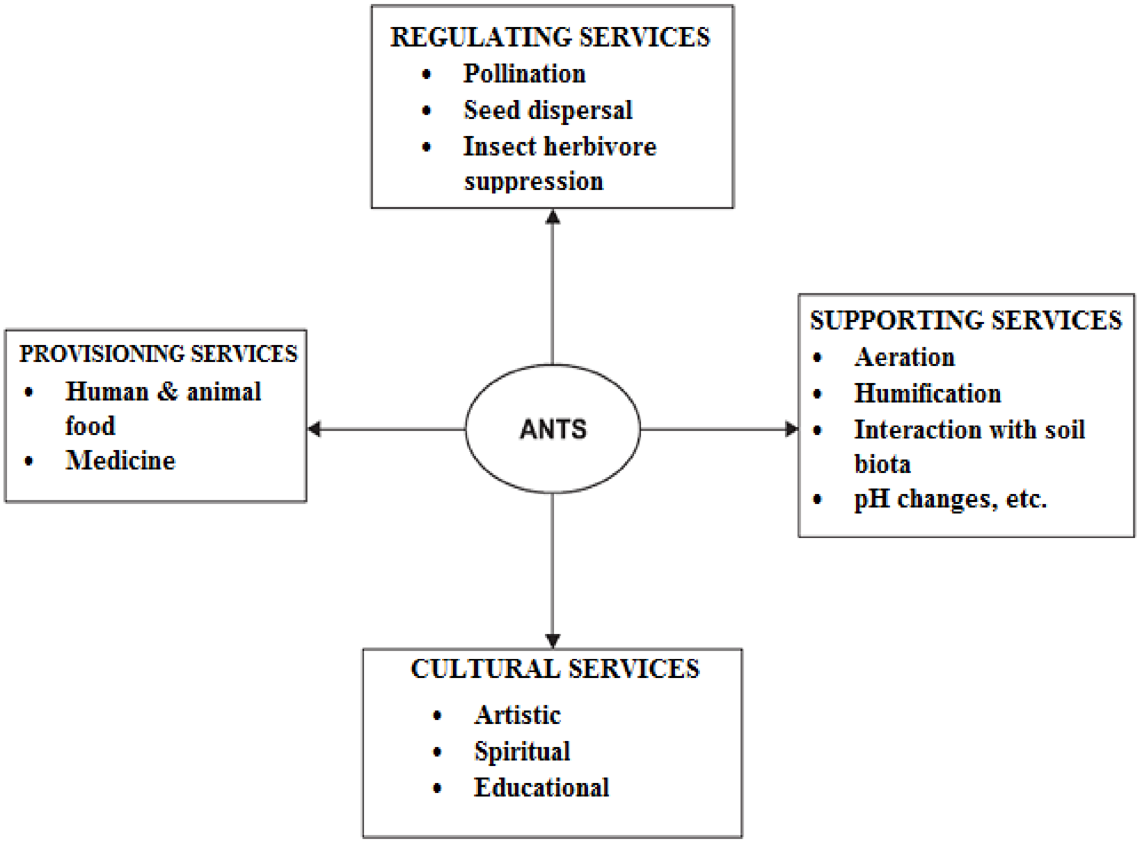

Figure 2: Ecosystem services (in the form of provisioning, regulating, supporting and cultural services) processes and economic resources provided by ants (Rastogi, 2011).

2.6.1 Seed dispersal by ants

Ants and seeds show a variety of interactions, from generalist seed harvesters to more selective elaiosome-eating ants (Azcárate *et. al.,* 2010). Harvester or granivore ants store seeds in underground granaries and consume them during the winter or the dry season. These ants are granivores, and the net outcome of the interactions is usually predation. Ants take advantage of the limited periods of time in which activity outside the nest is feasible to collect as many seeds as possible. This voracious strategy inflicts severe seed losses to certain plant species. However, harvester ants also behave as accidental seed dispersers (dyszoochory) by dropping or mislaying seeds in foraging areas, foraging trails, chaff piles and granary chambers (Sanchez *et. al.,* 2006).

In contrast to the less-discerning seed harvesters, some seed-feeding ants behave as true gourmets. These ants collect elaiosome-bearing seeds and disperse them after consuming the elaiosome (Handel and Beattie, 1990). Most of these plants produce seeds with elaiosomes, a term encompassing various external appendages or "food bodies" rich in lipids, amino acid, or other nutrients that are attractive to ants. Seed dispersal by ants is typically accomplished when foraging workers carry diaspores back to the ant colony after which the elaiosome is removed or fed directly to ant larvae.

Myrmecochory is a mechanism involving more than 3,000 plant species and hundreds of ant species across many ecosystems such as dry sclerophyll woodlands of Australia (Berg, 1975), sclerophyll shrubs in south Africa (Bond and Slingsby, 1983), tropical regions or temperate deciduous forests of Europe and North America. Many species in four subfamilies of the Formicidae (Formicinae, Myrmicinae, Ponerinae, and Dolichoderinae) gather seeds (Gómez and Espadaler, 2013).

2.6.2 Ants as pollinators

Although ants are present in most communities and are constant plant visitors and avid sugar collectors, there are few confirmed cases of pollination by ants. Ants do visit flowers and other reproductive parts of plants living in lowland dry tropics (Díaz-Castelazo *et. al.,* 2004), semiarid areas (Rico-Gray *et. al.,* 1998), Mediterranean regions (Bosch *et. al.,* 1997), and alpine environments but ant pollination is rare (Puterbaugh, 1998). Ant-pollinated species may be relatively more abundant in some types of desert. The basis for this was the observation that in some kinds of low-growing vegetation in arid environments winged pollinators such as bees seemed scarce, but ants were exceedingly abundant (Gomez *et. al.*, 1996). One of the reasons most often cited for the rarity of ant pollination is the combination of limited foraging areas and the lack of wings on worker ants; in this situation the ants cannot effect gene flow among the plants they service. However, pollen movement by ants is similar to that of many winged insects (Gomez and Zamora, 1992; Holland *et. al.,* 2011).

The main reason for the rarity of ant pollination is that some ant species possess metapleural glands whose secretions reduce pollen quality (Beattie *et. al.,* 1985). These glands, located on the thorax, secrete antibiotic substances that ants smear on themselves and their larvae for protection against fungal and bacterial infections. These are certainly useful for killing pathogenic bacteria and fungi infecting a colony; but just as they destroy the membranes of microorganisms, so they also damage grains of pollen (Currie *et. al.,* 1999). For example 3-hydroxydecanoic acid (myrmicacin) which is one of secretions from the metapleural glands of the ants *Atta sexdens* and *Myrmica laevinodis* inhibit germination, retard pollen-tube growth, and disrupt pollen-tube mitosis (Iwanami *et. al.,* 1981). But some ant groups like *Camponotus* ants living high in the Sierra Nevada in California, a habitat too arid for pollinating insects, have adapted to this situation. Having no metapleural gland, they have become very good at dispersing pollen (Holland *et. al.,* 2011).

2.6.3 Ants for pest management

Ants have important attributes that make them potentially useful biological control agents. They are diverse and abundant in most tropical and some temperate ecosystems, and most can be considered predators. Ants respond to changes in the density of prey and can remain abundant even when prey is scarce, because they can cannibalize their brood and/or use plant and insect exudates as stable sources of energy (Choate and Drummond, 2011). In addition, they can store food and hence continue to capture prey even if it is not immediately needed; i.e., predator satiation is not likely to limit the effectiveness of ants. Besides killing pests, they can deter many others including some too large to be successfully captured (Wilson, 1987). Although the use of ants in programs of pest control has certain drawbacks (e.g., tending Hemiptera, killing other predatory species, and irritating humans), ants have proved to be beneficial in suppressing many insect herbivores (Fernandes *et. al.,* 1994). Many ant species control cacao pests or fungal diseases. For example, the black cocoa ant (*Dolichoderus thoracicus* Smith, 1860) controls mirids, cacao pod borers, and rat attacks on cacao pods in South-east Asia (Way and Khoo, 1991). There are many species of natural enemies for mango insect pests. Among these, the green ant (*Rhytidoponera metallica,* Smith, 1858) is the most effective both as a predator and a deterrent of a range of mango insect pests (Peng and Christian, 2005).

2.6.4 Ants and soil property

Ground ants together with earthworms and termites; belong to the principal groups of invertebrates that influence soil processes in terrestrial ecosystems (Lavelle *et. al.,* 1997).

The soil of ant nests is usually significantly different, both chemically and physically, from surrounding soils (Wagner*,* 1997) and may increase the heterogeneity of the plant community. Through their activities, ants change physical, chemical and also biological properties of the soil (Boulton *et. al.,* 2003) by bioturbation and by accumulation of organic material. Due to the building of below-ground galleries, mounding and material mixing, the soil of ant nests is characterized by the impeded formation of soil horizons, increased porosity, drainage and aeration, reduced bulk density and modified texture and structure. Increased content of organic matter, P, N and K in the nests is due to food storage, aphid cultivation, and accumulation of faeces and ant remains (Folgarait, 1998).

Physical soil properties, including soil texture, bulk density, soil temperature, and soil moisture, are often different within ant mounds compared to surrounding soil (Wagner *et. al.,* 2004). Building of tunnels and chambers both above- and below- ground increases soil macroporosity and reduces bulk density. For example, bulk density in nests of *Pogonomyrmex occidentalis* was 1.47 g/cm3 compared with 1.54 in the surrounding soil. Reduced bulk density may increase soil aeration and permeability of soil for water (Eldridge, 1994).

Nests increase not only soil macroporosity but also organic matter content, which may increase water repellency at low soil moistures. Thus, ant nests increase water infiltration in moist or wet conditions but decreased water infiltration in dry conditions (Cammeraat *et. al.,* 2002). Macroporosity in nests of imported fire ants can increase drainage, quickly bringing water to deeper soil layers and ensuring higher moisture in soil below the nest while reducing moisture in the nest compared with that in the surrounding soil (Green *et. al.,* 1999; Veen and Olffa, 2011). *Camponotus punctulatus* nests are surrounded by a peripheral ditch where water accumulates, producing a constantly-wetted zone inside the anthill. Nest moisture is often significantly different from the moisture of surrounding soil and can be lower or higher. Soil moisture varies even within the same species (Frouz, 2000). For example, *Formica polyctena* can have wet and dry nests, which differ in temperature regime and also in the location and intensity of microbial activity, which is related to temperature and moisture content (Frouz and Finer, 2007).

Temperature is another physical factor that is altered in an ant nest, and regulation of internal nest temperature has been mostly described for nests with anthills. Soil temperature can be significantly higher in nest mounds than in surrounding soils (Brown and Human, 1997; Veen and Olffa, 2011). Pronounced above-ground parts of ant nests are the result of intensive mounding and serve to increase soil temperature needed for brood rearing. Maintenance of higher internal nest temperatures is possibly due to a combination of the insulation provided by the nest (Frouz, 2000), the trapping of solar radiation by the nest and by ant bodies, and the production of metabolic heat by the ants and by the microorganisms associated with the nest material (Coenen-Stass *et. al.,* 1980; Veen and Olffa, 2011).

Many studies have reported significant differences in chemical soil properties between ant nests and the surrounding soil. Ants increase pH in acidic soils and decrease it in basic soils (Frouz *et. al.,* 2003). Changes in N and P content in ant nests has often been reported (Lafleur *et. al.,* 2002). Besides affecting the total content of nutrients in nests, ants also affect the availability of nutrients. In addition to accumulating easily decomposable substances in the nest, ants can also increase the availability of P. Increases in total C and organic C as well as humus in the nest (Dmitrienko and Petrenko

1976 cited in Frouz and Jilkova, 2008) have often been reported. A decrease in N content was detected in nests in soil highly contaminated by nitrogen. This was explained by the increased numbers of soil bacteria that bound N in their biomass. Similarly, there was a lower salt content in nests than in the surrounding soil in soil with a high content of salt (Cox *et. al.,* 1992).

The soil biota is primarily responsible for chemical transformations in the soil. High mineralization rates and concentrations of mineral nutrients in ant nests suggest that ants alter the abundance, and perhaps the taxonomic identity, of the biotic agents of nutrient cycling. Bacteria and fungi typically occur at high densities in ant nest soils (Friese and Allen, 1993). Higher numbers of bacteria, including N_2 fixers, have been found in wood ant nests than in the surrounding soil. In some cases, however, microbial activity in an ant nest may be lower than in the surrounding soil, mainly because of lower moisture in the nest (Holec and Frouz, 2006).

Other groups of soil biota, including protozoa, were also more abundant in ant nests than in surrounding soil (Zaragoza *et. al.,* 2007). Ant nests may also serve as hot spots for soil invertebrates (Laakso and Setala, 1998).

2.6.5 Ants as food

In different parts of the world people use ants and their larvae as food. For example, the eggs of two species of ants are utilized in Mexican escamoles. They are considered a form of insect caviar and can sell for as much as $ 90/kg as they are seasonal and hard to find (DeFoliart, 1999).

Many species of ants constitute a cheap, unconventional but significant renewable protein source in human nutrition (Yhoung-Aree *et. al.,* 1997). Many families supplement their family income by harvesting and selling ant species in Thailand, Laos, Myanmar and Vietnam (Sribandit *et. al.,* 2008), Indonesia and Chhattisgarh in India. All stages including eggs, larvae, pupae and adults, particularly the reproductives, are utilized as food, although the particular stage used varies with species (Rastogi, 2011). In some parts of Africa, worker ants of *Camponotus* sp. are used as salad seasoning and relished for their sweet-sour flavour (Nonaka, 1996). In southern and eastern Africa, particularly in Zambia and Zimbabwe, winged sexual stages of *Carebara vidua* F. Smith are collected and eaten raw or cooked by boiling, roasting or frying (Mbata, 1995).

Consumption of ants by ethnic groups in parts of Asia, Australia, Africa, South and Central America is characterized by the ready availability of populous tropical ant colonies such as *Oecophylla* and *Atta* spp., subsistence farming conditions and age-old traditions of ant collection, particularly during periods of high seasonal availability, such as ant nuptial flights (Yhoung-Aree and Viwatpanich, 2005).

Annual sale of ant foods for human consumption in China amounted to approximately $100 million (DeFoliart, 1999). A more recent study *O. smaragdina* collection and marketing in Thailand revealed a daily income of US $12 per working day during the 4–5 month harvesting season (Sribandit *et. al.,* 2008). Ant products containing *Polyrhachis dives* are exported from China to south-east Asian countries including South Korea, Japan and Thailand (Shen and Ren, 1999). Reproductive ants of the *Eciton* genus, and leafcutter ants, *A. mexicana* and *A. cephalotes* are sold in many towns of Mexico, while the leafcutter ants are also in demand on the international market (Ramos-Elorduy and Pino, 2002).

2.6.6 Use of ants in traditional entomotherapy

Ethnoentomology has revealed some ant species are remarkable for their medicinal value, predominantly in Asian and Latin American countries and to a lesser extent in Australia and Africa. The use of biodegradable sutures in the form of ant mandibles (composed of chitin, a complex protein carbohydrate material) for internal surgery was well known in ancient India (Rastogi and Kaphle, 2008) and Africa (Gudger, 1925 cited in Rastogi, 2011). Soldiers of driver ants, *Dorylus (Anomma)* spp. found in Africa and tropical Asia have traditionally been used for suturing wounds. Soldier of black ants were held so that the open mandibles gripped the opposing edges of the perforated intestinal walls. Once the mandibles snapped shut, the thorax and gaster of the ants were pinched off, leaving behind a series of clamped mandibles in place (Rastogi and Kaphle, 2008).

In Brazil, the leafcutter ants *Atta cephalotes* and *Atta sexdens* are used in the treatment of a large variety of diseases like ear ache, sore throat (Branch and Silva, 1983), tonsillitis, stomach ache and dyspepsia (Alves, 2009). Ants have been used in China as complementary and/or alternative medicine to treat a wide range of medical conditions and diseases for more than three thousand years (Piao *et. al.,* 2009). They were utilized for a variety of purposes including improvement of the immune system, blood circulation and metabolism, reduction in inflammations and pain, treatment of asthma and rheumatoid arthritis, and slowing down of the aging process (Chen and Alue, 1994).

However, only a few of the attributed medical properties and treatments are supported by scientific evidence (Jiang *et. al.,* 2008).

2.6.7 Ants as potential sources of pharmaceuticals and antibiotics

Many species of ants are regarded as important sources of pharmaceuticals (Majer *et. al.,* 2004). Recent research demonstrated that social insects including ants possess well developed immune systems and disease resistance ability against fungi and bacteria. (Wilson-Rich *et. al*., 2009). Antibiotics produced by the paired metapleural glands of ants are secreted externally and provide protection against pathogenic fungi and bacteria (Poulsen *et. al.,* 2006). A variety of antimicrobial (bactericidal/fungicidal) compounds which provide protection against environmental pathogens were isolated and characterized from social insects, particularly ants (Schluns and Crozier, 2009).

Two antibacterial peptides synthesized in the ant *Myrmecia gulosa* (Fabr.) in response to bacterial infection were characterized (Mackintosh *et. al.,* 1998). Extracts of the Eurasian ant, *Formica aquilonia* Yarrow were found to exhibit antioxidant and anti-inflammatory properties (Piao *et. al.,* 2009). Also, fifteen novel peptides exhibiting antibacterial and insecticidal properties were isolated from the venom of the predatory ant *Pachycondyla goeldii* (Forel) (Orivel *et. al.,* 2001).

Certain components of the ant *Polyrhachis lamellidens* were active in the treatment of diseases including arthritis, rheumatism, liver ailments and asthma (Cheng *et. al.,* 2001). Extracts of the *P. dives* demonstrate inhibition in ferric-nitrilotriacetate-induced nephrotoxicity in laboratory rats (Ma *et. al.,* 1997). Further, the analgesic and anti-inflammatory activities of extracts of *P. lamellidens* were demonstrated by Liu and Jiang

(2005). From the same species Jiang *et. al.* (2008) identified two polyketides, suggested to have potential in the treatment of rheumatoid arthritis. These studies provide scientific evidence supporting the traditional uses of medicinal ants in the treatment of various diseases associated with inflammation (Rastogi, 2011). Recent studies show that natural silk fibres of the weaver ants *O. smaragdina* can serve as a cell matrix for cell adhesion and thus have application in tissue engineering and wound healing (Siri and Maensiri, 2010).

Metapleural glands are secretary glands that are unique to ants and basal in the evolutionary history of ants. They are responsible for the production of an antibiotic fluid that then collects in a reservoir on the posterior of the ant's alitrunk. These reservoirs are also referred to as the bulla and vary in size between ant species and also between castes of the same species (Hölldobler and Wilson, 1990). From the bulla, ants can groom the secretion onto the surface of their exoskeleton. This helps to prevent the growth of bacterial and fungal spores (Mueller *et. al.,* 2005) on the ants and inside their nest (Chapela *et. al.,* 1994).

2.6.8 Ants as biological indicators

Biological indicators, commonly abbreviated as "bioindicators", are species, groups of species, or other taxonomic units that can be used to measure some feature of the environment. In cases where they are used to measure the "health", "state" or "condition" of the environment they are termed environmental or ecological indicators (Mcgeoch, 1998). In addition, certain groups may be used as surrogates for the diversity or assemblage composition of other taxa; these are referred to as biodiversity indicators.

Bioindicators are used to measure the conservation potential of areas, progress with minesite restoration, the degree of degradation of areas, rangeland condition and impacts of tourism, as well as impacts of many other land uses (Holloway and Stork, 1991). Until recently, when considering terrestrial ecosystems, biologists have generally used plants as the primary source of bioindication, with a tendency to focus on the more charismatic birds, mammals, reptiles and amphibians if the fauna does happen to be considered (Buchs, 2003). Although invertebrates have long been used for the monitoring of the health of aquatic systems (Hawkins *et. al.,* 2000), they were not formally proposed for use in terrestrial ecosystems until about 25 years ago (Majer, 1983).

There is considerable interest in the identification of robust bioindicators for use in land monitoring and assessment programs (Spellerberg, 1993), and attention has recently focused on terrestrial invertebrates in this role (Williams, 1993). Ants scored the highest among insect groups as potential bioindicators (Brown, 1997). Several studies have recently focused on the use of ants as bioindicators of the conditions of degradation or recovery of terrestrial ecosystems in studies of biodiversity conservation. This is partially due to the fact that ants are easily sampled, relatively easily identifiable at a species level, and usually sensitive to environmental changes (Vasconcelos, 1999).

One success in using insect indicators in terrestrial ecosystems is that of ants in mine site reclamation in Australia (Andersen, 1993) and in Brazil (Majer, 1994) and as an indicator of habitat quality in Australian conservation parks. Ant species also effectively track environmental gradients (Andersen, 1993). This has been found to be true in agroecosystems. Ant populations were related to the nitrogen content of plant residues placed on soil surfaces to retain moisture and increase heat in tropical agroecosystems.

Because they are ubiquitous in agroecosystems, easily sampled, taxonomically well understood, and as demonstrated in this survey, correlated with soil, cropping, and management variables, ants may be the best place to start for the development of a terrestrial indicator in agroecosystems (Tian *et. al.,* 1993).

In the last decade, primarily in Australia, extensive development of a Rapid Bioassessment (RBA) using ant communities has shown great promise. Ants have the same advantage for terrestrial RBAs that fish do for aquatic systems in that they are an essential and ubiquitous component of virtually all terrestrial ecosystems (Andersen and Majer, 2004). They occupy a broad range of niches, functional groups, and trophic levels and they possess one very important characteristic that makes them ideal for RBA because, similar to the fishes, there is a wide range of tolerance to conditions within the larger taxa. Within ant communities there are certain groups, genera, or species that may be very robust and abundant under even the harshest impacts. There are also taxa that are very sensitive to disturbance and change and their presence or absence is also indicative of the local conditions (Wike *et. al.,* 2010).

Ant communities are highly responsive to human impact (Folgarait, 1998) and other changes, and even slight modifications to their ecosystem will produce changes in the ant community (Andersen, 1997). These changes can reflect direction and rate of specific impacts, and ants provide a reliable indicator of general change (King *et. al.,* 1998). In addition to ecosystem health, restoration, or recovery, ant communities have shown strong correlation to other variables. These variables include total and perennial plant cover (Seymour and Dean, 2002), vegetation type (Burbidge *et. al.,* 1992), litter and soil temperature, litter humidity, and forest stand structure, soil faunal richness, microclimate, and soil, agricultural management, and crop variables (Peck *et. al.,* 1998). Finally, ants are widely adopted as indicator organisms as a tool in land management (Andersen and Majer, 2004).

2.7 Diversity of ants in Ethiopia

Based on distribution of ant fauna of the world, Ethiopia is located in the Africa region, East Africa subregion and Afrotropical bioregion. To date there are 141 described species/subspecies of ants in Ethiopia which belong to seven subfamilies and 36 genera. Out of these 40 species (28.4%) are endemic to Ethiopia. Myrmicinae is the most diverse ant subfamily in Ethiopia which comprises 72 out of the total 141 species. This is followed Formicinae and Ponerinae which contain 27 and 25 species respectively. The subfamily Dorylinae contains nine species and Pseudomyrmecinae has six species. The remaining subfamily (Proceratiinae and Dolichoderinae) each contain one species. Table 1 shows the number of species and subspecies of ants that are known to occur in Ethiopia. This list is based upon the effort of many ant collectors as well as myrmecologists who have published on the taxonomy of Ethiopian ants (www.antwiki.org/wiki/Ethiopia).

Table 1: List of subfamilies and genus and number of species/subspecies of ants known to occur in Ethiopia (Adopted from www.antwiki.org/wiki/Ethiopia)

Subfamily	Genus	Number of species/subspecies
Dolichoderinae	*Technomyrmex*	1
Dorylinae	*Aenictus*	4

	Dorylus	5
Formicinae	*Acropyga*	1
	Camponotus	18
	Cataglyphis	2
	Cataulacus	1
	Lepisiota	3
	Plagiolepis	1
	Polyrhachis	2
	Tapinolepis	1
Myrmicinae	*Calyptomyrmex*	2
	Carebara	1
	Crematogaster	14
	Melissotarsus	2
	Messor	3
	Monomorium	11
	Myrmicaria	2
	Nesomyrmex	1
	Ocymyrmex	1
	Pheidole	12
	Solenopsis	2
	Strumigenys	5
	Tetramorium	12
	Trichomyrmex	2

	Anochetus	2
	Bothroponera	3
	Hypoponera	8
	Leptogenys	4
Ponerinae	*Megaponera*	2
	Mesoponera	3
	Ophthalmopone	1
	Platythyrea	1
	Plectroctena	1
Proceratiinae	*Probolomyrmex*	1
Pseudomyrmecinae	*Tetraponera*	6
Total Number of species/subspecies		**141**

Chapter 3: Materials and methods

3.1 Study area

3.1.1 Location

The Bale Mountains National Park (BMNP) is located 400 km Southeast of Addis Ababa in Oromia National Regional State in South-eastern Ethiopia. It was established by the Ethiopian Wildlife Conservation Organization (EWCO) in 1971 with the primary objective of conserving the wildlife and other valuable natural resources in the area. The park encompasses approximately 2,200 km^2 of mountains and forest. The park area is found within geographical coordinates of 6º29' – 7º10'N and 39º28' – 39º57'E. The local boundary of BMNP lies within five *woredas* (districts): Adaba (West), Dinsho (North), Goba (Northeast), Mana-Angetu (South) and Berbere (East) (OARDB, 2007). The BMNP is part of the Bale-Arsi massif, which forms the Western part of the South-eastern highlands of Ethiopian. The BMNP includes peaks like Tullu Demtu which is the second highest mountain in Ethiopia (4,377 m a.s.l.), and Mount Batu at an altitude of 4,307 m a.s.l. This is the largest area of afro-alpine region that comprises the high mountains of Ethiopia and Tropical East Africa (Kenya, Tanzania and Uganda), which were called ‘islands in the sky’ (Hedberg, 1970).

3.1.2 Parts of the park

The Bale Mountains form the single largest afro-alpine area, with a flora and vegetation being transient between East Africa and the drier Northern part of Ethiopia (Weinert and Mazurek, 1984). These are the central peaks and plateau area consisting of Afro-alpine vegetation; the Northern Gaysay area of grassland, swamp and woodlands; and the Southern Harenna forest of dense forest (Gashaw and Fetene, 1996).

The BMNP can be divided in to three major parts based on the landscapes and altitudinal differences. The northern Gaysay area has an altitude range of 3000 and 3550m a.s.l. and extends from one mountain range to another, with a central broad flat valley. The Southern part of the Gaysay area is mainly covered with *Juniperus procera* while the Northern ridge largely contains *Hagenia abyssinica* and *Hypericum revolutum*. In between the ridges are the flat valleys of Gaysay dominated by species of the genera *Artemisia, Helichrysum, Ferula* and *Kniphofia* (OARDB, 2007).

The afroalpine plateau is the central parts of the park. It lies between about 2800m a.s.l. up to 4377 m a.s.l. (Tulu Dimtu). The Northern escarpment of the Sanetti Plateau is dominated by *Juniperus spp.* with an altitude between 2800 and 3350 m a.s.l. (OARDB, 2007). Above this altitude, between 3350 up to 3500 m is covered by *Hagenia-Hypericum* woodland. The area between 3550 and 4000 m is covered by *Erica* shrub on ridges and *Helichrysum* moorland in the valleys. The mountain tips are flat and are covered by *Helichrysum* moorland and with scattered *Lobelia spp* (Gashaw and Fetene, 1996).

The Harenna forest is found in the southern escarpment and is covered by one of the most extensive and largely natural forests remaining in Ethiopia. This forest is is the second largest stand of moist tropical forest today in Ethiopia (Datson, 2002). BMNP can also be divided into five main vegetation zones: the northern Grasslands (Gaysay valley), the northern woodlands, ericaceous forest, the Afroalpine moorland and the Harenna Forest (Miehe and Miehe, 1994).

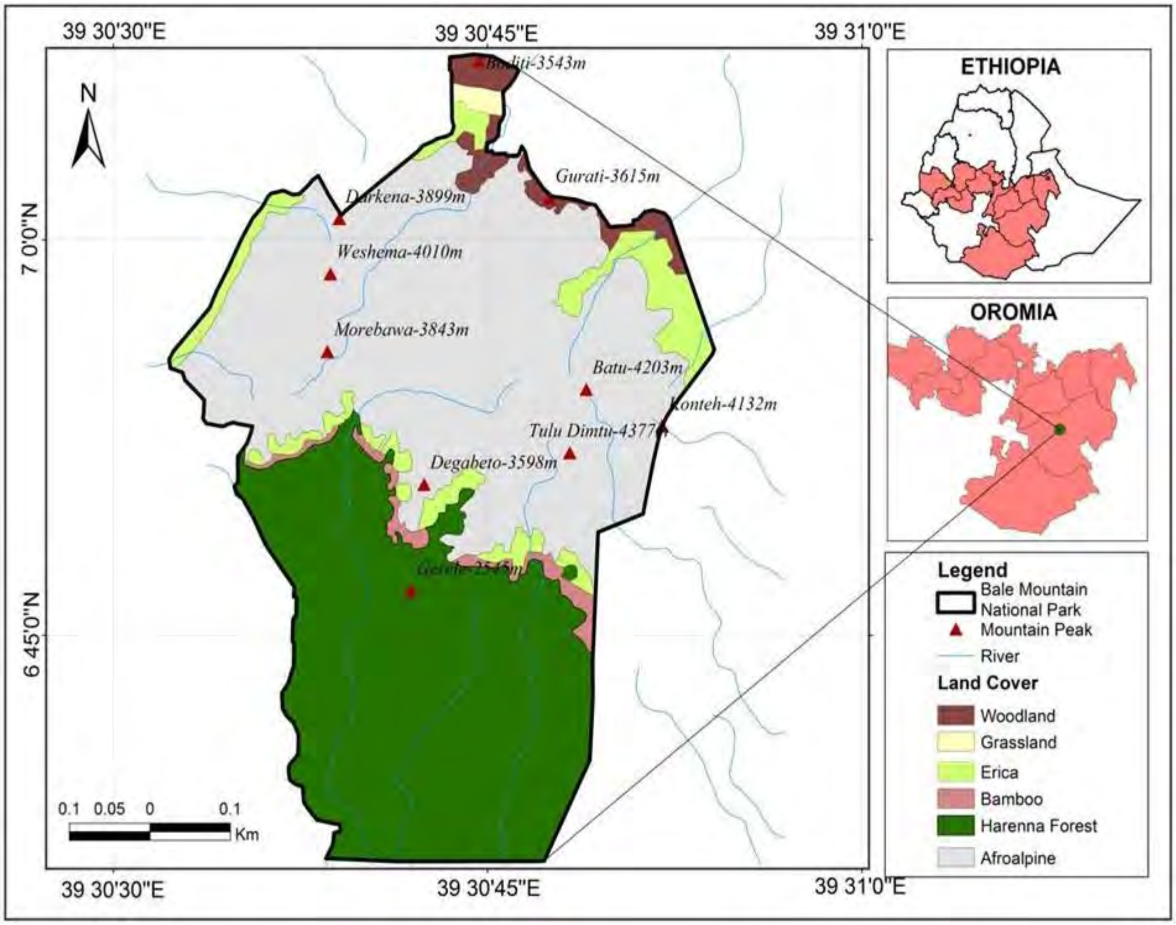

Figure 3: BMNP with its major habitat types and mountain peaks (Modified from OARDB, 2007).

3.1.3 Climate

3.1.3.1 Temperature

Because of the great altitudinal variation in the Bale Mountains massif, considerable variations of climatic conditions are recorded in the National Park. Air temperatures in

BMNP have a wide diurnal range but comparatively slight seasonal variation. The daily temperatures during the dry season show high variation. The lowest temperature that has been recorded in the mountains is -15 °C at night, with the highest recorded temperature the next day of +26 °C (Hillman, 1986). This is a range of 40 °C within a 24-hour period. On the contrary, the rainy season is mild and the temperature shows much less daily fluctuation. Freezing is rare during the rainy season and the temperature not often over 20 °C. The mean annual minimum temperature is 1.4 °C and the mean annual maximum temperature is 18.4 °C (Refera and Bekele, 2002).

At the highest altitudes of BMNP (Sanetti Plateau) having Afroalpine environment, there is marked diurnal changes with frosts at night time, while differences among seasons are generally only a few °C. Hedberg (1951) cited in Assefa *et. al.* (2011) characterized this phenomenon as “summer everyday and winter every night”. As thermal seasons are therefore weakly pronounced, wet season are often clear because most tropical mountains are located outside the permanently moist tropics (Rundel, 1994).

3.1.3.2 Rain fall

The Bale Mountains play a vital role in climate control in the region by attracting large amounts of orographic rainfall. BMNP has two rainy seasons, namely the small rainy season and the heavy rainy season. The small rain falls between March and June with its peak in April. The heavy rainy season is between July and October, with the highest peak in August. In the lower altitudes the amount of annual rainfall reaches 600 to 1000 mm while the higher altitudes get rainfall of 1000 up to 1400 mm annually (Miehe and Miehe, 1994). Rainfall is greatly seasonal on the northern slopes of the mountains and annual precipitation increases with altitude from 925 mm at Goba (2720 m) to 1061 mm at Koromi (3850 m), but is markedly lower at the highest altitudes (852 mm at Konteh, 4050 m). Mean annual rainfall on the southern slopes is not as much of the northern slopes (848 mm at Rira, 3000 m). However rainfall on the southern slopes is more uniformly distributed through the year (Umer *et. al.,* 2007). Presently, no permanent snow can be discovered, but precipitation in the form of hail could occur (Eggermont *et. al.,* 2011).

3.1.4 Hydrology

The Bale Mountains are important water supply that supports the life of millions of people and other organisms in the neighboring lowland areas. The watershed of the plateau is distinguished by flat, swampy areas as well as many small shallow lakes that are vital for stream/river flow regulation. Over 40 streams arise within BMNP. These streams contribute to form five major rivers namely, the Web, Wabe Shebelle, Welmel, Dumal and Genale. These rivers and springs are a single sources of recurrent water for the critical and lowlands of the East and Southeast of Ethiopia. These lowlands which include the Ogaden and Somali areas are extreme water deficit mainly during the dry season (OARDB, 2007).

The conservation of BMNP is vital to attain sustainable management of the hydrological cycle in the Bale Mountains massifs. This can be achieved by conservation of the various vegetation zones (Wolde-Selassie, 2004). The ericaceous belt which is dominated by Erica species covers the broad vertical belt of the Bale Mountains between 3000 to 4000 m a.s.l. extending over 1000 m vertically (Miehe and Miehe, 1994). This makes it an essential water catchments area in this maximum rainfall zone. The hydrological buffer function carried out by Erica vegetation benefits the water catchments of the mountains ecosystem. Furthermore, it has regional importance to in regulating the water flow critical for agriculture productively in the surrounding lowlands including Somalia and Kenya (Pocs, 1980).

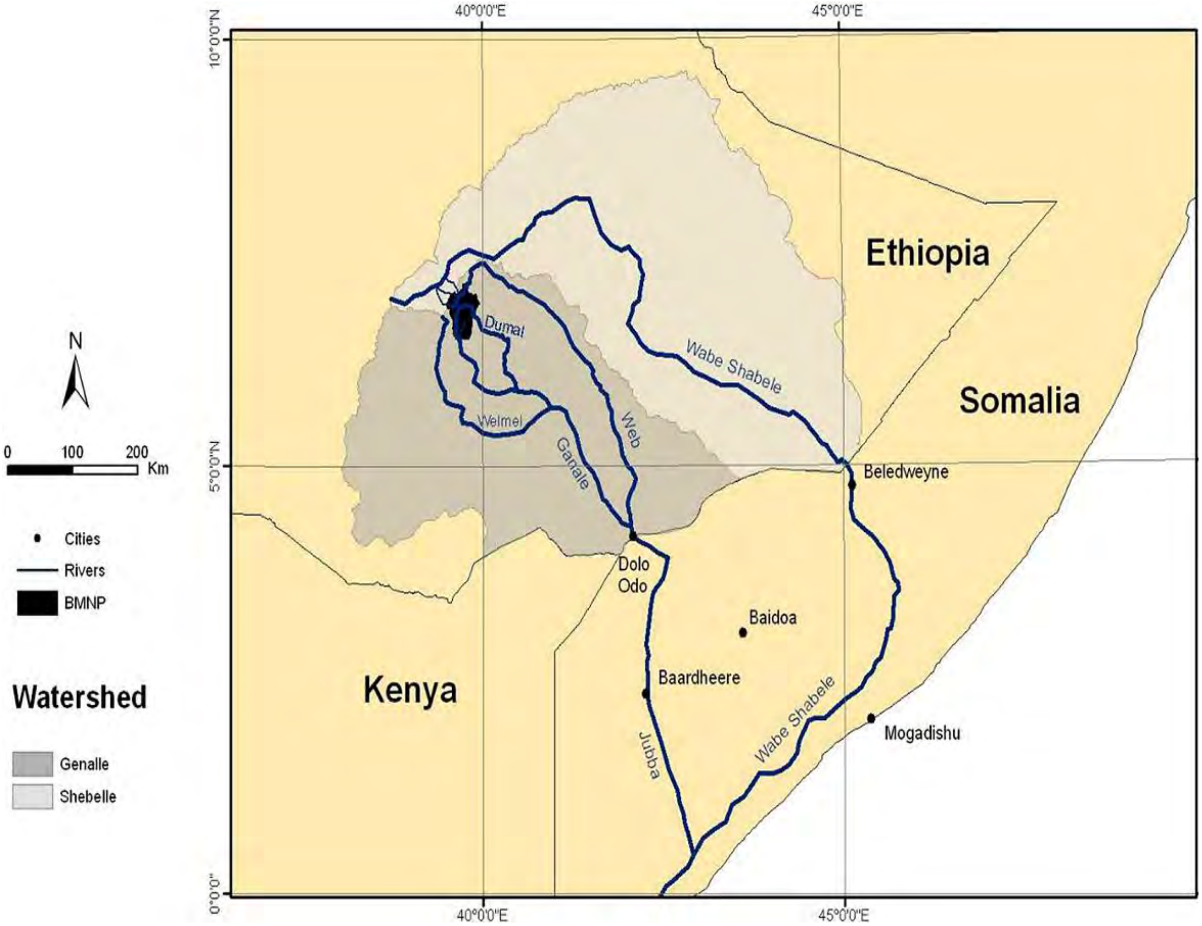

Figure 4: Major rivers and watersheds supplied by the Bale Mountains National Park (Source: OARDB, 2007)

3.1.5 Soil

The soil of BNMP is mainly derived from the basaltic and trachytic parent rock. The soil is fairly fertile silty loams of reddish-brown to black colour (Miehe and Miehe, 1994). There has been no comprehensive information about the soils in the Bale Mountains except from an initial soil survey. Yimer (2007) studied soil properties in relation to topographic aspects, vegetation communities and land use in the South-eastern highlands of Ethiopia. Soil samples collected under three vegetation communities showed that soil properties examined generally revealed significant variations with respect to vegetation and topographic aspect. Sand, silt and clay content was high under *Erica arborea*-dominated, *Hypericum revoltum/Erica arborea*/*Schefflera volkensii*-dominated and *Schefflera abyssinica/Hagenia abyssinica*-dominated soils respectively. The study also showed that soil organic carbon and total nitrogen stocks varied significantly with respect to topographic aspects and vegetation community. The top soils are progressively richer in organic matter with increasing altitude, and have a distinct humus layer at high altitudes (Yimer, 1996).

3.1.6 Flora and Fauna

3.1.6.1 Flora

Ethiopia has highly diverse flora and fauna resulting from its geographical location, diverse ecosystems and various climatic conditions (Yalden, 1983). The country is one of the top 25 biodiversity rich countries in the world (Woldemariam, 2007). Much of Ethiopia's wildlife diversity is found in the highlands (Williams *et. al.,* 2004). This is the result of the vast extent and isolation of Ethiopian highlands within the Afro-tropical region (Yalden, 1983). Vegetation type of the Bale Mountains physiognomically belongs mainly to the afro-montane and afro-alpine, showing a marked vegetation zonation along altitudes (Nigatu and Tadesse, 1989).

So far, more than 1,300 species of flowering plants have been documented in the Bale Mountains of which 400 species with medicinal value to the people (OARDB, 2007). The Ethiopian National Herbarium reports show that the total number of species of vascular plants of Ethiopia and Eritrea is about 6000. From these vascular plants, the total number of taxa for the Bale floristic region is estimated about 1650 species, from which about 1400 species occur between an altitude of 1500 and 4377 m a.s.l. There are about 600 endemic taxa in the Flora of Ethiopia and Eritrea. From these endemic taxa 177 (29.5%) are also endemic to Bale floristic region (www.balemountains.org).

3.1.6.2 Fauna

The Bale Mountains comprises one of the Key Biodiversity Areas which is included in Conservation International's Eastern Afromontane hotspot. To date, about 78 species of mammals are recorded in Bale Mountains, which represent 40% of mammals known from the whole Ethiopia. Out of these, 20 species are Ethiopian endemics, representing 26% of the total species reported in the area. From these endemic mammals three of them are found only in the Bale Mountains, namely: the giant mole rat *Tachyoryctes macrocephalus*, unstriped grass rat *Arvicanthis blicki* and harsh-furred mouse *Lophuromys melanonyx* (Admasu, 2011). The Bale Mountains are also home to the largest populations of endemic and endangered Mountain Nyala (*Tragelaphus buxtoni*) and the Ethiopian wolf (*Canis siemensis*) in the world. In BMNP lion and the wild dog populations inhabit the southern Harenna forest of the National Park, which represent unique forest populations of these savannah species (OARDB, 2007).

The BMNP is listed as one of the 69 designated Important Bird Area (IBA) in Ethiopia by Birdlife International. To date, about 280 species of birds have been recorded in BMNP, of which nearly 20% of the species recorded for Ethiopia. 57% of Ethiopia's endemic birds are found in the Bale Mountains. These endemic birds include: Rouget's rail, spot-breasted plover, blue-winged goose, the black headed siskin and white-backed black tit (EWNHS, 2001). The Bale Mountains is very important for many internationally rare large eagles, vultures and other raptors which depend on the abundant rodent community. This area is also breeding site for a number of Eurasian birds, such as the golden eagle, the ruddy shellduck and choughs. In addition, it is an important over-wintering place or passage station for migrants from Eurasia, in particular for passerines and waterfowl (OARDB, 2007).

The BMNP is offer an important refuge for at least 17 amphibian species of which 48% are endemic in Ethiopia and no fewer than five of the six endemic genera (*Altiphrynoides, Spinophrynoides, Balebreviceps, Ericabatrachus, Paracassina*). Thus, the region is a significant centre of amphibian diversity (Largen and Spawls, 2011). Reptiles are generally rare compared to amphibians at high elevations. Only three Ethiopian snakes have yet been recorded at altitudes more than of 2800 m. All of these high altitude snakes are found in the Bale Mountains (*Psammophylax variabilis*: 1900 to 3000 m, *Duberria lutrix*: 1800 to 3100 m, *Pseudoboodon lemniscatus*: 1750 to 3300 m) (Largen, 2001). The knowledge of lizards in Bale Mountains is insufficient and it can be predicted that there are many more montane and forest specialists, other than the two endemic chameleons already identified (Largen and Rasmussen, 1993). There is no information on invertebrates of the study area except some studies on butterflies and moths (www.balemountainlodge.com).

3.1.7 Threats of the BMNP

Current the Park is under serious threat. Increasing human pressures have resulted in unsustainable and unmanaged use of natural resources. Human settlement, livestock overstocking, agricultural expansion and wood extraction resulting from immigration and population growth are the severe problems and the most destructive to ecosystem health in BMNP. For example, grazing within the Web Valley, a prime Ethiopian wolf habitat, cattle density is estimated at 250 per square kilometer. With regard to increasing settlements within the park, currently over 40,000 people live within the park's boundaries. This is increasing pressure on the natural resources of the area and reducing natural habitats of wild animals. Associated with these settlements, domestic dogs come in contact with the Ethiopian wolf and transmit rabies and canine distemper. In 2010 alone these diseases killed 106 individuals (approximately 40% of the Bale population). The use of the wolf habitat by livestock for grazing significantly reduces the availability of rodent prey (OARDB, 2007). Other major threat is recurrent and extensive forest fire which is causing severe habitat destruction. Recently, fire accidents (most of which were human induced) have burnt 60% of the "Ericaceous belt", the major water catchment area of the Bale Mountains massif. If forest fire continues it may lead to the destruction of the entire hot spot, suggesting the urgent need to design an integrated forest fire management plan which appears non-existent in the country (Belayneh *et. al.,* 2013).

3.2 Sampling procedures

Sampling was conducted from December 14, 2013 to October 08, 2015. Ants were collected from different altitudes and habitats of BMNP. Ant samples were collected from the Harenna forest (1500 m to 2400 m), Northern woodland (2400 to 3300 m), Gaysay grassland (3000 to 3100 m), Erica moorland (3200 to 3400) and Afro-alpine habitat (3900 to 4000 m). Three collection methods, namely manual search, baits and pitfall traps were used for sample collections. During manual search, ants were searched on the ground, on vegetation, under stones and fallen trees. They were collected by hand and aspirator. Ant nests were also dug up when found and workers were collected. Reproductive castes were also collected when available. Individuals were picked directly from the nest, or a portion of the nest substrate was transferred to a white plastic bucket, from which the ants were transferred to small plastic vials containing 95% ethanol. Collection was done for three days in each habitat from 10:00 – 11:50 am and 3:00 – 4:30 pm.

Four line transects (two transect for baits and two for pitfall trap) were randomly set up on the ground floor for baits and pitfall traps. Transects for baits and pitfall trap were 10 meters apart, and 90 meters in length (Fisher and Robertson, 2002). A 10 m distance was kept between the traps and between the baits in the line transect. The second transects were set 500 m away from the first. We sampled ants with pitfall traps constructed from 120 ml plastic cups (6 cm diameter) embedded in soil so that the lip of the cup was flush with the soil surface. Cups were filled to two third of their volume with 4 percent formaldehyde mixed with a small amount of detergent. After 24 h the traps were retrieved and the contents of were strained through fine mesh and stored in 95% ethanol until identification (Watanasit *et. al.,* 2005). Baits were prepared in a 12 by 75 mm test tubes with a piece of tuna (StarKist Selects Solid Light Yellowfin Tuna in Extra Virgin Olive Oil, 4.5 oz) inserted approximately 2 cm into the tube. The bait tube was placed directly on the ground at each sampling point and shaded with one half of a Styrofoam plate. The baits were left for 24 h and collected, and the ends were plugged with small cotton to prevent escaping (king and Porter, 2005). Samples were transferred in to plastic vials containing 95% ethanol. All sampling methods were replicated four times during the study period.

3.3 Storage of ant specimens

All ant samples were stored in 5 ml plastic vials containing 95% ethanol (King and Porter, 2003). The specimens were transported to Addis Ababa University and any dirt, plant material or other debris that was collected with the ants were removed. The alcohol was changed for each vial to prevent dilution by body fluids during first preservation. Ants were kept inside a refrigerator until transported to University of Utah, USA and University of Nizhni Novgorod, Russia for identification. The sample collections with collection code starting with Ayele-0001up to Ayele-0101were taken to the United States of America (USA), and identified in Longino laboratory, University of Utah, USA and in University of Nizhni Novgorod, Russia. The remaining samples with collection code starting from Ayele-0139 to Ayele-0199 were identified in Addis Ababa University.

3.4 Dry-mount (point-mount) preparation

From a total of 101 sample collections taken to USA, 205 dry mount specimens were prepared following a dry mount preparation online manual prepared by John T. Longino (www.academic.evergreen.edu/projects/ants /HOWTOMOUNTANTS). Ants stored in 95% ethanol were taken to University of Utah, USA and University of Nizhni, Novgorod, Russia for identification. Specimens transported to University of Utah, at Longino lab were placed in a Petri dish containing ethanol and were examined under a dissecting microscope. Specimens of all castes in the collection (workers, queens, males) were selected when available. When there was worker size variation, or two distinct worker size classes, selection was made from a range of sizes or of both worker castes (minor and major workers). Curled up specimen legs, head, and abdomen were extended gently while it was in alcohol using forceps.

Then the selected specimens were placed on blotter paper to dry for a few minutes, until the integument was dry. For alate queens and males the specimens were floated out on a small piece of paper, in order that the wings lie flat on the paper. A 3x5cm card and a microscope slide were put under the microscope. The specimens were placed on the card, on their backs, with heads pointing down, legs spread and head and abdomen extended. Points were prepared from strips of stiff, white, acid-free paper by punching with a specially designed hand-punch. A drop of glue was placed on the slide and a point was carried by grasping it at the sides with forceps. The glue, the specimen, and the point all were adjusted within the field of view under the scope. A small amount of glue, its quantity decided by the size of the specimen was taken by the tip of the point and was placed on the platform formed by the middle and hind coxae, inserting the point from the right, between the mid and hind legs. When the glue was dried sufficiently the point was picked up with forceps and the specimen was pinned using a pinning block.

3.5 Labeling

Once the specimens were properly mounted labels were put on each specimen that makes identification easy. Labels were made on acid-free archival quality paper and were printed with an archival quality ink printer. Labels included information like the location, the latitudes, longitudes and altitudes as well as the date of collection and collection code and habitat type. Barcodes were attached to each mounted specimens. The barcodes are standard labels to keep a database inventory of ant specimens and are important the materials were to be deposited in the Zoological Natural History Museum, Addis Ababa University (www.antwiki.org/wiki/ Preparing_Ants_for_Study).

3.6 Identification of Ants

Identification guides of the ant genera of the world by Bolton (1994) and Hölldobler and Wilson (1990) were used for identification to genus. All identifications of ants to species level were confirmed by John T. Longino, at University of Utah and by Vladimir Zryanin, University of Nizhni Novgorod. Pinned voucher specimens from University of Utah were brought back to Ethiopia and were deposited at the Zoological Natural History Museum, Addis Ababa University. All unpinned specimens were stored in plastic vials containing 95% ethanol and deposited in the Museum. Those specimens identified to species level were incorporated in AntWeb, which is the world's largest online database of images, specimen records, and natural history information on ants.

3.7 Ant species selection for thermal experiments

To determine the heat and cold tolerance of ants with elevation, ant species with widest altitude distribution range were selected for heat and cold tolerance experiments. As was evident from the results of ant species diversity and distribution with elevation and habitat types, *C. negus* had the widest altitude distribution range. This species was found to be distributed from altitude of 2746 m up to 3981 m above sea level (a.s.l.), and was the only species which was found above 3250 m a.s.l. It was found in all habitat types except the rainforest, and is the only species occupying Erica moorland and afro-alpine habitats. Thus, *C. negus* was chosen to be best candidate for the study of the effect of altitude on heat and cold tolerance of ants. Due to resource and time constraints, colony samples were taken from two altitudes ≈ 3040 (Gaysay) and 3970 m (Sanetti).

As there was no other ant species under the genus *Camponotus* in the study area, *C. acvapimensis* from Dilla, Ethiopia was used to determine differences in heat and cold tolerance between species. Since, *C. acvapimensis* was located in farther lower altitude ranges (1430–1450 m) than *C. negus*; it was a better prospect to determine the effect of altitude on heat and cold tolerance of ants. Heat and cold tolerance experiment was conducted from March 27 to April 20, 2015. Experiments on ant colonies from BMNP were conducted in Biological Sciences laboratory, Mede Welabu University, Bale-Robe, Oromia administrative region and for colonies from Dilla University at Chemistry research laboratory, Dilla University, SNNPR, Ethiopia. All experiments were done within three hours of collection to reduce acclimatization effects. Ant colony collection sites for thermal experiments are shown in Table 2.

Table 2: Ant colony collection sites for thermal experiment

Place	Site	Colony	Latitude	Longitude	Elevation (m)	Habitat
BMNP	Gaysay	1	7.65716	39.44347	3043	Grassland
BMNP	Gaysay	2	7.65771	39.44342	3043	Grassland
BMNP	Gaysay	3	7.65666	39.44353	3040	Grassland
BMNP	Sanetti	1	6.47464	39.48429	3980	Afro-alpine
BMNP	Sanetti	2	6.47545	39.48363	3975	Afro-alpine
BMNP	Sanetti	3	6.47375	39.48402	3969	Afro-alpine
Dilla	Dilla	1	6.25168	38.16429	1452	Woodland
Dilla	Dilla	2	6.25171	38.16429	1451	Woodland
Dilla	Dilla	3	6.25166	38.16429	1451	Woodland

3.8 Determination of heat tolerance

To determine of the heat tolerance of ant colonies, three colonies were taken from each site, 22 to 24 worker ants per colony. The time taken (minutes) by ant colonies to lose mobility at a stressfully high temperature (knock-down resistance) of colonies were measured from each site as an index of heat tolerance. During the experiment 10 to 12 individuals from each colony (for a minimum of 24 individuals per colony) were placed into two Petri dishes in identical water baths (Stuart digital water bath, RE300DB, Keison International Ltd, UK). Laboratory tests were conducted in a room temperature of approximately 20–22 °C, which created a thermal gradient from the bottom surface of the Petri dish touching the water (hot) to the top surface surrounded by air (cool). This gradient allowed ants to hang on the top of the Petri dish where the temperature was less stressful. Temperature of the water bath was constantly monitored by an infrared thermal gun (Raynger ST30, Raytek). Initially the temperature in both baths was set and maintained at 45 °C for 90 minutes. After 90 minutes, the temperature was raised by 5 °C, and continued to rise by 5 °C every 30 minutes thereafter until all ants had succumbed to heat stress. The Petri dishes in each water bath were monitored continuously by two observers. We considered an individual's tolerance to end when it became completely immobile. Time was recorded starting from the time the first ant completely lost mobility till the last ant stopped moving. The methods generally followed were those of Angilletta *et. al.* (2007).

3.9 Determination of cold tolerance

The time required for an individual to recover from exposure to extremely low temperatures (chill-coma recovery) was used to assess cold tolerance of ants. From each colony 24 to 27 ants were placed in a Petri dish covered in ice for 20 minutes. At the end of this period, ants were removed from Petri dish and were placed on their backs in the center of a printed 32 mm diameter circle. The experiments were conducted in a room temperature of approximately 20–22 °C. Samsung HD digital camcorder, model HMX-F80 was used to record the experiment. The time for each individual to come to upright position and move out of the circle was noted. When an individual was fully out of the circle, it was removed from the experiment to ensure it did not influence recovery time of others (Angilletta *et. al.*, 2007). Data was retrieved from the camcorder by replay.

3.10 Statistical data analysis

All analyses were performed using SPSS software (version 20). From a total of 162 ant samples collected, 170 sample identifications were made (Appendix 4) for the analysis of species distribution and abundance. The list of sample identification was greater than the total number of sample collections, because some sample collections were found to contain more than one species. Species diversity and distribution with elevation was analyzed in five altitude ranges starting from 1500 m and adding 500 m for each range up to a range of 4000 m. Relative species abundance was analyzed for each altitude range. It indicated the abundance of a species relative to other species found in that particular altitude range. Thus, the relative species abundance of a species for an altitude range was analyzed by dividing the frequency (count) of a species in a given altitude range divided by to the total frequency of all species in that particular altitude range multiplied by hundred. The diversity, distribution and abundance of ant species for each habitat were also analyzed with the same method used for analyzing diversity, distribution and abundance with altitude. A Cox proportional hazard model was performed to test the differences in knock-down resistance as well as chill coma recovery time among colonies, between sites and between species (*C. negus* and *C. acvapimensis*) (Angilletta *et. al.,* 2007). The formula of Cox proportional hazard model is shown below.

$$h(t;x) = h_o(t)\exp\{\beta_1 x_1+\ldots+\beta_k x_k\}$$

Where

$h(t; x)$ = the hazard function at time t for a subject with covariate values $x_1, \ldots x_k$,

$h_0(t)$ = the baseline hazard function, i.e., the hazard function when all covariates equal zero

exp = the exponential function ($\exp(x) = e^x$),

x_i = the **ith** covariate in the model, and

β = the regression coefficient for the i^{th} covariate, $\mathbf{x_i}$

All values were expressed as the mean value ± standard error of the mean (SEM) and the level of significance was calculated by one way analysis of variance (ANOVA). This was followed by Scheffe post-hoc test for comparisons of the means. A probability level of less than 5% ($p<0.05$) was considered statistically significant difference among test groups.

Chapter 4: Results

4.1 Ant species richness and abundance

From a total of 162 sample collections made in the study area, 16 species were identified (Table 3).

4.1.1 Ant species richness with subfamilies

As can be seen from Table 3, there are a total of 8 genera under four subfamilies namely; Dorylinae, Formicinae, Myrmicinae and Ponerinae identified. Subfamily Myrmicinae was the most diverse subfamily in the study area with nine species (56.25%) and subfamily Dorylinae and Ponerinae were the second diverse subfamilies, each containing three species (18.75%). Subfamily Formicinae was the least diverse which is represented by only one species (6.25%).

Table 3: Summary table of ant species identified from the study area

No. of species	Subfamily	Species
3	Dorylinae	*Dorylus affinis* (Shuckard, 1840) *Dorylus fimbriatus* (Shuckard, 1840) *Dorylus nigricans* (Illiger, 1802)
1	Formicinae	*Camponotus negus* (Forel, 1907) *
9	Myrmicinae	*Messor galla* (Mayr, 1904) *Monomorium* sp. 01 *Pheidole* cf. *aeberlii* (Forel, 1894) *

		Pheidole megacephala (Fabricius, 1793) *
		Tetramorium cf. *eminii* (Forel, 1894) *
		Tetramorium edouardi (Forel, 1894)
		Tetramorium nr. *simillimum* (Smith, 1851) *
		Tetramorium sp. 01
		Tetramorium zambezium (Santschi, 1939) *
3	Ponerinae	*Bothroponera soror* (Emery, 1899)
		Hypoponera exigua (Bolton & Fisher, 2011) *
		Hypoponera faex (Bolton & Fisher, 2011) *

* Species identified in Russia

4.1.2 Ant species richness with genus

As can be observed from Table 3, the genus *Tetramorium* was found to be the most speciose genus in the study area containing five species followed by *Dorylus* with three species, which in turn was followed by *Pheidole* and *Hypoponera* each having two species. The remaining ant genera (*Camponotus, Messor, Monomorium* and *Bothroponera*) had the least species richness, each with a single species.

4.1.3 Ant species abundance

Abundance of each species is shown in Figure 5. *C. negus* was the most abundant species and occurred 92 times (54.12%). *P. megacephala* was the second which occurred 38 times (22.35%). *C. negus* and *P. megacephala* together make more than three fourth of the total samples identified. Following the two most abundant species, *Dorylus affinis* was the third in its abundance which occurred 11 times (6.47%). The rest 13 species together made only 17% of the total samples identified.

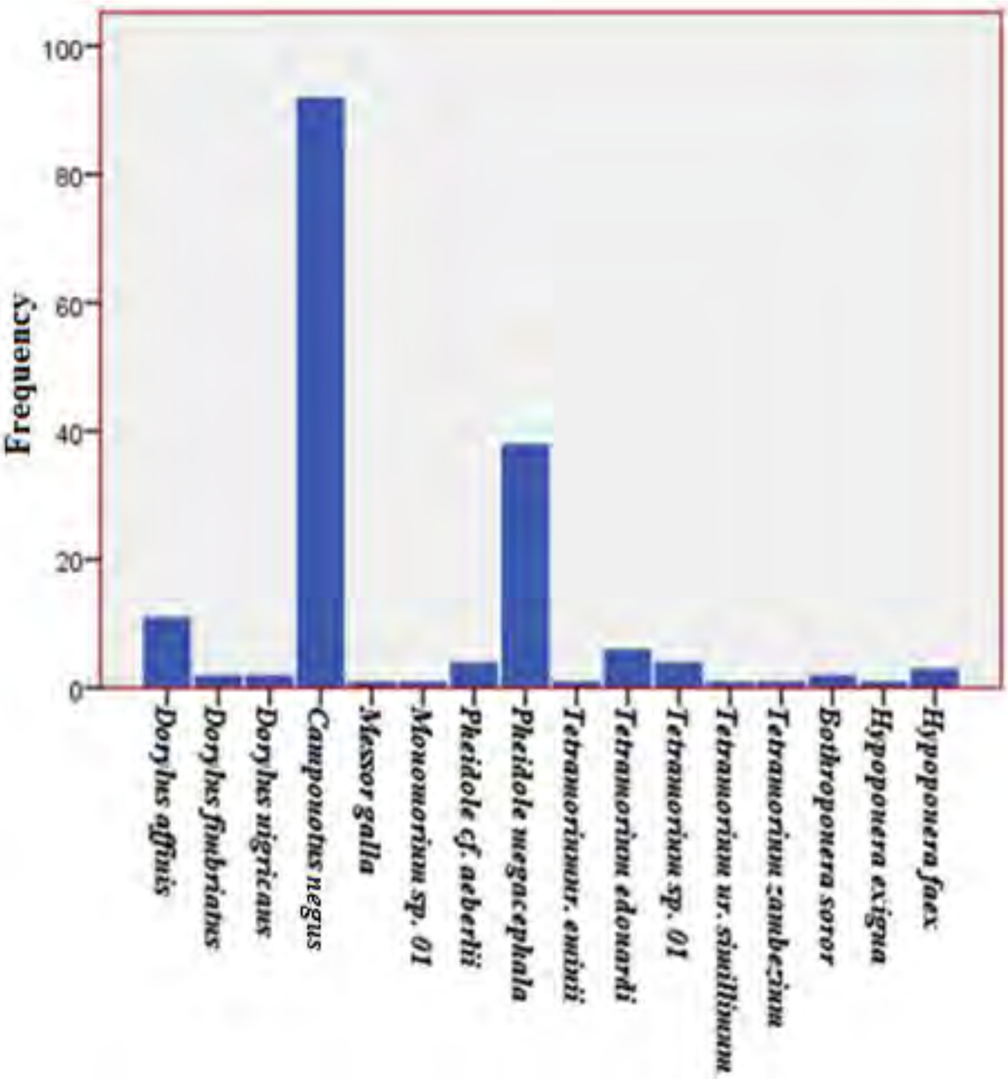

Figure 5: Species abundance in the study area

4.2 Ant species richness, distribution and abundance with elevation

4.2.1 Species richness and distribution with elevation

The result of the analysis of species richness and distribution with elevation is shown in Figure 6.

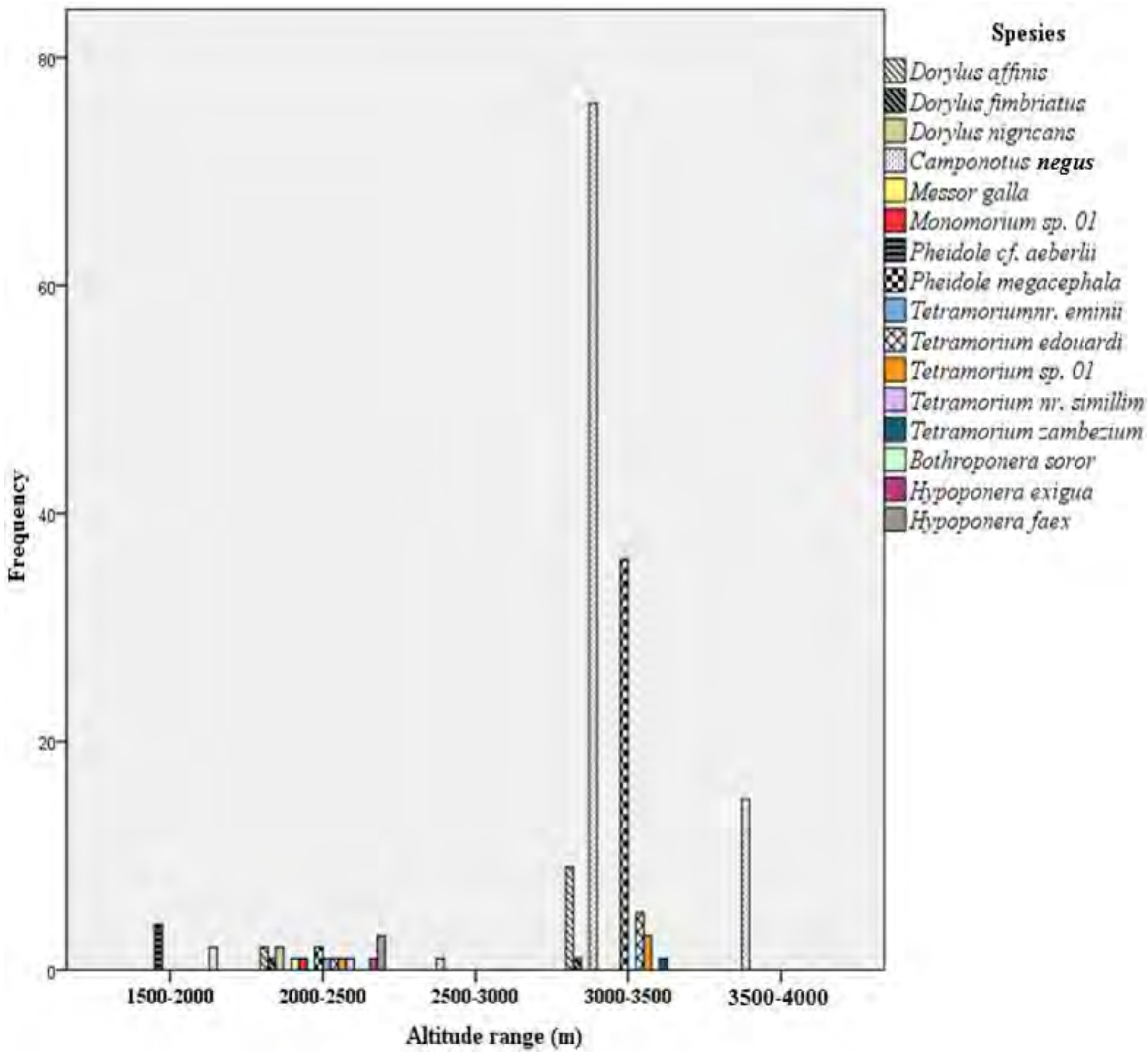

Figure 6: Species richness and distribution with elevation

The highest species richness was found in the altitude range of 2000–2500 m (12 species) followed by 3000–3500 m (7 species). The lowest was found in altitude range of 2500–3000 m and 3500–4000 m. These two altitude ranges were occupied by, *C. negus* and there was no uniform trend of species richness pattern with elevations.

Most of the species in the study area were found within the two altitude ranges, namely 2000–2500 m (12 species) and 3000–3500 m (7 species). *Bothroponera soror* and *Pheidole* cf. *aeberlii* occupied the lowest altitude range while *C. negus* occupied the highest altitude range. The altitude ranges 2500–3000 m and 3500–4000 m were represented by a single species, *C. negus*. This species is distributed in all altitude ranges above 2500 m. On the other hand, the distributions of *Dorylus affinis, Dorylus fimbriatus, P. megacephala, Tetramorium edouardi* and *Tetramorium* sp.01 were starting from the altitude range of 2000–2500 m, then were missing in the range of 2500–3000 m and reappeared in the range of 3000–3500 m. The distribution of *Dorylus nigricans, Hypoponera faex, Tetramorium* nr. *eminii, Tetramorium* nr. *simillimum, Monomorium* sp. 01, *Hypoponera exigua* and *Messor galla* were restricted only to the altitude range of 2000–2500 m.

4.2.2 Species abundance with elevation

Table 4 shows the relative species abundance in each altitude range.

Table 4: Relative ant species abundance with elevation

Altitude range	No. of species	Species name	Frequency	Relative species abundance (%)
1500–2000 m	2	*Bothroponera soror*	2	33.33
		Pheidole cf. *aeberlii*	4	66.67
2000–2500 m	12	*Dorylus affinis*	2	11.77
		Dorylus nigricans	2	11.77
		Hypoponera faex	3	17.65
		Tetramorium edouardi	1	5.88
		Tetramorium nr. *eminii*	1	5.88
		Tetramorium nr. *simillimum*	1	5.88
		Dorylus fimbriatus	1	5.88
		Monomorium sp. 01	1	5.88
		Hypoponera exigua	1	5.88
		Tetramorium sp.01	1	5.88
		Pheidole megacephala	2	11.77
		Messor galla	1	5.88
2500–3000 m	1	*C. negus*	1	100
3000–3500 m	7	*C. negus*	76	58.02
		Dorylus affinis	9	6.87
		Dorylus fimbriatus	1	0.76
		Pheidole megacephala	36	27.48
		Tetramorium edouardi	5	3.82
		Tetramorium sp.01	3	2.29
		Tetramorium zambezium	1	0.76
3500–4000 m	1	*C. negus*	15	100

As can be observed from Table 4, *C. negus* was the most abundant species in all altitude ranges above 2500 m while, *Hypoponera faex* was the most abundant in the altitude range of 2000–2500 m. *P. megacephala* was the second most abundant in 2000–2500 m and 3000–3500 m and *Pheidole* cf. *aeberlii* was the most abundant in the lowest altitudes (1500–2000 m).

4.3 Species richness, distribution and abundance with habitats

4.3.1 Species richness and distribution with habitats

The result of analysis of species richness and distribution with habitats is shown in Figure 7.

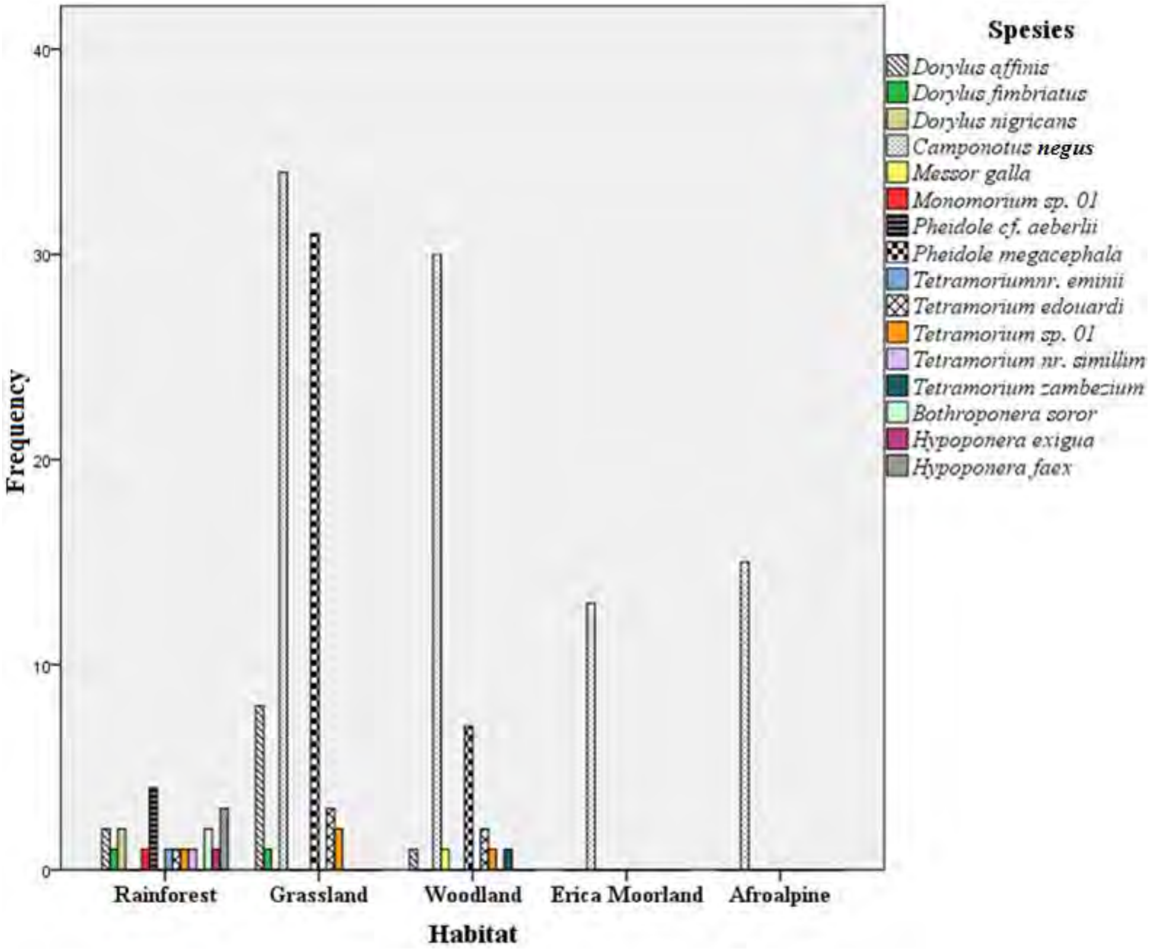

Figure 7: Species richness and distribution with habitats

Ants were most diverse in rainforest with a total of 12 species. Woodland was the second habitat in ant species richness with seven species which was followed by grassland. Erica moorland and afro-alpine habitats had the lowest with only a single species, *C. negus*. Species distribution varied with habitats. *C. negus* had the widest habitat distribution. This species was distributed in all habitat types except rainforest. *Dorylus affinis, Tetramorium edouardi* and *Tetramorium* sp.01 were with the second widest habitat distribution. These three species were found in three habitat types; rainforest, grassland and woodland. *Dorylus fimbriatus* was found in rainforest and grassland while, *P. megacephala* was found in grassland and woodland. All the remaining species were restricted to only one habitat type, either rainforest (*Dorylus nigricans, Monomorium* sp. 01, *Pheidole* cf. *aeberlii, Tetramorium* nr. *eminii* and *Tetramorium* nr. s*imillimum*), or woodland (*Messor galla* and *Tetramorium zambezium*).

4.3.2 Species abundance with habitats

Table 5 illustrates the relative abundance of a species in each habitat.

Table 5: Relative ant species abundance with habitats

Habitat	No. of spp.	Species	Frequency	Relative spp. abundance (%)
Rainforest	12	*Bothroponera soror*	2	10
		Dorylus affinis	2	10
		Dorylus fimbriatus	1	5
		Dorylus nigricans	2	10
		Hypoponera exigua	1	5
		Hypoponera faex	3	15
		Monomorium sp. 01	1	5

		Pheidole cf. *aeberlii*	4	20
		Tetramorium edouardi	1	5
		Tetramorium nr. *eminii*	1	5
		Tetramorium nr. *simillimum*	1	5
		Tetramorium sp.01	1	5
Grassland	6	*C. negus*	34	43.03
		Dorylus affinis	8	10.13
		Dorylus fimbriatus	1	1.27
		Pheidole megacephala	31	39.24
		Tetramorium edouardi	3	3.8
		Tetramorium sp.01	2	2.53
Woodland	7	*C. negus*	30	69.76
		Dorylus affinis	1	2.33
		Messor galla	1	2.33
		Pheidole megacephala	7	16.27
		Tetramorium edouardi	2	4.65
		Tetramorium sp.01	1	2.33
		Tetramorium zambezium	1	2.33
Erica moorland	1	*C. negus*	13	100
Afro-alpine	1	*C. negus*	15	100

As can be viewed from Table 5, *C. negus* was the most abundant species in all habitats except its absence in rainforest. *P. megacephala* was the second most abundant species in grassland and woodland and *Pheidole* cf. *aeberlii* was the most abundant species in rainforest followed by *Hypoponera faex*.

4.4 Heat and cold tolerance of ants

4.4.1 Heat tolerance

4.4.1.1 Heat tolerance among colonies of *C. negus*

Results of Cox proportional hazard model of heat tolerance among colonies of *C. negus* from Gaysay and Sanetti are shown in Table 6. The time for knock-down resistance did not significantly differ among colonies of *C. negus* in the same site. But time for knock-down resistance significantly differed among colonies from different altitudes. Ant colonies from Sanetti had significantly higher knock-down resistance than ant colonies from Gaysay. Thus, colonies of *C. negus* from Sanetti had high heat tolerance than colonies from Gaysay. Graph of percentage survival of ant colonies from Gaysay and Sanetti is shown in Figure 8.

Table 6: Knock-down resistance (in minutes) of ant colonies of *C. negus*

Site	Colony	Elevation (m)	Mean ± SEM
Gaysay	1	3043	94.17 ± 3.08^a
	2	3043	95.18 ± 3.15^a
	3	3040	105.54 ± 3.02^a
Sanetti	1	3980	141.48 ± 3.08^b
	2	3975	143.83 ± 3.02^b
	3	3969	145.26 ± 3.08^b

Values are mean ± SEM (Means with the same letters in the same column are not significantly different), ($p>0.05$)

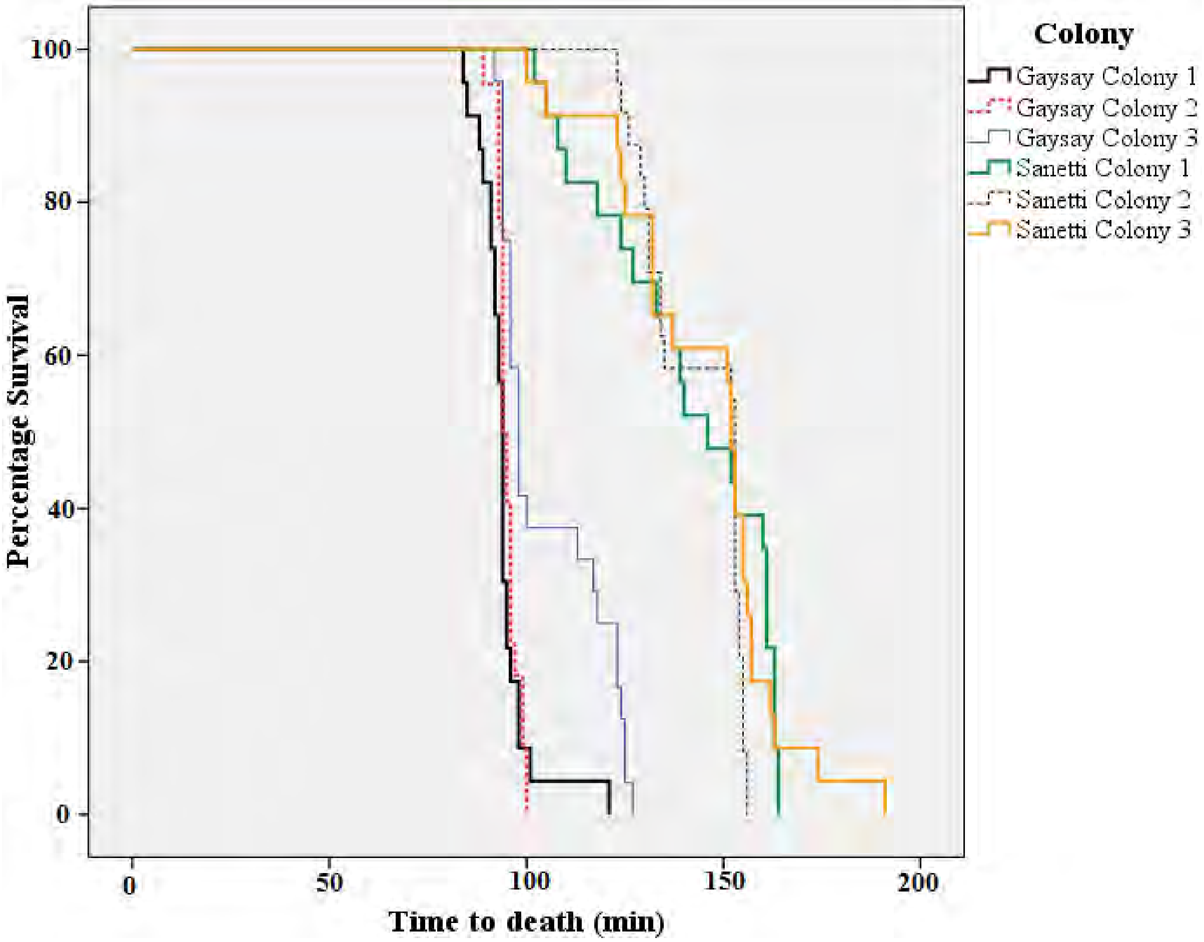

Figure 8: Percentage survival curve of colonies of *C. negus* from Gaysay and Sanetti.

4.4.1.2 Heat tolerance of ants between sites

Results of Cox proportional hazard model of heat tolerance of ant colonies from Gaysay and Sanetti respectively were 98.30 ± 1.78 and 143.52 ± 1.77 minutes. There was significant difference in heat tolerance between sites. Colonies from Sanetti on average tolerated heat for additional 45 minutes compared to Gaysay.

4.4.1.3 Heat tolerance of *C. negus* and *C. acvapimensis*

The heat tolerance for colonies of *C. negus* and *C. acvapimensis* is shown in Table 7. There was no significant difference in heat tolerance between *C. negus* from Sanetti and *C. acvapimensis* from Dilla. But heat tolerance significantly differed between *C. negus* from Gaysay with *C. acvapimensis* from Dilla and *C. negus* from Sanetti. Thus, heat tolerance is best explained in terms of colony sites rather than the species. Percentage survival curve of ant colonies from the three sites is shown in Figure 9.

Table 7: Knock-down resistance (minutes) of *C. negus* and *C. acvapimensis*

Place	**Site**	**Species**	**Mean ± SEM**
BMNP	Gaysay	*C. negus*	98.30 ± 1.82[a]
BMNP	Sanetti	*C. negus*	143.52 ± 1.80[b]
Dilla	Dilla	*C. acvapimensis*	146.6 ± 11.81[b]

Values are mean ± SEM (Means with the same letters in the same column are not significantly different), ($p>0.05$)

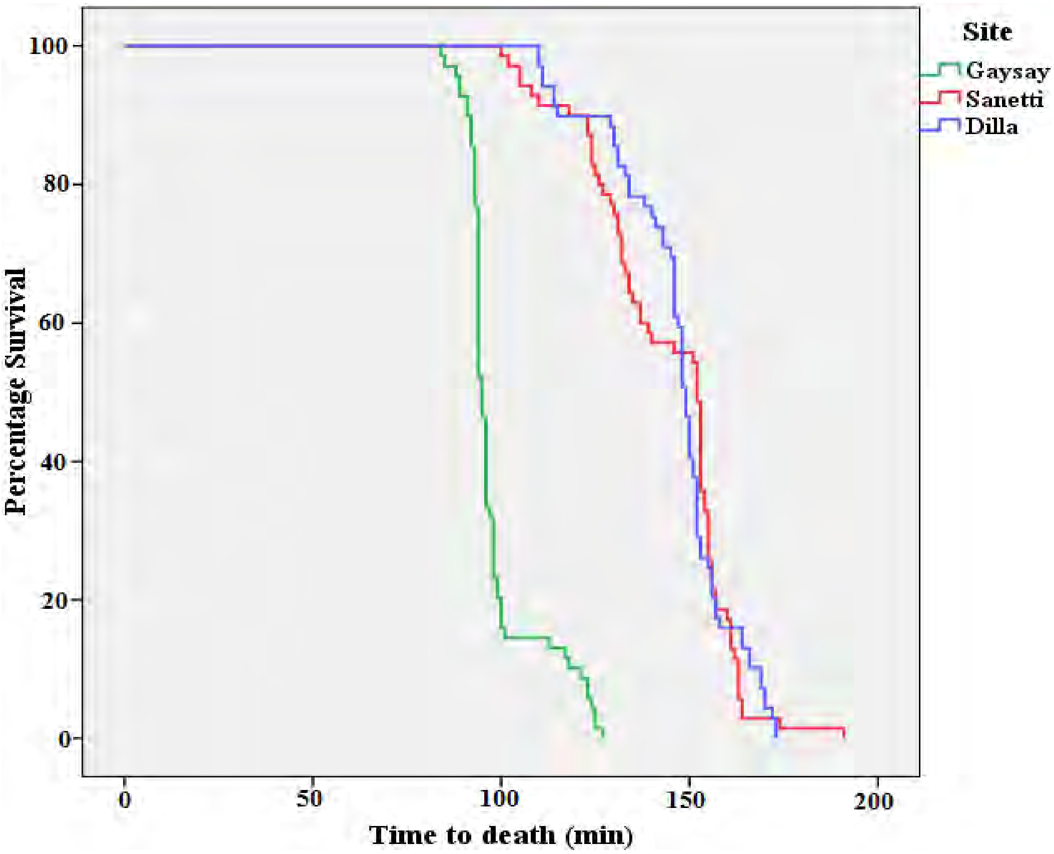

Figure 9: Percentage survival curve of ant colonies of *C. negus* and *C. acvapimensis*

4.4.2 Cold tolerance

4.4.2.1 Cold tolerance of colonies of *C. negus*

The results of Cox proportional hazard model of chill-coma recovery time of ant colonies from Gaysay and Sanetti is shown in Table 8. The results of Cox proportional hazard analysis showed that there was no significant difference in chill-coma recovery time among colonies of *C. negus*. Thus, there was similar low temperature tolerance among colonies of *C. negus*.

Table 8: Chill-coma recovery time (in seconds) of colonies from Gaysay and Sanetti

Site	Elevation (m)	Colony	No. of ants per colony	Mean ± SEM
Gaysay	3043	1	27	112.67 ± 18.72^{a}
Gaysay	3043	2	25	113.24 ± 19.46^{a}
Gaysay	3040	3	24	115.92 ± 19.86^{a}
Sanetti	3980	1	27	67.85 ± 18.72^{a}
Sanetti	3975	2	27	76.89 ± 18.72^{a}
Sanetti	3969	3	27	107.37 ± 18.72^{a}

Values are mean ± SEM (Means with the same letters in the same column are not significantly different), ($p>0.05$)

Graph of percentage recovery from chill-coma of colonies from Gaysay and Sanetti is shown in Figure 10.

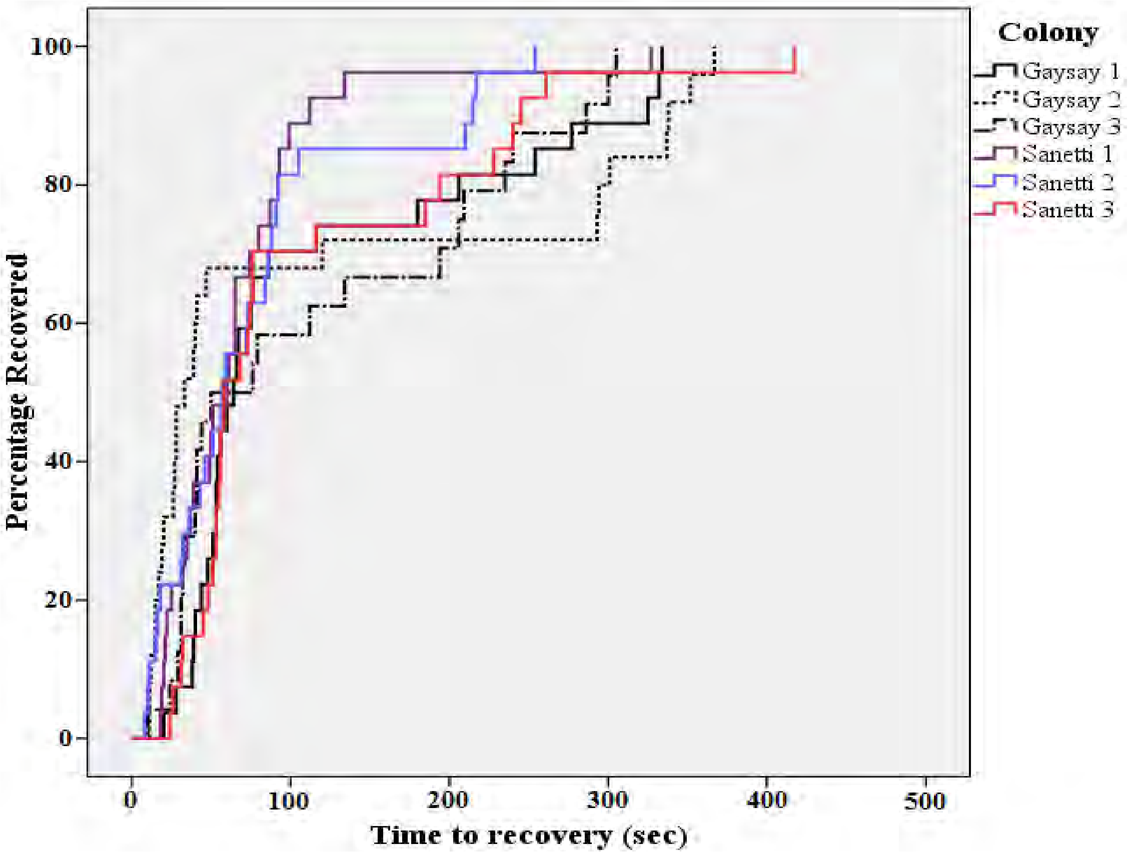

Figure 10: Percentage recovery time (in seconds) of ant colonies of *C. negus*

4.4.2.2 Cold tolerance between sites

The results of Cox proportional hazard model of chill-coma recovery time of colonies from Gaysay and Sanetti respectively were 113.94 ± 11.17 and 84.04 ± 10.81 seconds and there was no significant difference between sites. The graph of percentage of ant colonies recovered from chill-coma for the two sites is shown in Figure 11.

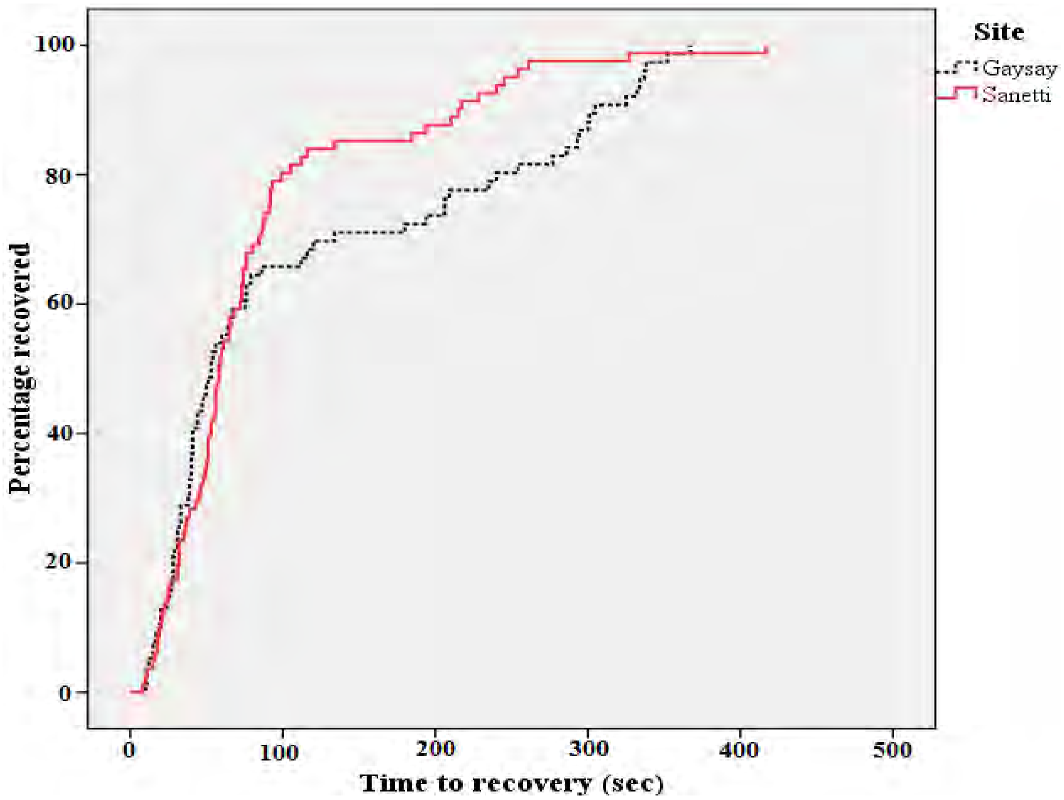

Figure 11: Graph of percentage recovery from chill-coma for colonies of *C. negus* from Gaysay and Sanetti

4.4.2.3 Cold tolerance of *C. negus* and *C. acvapimensis*

Results of chill-coma recovery time for colonies of *C. negus* and *C. acvapimensis* is presented in Table 9. Recovery time of ant colonies of *C. acvapimensis* was significantly higher than *C. negus*. Colonies of *C. acvapimensis* needed nearly five times more to recover from chill-coma compared to colonies of *C. negus* from Gaysay and six times more than *C. negus* from Sanetti. Thus colonies of *C. negus* had significantly higher low temperature tolerance compared to *C. acvapimensis*. Percentage recovery of ant colonies from chill-coma for *C. negus* and *C. acvapimensis* is shown in Figure 12.

Table 9: Chill-coma recovery time (seconds) of *C. negus* and *C. acvapimensis*

Place	Species	Site	Mean ± SEM
BMNP	*C. negus*	Gaysay	113.94 ± 19.40^{a}
		Sanetti	84.04 ± 18.77^{a}
Dilla	*C. acvapimensis*	Dilla	549.79 ± 18.77^{b}

Values are mean ± SEM (Means with the same letters in the same column are not significantly different), ($p>0.05$)

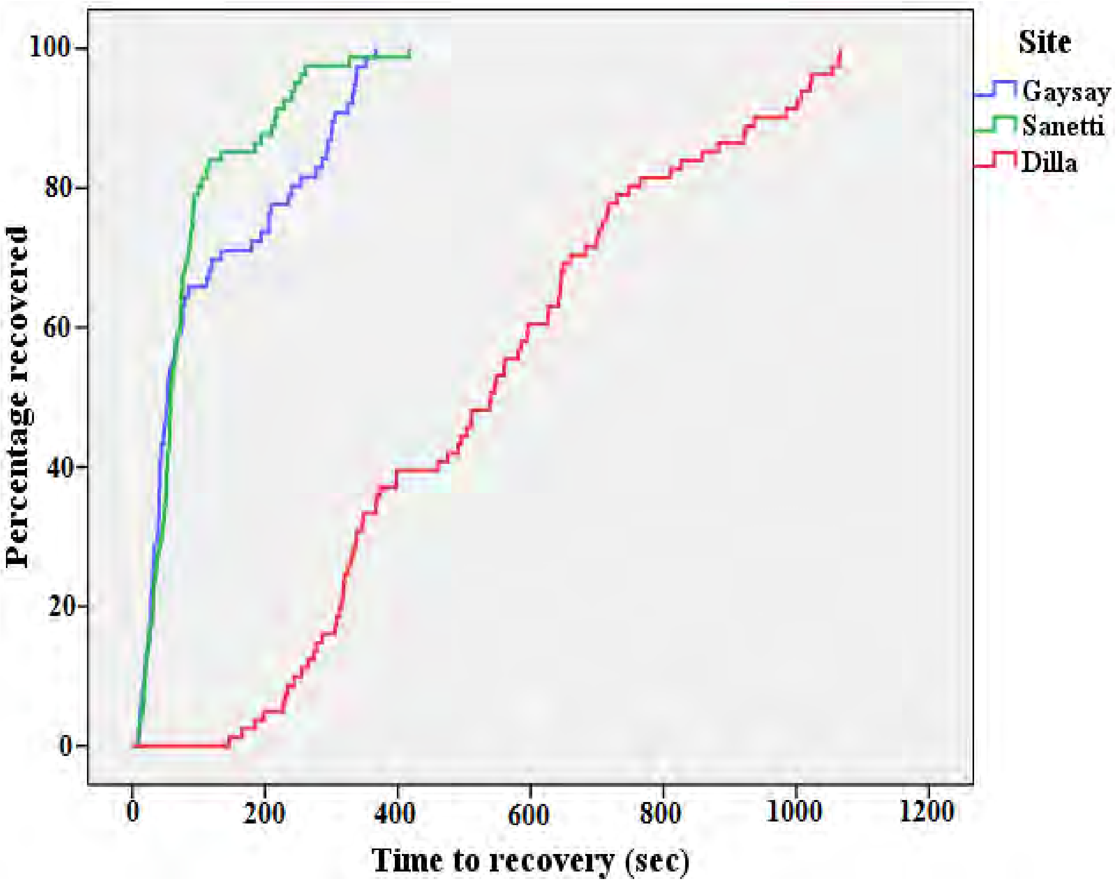

Figure 12: Graph of percentage recovery time of ant colonies from chill-coma for *C. negus* and *C. acvapimensis*

Chapter 5: Discussion, Conclusion and Recommendations

5.1 Discussion

5.1.1 Ant species richness and abundance

The subfamily Myrmicinae was the most diverse subfamily in the study area with nine identified species. This may be due to the fact that this subfamily is considered to be the most diverse subfamily worldwide (Hölldobler and Wilson, 1990; Ward *et. al.,* 2015). Consequently, Myrmicinae is the most diverse in Ethiopia which comprises 72 out of the total 141 species identified (www.antwiki.org/wiki/Ethiopia). This result is in agreement with many other studies (Mustafa *et. al.,* 2011; Patkar and Chavan, 2014). Patkar and Chavan also showed that among the subfamilies reported from India Myrmicinae were dominant with 7 species (35 %) out of the 20 ant species identified.

In a study of ant species richness along elevation gradient in Jammu-Kashmir Himalaya by Bharti and Sharma (2009), 66% of the specimens identified belonged to the subfamily Myrmicinae. This study also showed that subfamily Formicinae was the second abundant. This is not true in the present study, because this subfamily was the least diverse subfamily. Dorylinae and Ponerinae rather than Formicinae, were the second in their species richness in the present study. These differences might be due to factors like habitat, elevation, microhabitat (Savitha *et. al.,* 2008) or the sampling methods used in data collection. Bharti and Sharma used Winkler’s bags in addition to pitfall traps and hand collection, but we used pitfall traps, hand collection and transect/baits (Bharti and Sharma, 2009).

Tetramorium was the most diverse ant genus in the study area containing five species. This may be partly because this genus is widespread throughout all zoogeographical regions and can be considered as hyper-diverse, with currently more than 545 valid species (Bolton, 2013). In addition, it is predominantly found in Afrotropical regions, with 230 described species. This genus also occupies a wide range of habitats, microhabitats and lifestyles in Afrotropical region not seen in any other region (Hita Garcia *et. al*., 2009).

Chantarasawat and his colleagues found similar results in a study of ant species richness in Dry Dipterocarp and Mixed-Deciduous Forests at Sri Nan National Park, Northern Thailand. Their study results showed that *Tetramorium* was among the richest genera in the National Park (Chantarasawat *et. al.,* 2013). Another study of ant species richness in Sinharaja Forest Reserve in southwestern Sri Lanka, *Tetramorium* was found to be the most speciose genera together with *Pheidole* (Gunawardene *et. al.,* 2008).

C. negus was the most abundant species in the study area and occurred in more than half of the total sample collections. It was collected by all sampling methods. Moreover, this species was observed to nest in soil, under stones and within rock layers. In addition, it was found in all habitat types of BMNP except in the rainforest and it was the only species found in highest elevation (above 3250 m), Erica moorlands and the Sanetti plateau. Therefore, this species is the most dominant in terms of abundance and distribution. This may be a result of unique adaptations of this species to highlands, which needs further study on its biology and ecology.

Next to *C. negus, P. megacephala* was the second abundant species in the study area. *P. megacephala,* also commonly called the bigheaded ant, is believed to be native to southern Africa and has invaded various types of habitats worldwide (www.antweb.org). Its invasion displaced dominant ant species (Holway *et. al.,* 2002) and has resulted in loss of several functional groups of local ants (Vanderwoude *et. al.*, 2000). But this is not partly true in the present study area, in which *P. megacephala* was found to be dominated by species *C. negus,* in both grassland and woodland habitats where the two species were found together. Since there was no ant species record in the study area, it is not known whether other native ant species were affected by this invasive ant or not (Bertelsmeier *et. al.,* 2012).

5.1.2 Ant species richness, distribution and abundance with elevation and habitats

Highest species richness along elevation gradient was observed in the altitude ranges of 2000–2500 m followed by 3000–3500 m and the lowest in altitude range of 2500– 3000 m and above 3500 m. It seemed that, there was no uniform trend of species richness pattern with elevation. This might be due to habitat, microhabitat, soil type or other environmental factors (Savitha *et. al.,* 2008). Indeed, it was not possible to find ants in the southern slope of the Sanetti plateau from an altitude of about 2700 m to 3000 m, which is predominantly covered with *Erica arborea* shrub. All the three methods employed could not catch ants in this site. Ants were also absent in altitude range of about 3300 m to 3900 m. These areas are wetlands, with high altitude lakes and streams that are flooded during the wet seasons. Though, flooding reduces soil ant biodiversity (Majer and Delabie, 1994), it may not be the reason for their complete absence. Thus, finding the factors affecting ant distributions in these sites needs further study.

There are few studies of ant species richness along an elevational gradient in Africa. Among these, one is the study of diversity patterns of ants along an elevational Gradient on Monts Doudou in southwestern Gabon (Fisher, 2002b). The result of this study showed that species richness was relatively constant along the elevational gradient. But this study was done at elevation of 110, 375, and 640 m, in altitude ranges much less than the present study which could end up in a different result. Another study in Africa was on ant diversity patterns along an elevational gradient in the Reserve Speciale de Manongarivo, Madagascar (Fisher, 2002a). He sampled ants at elevation of 400, 780, 1240, 1580, and 1860 m and found that species richness was greatest at 780 m and lowest at 1860 m. It seemed that his study results are similar to the present finding for the reason that species richness was observed to be low in the altitude of 1500- 2000m. However, the altitudes sampled for the two studies were different. Indeed the highest altitude range considered by Fisher (2002a) was similar to the lowest altitude range considered in the present study. Thus, the altitude considered for the study of elevation gradient in the present study was much higher, which could have resulted in a different conclusion.

Most of other studies on ant species richness along an elevational gradient worldwide were from lower elevations as compared to the present study (Brühl *et. al.,* 1999; Araújo and Fernandes, 2003; Bharti and Sharma, 2009; Sabu, *et. al.,* 2008; Burwell and Nakamura, 2011; Longino and Colwell, 2011; Orabi *et. al.,* 2011; Smith *et. al.,* 2014; Nowrouzi *et. al.,* 2016). Most of the studies were carried out at elevation less than 2000 m. Indeed, the present study was done at altitude range twice higher. In most cases, the results of these studies illustrated that the number of ant species decreased with increase in elevation (Brühl *et. al.,* 1999; Araújo and Fernandes, 2003; Bharti and Sharma, 2009;

Burwell and Nakamura, 2011; Longino and Colwell, 2011). This decline in species richness with increasing elevation on mountains is the most widely observed pattern of species diversity (Brown and Gibson, 1983). Samson *et. al.* (1997) surveyed ant communities along an elevational gradient in the Philippines extending from lowland forest to high elevation and found that very few ants occur at high elevations in the tropics. On the other hand, many studies show that ant species richness is high at mid-elevations (Sabu, *et. al.,* 2008; Smith *et. al.,* 2014 Nowrouzi *et. al.,* 2016). But an increase in species richness with elevation was rarely observed (Sanders *et. al.,* 2003).

A study on patterns of ant diversity and abundance along elevational gradients in Venezuela showed that fewer individuals were present at the higher elevations and none at elevations above 3550 m (Janzen *et. al.*, 1976). In another study by Janzen (1973) in Costa Rica, ants were absent above 3380 m. Similarly, in tropical Africa, only three species were found above 2200 m and none at 3200 m (Weber, 1943cited in Samson *et. al.,* 1997). These results show that ants were not common at highest elevations, for example above 3500 m. In contrast, ants were found in the present study up to an elevation of nearly 4000 m. However, this represented by only a single species.

The presence of ants in an altitude of 4000 m was documented in the Himalayas. A study of ant species richness along an elevational gradient in the Himalayas by Bharti *et. al.*, 2013 showed that species richness was the highest at 1000 and 2000 m but decreased at an altitude of 3000 m and becomes the lowest at 4000 m. They illustrated that species richness was the highest at mid elevations and then decreased as altitude further increased to 4000 m. The result of the present study was partly similar to these results in the fact that species richness was the highest at elevations of 2000 m and lowest at 4000 m. But high species richness observed at 1000 m in their study was not seen at least at 1500 m in the present study. This might be due to the disturbance or other factors which could decrease ant species richness in the present study. During the study period it was observed that this area was within the park boundary where grazing and farming was practiced. Kwon *et. al.* (2014) proved that forest disturbance decreased ant species richness.

Species abundance along elevation gradient showed that *C. negus* was the most dominant species in all altitude ranges above 2500 m. It was the only species found in an elevation above 3250 m to nearly 4000 m. Sanders *et. al.* (2003) in their study of patterns of ant species richness along elevational gradients found that species in the subfamily Formicinae (e.g. *Formica* and *Lasius* spp.) were better able to tolerate colder temperatures at higher elevations than were species in other subfamilies like Myrmicinae. This was also true in the present study because the only species from subfamily Formicinae (*C. negus*) was dominating the high elevations. This makes it probably the only ant species able to adapt in the afro-alpine habitats of BMNP. Further study is needed to know how this species is adapted to the highlands.

Ant species richness in the rain forest was the highest which contained 12 species. This can be explained by the fact that tropical rainforest support higher levels of primary production and are the most species-diverse terrestrial ecosystems (Hölldobler and Wilson, 1990). Similar finding was observed in a study of distribution of ant species along an altitudinal transect in subtropical Queensland, Australia (Burwell and Nakamura, 2011). Their study results showed that majority of recorded species were from the rainforest. In addition, ants are most abundant in tropical rainforest canopies comprising up to 94% of arthropods in insecticidal fogging samples and 86% of the biomass of those samples (Tobin, 1997; Davidson *et. al.,* 2003). Moreover, a study of diversity of soil fauna in the canopy and forest floor of a Venezuelan cloud forest showed that ants were the most numerically dominant group (Paoletti *et. al.,* 1991).

5.1.3 Thermal tolerance of *C. negus* and *C. acvapimensis*

Study of heat tolerance among colonies of *C. negus* showed that the time for knock-down resistance did not significantly differ among colonies in the same site. The similarity in heat tolerance among ant colonies in the same site may be due to exposure to related environments which could result in similar heat capacities. In fact samples of ant colonies in the same site were taken from nearly homogenous habitat with similar altitude and little distances apart. Such similarity of heat tolerance among colonies from sites of homogenous habitats was observed in other studies (Boyles *et. al.,* 2009).

But time for knock- down resistance significantly differed among colonies from different sites. Ant colonies from Sanetti (nearly 3970 m a.s.l.) had a significantly higher heat tolerance than Gaysay (nearly 3040 m a.s.l.). That is, ants from a higher altitude had significantly higher heat tolerance than from a lower altitude. Because temperature decreases by an average of approximately 0.68 °C for each 100 m increase in elevation (Barry, 2008), the temperature at Sanetti is expected to be 6 °C lower than that of Gaysay. Thus, the ants at Gaysay, which were located in a habitat with a higher environmental temperature, should have had a higher thermal tolerance than the ants from Sanetti. Therefore, this result was different from what was hypothesized in the present study. However, this may be partly due to microclimatic factors which can affect the thermal tolerance of ants from the two habitats. In fact, ants from Gaysay grassland live in a habitat covered by dense grasses which provide shade and can help as a refugee from direct sunlight. Consequently, ants in this habitat may live in a micro habitat cooler than it is expected. On the other hand, the habitat in Sanetti does not have plant cover to give shade and hide them from direct sunlight. Therefore, ants from Sanetti may have higher temperature exposure than ants from Gaysay and thus have had elevated thermal adaptation. Boyles and his colleagues (2009) tested the variation in physiological response of red imported fire ants (*Solenopsis invicta*) to small-scale thermal heterogeneity. The result of their study indicated that colonies from an unshaded, warmer site had higher heat tolerance than colonies from a shaded, cooler site. Similarly, Meisel (2006) showed that thermal tolerance of an ant species commonly found in open, hot environments (*Atta cephalotes*) is higher than restricted to forest fragments (*Eciton burchellii*). Thus, microhabitat has significant effect on upper thermal tolerance of ants and results in a differed thermal adaptation (Baudier *et. al.,* 2015). Whether other factors play a role for higher heat tolerance of ants from Sanetti is not known. Moreover, this higher heat tolerance of Sanetti ants has genetic basis, results from plastic responses to environmental conditions, or both needs further study.

This result imply that *C. negus* from lower altitudes (Gaysay) which had low heat tolerance may be more affected by future climate warming than higher heat tolerant from higher altitudes (Sanetti). Indeed, the climate of Gaysay has already shown an increase in both maximum and minimum temperature within the last three decades. Moreover, the habitat is also under pressure by human activity such as livestock grazing (OARDB, 2007), which is changing the habitat. Consequently, this can seriously affect this habitat as well as the ants and other animals living in the shady grasses. One of the expected responses of ants to increased environmental temperature is shifting their distribution range to higher elevation (Chen *et. al.,* 2011). It was found that ant species, for example *Aphaenogaster rudis* shift their distribution upward in elevation in response to warming (Warren and Chick, 2013). The expected upward distribution shift to an increased warming seems less likely to be the response for *C. negus,* because ants from Gaysay may not benefit from shifting their range to higher altitudes as they may not endure the habitat where ants with higher heat tolerance are able to adapt.

Knock-down resistance tests showed that heat tolerance of colonies of *C. negus* from Sanetti had similar high temperature tolerance with *C. acvapimensis* from Dilla. But this is not true for *C. negus* from Gaysay, which had lower thermal tolerance. This demonstrates that *C. negus* from Sanetti developed a high temperature tolerance equivalent to *C. acvapimensis* from Dilla which is found in a warmer environment.

There was similar cold tolerance among colonies of *C. negus* of the same site and between sites. Thus, the higher heat tolerance of ant colonies of Sanetti came without a decrease in cold tolerance; as ants both from Sanetti and Gaysay had similar recovery time from chill-coma. A similar result was observed in a study by Angilletta *et. al.* (2007) that compared thermal tolerances of leaf-cutter ants (*Atta sexdens rubropilosa*) from inside and outside of the city of Sao Paulo, Brazil. Their study results showed that ants from within the city had higher heat tolerance than ants from outside the city. But the observed heat tolerance of ants within the city came at no apparent cost to cold tolerance, because ants from both habitats had similar chill-coma recovery times. Similarly, in the study of Boyles *et. al.* (2009), the higher heat tolerance of colonies from an unshaded warmer site came at no cost to cold tolerance, since ants from both unshaded and shaded habitats took similar time to recover from chill-coma.

Chill-coma recovery time of ant colonies of *C. acvapimensis* was significantly higher than *C. negus.* Colonies of *C. acvapimensis* needed nearly five times more time to recover from chill-coma compared with colonies of *C. negus* from Gaysay and six times more time than *C. negus* from Sanetti. Thus, colonies of *C. negus* had significantly higher cold tolerance compared with *C. acvapimensis.* This may be because *C. negus* is found in areas where the mean annual minimum temperature is 1.4°C and the mean annual temperature is 10.80 °C. In addition, night temperature is very low and sometimes it falls below freezing during the dry season (Refera and Bekele, 2002). Thus, an adaptation developed as a result of living in this low temperature environment may have helped *C. negus* to have higher cold tolerance. In the contrary, *C. acvapimensis* is located at hot environment where the mean annual temperature is 21 °C (Fantahun and Gashaw, 2014) and thus had poor cold tolerance.

5.2 Conclusions

The study showed that subfamily Myrmicinae was the most diverse subfamily followed by Dorylinae and Ponerinae, which are the second and the third diverse subfamily in the study area, respectively. The genus *Tetramorium* was the most diverse in the study area. *C. negus* is the most abundant species and occurred in more than half of the total sample collections. This species was the most dominant in terms of both abundance and distribution in all altitude ranges above 2500 m and in all habitat types except the rainforest. Following *C. negus, P. megacephala* (the bigheaded ant) is the second most abundant species. *P. megacephala* is an invasive species displacing native ant species in many parts of the world. But this did not happen to date in the present study in which native species *C. negus* dominated *P. megacephala*. However, it is not known whether other native ant species in our study area were affected by this invasion. The highest ant species richness was observed in the altitude range of 2000–2500 m followed by 3000–3500 m while species richness was the lowest in altitude range of 2500– 3000 m and above 3500 m. Therefore, there was no clear pattern of species richness with elevation. The highest species richness in the study area was recorded from the rainforest.

There was similar heat tolerance of colonies of *C. negus* from the same site but heat tolerance differed among colonies between sites. Ant colonies from Sanetti (elevation ≈3970 m) had higher heat tolerance than that of Gaysay (elevation ≈3040 m). *C. negus* from lower altitudes (Gaysay) which had low heat tolerance may be more affected by future climate warming than higher heat tolerant *C. negus* from higher altitudes (Sanetti). Heat tolerance of colonies of *C. negus* from Sanetti had similar high temperature tolerance as *C. acvapimensis* from Dilla University main campus. *C. negus* from Gaysay had a lower heat tolerance than ants from the other two sites. There was similar cold tolerance among colonies of *C. negus* of the same site and between sites. But *C. acvapimensis* had very low cold tolerance compared to *C. negus*.

5.3. Recommendations

Some of the major recommendations to be considered are:

- awareness creation on climate change and proper utilization of resources by the local community living alongside the BMNP;

- protection of the park to reduce pressure on biodiversity;
- additional Meteorological stations in the park, mainly at Sanetti plateau;
- well organized and intensive study on climate change and its effect on the biodiversity of the BMNP;
- more study on diversity of invertebrates and lower plants to produce reliable information on species distribution, abundance, and conservation status more study on thermal response of animals and plants in the changing climate of BMNP;
- studies on the biology and ecology of diverse species should be initiated and
- the effect of climate warming in relation to ants abundance should be addressed

6. References

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7. Appendixes

Appendix 1: Collected ant samples from BMNP

No.	Collection Code	Habitat	Method	Collection Date	Latitude	Longitude	Elevation (m)
1	Ayele-0001	grassland	search	14/12/13	7.12554	39.74235	3049
2	Ayele-0002	grassland	search	14/12/13	7.12317	39.74004	3045
3	Ayele-0003	grassland	search	14/12/13	7.12121	39.73599	3048
4	Ayele-0004	grassland	search	14/12/13	7.10975	39.75968	3036
5	Ayele-0005	grassland	search	14/12/13	7.11033	39.75823	3044
6	Ayele-0006	grassland	search	14/12/13	7.11027	39.75787	3042
7	Ayele-0007	grassland	search	14/12/13	7.10991	39.75741	3044
8	Ayele-0008	grassland	search	14/12/13	7.10954	39.75592	3054
9	Ayele-0009	grassland	search	14/12/13	7.10922	39.75363	3061
10	Ayele-0010	grassland	search	14/12/13	7.10919	39.7535	3058
11	Ayele-0011	grassland	bait	16/12/13	7.12195	39.73748	3051
12	Ayele-0012	grassland	bait	16/12/13	7.12224	39.73735	3051
13	Ayele-0013	grassland	bait	16/12/13	7.12233	39.73732	3051
14	Ayele-0014	grassland	bait	16/12/13	7.1225	39.73726	3052
15	Ayele-0015	grassland	bait	16/12/13	7.12266	39.73718	3052
16	Ayele-0016	grassland	bait	16/12/13	7.12426	39.74138	3046
17	Ayele-0017	grassland	bait	16/12/13	7.12445	39.74155	3050
18	Ayele-0018	grassland	bait	16/12/13	7.12462	39.74163	3050
19	Ayele-0019	grassland	bait	16/12/13	7.12485	39.74177	3052
20	Ayele-0020	rainforest	search	15/12/13	6.6988	39.72931	2191
21	Ayele-0021	rainforest	search	15/12/13	6.69821	39.72952	2176
22	Ayele-0022	rainforest	search	15/12/13	6.69922	39.72624	2204
23	Ayele-0023	rainforest	search	15/12/13	6.7027	39.72625	2275
24	Ayele-0024	woodland	search	16/12/13	7.09468	39.79142	3205
25	Ayele-0025	woodland	search	16/12/13	7.09468	39.79148	3210
26	Ayele-0026	woodland	search	16/12/13	7.09439	39.79171	3213
27	Ayele-0027	woodland	search	16/12/13	7.09394	39.79216	3208
28	Ayele-0028	woodland	search	16/12/13	7.09304	39.79265	3207
29	Ayele-0029	woodland	search	16/12/13	7.09261	39.79255	3201
30	Ayele-0030	woodland	search	16/12/13	7.09313	39.79165	3182
31	Ayele-0031	woodland	search	16/12/13	7.09403	39.79096	3191
32	Ayele-0032	woodland	search	16/12/13	7.09398	39.79127	3199
33	Ayele-0033	rainforest	search	25/01/14	6.4265	39.43103	2400
34	Ayele-0034	rainforest	search	25/01/14	6.42673	39.43184	2373

35	Ayele-0035	rainforest	search	25/01/14	6.42711	39.43185	2392
36	Ayele-0036	rainforest	search	25/01/14	6.42792	39.43179	2377
37	Ayele-0037	rainforest	search	25/01/14	6.42864	39.43102	2380
38	Ayele-0038	rainforest	search	25/01/14	6.42744	39.43124	2380
39	Ayele-0039	rainforest	search	25/01/14	6.29607	39.44863	1557
40	Ayele-0040	rainforest	search	25/01/14	6.29585	39.44862	1542
41	Ayele-0041	rainforest	search	25/01/14	6.29586	39.44919	1536
42	Ayele-0042	rainforest	search	25/01/14	6.29593	39.44936	1542
43	Ayele-0043	rainforest	search	25/01/14	6.2964	39.4498	1544
44	Ayele-0044	rainforest	search	25/01/14	6.2967	39.44978	1549
45	Ayele-0045	afro-alpine	search	26/01/14	6.85978	39.70432	3950
46	Ayele-0046	afro-alpine	search	26/01/14	6.85787	39.70828	3981
47	Ayele-0047	afro-alpine	search	26/01/14	6.86286	39.7144	3975
48	Ayele-0048	grassland	search	2/3/2014	7.10806	39.74818	3056
49	Ayele-0049	grassland	search	2/3/2014	7.10819	39.74828	3056
50	Ayele-0050	grassland	search	2/3/2014	7.10848	39.74732	3050
51	Ayele-0051	grassland	search	2/3/2014	7.10925	39.74701	3061
52	Ayele-0052	grassland	search	2/3/2014	7.108	39.74589	3070
53	Ayele-0053	grassland	search	2/3/2014	7.10784	39.74727	3068
54	Ayele-0054	grassland	search	3/3/2014	7.10867	39.74421	3035
55	Ayele-0055	grassland	search	3/3/2014	7.1118	39.73932	3044
56	Ayele-0056	grassland	search	3/3/2014	7.11225	39.7437	3034
57	Ayele-0057	grassland	search	3/3/2014	7.11299	39.7449	3051
58	Ayele-0058	grassland	search	3/3/2014	7.11389	39.74233	3041
59	Ayele-0059	grassland	search	3/3/2014	7.11968	39.74651	3051
60	Ayele-0060	grassland	search	3/3/2014	7.12305	39.74334	3054
61	Ayele-0061	grassland	search	3/3/2014	7.12435	39.7444	3057
62	Ayele-0062	grassland	search	4/3/2014	7.11669	39.72748	3064
63	Ayele-0063	grassland	search	4/3/2014	7.12576	39.72816	3061
64	Ayele-0064	grassland	search	4/3/2014	7.12539	39.71474	3090
65	Ayele-0065	grassland	search	4/3/2014	7.11822	39.72392	3050
66	Ayele-0066	grassland	search	4/3/2014	7.12821	39.71609	3071
67	Ayele-0067	grassland	search	4/3/2014	7.12259	39.75437	3044
68	Ayele-0068	grassland	search	4/3/2014	7.12688	39.72564	3058
69	Ayele-0069	grassland	search	4/3/2014	7.11686	39.73555	3046
70	Ayele-0070	grassland	search	4/3/2014	7.12685	39.72522	3050
71	Ayele-0071	grassland	search	4/3/2014	7.11701	39.72519	3044
72	Ayele-0072	woodland	search	5/3/2014	7.09488	39.79102	3200
73	Ayele-0073	woodland	search	5/3/2014	7.09624	39.79489	3200
74	Ayele-0074	woodland	search	5/3/2014	7.09638	39.79501	3209
75	Ayele-0075	woodland	search	5/3/2014	7.09629	39.7936	3217
76	Ayele-0076	woodland	search	5/3/2014	7.09732	39.79522	3217
77	Ayele-0077	woodland	search	5/3/2014	7.09791	39.79442	3217

78	Ayele-0078	woodland	search	5/3/2014	7.09896	39.79627	3217
79	Ayele-0079	woodland	search	5/3/2014	7.09977	39.7961	3217
80	Ayele-0080	woodland	search	5/3/2014	7.0998	39.79609	3217
81	Ayele-0081	woodland	search	6/3/2014	7.09509	39.7947	3217
82	Ayele-0082	woodland	search	6/3/2014	7.09505	39.79479	3217
83	Ayele-0083	woodland	search	6/3/2014	7.09515	39.7948	3217
84	Ayele-0084	woodland	search	6/3/2014	7.09514	39.79476	3217
85	Ayele-0085	woodland	search	6/3/2014	7.09511	39.79471	3221
86	Ayele-0086	Erica moorland	search	6/3/2014	6.54729	39.5526	3217
87	Ayele-0087	Erica moorland	search	6/3/2014	6.54742	39.55227	3217
88	Ayele-0088	Erica moorland	search	6/3/2014	6.54865	39.54937	3217
89	Ayele-0089	Erica moorland	search	6/3/2014	6.54558	39.54939	3217
90	Ayele-0090	woodland	search	6/3/2014	7.09547	39.79146	3217
91	Ayele-0091	woodland	search	6/3/2014	7.0969	39.79214	3230
92	Ayele-0092	woodland	search	6/3/2014	7.0972	39.79364	3219
93	Ayele-0093	woodland	search	6/3/2014	7.09675	39.79752	3175
94	Ayele-0094	grassland	search	7/3/2014	7.12367	39.75347	3070
95	Ayele-0095	grassland	search	7/3/2014	7.12152	39.75927	3047
96	Ayele-0096	grassland	search	7/3/2014	7.11981	39.76038	3041
97	Ayele-0097	grassland	search	7/3/2014	7.10884	39.75805	3052
98	Ayele-0098	woodland	search	6/3/2014	7.16683	39.96136	2425
99	Ayele-0099	woodland	search	6/3/2014	7.16592	39.96086	2420
100	Ayele-0100	woodland	search	6/3/2014	7.16579	39.96086	2413
101	Ayele-0101	woodland	search	6/3/2014	7.16568	39.52689	2746
102	Ayele-0139	grassland	bait	27/03/15	7.07102	39.44527	3053
103	Ayele-0140	grassland	bait	27/03/15	7.07103	39.44542	3053
104	Ayele-0141	grassland	bait	27/03/15	7.07116	39.44553	3053
105	Ayele-0142	grassland	bait	27/03/15	7.07117	39.44202	3045
106	Ayele-0143	grassland	bait	27/03/15	7.06195	39.44523	3036
107	Ayele-0144	grassland	bait	27/03/15	7.066	39.44338	3048
108	Ayele-0145	grassland	bait	27/03/15	7.06512	39.44478	3046
109	Ayele-0146	grassland	bait	27/03/15	7.06329	39.44393	3060
110	Ayele-0147	grassland	bait	27/03/15	7.06316	39.44465	3057
111	Ayele-0148	grassland	search	28/03/15	7.06369	39.44224	3073
112	Ayele-0149	grassland	search	28/03/15	7.06368	39.44248	3070
113	Ayele-0150	grassland	pitfall trap	28/03/15	7.06363	39.44259	3070
114	Ayele-0151	grassland	pitfall trap	28/03/15	7.06358	39.44282	3068
115	Ayele-0152	grassland	pitfall trap	28/03/15	7.06362	39.44296	3066
116	Ayele-0153	grassland	search	28/03/15	7.06362	39.44337	3062
117	Ayele-0154	grassland	search	28/03/15	7.0635	39.44358	3060
118	Ayele-0155	grassland	pitfall trap	28/03/15	7.07113	39.4423	3043
119	Ayele-0156	grassland	pitfall trap	28/03/15	7.07067	39.44314	3047
120	Ayele-0157	grassland	search	28/03/15	7.07095	39.44398	3053

121	Ayele-0158	woodland	search	29/03/15	7.06233	39.45175	3129
122	Ayele-0159	woodland	search	29/03/15	7.06114	39.44091	3230
123	Ayele-0160	woodland	search	29/03/15	7.06193	39.4412	3250
124	Ayele-0161	woodland	pitfall trap	29/03/15	7.06255	39.44338	3124
125	Ayele-0162	woodland	search	29/03/15	7.06218	39.44391	3135
126	Ayele-0163	woodland	search	29/03/15	7.06185	39.44546	3134
127	Ayele-0164	woodland	pitfall trap	29/03/15	7.06199	39.45094	3148
128	Ayele-0165	woodland	search	29/03/15	7.0624	39.45162	3129
129	Ayele-0166	afro-alpine	search	30/03/15	6.47592	39.48702	3921
130	Ayele-0167	afro-alpine	search	31/03/15	6.47589	39.48671	3911
131	Ayele-0168	Erica moorland	search	31/03/15	6.54865	39.54937	3217
132	Ayele-0169	Erica moorland	search	31/03/15	6.54806	39.54976	3289
133	Ayele-0170	Erica moorland	search	31/03/15	6.54823	39.54955	3311
134	Ayele-0171	Erica moorland	search	31/03/15	6.54844	39.54945	3255
135	Ayele-0172	grassland	search	7/10/2015	7.09502	39.45525	3040
136	Ayele-0173	woodland	search	7/10/2015	7.06244	39.45101	3115
137	Ayele-0174	woodland	search	7/10/2015	7.06246	39.45158	3127
138	Ayele-0175	afro-alpine	search	7/10/2015	6.47591	39.48709	3922
139	Ayele-0176	afro-alpine	search	7/10/2015	6.47579	39.48764	3918
140	Ayele-0177	afro-alpine	search	7/10/2015	6.47521	39.48755	3902
141	Ayele-0178	afro-alpine	search	7/10/2015	6.47558	39.4877	3908
142	Ayele-0179	afro-alpine	search	7/10/2015	6.47579	39.49138	3913
143	Ayele-0180	afro-alpine	search	7/10/2015	6.4755	39.48804	3907
144	Ayele-0181	afro-alpine	search	7/10/2015	6.47568	39.4881	3917
145	Ayele-0182	afro-alpine	search	7/10/2015	6.47556	39.48849	3914
146	Ayele-0183	afro-alpine	search	7/10/2015	6.47552	39.48887	3913
147	Ayele-0184	afro-alpine	search	7/10/2015	6.47611	39.48608	3899
148	Ayele-0185	Erica moorland	search	7/10/2015	6.54851	39.54907	3276
149	Ayele-0186	Erica moorland	search	7/10/2015	6.54877	39.54911	3321
150	Ayele-0187	Erica moorland	search	7/10/2015	6.5484	39.54932	3318
151	Ayele-0188	Erica moorland	search	7/10/2015	6.54883	39.5491	3305
152	Ayele-0189	Erica moorland	search	7/10/2015	6.54889	39.54909	3309
153	Ayele-0190	grassland	search	8/10/2015	7.07125	39.44569	3054
154	Ayele-0191	grassland	search	8/10/2015	7.07113	39.44575	3053
155	Ayele-0192	grassland	search	8/10/2015	7.07131	39.44036	3053
156	Ayele-0193	grassland	search	8/10/2015	7.07127	39.44397	3054
157	Ayele-0194	grassland	search	8/10/2015	7.07019	39.44367	3047
158	Ayele-0195	grassland	search	8/10/2015	7.06316	39.44493	3055
159	Ayele-0196	grassland	search	8/10/2015	7.07151	39.4528	3043
160	Ayele-0197	grassland	search	8/10/2015	7.07127	39.45337	3036
161	Ayele-0198	grassland	search	8/10/2015	7.07097	39.45363	3032
162	Ayele-0199	grassland	search	8/10/2015	7.07069	39.454	3030

Appendix 2: List of dry mount specimens prepared in the University of Utah, USA

No.	Collection code	Specimen barcode	Species	Caste
1	Ayele-0001	CASENT0634320	*Camponotus* sp. 01	adult worker
2	Ayele-0001	CASENT0634321	*Camponotus* sp. 01	adult worker
3	Ayele-0001	CASENT0634322	*Camponotus* sp. 01	adult worker
4	Ayele-0001	CASENT0634323	*Camponotus* sp. 01	adult worker
5	Ayele-0002	CASENT0634324	*Pheidole* sp. 01	major worker
6	Ayele-0002	CASENT0634325	*Pheidole* sp. 01	major worker
7	Ayele-0003	CASENT0634326	*Dorylus affinis*	adult worker
8	Ayele-0003	CASENT0634327	*Dorylus affinis*	adult worker
9	Ayele-0003	CASENT0634328	*Dorylus fimbriatus*	adult worker
10	Ayele-0004	CASENT0634329	*Pheidole* sp. 01	major worker
11	Ayele-0004	CASENT0634330	*Pheidole* sp. 01	major worker
12	Ayele-0004	CASENT0634331	*Pheidole* sp. 01	major worker
13	Ayele-0005	CASENT0634332	*Pheidole* sp. 01	major worker
14	Ayele-0005	CASENT0634333	*Pheidole* sp. 01	major worker
15	Ayele-0006	CASENT0634334	*Pheidole* sp. 01	major worker
16	Ayele-0006	CASENT0634335	*Pheidole* sp. 01	major worker
17	Ayele-0007	CASENT0634336	*Dorylus affinis*	adult worker
18	Ayele-0007	CASENT0634337	*Dorylus affinis*	adult worker
19	Ayele-0008	CASENT0634338	*Dorylus affinis*	adult worker
20	Ayele-0008	CASENT0634339	*Dorylus affinis*	adult worker
21	Ayele-0009	CASENT0634340	*Dorylus affinis*	adult worker
22	Ayele-0009	CASENT0634341	*Dorylus affinis*	adult worker
23	Ayele-0010	CASENT0634342	*Pheidole* sp. 01	major worker
24	Ayele-0010	CASENT0634343	*Pheidole* sp. 01	major worker
25	Ayele-0010	CASENT0634344	*Pheidole* sp. 01	major worker
26	Ayele-0011	CASENT0634345	*Tetramorium edouardi*	adult worker
27	Ayele-0012	CASENT0634346	*Pheidole* sp. 01	minor worker
28	Ayele-0013	CASENT0634347	*Pheidole* sp. 01	major worker
29	Ayele-0014	CASENT0634348	*Tetramorium* sp. 01	adult worker
30	Ayele-0015	CASENT0634349	*Camponotus* sp. 01	adult worker
31	Ayele-0015	CASENT0634350	*Camponotus* sp. 01	adult worker
32	Ayele-0016	CASENT0634351	*Tetramorium edouardi*	adult worker
33	Ayele-0017	CASENT0634352	*Pheidole* sp. 01	major worker
34	Ayele-0018	CASENT0634353	*Pheidole* sp. 01	major worker
35	Ayele-0019	CASENT0634354	*Tetramorium edouardi*	adult worker
36	Ayele-0020	CASENT0634355	*Dorylus nigricans*	adult worker
37	Ayele-0020	CASENT0634356	*Dorylus nigricans*	adult worker
38	Ayele-0021	CASENT0634357	*Dorylus affinis*	adult worker

39	Ayele-0021	CASENT0634358	*Dorylus affinis*	adult worker
40	Ayele-0022	CASENT0634359	*Dorylus affinis*	adult worker
41	Ayele-0022	CASENT0634360	*Dorylus affinis*	adult worker
42	Ayele-0023	CASENT0634361	*Hypoponera* sp. 01	adult worker
43	Ayele-0023	CASENT0634362	*Hypoponera* sp. 01	adult worker
44	Ayele-0024	CASENT0634363	*Camponotus* sp. 01	adult worker
45	Ayele-0024	CASENT0634364	*Camponotus* sp. 01	adult worker
46	Ayele-0025	CASENT0634365	*Camponotus* sp. 01	adult worker
47	Ayele-0026	CASENT0634366	*Camponotus* sp. 01	adult worker
48	Ayele-0026	CASENT0634367	*Camponotus* sp. 01	adult worker
49	Ayele-0027	CASENT0634368	*Camponotus* sp. 01	adult worker
50	Ayele-0028	CASENT0634369	*Camponotus* sp. 01	adult worker
51	Ayele-0028	CASENT0634370	*Camponotus* sp. 01	adult worker
52	Ayele-0029	CASENT0634371	*Camponotus* sp. 01	adult worker
53	Ayele-0029	CASENT0634372	*Camponotus* sp. 01	adult worker
54	Ayele-0029	CASENT0634373	*Camponotus* sp. 01	adult worker
55	Ayele-0030	CASENT0634374	*Camponotus* sp. 01	adult worker
56	Ayele-0030	CASENT0634375	*Camponotus* sp. 01	adult worker
57	Ayele-0031	CASENT0634376	*Camponotus* sp. 01	adult worker
58	Ayele-0031	CASENT0634377	*Camponotus* sp. 01	adult worker
59	Ayele-0032	CASENT0634378	*Camponotus* sp. 01	adult worker
60	Ayele-0032	CASENT0634379	*Camponotus* sp. 01	adult worker
61	Ayele-0033	CASENT0634380	*Hypoponera* sp. 01	adult worker
62	Ayele-0033	CASENT0634381	*Hypoponera* sp. 01	adult worker
63	Ayele-0034	CASENT0634382	*Tetramorium edouardi*	adult worker
64	Ayele-0034	CASENT0634383	*Tetramorium* cf. *eminii*	adult worker
65	Ayele-0035	CASENT0634385	*Hypoponera* sp. 02	adult worker
66	Ayele-0035	CASENT0634384	*Tetramorium* sp. 01	adult worker
67	Ayele-0036	CASENT0634386	*Dorylus fimbriatus*	adult worker
68	Ayele-0036	CASENT0634387	*Dorylus fimbriatus*	adult worker
69	Ayele-0037	CASENT0634389	*Hypoponera* sp. 01	adult worker
70	Ayele-0037	CASENT0634388	*Monomorium* sp. 01	adult worker
71	Ayele-0038	CASENT0634390	*Dorylus nigricans*	adult worker
72	Ayele-0038	CASENT0634391	*Dorylus nigricans*	adult worker
73	Ayele-0039	CASENT0634392	*Pheidole* sp. 02	minor worker
74	Ayele-0039	CASENT0634393	*Pheidole* sp. 02	minor worker
75	Ayele-0040	CASENT0634394	*Pheidole* sp. 02	minor worker
76	Ayele-0040	CASENT0634395	*Pheidole* sp. 02	minor worker
77	Ayele-0041	CASENT0634396	*Bothroponera soror*	adult worker
78	Ayele-0042	CASENT0634397	*Pheidole* sp. 02	minor worker
79	Ayele-0043	CASENT0634398	*Pheidole* sp. 02	major worker

80	Ayele-0043	CASENT0634399	*Pheidole* sp. 02	major worker
81	Ayele-0044	CASENT0634400	*Bothroponera soror*	adult worker
82	Ayele-0044	CASENT0634401	*Bothroponera soror*	adult worker
83	Ayele-0044	CASENT0634402	*Bothroponera soror*	adult worker
84	Ayele-0044	CASENT0634403	*Bothroponera soror*	adult worker
85	Ayele-0045	CASENT0634404	*Camponotus* sp. 01	adult worker
86	Ayele-0045	CASENT0634405	*Camponotus* sp. 01	adult worker
87	Ayele-0046	CASENT0634406	*Camponotus* sp. 01	adult worker
88	Ayele-0046	CASENT0634407	*Camponotus* sp. 01	adult worker
89	Ayele-0047	CASENT0634408	*Camponotus* sp. 01	adult worker
90	Ayele-0047	CASENT0634409	*Camponotus* sp. 01	adult worker
91	Ayele-0048	CASENT0634410	*Camponotus* sp. 01	male
92	Ayele-0048	CASENT0634411	*Camponotus* sp. 01	male
93	Ayele-0048	CASENT0634412	*Camponotus* sp. 01	adult worker
94	Ayele-0049	CASENT0634413	*Camponotus* sp. 01	adult worker
95	Ayele-0049	CASENT0634414	*Camponotus* sp. 01	adult worker
96	Ayele-0050	CASENT0634416	*Dorylus affinis*	adult worker
97	Ayele-0050	CASENT0634415	*Tetramorium* sp. 01	adult worker
98	Ayele-0051	CASENT0634417	*Pheidole* sp. 01	male
99	Ayele-0051	CASENT0634418	*Pheidole* sp. 01	adult worker
100	Ayele-0052	CASENT0634419	*Camponotus* sp. 01	alate queen
101	Ayele-0052	CASENT0634420	*Camponotus* sp. 01	male
102	Ayele-0052	CASENT0634421	*Camponotus* sp. 01	adult worker
103	Ayele-0053	CASENT0634422	*Pheidole* sp. 01	major worker
104	Ayele-0053	CASENT0634423	*Pheidole* sp. 01	major worker
105	Ayele-0054	CASENT0634424	*Pheidole* sp. 01	major worker
106	Ayele-0054	CASENT0634425	*Pheidole* sp. 01	major worker
107	Ayele-0055	CASENT0634426	*Pheidole* sp. 01	minor worker
108	Ayele-0056	CASENT0634427	*Pheidole* sp. 01	major worker
109	Ayele-0056	CASENT0634428	*Pheidole* sp. 01	major worker
110	Ayele-0057	CASENT0634429	*Pheidole* sp. 01	male
111	Ayele-0057	CASENT0634430	*Pheidole* sp. 01	major worker
112	Ayele-0058	CASENT0634431	*Camponotus* sp. 01	adult worker
113	Ayele-0058	CASENT0634432	*Camponotus* sp. 01	adult worker
114	Ayele-0059	CASENT0634433	*Pheidole* sp. 01	major worker
115	Ayele-0059	CASENT0634434	*Pheidole* sp. 01	major worker
116	Ayele-0060	CASENT0634435	*Camponotus* sp. 01	adult worker
117	Ayele-0061	CASENT0634436	*Pheidole* sp. 01	male
118	Ayele-0061	CASENT0634437	*Pheidole* sp. 01	major worker
119	Ayele-0062	CASENT0634438	*Dorylus affinis*	adult worker
120	Ayele-0062	CASENT0634439	*Dorylus affinis*	adult worker

121	Ayele-0063	CASENT0634440	*Camponotus* sp. 01	adult worker
122	Ayele-0064	CASENT0634441	*Dorylus affinis*	adult worker
123	Ayele-0065	CASENT0634442	*Pheidole* sp. 01	alate queen
124	Ayele-0065	CASENT0634443	*Pheidole* sp. 01	major worker
125	Ayele-0066	CASENT0634444	*Camponotus* sp. 01	male
126	Ayele-0066	CASENT0634445	*Camponotus* sp. 01	adult worker
127	Ayele-0067	CASENT0634446	*Camponotus* sp. 01	adult worker
128	Ayele-0067	CASENT0634447	*Camponotus* sp. 01	adult worker
129	Ayele-0068	CASENT0634448	*Dorylus affinis*	adult worker
130	Ayele-0068	CASENT0634449	*Dorylus affinis*	adult worker
131	Ayele-0069	CASENT0634450	*Camponotus* sp. 01	alate queen
132	Ayele-0069	CASENT0634451	*Camponotus* sp. 01	male
133	Ayele-0069	CASENT0634452	*Camponotus* sp. 01	adult worker
134	Ayele-0070	CASENT0634453	*Pheidole* sp. 01	male
135	Ayele-0070	CASENT0634454	*Pheidole* sp. 01	major worker
136	Ayele-0071	CASENT0634455	*Pheidole* sp. 01	major worker
137	Ayele-0072	CASENT0634456	*Camponotus* sp. 01	adult worker
138	Ayele-0072	CASENT0634457	*Camponotus* sp. 01	adult worker
139	Ayele-0073	CASENT0634458	*Camponotus* sp. 01	male
140	Ayele-0073	CASENT0634459	*Camponotus* sp. 01	male
141	Ayele-0073	CASENT0634460	*Camponotus* sp. 01	alate queen
142	Ayele-0073	CASENT0634461	*Camponotus* sp. 01	adult worker
143	Ayele-0074	CASENT0634462	*Tetramorium edouardi*	adult worker
144	Ayele-0075	CASENT0634463	*Dorylus affinis*	adult worker
145	Ayele-0075	CASENT0634464	*Dorylus affinis*	adult worker
146	Ayele-0076	CASENT0634465	*Camponotus* sp. 01	adult worker
147	Ayele-0077	CASENT0634466	*Camponotus* sp. 01	adult worker
148	Ayele-0077	CASENT0634467	*Camponotus* sp. 01	adult worker
149	Ayele-0078	CASENT0634469	*Tetramorium edouardi*	adult worker
150	Ayele-0078	CASENT0634468	*Tetramorium* sp. 01	adult worker
151	Ayele-0079	CASENT0634470	*Camponotus* sp. 01	adult worker
152	Ayele-0079	CASENT0634471	*Camponotus* sp. 01	adult worker
153	Ayele-0079	CASENT0634472	*Camponotus* sp. 01	adult worker
154	Ayele-0080	CASENT0634473	*Camponotus* sp. 01	alate queen
155	Ayele-0080	CASENT0634474	*Camponotus* sp. 01	adult worker
156	Ayele-0081	CASENT0634475	*Camponotus* sp. 01	adult worker
157	Ayele-0081	CASENT0634476	*Camponotus* sp. 01	adult worker
158	Ayele-0082	CASENT0634477	*Camponotus* sp. 01	adult worker
159	Ayele-0082	CASENT0634478	*Camponotus* sp. 01	adult worker
160	Ayele-0082	CASENT0634479	*Camponotus* sp. 01	adult worker
161	Ayele-0083	CASENT0634480	*Camponotus* sp. 01	adult worker

162	Ayele-0083	CASENT0634481	*Camponotus* sp. 01	adult worker
163	Ayele-0084	CASENT0634482	*Camponotus* sp. 01	male
164	Ayele-0084	CASENT0634483	*Camponotus* sp. 01	adult worker
165	Ayele-0084	CASENT0634484	*Camponotus* sp. 01	adult worker
166	Ayele-0085	CASENT0634485	*Camponotus* sp. 01	adult worker
167	Ayele-0085	CASENT0634486	*Camponotus* sp. 01	adult worker
168	Ayele-0086	CASENT0634487	*Camponotus* sp. 01	adult worker
169	Ayele-0086	CASENT0634488	*Camponotus* sp. 01	adult worker
170	Ayele-0087	CASENT0634489	*Camponotus* sp. 01	adult worker
171	Ayele-0087	CASENT0634490	*Camponotus* sp. 01	adult worker
172	Ayele-0088	CASENT0634491	*Camponotus* sp. 01	adult worker
173	Ayele-0088	CASENT0634492	*Camponotus* sp. 01	adult worker
174	Ayele-0089	CASENT0634493	*Camponotus* sp. 01	adult worker
175	Ayele-0089	CASENT0634494	*Camponotus* sp. 01	adult worker
176	Ayele-0090	CASENT0634495	*Camponotus* sp. 01	adult worker
177	Ayele-0090	CASENT0634496	*Camponotus* sp. 01	adult worker
178	Ayele-0090	CASENT0634497	*Camponotus* sp. 01	adult worker
179	Ayele-0091	CASENT0634498	*Camponotus* sp. 01	alate queen
180	Ayele-0091	CASENT0634499	*Camponotus* sp. 01	adult worker
181	Ayele-0091	CASENT0634500	*Camponotus* sp. 01	adult worker
182	Ayele-0092	CASENT0634501	*Camponotus* sp. 01	adult worker
183	Ayele-0092	CASENT0634502	*Camponotus* sp. 01	male
184	Ayele-0092	CASENT0634503	*Camponotus* sp. 01	adult worker
185	Ayele-0093	CASENT0634504	*Camponotus* sp. 01	male
186	Ayele-0093	CASENT0634505	*Camponotus* sp. 01	adult worker
187	Ayele-0093	CASENT0634506	*Camponotus* sp. 01	adult worker
188	Ayele-0094	CASENT0634507	*Camponotus* sp. 01	adult worker
189	Ayele-0094	CASENT0634508	*Camponotus* sp. 01	adult worker
190	Ayele-0095	CASENT0634509	*Camponotus* sp. 01	adult worker
191	Ayele-0096	CASENT0634510	*Camponotus* sp. 01	adult worker
192	Ayele-0096	CASENT0634511	*Camponotus* sp. 01	adult worker
193	Ayele-0097	CASENT0634512	*Pheidole* sp. 01	major worker
194	Ayele-0098	CASENT0634513	*Pheidole* sp. 01	alate queen
195	Ayele-0098	CASENT0634514	*Pheidole* sp. 01	major worker
196	Ayele-0099	CASENT0634515	*Messor galla*	male
197	Ayele-0099	CASENT0634516	*Messor galla*	alate queen
198	Ayele-0099	CASENT0634517	*Messor galla*	adult worker
199	Ayele-0099	CASENT0634518	*Messor galla*	adult worker
200	Ayele-0099	CASENT0634519	*Messor galla*	adult worker
201	Ayele-0099	CASENT0634520	*Messor galla*	adult worker
202	Ayele-0100	CASENT0634521	*Pheidole* sp. 01	major worker

203	Ayele-0100	CASENT0634522	*Pheidole* sp. 01	alate queen
204	Ayele-0101	CASENT0634523	*Camponotus* sp. 01	adult worker
205	Ayele-0101	CASENT0 634524	*Camponotus* sp. 01	adult worker

Appendix 3: Knock-down resistance (in minutes) of ant colonies

Petri dish No.	**Number of ants per Petri dish**	**Gaysay**			**Sanetti**			**Dilla**		
		Col. 1	**Col. 2**	**Col. 3**	**Col. 1**	**Col. 2**	**Col. 3**	**Col. 1**	**Col. 2**	**Col. 3**
1	1st	93	93	92	102	131	123	141	131	156
	2nd	93	94	94	105	134	124	148	131	164
	3rd	94	95	94	108	135	126	148	138	164
	4th	94	95	94	110	152	129	148	140	166
	5th	94	96	96	118	153	130	149	151	169
	6th	94	96	96	124	153	131	149	152	169
	7th	94	97	96	133	154	134	149	155	170
	8th	94	99	98	137	155	153	150	156	170
	9th	95	100	117	139	155	153	150	156	172
	10th	95	100	118	140	155	153	150	157	173
	11th	96	-	125	152	156	153	150	157	173
	12th	98	-	127	153	156	154	151	158	-
2	1st	91	91	94	123	100	127	110	111	110
	2nd	91	93	94	125	105	146	129	114	111
	3rd	92	93	96	151	124	160	130	115	114
	4th	94	93	98	152	132	161	133	134	130
	5th	95	94	98	152	132	161	143	146	134

6th	98	94	98	153	132	161	145	146	143
7th	99	94	100	153	137	163	146	146	146
8th	99	94	113	155	162	163	147	152	146
9th	101	94	123	155	163	163	148	152	152
10th	103	96	123	156	174	164	148	152	148
11th	121	96	124	157	191	164	153	166	152
12th	-	99	125	157	-	-	-	-	-

Appendix 4: Chill-coma recovery time (seconds) of ant colonies starting from the first ant

	Gaysay			**Sanetti**			**Dilla**		
No. of ants used	**Col. 1**	**Col. 2**	**Col. 3**	**Col. 1**	**Col. 2**	**Col. 3**	**Col. 1**	**Col. 2**	**Col. 3**
1st	20	11	10	18	8	24	146	165	286
2nd	28	11	24	19	10	26	198	185	315
3rd	38	12	29	20	11	31	227	265	338
4th	39	15	31	21	15	32	230	278	346
5th	40	15	31	22	16	45	234	308	348
6th	44	17	32	25	18	48	244	312	367
7th	48	19	33	32	31	51	255	318	398
8th	51	20	40	35	32	53	273	319	475
9th	53	26	41	37	36	53	305	320	491
10th	53	27	41	39	43	55	373	325	495
11th	54	28	44	49	46	56	398	330	504
12th	56	28	50	50	51	56	511	333	511
13th	60	33	76	51	56	58	596	337	538
14th	64	39	79	60	57	58	626	368	540
15th	66	40	112	61	59	68	642	460	546
16th	67	41	134	65	72	73	649	595	548
17th	75	47	194	65	73	74	702	627	560

18th	76	120	206	65	84	76	715	645	561
19th	86	293	209	74	86	76	717	660	581
20th	116	294	235	80	88	116	883	699	586
21st	180	301	240	87	91	185	937	708	644
22nd	206	337	286	92	92	194	985	811	646
23rd	254	338	300	93	105	228	1002	827	683
24th	277	352	305	99	210	240	1021	920	730
25th	325	367	-	112	215	245	1023	924	748
26th	332	-	-	134	217	261	1055	1008	765
27th	334	-	-	327	254	417	1065	1066	858

Appendix 5: Sample photographs showing the habitats of the study area and the study process

Sanetti plateau (a) alpine lakes (wet season) (b) Ethiopian wolf (*Canis simensis*) chasing rodents (dry season) (c) The vast expanse of the plateau showing the road through, from Goba to Dolo Manna (d) Sample collection

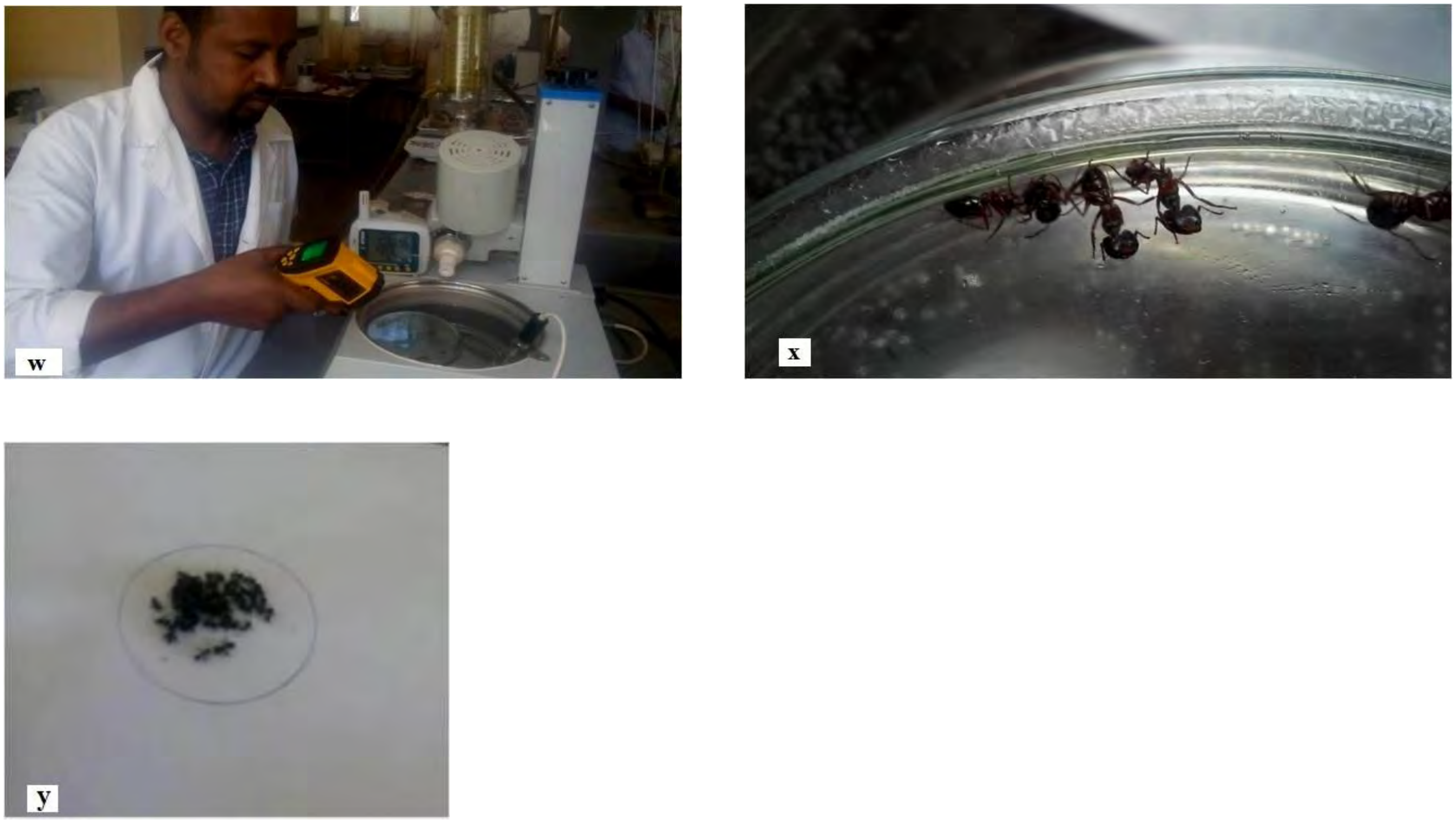
Gaysay grassland (e) mount Bodidi (3543 m a.s.l.) on the side (dry season) (f) Mountain Nyala (*Tragelaphus buxtoni*) in the grassland (wet season) (g) Sample collection (wet season) (h) Preparing baits (dry season)

(i) Harenna forest (roadside) (j) Sample collection (Harenna forest) (k) Setting transect (the northern woodland) (l) Pitfall trap set for sampling (the northern woodland)

(m) Sample collection at Erica moorland (n) Most of the Erica moorland was destroyed during March, 2015 fire accident in BMBP

(o) Sample preservation in 95% ethanol (AAU) (p) samples packed for storage (AAU) (q) Dry mount specimens (University of Utah) (r) Sample ready for identification (University of Utah)

University of Utah (s) Sample identification (t) Samples with barcodes, ready for incorporation to AntWeb (u) incorporating samples to AntWeb (v) I, Prof. John T. Longino and his wife Prof. Nalini Nadkarni, after a goodbye dinner



Thermal experiments (w) Temperature of the water bath was constantly monitored by an infrared thermal gun (knock-down resistance test) (x) ants hanging on the top of the Petri dish during heat where the temperature was less stressful during knock-down resistance test (y) ants placed on their backs in the center of a printed 32 mm diameter circle during chill-coma recovery experiment

Pictures of the ant species used for thermal experiments (z-1) *C. negus* soldier in full front view (z-2) *C. negus* soldier lateral view (z-3) *C. acvapimensis* soldier in full front view (z-4) *C. acvapimensis* soldier lateral view