Morphological and Molecular Characterization, Diversity and Ethnomycological Studies on Wild Mushrooms of Central and Northwest Ethiopia

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Abstract

Morphological and Molecular Characterization, Diversity and Ethnomycological Studies on Wild Mushrooms of Central and Northwest Ethiopia

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The diversity and ecology of fungi in general and macrofungi in particular, have been largely neglected in Ethiopia. In this context, this research aimed at providing documentation and analysis of wild mushroom diversity along with the ethnomycological knowledge associated with wild edible and medicinal mushrooms. Mushroom samples were collected from 48 plots (30 m × 30 m) of three different habitat type (forest, grazing land and farming area) over three years (2012 – 2014) from two study sites located in central (Welmera district) and Northwest (Menge district) parts of Ethiopia. Both morphological and molecular (ITS and partial LSU rDNA sequences) data were used for specimen identification. Maximum parsimony (MP), Maximum likelihood (ML) and Bayesian posterior probabilities (PP) were used for phylogenetic analysis. A hierarchical cluster analysis, with statistical program R was used to identify macrofungal community type and synoptic values to indicate dominant species in each community. Shannon-Wiener diversity index and Sorensen's similarity coefficient were used to assess species richness and evenness and to measure similarities among communities. Ethnomycological data were collected using semi-structured interviews, focus group discussions, participant observation and walk-in-the-woods methods. Ethnomycological knowledge held by different informant categories was compared using One-way ANOVA and t-tests. Results in this study showed, a total of 105 species belong to 23 families and 49 genera. About 95% of the species identified in this study are new records for Ethiopia. Family Agaricaceae (49), Lyophyllaceae (12), Tricholomataceae (9) and Psathyrellaceae (6) were represented by more species which together accounted for 74.4% of the total. The observed Shannon-Wiener diversity index (H'=3.76) of WFO showed the highest species diversity and the highest species richness (S= 62). Results of the ethnomycological study revealed 15 wild edible and medicinal mushroom species belonging to 7 genera and 5 families that are used mainly as food in the Menge District. Family Lyophyllaceae with 9 species (45%) was found to be best represented in the area. Ethnomycological knowledge is significantly influenced by gender, age,

experience and litracy level parameters. Preference ranking exercise has indicated *Termitomyces schimperi* was ranked first followed by *T. letestui, T. microcarpus* and *T. eurhizus* as the second, third and fourth preferred edible mushrooms respectively. The phylogenetic relationship of 33 Ethiopian *Agaricus* collection with other members of the genus from tropical/subtropical and temprate region was compared based on morphological and molecular (ITS 1+2 rDNA sequences) characteristics. More than two-third of the Ethiopian *Agaricus* sequences examined in this study was distributed amongst four of the eight well accepted/temprate sections of the subgenus *Agaricus*. The remaining six Ethiopian sequences group together with four distinct and exclusively tropical (African and/or Asian) clades with ML/MP/PP branch support 99/100/100, 100/100/100, 83/75/90, 85/75/90 respectively. None of the Ethiopian sequences in the dataset belongs to section Arvenses, Bivelares, Chitonioides and Spissicaules. This study also shown that *A. campestris, A. cupreobrunneus, A.bohusii, A. purpurellus, A. subsaharianus* and *A. heterocystis*, which are known to be edible are highly recommended for domestication and cultivation because of their good nutritional and medicinal value. Generally, the present study indicated that country is rich in wild mushroom diversity and associated indigenous knowledge. However, anthropogenic factors together with loss of indiginous knowledge and very poor conservation efforts threaten economically and ecologically important mushrooms survival in the area. Thus, complementary *in-situ* and *ex-situ* conservation strategy at national level is highly recommended.

Key words: Welmera, Menge, wild mushrooms, diversity, molecular phylogeny, traditional knowledge

Dedication

This thesis is dedicated to the people living in Menge District who conserved their rich indignous knowledge and practices for generations.

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List of Acronyms

AIDS	Acquired immuno deficiency syndrome
AMA	African Mycology Association
ASEMM	African Society for Edible and Medicinal Mushrooms
BRFSS	Benishangul-gumuz region Food Security Strategy
CBD	Convention on Biological Diversity
CTAB	Cetyltrimethylammonium Bromide
EF-1α	Eelongation factor 1-alpha
FAO	Food and Agricultural Organization of the United Nations
HIV	The human immunodeficiency virus
HMAS	Herbarium Mycologicum Academiae Sinicae
IBC	Institute of Biodiversity Conservation
IMCAS	Institute of Microbiology, Chinese Academy of Sciences
ITS	Internal Transcribed Spacer
IUCN	The International Union for the Conservation of Nature
LSU	Large Subunit
MCM	minichromosome maintenance protein
MOARD	Ministry of Agriculture and Rural Development
ML	Maximum Likelihood
MP	Maximum Parsimony
NCBI	National Center for Biological Information
NMA	National Meteorological Agency
NWFP	Non-Wood Forest Products
PCR	Polimerase Chain Reaction
PHCE	Population and Housing Census of Ethiopia
PP	Posterior Probabilities
RPB1	RNA polymerase I subunit
SSU	Small subunit
USD	United States Dollar
WEM	Wild Edible Mushrooms

This dissertation is prepared based on the following papers which are included in different chapters:

1. Rediet Sitotaw R., Li, Y., Wei, T.-Z., Dawit Abate and Yao, Y-J. (2015). Two new records of *Agaricus* spp. from Ethiopia. *Mycotaxon* **133:** 1171–1183.
2. Rediet Sitotaw R., Anteneh M. and Dawit A. (2015). Morphological and Molecular Studies on *Termitomyces* species of Menge District, Asossa Zone, Northwest Ethiopia. *Sci. Technol. Arts Res. J.* **4(4):** 49-57.
3. Rediet Sitotaw and Dawit Abate (2016). Ethnomycological study of mushrooms used for food and medcine in Menge District, Asossa Zone, Benshangul Gumuz Region, Ethiopia. *Journal of Ethnobiology and Ethnomedicine* (In preparation).
4. Rediet Sitotaw and Dawit Abate (2016). A Comparative Analysis on Diversity, species composition and community type macrofungi in selected habitat type of Central highland and North-west Ethiopia. *Fungal Ecology* (In preparation)

1. Introduction

1.1. Background

Fungi are an extremely diverse group of microorganisms that have a major influence on terrestrial ecosystems that serve as major decomposers, provide enzymes, food, drugs and serve as experimental organisms. In well-studied systems, the number of fungal species is about six times higher than plant species. Since the number of plant species is known to be around 270,000, thus the total number of fungal species estimated to be around 1.5 million (Hawksworth, 1991). This estimate now is considered to be conservative by many mycologists since it did not take into account fungi that might be associated with other hosts (insects, mosses, liverworts and lichens), fungi in different environments (anaerobes, antarctic rocks, marine and freshwater) and cryptic species (Hawksworth, 2001). More recent estimates based on high-throughput sequencing data of clone libraries from different environmental samples supported an approximate of 3.5 to 5.1 million fungal species to exist (Blackwell, 2011)

From the approximately 70,500 described species of fungi, about 10,000 belong to macrofungi recognised by their distinguished fruiting bodies, called mushrooms. Macrofungi are a group of fungi which produce mature spore-bearing and morphologically distinct fruiting bodies, which are visible to the unaided eye and generally are $\geq$1 cm in size (Arnolds 1992). Among the 10,000 species of macrofungi nearly 5,000 species are regard as edible. Over 1,800 species are considered as medicinal mushrooms (Boa, 2004).

Macrofungal genetic resources are certainly the most abundant in tropical and sub-

tropical areas of the world but it has been the least known (Arnold and Lutzoni, 2007) The first serious attempt to provide a methodical description of East African species in order Agaricales using modern taxonomic criteria was published by Pegler (1977). In his preliminary study Pegler (1977) has identified 389 species of mushrooms belonging to 94 genera in this region particularly Kenya, Tanzania and Uganda. About 60 species of edible mushrooms have been documented in Malawi (Morris, 1984; 1987), and about 150 species were identified in South Africa (Westhuizen and Eicker, 1990). From an extensive literature survey by Rameloo and Walleyn (1993) about 300 of wild edible mushroom were reported to be found in Africa which account much less when compared with the total number of edible species identified globally. There are obvious evidences that gaps in taxonomic knowledge are limiting the utilization of wild fungi in Africa. However, there is still much left to do, progress has been made in naming new species of mushroom in the region (Boa, 2004).

Macrofungal research in Ethiopia is a very recent activity and not well developed. It mainly focus on domesticated, commercially and ethnomycologically important group of mushrooms. The first small scale commercial mushroom cultivation, started in 1997 by the cultivation of the Oyster (*Pleurotus ostreatus*) mushroom. Later, the Button (*Agaricus bisporus*) followed by Shiitake (*Lentinula edodes*) mushrooms were introduced to the local market (Dawit Abate, 1998). All cultivated mushrooms are sold in fresh to local supermarkets, restaurants and hotels in the capital city, Addis Ababa.

In Ethiopia, wild mushroom consumption is a common practice among some ethnic groups, though; the habit differs from region to region. Dawit Abate (1999) described

that the country has clearly visible mycophilic and mycophobic regions. Many tribes in south and southwest Ethiopia are mycophilic and have a well-developed traditional knowledge and habit of picking and using wild mushrooms from nearby environment during the wet season (Tuno, 2001; Taddesse Yebo, 2010; Teferi Yenealem, *et al*., 2013). On the other hand, the North, Northeast and East societies are mostly mycophobic and have a minor regards for mushrooms.

As compared to studies on the flora and fauna of the country, there is scanty information on the macrofungal diversity and composition in the country. Thus, it is highly imperative to describe the macrofungal diversity and species composition of different habitat in the country. The output of this study will help addressing the general lack of knowledge pertaining to fungal biodiversity, to integrate macrofungi in biodiversity conservation strategies of the country, to identify the edible and medicinal mushroom resource of the country for their attributes as direct sources of protein and bioactive compounds (medicinal substances) while generating livelihood. So that these important organisms should be saved before, they get lost due to deforestation and environmental degradation now taking place in the country.

Therefore, this study was designed with the aim of increasing the taxonomic and diversity knowledge of macrofungi of some parts of Ethiopia and compile traditional knowledge of indigenous people residing in the Northwest parts of the country.

In this particular study, macrofungi refers to an agaric fungi/gilled mushroom with fruiting body characterized by the presence of a pileus (cap) that is clearly differentiated from the stipe (stalk), with lamellae (gills) on the underside of the pileus

1.2. Research Questions, Hypotheses and Objectives

1.2.1. Research questions

Since anthropogenic and other environmental factors, are known to damage the habitats available for native macrofungi, recording the status of macrofungal diversity of different habitat is necessary. Given that the country does not currently have a macrofungal species list, an information on the rarity or commonness of the different species and information on macrofungal communities will be very informative for the establishment of biodiversity information base.

Thus, it is important to address the following points/ research questions:

- What is the status of macrofungal diversity and how can data on macrofungi be used to inform better management of some habitats, and create awareness for conservation of fungi and their habitats?

- How can fungal community of a natural habitat can be affected by biotic (vegetation type, human disturbance, land management practice) and abiotic (altitude, temperature and precipitation) environmental variables?
- Macrofungi have different geographic distribution patterns. It may also be expected that certain macrofungal species would be much more common and possibly totally or semi-restricted to a single habitat type, and thus may have potential for use as indicator species.
- Documenting the indigenous knowledge is very important since the result will serve as source of primary information for further research in the field of nutrition and mycomedicine.

1.2.2. Research hypotheses

- Macrofungal diversity and species composition is habitat dependent and showed comparative degree variation along with different habitat within the study sites.

- Forests have high species diversity and are reservoirs for wild mushrooms in the country and a major source of edible and medicinal mushrooms for the community in the study sites.

- Wild macrofungal species composition of Benishangul Gumuz region (low land) is different from those found in the central highland of Ethiopia due to difference in vegetation type, climate and geography of the regions.

- The Ethnic groups in Benishangul Gumuz region are mycophilic and have a good traditional knowledge and habit of using wild mushrooms as food and medicine.

1.2.3. Research Objectives

General objective

This research is aimed at documenting and analyzing macrofungal diversity of different habitat type along with morphological and molecular description and the traditional use of wild edible and medicinal mushrooms in northwest and central Ethiopia.

Specific objectives

The specific objectives of the research were:

- To collect, identify, record and preserve macrofungal specimens from the two regions.

- To evaluate the morphological and molecular characteristics of Ethiopian macrofungal species based on sequences of internal transcribed spaces (ITS) and large subunit ribosomal DNA genes.
- To identify macrofungal species diversity, composition, community type and phenology in the two study sites.
- To record local knowledge, attitude and practice among different groups of indigenous people on wild edible and medicinal mushroom utilization.
- To identify major threats of local conservation practices of wild mushroom species of the study site
- To develop a baseline data, which will contribute to establish mushroom traditional uses depository and sustainable use of these genetic resources.

2. Literature Review

2.1. Global Overview of the Use of Mushrooms

Wild edible mushroom (WEM) have been collected and utilized by people in different part of the world for thousands of years. The archaeological record reveals edible species associated with people living 13, 000 years ago in Chile (Rojas and Mansur, 1995) but it is in China where the consumption of WEF was first reliably noted, several years before the birth of Christ (Aaronson, 2000). Edible fungi were collected from forests in ancient Greek and Roman times and highly valued, though more by high-ranking people than by peasants (Buller, 1914). Many countries in Europe and Asia have for centuries valued various species of WEF, not only for food but also for their healing properties. Currently, China is the leading producer and exporter of cultivated mushrooms (Boa, 2004).

Table 2-1 Mushroom tradition in 210 countries according to FAO-defined regions

Region	Countries	Strong mushroom tradition	Weak mushroom tradition	No information
Africa	55	10 (18%)	28 (51%)	17 (31%)
Asia	51	15 (30%)	18 (35%)	18 (35%)
Europe	37	14 (62%)	9 (29%)	3 (9%)
America	47	7(15%)	11 (23%)	29 (62%)
Oceania	20	1 (5%)	3 (15%)	16 (79%)
TOTAL	210	47	69	83

Note: Tradition refers to cultural use and collecting to sell (Boa, 2004)

Mushrooms have wide ranges of health benefits; their medicinal values include enhancing immunity, reducing cholesterol accumulation in blood, wound-healing and

tumor-retarding effects (Garibay-orijel and Cifuentes, 2006; Gregori and Pohleven, 2007; Oyetayo, 2011). Recently their value has been promoted to tremendous levels with medicinal mushroom trials conducted for HIV/AIDS patients, which have been making encouraging results (Zhang *et al.,* 2014). Wild useful fungi, therefore, contribute towards diet, health, income and ecological role as a tool in the management of healthy ecosystems.

2.2. Potential and Emerging Economic Benefits of Mushroom Cultivation

Mushrooms are now getting significant attention all over the world due to their nutritional value such as high proteins, vitamins and fibre content apart from having certain medicinal properties. Today the cultivation of various species is being done in about 100 countries (Boa, 2004). At present, world production of mushroom is estimated to be around five million tonnes and is ever increasing (Miriam *et al.,* 2006; Ayodele *et al.,* 2011; Polashree and Joshi, 2013). Oyster (*Pleurotus ostreatus*), Shiitake (*Lentinula edodes*), Black fungus (*Auricularia polytricha*), Velvet Foot (*Flammulina velutipes*) and Button mushroom (*Agaricus bisporus*) contribute 99% of the total world production (Boa, 2004).

Mushroom cultivation offers prospects for converting lignocellulosic residues from agricultural fields and forests into protein rich biomass (Miriam *et al.,* 2006). Such processing of agro-waste not only reduces environmental pollution but the by-product of mushroom cultivation is also a good source of manure, animal feed supplement and soil conditioner (Kakon, *et al*., 2012).

Table 2-2 Estimated number of species of wild edible and medicinal fungi

No.	Use Category*	No. of Species	Percentage total
1	Edible only	1 009	43
2	Edible and medicinal	88	4
3	Food only	820	35
4	Food and medicinal	249	11
5	Medicinal only	133	6
6	Other uses (none of above)	29	1
	Total	**2,328**	**100**

Source (Boa, 2004)

2.3. Overview of African Mycota

Most of the information on the biology and ecology of macrofungi is based on studies carried out in developed countries (Zhao *et al.,* 2011). There is still no correct estimate of the diversity value of macrofungi represented in Africa. However, it has been estimated that the continent constitutes at least 25% of the total mushroom biodiversity worldwide but contributes barely 0.4% of total mushroom sales and new mushroom products to the global market (Miriam*et al.,* 2006).

The first account of the order Agaricales using modern taxonomic criteria, rather than an enumeration of species in Africa were published by Pegler (1977) followed by Morris (1987) and Westhuizen and Eicker, (1996). From an extensive literature survey by Rameloo and Walleyn (1993), about 300 edible species of wild mushroom are reported to be found in Africa which accounts much less when compared with the total number of species identified. Thereafter some fragmental studies of agarics have been reported from different part of Africa (Boa, 2004; Miriam *et al*., 2006; Mueller *et al*., 2007; Olusegun, 2012; Zhao *et al*., 2011).

Wild edible mushrooms are well known in most indigenous African recipes (Tibuhwa, 2012a). During the rainy seasons, it is customary to find rural people

across many African countries (including Benin Republic, Cameroon, Equatorial Guinea, Ethiopia, Ghana, Kenya, Malawi, Nigeria, Togo, Uganda and Zimbabwe) going out to search mushrooms on decaying wood and in the nearby forest (Yongabi *et al*., 2004). The importance of wild edible fungi to people in Africa may also have gone unremarked for the simple reason that most of the collections are for personal use, reports from Ghana and Sierra Leone also indicated that local use is widespread (Okhuoya *et al*., 2010).

There are obvious evidence that gaps in taxonomic knowledge limit the utilization of wild fungi (Boa, 2004). The studies on mushrooms are mainly aimed at describing them and validating its status as new record or species new to science, but, there are relatively a few studies that have been done to understand the ecology of this group of fungi mainly due to short life span of their fruiting bodies (Miriam *et al.,* 2006). For the full benefit of mushrooms to be realized in Africa, a detailed understanding of their taxonomy, diversity, development of indigenous species domestication, cultivation strategies and marketing should be aggressively followed.

2.4. The Status of Mushroom Research in Ethiopia

Study on macrofungal biology and ecology is a very recent research area in Ethiopia and not well established. The mycobiota of different ecosystems and regions of Ethiopia have not been well explored and documented. There have been very few literature regarding macrofungal research in the country these include Ryvarden and Johansen (1980) and Pegler (1977) has described the bracket and agaric fungi of East Africa including few parts of South and Eastern Ethiopia. Dawit Abate (1999) has reported the presence of *Agaricus campestris* in some highland areas of the country.

According to Boa (2004), a preliminary database record of published information of 2, 800 records from 85 countries of wild useful fungi, Ethiopia was represented only with *Lentinus* sp. and *Schizophyllum commune*.

Regarding commercial production of mushroom, small-scale commercial mushroom cultivation was started in 1997 in the country, however, it has been restricted to only three types; the Oyster (*Pleurotus ostreatus*), Button (*Agaricus bisporus*) and Shiitake (*Lentinula edodes*) mushrooms (Dawit Abate, 1998).

The fatty acid profile (Woldegiorgis *et al*., 2015a), antioxidant property (Woldegiorgis, *et al*., 2014) of some wild and cultivated edible mushrooms and medicinal property of *Laetiporus sulphureus* (Woldegiorgis *et al*., 2015b) collected from Ethiopia have been evaluated. This study include cultivated *Pleurotus ostreatus, Lentinula edodes, Agaricus bisporus,* and eight wild species of *A. campestris, Laetiporus sulphureus, Termitomyces clypeatus, T. microcarpus, T. aurantiacus* and *T. letestui*. These studies revealed that *A. campestris* had highest antioxidant activity in all assays with lower EC_{50} values and exhibited greater total phenolics and total flavonoids content.

In addition, all mushrooms contained various amounts of ergothioneine, with *Pleurotus ostreatus* (Oyster mushroom) containing substantially higher amount of ergothioneine. Eventhough all the edible mushrooms evaluated in this study were generally low in lipids, their fat quality is good, mostly consisting of unsaturated fatty acids with hign polyunsaturated linoleic acid. Thus, local people eating mushrooms might be beneficial to protect themselves against oxidative damage (Woldegiorgis *et*

al., 2014) and reduce blood cholesterol (Woldegiorgis *et al.*, 2015a). Moreover, Woldegiorgis *et al*., (2015b) reported that glycyrrhetinic acid (Enoxolone) might be one of the biomarker responsible for ethnopharmacological health benefits suggested by the kaffa people that uses the fruiting body of *Laetiporus sulphureus* to relieve stomach pain and to expel a woman's retained placenta following delivery.

Ethnomycological studies among some ethnic groups in southwestern part has been reported (Teferi Yenealem *et al*., 2013; Tuno, 2001) that *Termitomyces* sp. and *Schizophyllum commune* were commonly collected and consumed by the local people. Another ethnomycological study in southwestern was condacted by Taddesse Yebo (2010) that has shown the three ethnic groups in Kaffa zone, known as the Kaffecho, Chara and Nao from the three selected woredas Gimbo, Decha and Bonga areas often collect and consume WEMs for food and medicine. In his study five species of WEMs (three *Termitomyces* sp: *T. microcarpus*, *T. clypeatus* and other medium and big size unidentified *Termitomyces* species) and two wood inhabiting mushrooms (*L. sulphureus* and *Pleurotus* sp.) were recorded as edible mushroom.

2.5. Morphological Vs Molecular Characterization in fungal Systematics

2.5.1. Morphology based identification

Morphological characters have been used for the last 300 years to identify, classify, and infer phylogenies of fungi (Seifert *et al.*, 2007). Most of the time, morphology-based identification of a species in kingdom fungi is not easy. This can be attributed to the lack of distinctive morphological characters and predominance of microscopic species in the Kingdom (Schoch *et al.*, 2012). Moreover, morphology-based

identification have limitations which leads to incorrect identification,these include: i) parallel evolution, phenotypic plasticity and reversal (homoplasy) (Judd *et al.,* 2002) due to different environmental factors, ii) ineffectiveness in discriminating morphologically cryptic taxa (Schoch *et al.,* 2012), iii) poorly known life-cycle of pleomorphic organisms (Seifert *et al.,* 2007) and iv) needs a high level of identification expertise. Consequently, many modern genera and families are not well established and mycologists still disagree about the identity of the majority of taxa in the Kingdom. In the present era of phylogenetic molecular analyses, the coordination of the anamorphic and the teleomorphic element of a fungus can in principle clearly be established and molecular mycologists have therefore been the driving forces striving for a consistent unification of fungal nomenclature (Gams, 2013).

2.5.2. Molecular characters and DNA barcoding

Molecular characters such as DNA sequence-data are mainly helpful in offer a greater number of distinctive characters, which can be analysed statistically to infer phylogenetic relationships (Shenoy, *et al.,* 2007). Molecular phylogenetic analysis in fungi has a short history of 15-25 years (Blackwell, 2011) and it is now becoming avital part of fungal systematics. However, it is unreasonable to claim that molecular data are more significant than morphological data in phylogenetic inference as molecular characters are also known to be subject to convergence and parallelism (Judd *et al.*, 2002).

The protein-coding gene regions (largest subunit of RNA polymerase I (RPB1), second largest subunit of RNA polymerase II (RPB 2), and minichromosome maintenance protein (MCM7)) often had a higher percent of correct identification

compared with ribosomal markers (ITS, LSU and SSU (Eberhardt, 2010; Schoch *et al.,* 2012)). The low PCR amplification and sequencing success eliminated protein-coding gene as candidates for a universal fungal barcode (Lindner and Banik, 2011; Schoch *et al.,* 2012). In some cases,partial β-tubulin gene sequences (Samson *et al.,* 2004) and partial elongation factor 1-alpha (EF-1α) sequences (Geiser *et al.,* 2004) have been used to identify fungi to the species level.

The internal transcribed spacer (ITS) region has the highest probability of successful identification for the broadest range of fungi, (Lindner and Banik 2011; Schoch *et al.,* 2012; Lodge *et al.,* 2014). However, LSU is a popular phylogenetic marker in certain taxonomic groups, such as the early diverging lineages and the ascomycete yeasts, it is slightly inferior to the ITS. The SSU has a poor species-level resolution in fungi (Schoch *et al.,* 2012). Thus, ITS is formally proposed for adoption as the primary fungal barcode marker to the Consortium for the Barcode of Life, with the chance that additional barcodes can be developed for specific circumscribed taxonomic groups (Laursen and Taylor, 2008; Lindner and Banik 2011; Raspé *et al.,* 2011; Schoch *et al.,* 2012; Voigt and Kirk, 2011).

DNA barcoding approach, however, has come under some criticism, due to 1) its reliance on the reference databases for comparison since about 20-30% of the entries may be incorrectly identified to species level (Nilsson *et al.,* 2006). 2) the taxon sampling of fungi is far from complete and 3) the absence of description of majority of entries (Voigt and Kirk, 2011). Due to these drawbacks, there is a need to take account of both molecular and morphological data sets in phylogenetic studies. This general practice in macrofungal systematics has raised many unresolved problems and

arguments concerning how to best incorporate information revealed by morphological and molecular characters (Zhao *et al.*, 2011).

2.6. Factors Influencing Macrofungal Diversity and Community Structure

2.6.1. Vegetation

Since plants constitute the habitat and energy source for most macrofungi, vegetation type influences the composition and the quantity of species (Li *et al.*, 2012; Anderson *et al.*, 2013). Plant associations or vegetation zones are valuable standards to use when dividing a land for sampling (Laursen and Taylor, 2008; Hanlon *et al.*, 2012). Some earlier studies found that the distribution of macrofungal species (especially ectomycorrhizal fungi) to be correlated with forest type (deciduous trees or conifers), since every fungus shows a certain degree of host or substratum specificity (Anderson *et al*., 2013). However, in other studies it was believed that precipitation to be better than vegetation type as a predictor of species richness of macrofungi (O'Dell *et al.*, 1999).

2.6.2. Succession

There are several viewpoints to consider succession of macrofungi. The first involves changes in community structure frequently are associated with changes in the quality of the substratum. Certain species only grow well on leaf litter that previously has been decomposed by other fungi (Hedger, 1985). Similarly, trunks of fallen trees host a group of fungi that fruit at the beginning of log decomposition and others that fruit only later (Heilmann-Clausen 2001). The second perspective of succession happens during changes in the vegetation that has a direct influence on fungi with the

establishment of an association with new host and changes in the amount and quality of accessible organic matter (Hedger, 1985). Thus, from a successional point of view, including diverse microhabitats and a variety of stand ages among the sampling sites are very important for studying the macrofungal diversity of a landscape.

2.6.3. Geography

The diversity of habitats rather than geography was believed to have the strongest impact on fungal species richness, however, currentely it has been reported that the effects of elevation on temperature and precipitation influence fruiting within a geographic region (Mueller *et al.*, 2007; Lee and Lee, 2004). Consequently, certain species may fruit at different seasons of the year across wide geographic distances or along strong elevational gradients (Ohenoja, 1993). Some ectomycorrhizal fungi (e.g., *Cortinariaceae*) was thought to be most diverse at middle and high latitudes, whereas Agaricales in general (especially saprobic *Tricholomataceae*) were considered most diverse at low latitudes (Sapphire *et al.*, 2002).

The aspect of the site may also have an impact on the fungi appearing, as south-facing slopes will generally get more sunlight than north-facing slopes, so a south-facing slope can expect higher soil temperatures and lower moisture contents of the soil (Ohenoja 1993). Lee and Lee, (2004) found that fungal diversity increased in their plots which faced north, due to higher relative moisture levels in the soil.

2.6.4. Seasonality and Year-to-Year Variation

Yearly difference in the presence of sporocarps along with diverse seasonal peaks of abundance for distinct species, can occure several times (Hanlon *et al.*, 2012). Lodge

(1996) also reported that several species in the *Entolomataceae* fruited every second or third year in a wet subtropical forest in Puerto Rico, whereas a limited number of other species were found only once during 13-years of survey.

Erratic weather conditions can cause unusual patterns of fruiting, however; in regions with dry summers, for instance, fruiting of species rarely follow a summer shower (Hanlon *et al.,* 2012). Although, the fruiting of macrofungi is most often limited by an absence of precipitation, excess moisture can hinder fruiting in most species (Ohenoja, 1993). Typical data for measuring species richness in a location may involve intense collecting over a period of several days/years when sporocarp production is high (Lodge, 1996).

2.7. Ethnomycology and Indigenous mushroom use

Humans-mushroom interaction has dated back to many millennia, however, the written evidence of ethnomycological knowledge is a recent conception (Miriam *et al.,* 2006). Ethnomycology is a multidisciplinary field of study involving many aspects of mycology (collecting, describing, and taxonomy), linguistics (vernacular names) and anthropology and sociology (investigations) (Lampman, 2004; Aryal and Budathoki, 2012). This field of study like other ethnobiology fields derived from insatiable and dynamic need of human to improve food and medicine base.

A number of ethnomycological works have been published from Asia, Africa, South America, North America and Canada. Most of these studies represent diverse areas of mushroom knowledge that includes collection, income generation (Garibay-orijel and Cifuentes, 2006; Miriam *et al.,* 2006), entheogenic (spirituality and mythological

values) (Am *et al.,* 2012; Lampman, 2004), mycopharmaceuticals, nutriceuticals, folk mushroom taxonomy and the dynamics of inherited folk knowledge of mushrooms (Morris, 1984; Montoya and Torres, 2002; Adhikari *et al.,* 2005; Flatie *et al.,* 2009; Garibay-orijel *et al.,* 2012; Osarenkhoe *et al.,* 2014).

However, our understanding on African ethnomycology is still limited, unevenly distributed and information has become available the last few decade (Rammeloo and Walleyn, 1993). In the present day only a few African countries, or regions, have been really benefited from an extensive ethnomycological survey, the most important ones include; Bénin (De Kesel *et al.,* 2008), Burkina Faso (Guissou *et al.,* 2008), Burundi (Buyck, 1994), Cameroun (Yongabi *et al.,* 2004), Central African Republic and D.R. Congo (Degreef *et al.,* 1997), Malawi (Morris 1984), Nigeria (Oso, 1985), Tanzania (Härkönen *et al.,* 1995), Togo,Gabon (Osarenkhoe *et al.,* 2014), and Zambia (Piearce, 1981). Joint efforts are therefore necessary to capture the representative macrofungi species of Africa with a view to revising their global representation (Osarenkhoe *et al.,* 2014) may be under the platform of either the African Mycology Association (AMA) or the African Society for Edible and Medicinal Mushrooms (ASEMM).

Indigenous knowledge, nutrition and health care

Mushroom hunting refers to the habit of picking edible and medicinal mushrooms from the wild and surrounding woodlands (forests, plantations), farmlands and grasslands along with other non-wood forest products (NWFP) (Boa, 2004). Wild mushroom collection practice and the consequences of over-exploitation of mushrooms in forest ecosystems may affect species composition, richness and diversity (Garibay-orijel *et al.,* 2012). Economically feasible and sustainable

beginning of domesticated mushrooms in developed countries as the primary source of edible mushrooms, gradually substituted the ancient practice of their collection from the wild (Miriam*et al.,* 2006). In these countries, the multidimensional use of mushrooms has elevated them to cash crops status that is well priced as food, medicine and tool in the healthy management of ecosystem, especially agroforests.

The rural people in most parts of developing countries still rely on wild edible mushrooms for their livelihood especially as a low-cost alternative to animal proteins and flavouring in diets, source of income and raw material in local traditional medicine practice (Osarenkhoe *et al.,* 2014). The appearance of the majority of edible mushrooms, in the tropics, is restricted to the rainy season, a season which represents the low availability of food hence mushrooms were the most favourable option for overwintering the food shortage season (Boa, 2004).

Many ethnomycological studies showed that women and children are more involved in collecting wild edible mushrooms either for food preparation or sale at local markets, dye extraction for rural cosmetics or for religious rites (Miriam *et al.,* 2006; Garibay-orijel *et al.,* 2012; Treu and Adamson, 2006; Yongabi *et al.,* 2004). It is, therefore, rational to assume that women have better knowledge than men in the identification of edible andpoisonous mushrooms as well as, their characteristic and identification features, which are mostly macroscopic. In addition, women have a better knowledge on their spatial distribution in terms of habitat, phenology and associated substrate(s), processing (handling, drying and cooking) and appropriate local uses (Adhikari *et al.,* 2006; Garibay-orijel *et al.,* 2012). On the contrary, a study by Akpaja *et al*., (2003) reported that aged (50 - 75 years old) men have relatively sufficient knowledge of indigenous wild edible and poisonous mushrooms.

However, the influence of occupation type on the depth of indigenous knowledge of wild edible mushrooms remains unclear, observation at different parts of Africa affirmed high knowledge of mushroom heritage in local men linked to forest-related occupations like game hunting, farming, palmwine tapping, foresters, firewood collector and herbalists. Different studies have showen that mushroom collectors derive local (indigenous) names of various edible mushrooms from their habitat, season/phenology, shape, colour, myth and uses (Garibay-orijel *et al.,* 2012; Lampman, 2004; Tibuhwa, 2012).

There are inadequate data on the identity and medicinal properties of the majority of wild mushrooms in Africa. Information on the ethnomedicinal uses of some mushrooms such as *Pleurotus tuberregium* used for headache, stomach pain, fever, cold, constipation (John and Akpaja, 2005); *Lentinus squarullosus* for mumps, heart diseases (Osarenkhoe *et al.,* 2014); *Termitomyces microcarpus* for gonorrhea; *Calvatia cyathiformis* for leucorrhea (Oyetayo, 2011); *Ganoderma lucidum* for treating arthritis, neoplasia; *G. resinaceum* used for hyperglycemia, liver diseases (hepatoprotector) (Treu and Adamson, 2006) have been reported. These informations are mainly obtained from traditional herbalists in west and central Africa and many of these mushrooms are obtained only in the wild, which need urgent understanding, documenting and detail study of their medicinal properties. With regard to nutritional composition of mushrooms, in general contains protein (2–40%), fat (2–8%), carbohydrates (1–55%), fiber (3–32%) and ash (8–10%) (Firenzuoli *et al*., 2008).

2.8. Threats to wild mushrooms, associated knowledge and conservation plan

Fungi are certainly poorly understood and appreciated compared to plants and animals, moreover, currently they are highly threatened by habitat loss, pollution, over-exploitation and climate change. From the total estimated species of fungi, only 8–10% have been discovered and described (Blackwell, 2011). The International Union for the Conservation of Nature (IUCN) assesses threats to biodiversity by analyse the status of individual species and provides a report of Red Lists in order to catalyse action for biodiversity conservation, however, these lists have a bias towards well-known groups of species (Moore, 2001). The global red lists comprise almost 77,000 species, of which 26,000 are vertebrates. In contrast, as of July 2016, IUCN has evaluated the conservation status of 34 fungus species. The list includes only nine from Ascomycota and 25 Basidiomycota (IUCN, 2016). This actually indicates that fungi are the most under-researched and under-funded group of organisms indicating a critical and immediate action to collect more information on their conservation status.

However, in recent decades, enormous advances in the knowledge of taxonomy, distribution, ecology, and conservation status of macrofungi in developed countries enable this biodiversity to be appreciated, considered and incorporated into conservation actions at both the national and global level (Moore, 2001). The main causes are inappropriate forest and farmland management and air pollution; however, fungal Red-list analysis rarely seems to be considered in national programmes (Blackwell, 2011).

Currently, concerns for the diversity and conservation of macrofungi such as identification and designation of protected areas, inclusion in monitoring programmes, and production of management guidelines are starting to be considered in a few developing countries (Crabtree *et al*., 2010; Sysouphanthong *et al.,* 2013). The plant conservation strategy, endorsed by the Convention on Biological Diversity (CBD), is an obvious sister strategy because of the close interrelationship between fungal and plant diversity. The modified objectives are: 1) understanding and documenting fungal biodiversity; 2) conserving fungal biodiversity; 3) using fungal diversity sustainable; 4) promoting education and awareness about fungal diversity; 5) building capacity for fungal conservation (Garibay-orijel *et al*., 2012).

Local knowledge has also been found useful for ecosystem restoration and often has ingredients of adaptive management (Garibay-orijel *et al.,* 2012). Local knowledge helps in scenario analysis, data collection, management planning, designing of the adaptive strategies and institutional support to put policies into practice (Anderson *et al.,* 2013). Local knowledge systems are disappearing at a rate that may not allow us even to know what value, such systems had. Thus application of scientific research and local knowledge contributes both to the equity, opportunity, security and empowerment of local communities, as well as to the sustainability of the natural resources (Lampman, 2004) and this issue could possibly be accomplished with the use of ethnoeconomics or ethnoecological studies.

3. Macrofungal Diversity, Species Composition and Community Type in Selected Habitat of Central Highland and Northwest Ethiopia

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Abstract

The diversity and ecology of fungi in general and macrofungi, in particular, has been largely neglected in Ethiopia. In this context, this study was designed to examine the macrofungal diversity of three different habitat type (forest, grazing land and farming area) through the collection of sporocarps over three years (2012 – 2014) from two study sites located in central (Welmera district) and Northwest (Menge district) parts of Ethiopia. Shannon-Wiener diversity index was used to assess species richness and evenness. A hierarchical cluster analysis, with statistical program R version 3.2.2 was used to identify macrofungal communities and synoptic values for identification of the dominant species. Sorensen's similarity coefficient was used to measure similarities among communities. A sampling of 48 plots (30 m × 30 m) revealed 105 taxa belong to 23 families and 49 genera. The total family/genus ratio 0.47 and genus/species ratio 0.45 suggest the presence of moderate family and generic diversity in the collections. About 95% of species in this study were new records for Ethiopia. The families that contained the most number of species were Agaricaceae (49), Lyophyllaceae (12), Tricholomataceae (9), Psathyrellaceae (6), and Hymenogastraceae (4), which together accounted for 76.2% of the total surveyed species, while Amanitaceae, Cystofilobasidiaceae, Geastraceae, Inocybaceae, Mycenaceae, Physalacriaceae, Pleurotaceae and Strophariaceae were each represented by one genus and one species. The mean species per sample of WFO (13.3) were significantly different from that of MFO (8.3) ($p<0.05$). The mean species per sample of WGR (8) were significantly different from that of MGR (6.1) ($p<0.05$). However, the mean species per sample of WFA did not show any significant difference as compared to MFA ($p = 0.054$). The observed Shannon-Wiener diversity index ($H'=3.76$) of WFO showed the highest species diversity among the whole habitat investigated followed by MFO with ($H' = 3.57$). Results also revealed WGR, MGR and MFA are the 3rd , 4th and 5th with $H'= 2.8, 2.9, 2.7$ respectively. Similarly WFO has the highest species richness ($S= 62$) followed by MFO with $S = 44$, WFA and

MGR each with S = 26 indicating local climatic variations, land use/land cover and habitat disturbances could be among the factors most responsible for variations in species diversity and evenness in a given forest and disturbed area surrounding the forests. Based on this, therefore, recommendations are made to include monitoring of fungi, particularly macrofungi, in biodiversity management plans of the country.

Key words: Macrofungi, Diversity, species richness, Community

3.1. Introduction

Macrofungi are well known to inhabit diverse types of habitats, varying principally in the composition of vegetation, substrates, temperature and moisture (Hawksworth, 1991). The associative nature of some macrofungi can be described as their relationship with a specific species of plant, insect or other organisms, connection in the breakdown of a particular types of substrates i.e. litter, dung or wood, as well as colonisation upon a particular types of soil (Blackwell, 2011). The specificity of a macrofungal species to its sustaining environment make the conservation of their habitat very important. With the current status of increasing dependence of societies on genetic resources in the environment, degradation of habitat and poor conservation practice put most of these macrofungi in threat (Sapphire *et al*., 2002).

Tropical regions of the world are considered to have the highest diversity for most groups of organisms (Hillebrand, 2004), and this is in general true for fungi as well (Arnold and Lutzoni, 2007). However, understanding the macrofungal flora of the region has been limited to a small portion (Boa, 2004). This could possibly due to diverse physiography, agro-climatic variations and different topography, a higher diversity of vascular plants that make niches which play a significant role to macrofungal richness of a region (Packham *et al.,* 2002; Kirk *et al*., 2008).

Study on macrofungal communities in different regions has typically been carried out using visual examination of species from different sites (Winterhoff, 1992). Multivariate ecological data analysis which have been used for plant community data (Whittaker, 1975) for many years, has now become one of the methods to the study of macrofungal communities (Arnolds, 1992; Villeneuve *et al*. 1991). Many authors

since then have used multivariate ecological data analysis in different mycological studies such as; replicated plot-based data for comparison of different forests used in Australia (Packham *et al.,* 2002); comparing the community of fungi on logs differing in decay stage and other characteristics New Zealand (Allen *et al.,* 2000).

Ethiopia, because of its rainfall pattern, ranges of altitude, soil variability and geographical position, has ecological diversity and enormous wealth of biological resources (IBC, 2007). However, our knowledge on macrofungal diversity and their ecological function in the country is limited. The ecological interaction and contribution of macrofungi with other organisms in different habitat type of the country is poorly understood due to lack of, appropriate governmental and non-governmental organizations to take the initiative for mycological research, lack of experts in the field of macrofungal systematics, the ephemeral nature of many fungal species. Incomplete information on the biodiversity of macrofungi from such ecosystems is only available from very few scattered sources (Dawit Abate, 1998; Teferi Yenealem *et al.,* 2013; Rediet Sitotaw *et al.,* 2015 a, b).

The aims of this study are to compare the macrofungal species richness and community assemblages in three different habitat types: forest, grazing land and farmland in the two study areas. The two study sites are in distantly located regions, which are > 600 km apart. This information can be interpreted ecologically to assess the role of different habitat type in maintaining macrofungal diversity. In addition, this study was designed to strengthening awareness about the diversity, value and better management of the macrofungi.

3.2. Materials and Methods

3.2.1. Study area description

The study was conducted in two Regional States, Oromiya (Welmera District) and Benishangul Gumuz (Menge District), Ethiopia, located in the central and northwest part of the country respectively (Figure 3.1). From each site, three different habitat types were selected (forest, farmland and grazing fields) and served as study locations.

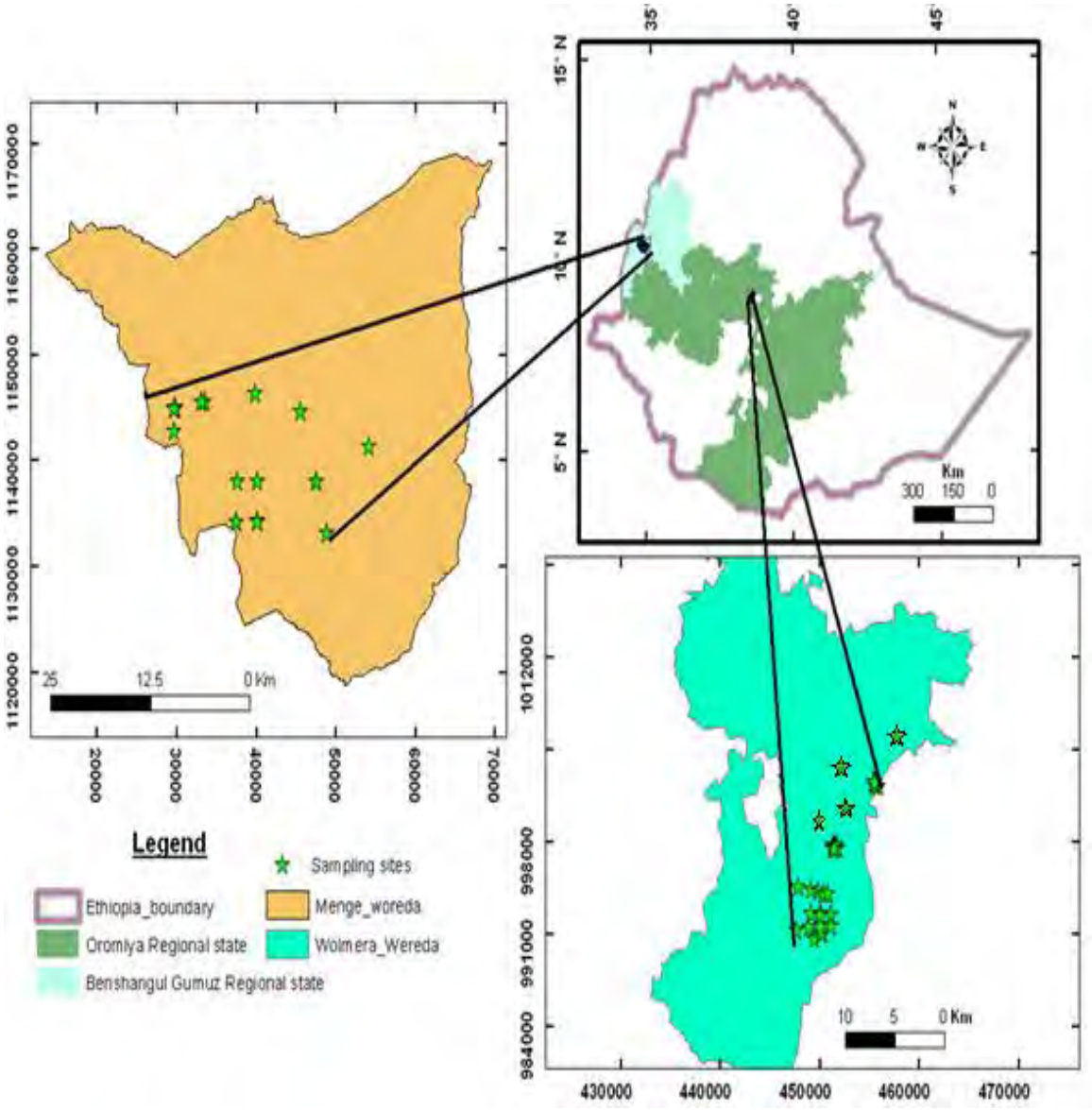

Figure 3-1 Map of Ethiopia and the study areas

3.2.2. Physical and biological settings of Welmera and Menge District

Location and physiography of Welmera District

Welmera District is located about 35 km south-west of Addis Ababa, between longitudes 38° 32' and 38°56' East and latitudes 8°56' and 9°00' North in Oromiya Special Zone surrounding Addis Ababa, Ethiopia. It includes a mountainous topography that ranges from 2050 to 3390 m above sea level (Figure 3.2). The area covers forest, farmland, grazing land and settlement areas. Menagesha state forest, which is considered as the 'the Oldest Park in Africa' is one of the few protected natural forests in Ethiopia which used to be 9,248 ha, but only about 2,500 ha of the original forest now remains with a further 1,000 ha under plantation (Sebsebe Demissew, 1988; Abate Zewdie, 2007).

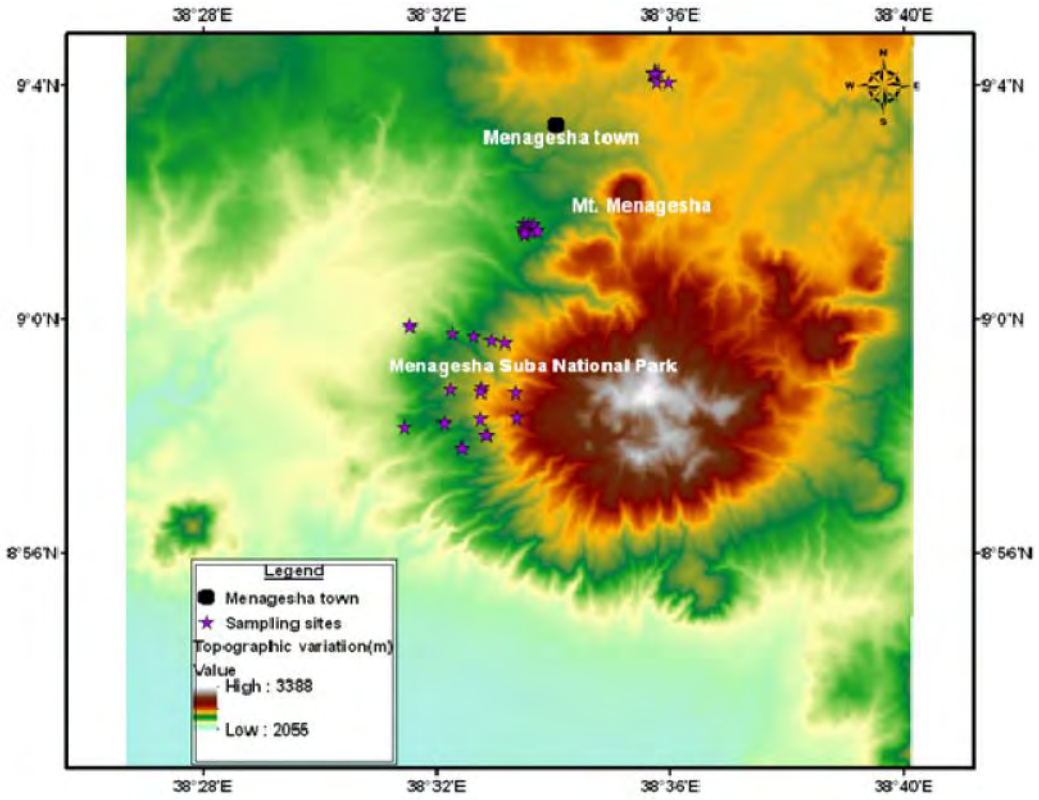

Figure 3-2 Topographic variation of the study site in welmera District

Vegetation of Welmera District

According to MOARD (2002), the forest is dominated by *Juniperus procera*, *Olea europaea* subsp *cuspidata*, *Allophyllus abyssinicus*, *Maytenus* sp. and *Euphorbia ampliphylla* and some *Podocarpus falcatus* trees are scattered throughout the forest. At higher altitudes, smaller *Juniperus procera* trees are mixed with *Erica arborea*, *Rosa abyssinica* and the endemic *Jasminum stans*. Below the summit is an intensively farmed basin with scattered homesteads and fields of barley, maize, beans and potatoes, however, this farm and grazing lands are now being built up as a suburb of Addis Ababa (Dinkissa Beche, 2011).

Climate of Welmera District

The rainfall and the temperature condition of the area was described based on the data from 2003-2013 by the National Meteorological Agency (NMA). According to this data, mean annual rainfall of the area was around 1100 mm with June to September the main rainy season. However, inside the forest, it can rain in any month of the year and the forest gets additional moisture from low clouds and mist. The mean annual temperature of the surrounding area is 18°C with a mean maximum of 26°C and mean minimum of 10°C (Figure 3-3). In the forest, overall temperatures are cooler with an average of 11°C in the upper parts (MOARD, 2002).

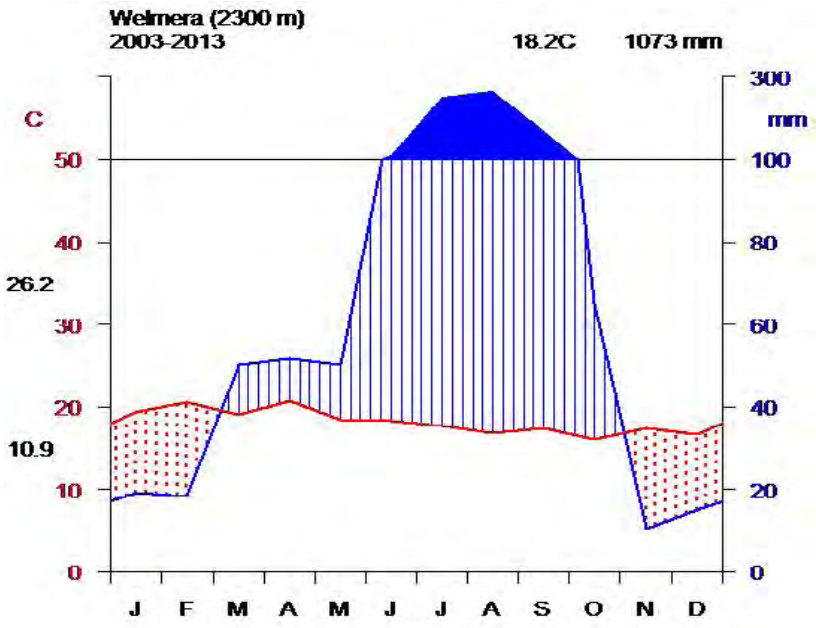

Figure 3-3 Climadiagram of Welmera station

Geology and soil of Welmera District

The geological aspect of the area around the forest could be defined based on volcanic dome of Mountian Wochacha. The topography of the area was the result of the siliceous volcanic cone. There are various rock types, including trachytes and basalt trap series. The basalts are the main rock types from which the soil parent material of this area was derived (Mohr, 1971). According to Tamrat Bekele (1993), the soil of the forest at lower altitudes was reddish brown, deep and less gravelly, whereas at higher altitudes light brown and shallow and the substrate is locally rocky.

Location and physiography of Menge District

Benishangul Gumuz Region is situated in the Blue Nile River Basin. With the reference to the country, the region is located in the northwest part of Ethiopia. Moreover, the Region is bounded by Amhara, Oromia, Gambella Regional States and the Republic of Sudan in the north, east, south and west respectively (BRFSS, 2004). Menge District is one the eight Districts in Asossa zone, which is located 720 km

northwest of Addis Ababa and 40 km to the North of Asossa town (the capital of the region). It is geographically located between 34° 30` to 35° 10`E and 10° 00` to 10° 30`N. Topography of the region is composed of mainly lowland and plains and a few mountainous and gorges, altitude ranges from about 600 -1700 m.a.s.l (Figure 3-4).

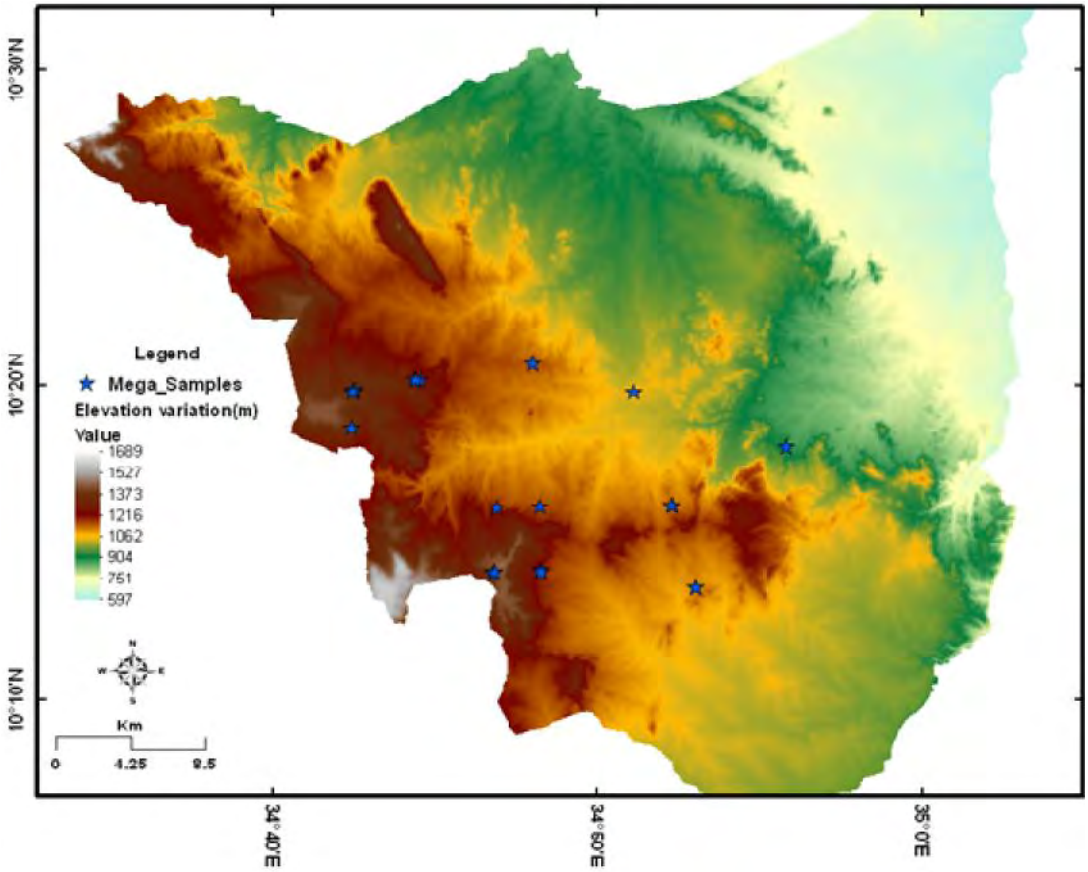

Figure 3-4 Topographic variation of the study sites in Menge District

Vegetation of Menge District

About 55% of the total land area of the District is covered with different vegetation. The major part is covered by natural vegetation, especially bamboo thickets (*Oxytenanthera abyssinica*), broad-leaved deciduous woodlands dominated by *Anogeissus leiocarpa*, *Balanites aegypticus*, *Boswellia papyrifera*, *Combretum collinum*, *Dalbergia melanoxylon*, *Lannea fruticosa*, *Lonchocarpus laxiflorus*,

Pterocarpus lucens, *Piliostigma thonningii*, *Stereospermum kunthianum* and *Terminalia laxiflora* (Sebsebe Demissew *et al*., 2005) and parts of it with grazing and cultivated land. The lowland bamboo tree is one of the dominant species covering large areas (BRFSS, 2004).

The local communities in the district highly depend on forests as sources of construction material, fuel wood, bee keeping and wild foods. Sorghum, millet and maize (covering over 70% of the cultivated land) are the most dominant food crops grown in the area. In addition, pulses, vegetables, fruits, ginger and fibre crops are also grown (BRFSS, 2004).

Climate of Menge District

According to the data obtained from the NMA, Asossa branch office, the surrounding area has mean annual minimum and maximum temperatures of 12°C and 32°C, respectively. The rainfall in the area is unimodal and obtains high rainfall from May to October (Figure 3-5). Generally, the rainfall is erratic from year to year. However, the highest rainfall occurs usually in August. With regard to temperature, the lowest occurs in August while the maximum occurred during the month of January. Annual rainfall varies from 800 to 2000 mm. The temperature reaches a daily maximum of 20°C to 25°C in the rainy season and rises to 35°C to 40°C in the dry season (February to April) (BRFSS, 2004).

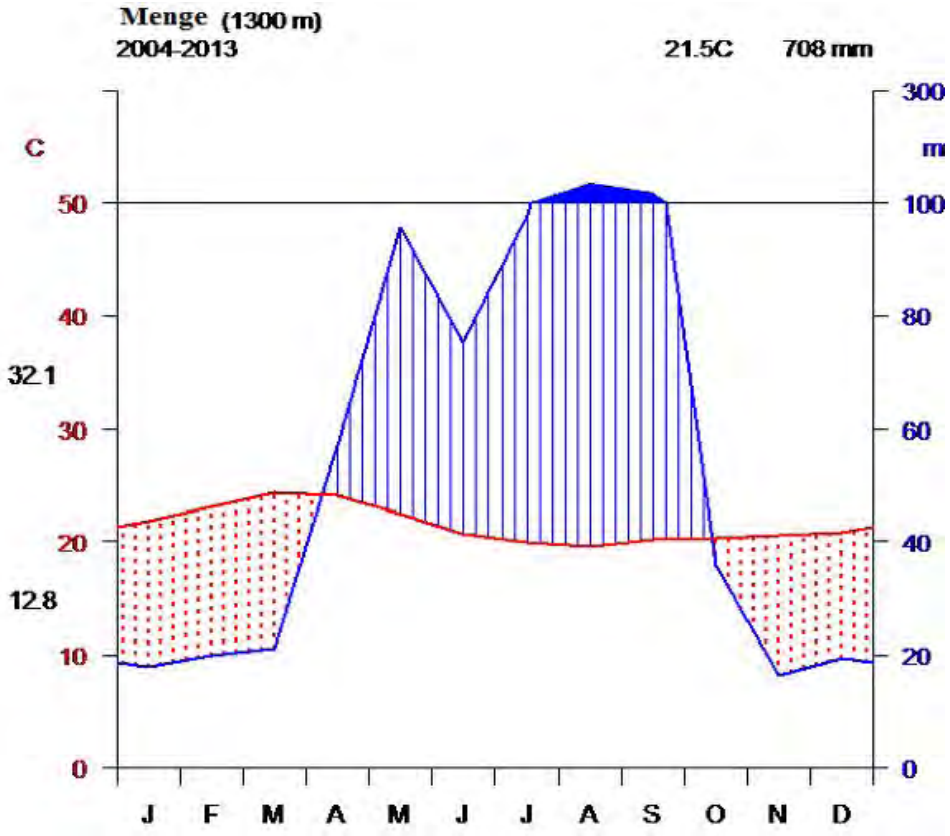

Figure 3-5 Climadiagram of Asossa station

3.2.3. Site selection, establishment of plot and sample collection

During June 15–30, 2011, reconnaissance survey was conducted to get an insight of the vegetation pattern, topography and other environmental conditions in order to locate sampling plots. This preliminary investigation suggested the presence of three characteristic habitat types in the study areas, i.e. forest, grazing land and farming lands with villages. A total of 48 plots, eight from each (Welmera forest (WFO), Welmera grazing land (WGR), Welmera farmland (WFA) and Menge forest (MFO), Menge grazing (MGR) land and Menge farmland (MFA) were chosen.

A plot size of 30 × 30 m each 20 m apart was established for sampling (Gates and Ratkowsky, 2009). Repeated field surveys (every two weeks/plot) were conducted during May –September of 2012–2014 in the study areas.

All visibly present basidiomata were collected, photographed (Canon A470) and their morphological and ecological characteristics were noted along with the range of associated substrates. Methuen Handbook of colour (Kornerp and Wanscher, 1978) was used to compare colors. In this study, irrespective of the number of sporocarps in a cluster, samples have recorded as one observation. A brief note on distinguishing macroscopic features and microhabitat as unique identity were made during collection on the field. The altitude and location of each site were obtained from global positioning system (GPS) readings with Garmin eTrex GPS.

All collected specimens were then labelled, placed in paper bags, and brought to Mycology Laboratory, Addis Ababa University for detail microscopic and macroscopic characterization. The inner tissues of mushroom were taken using sterile forceps and stored at – 20°C using the method of Lee and Taylor (1990) for DNA extraction.

Specimens identification was assisted with standard texts/litratures as a reference, taxonomic classification of species was made according to Kirk *et al*. (2008), and the valid names for the species mentioned in the Index fungorum were reviewed (http://www.indexfungorum.org/names/names). When specimen could not be matched to known species descriptions, it was assigned to a genus and given a species number (e.g. *Agaricus* sp1 (*Agaricus* species. number. 1)). In addition, basidiomata were categorized according to their trophic group (Symbiotic, parasitic or saprophytic). Specimens were dryed, preserved using standard methods (Largent *et al*., 1986) and deposited in Mycology laboratory, Addis Ababa University, Ethiopia,

and duplicates are preserved in the Fungarium (HMAS), Institute of Microbiology, Chinese Academy of Sciences, China.

3.2.4. Macrofungal diversity data analysis

Analysis of species composition and frequency

The dataset used for this analysis was 105 taxa × 48 sites. The frequency, the number of plots in which a species occurs, is computed for each habitat type separately. Percent frequency of each species was calculated by the following formula as suggested by (Aung *et al.*, 2008)

$$\text{Percent frequency of a species} = \frac{\text{Number of plots containing Species}_X \text{ in Site}_n}{\text{Total number of plots examined in Site}_n} \times 100$$

The following frequency classes were used to designate the frequency status of species in each habitat type (Kumelachew Yeshitela, 2008)

≤ 10% occurrence = Rare,
11-20% occurrence = Occasional,
21-30% occurrence = Less frequent,
31-40% occurrence = Frequent,
41-50% occurrence = Very frequent,
>50% occurrence = Dominant

Cluster Analysis

The analysis was done following agglomerative hierarchial classification which depends on how similar (or dissimilar) the plots are to each other. Similar plotes are treated as a homogeneous class or group, whereas dissimilar items form additional classes or groups. The community types distinguished were further refined in a synoptic table with a species having at least 2.5 cover-abundance value in one of the

communities. The macrofungal community types were described and named as 'type' by dominant characteristic species with high cover abundance values.

Macrofungal diversity: -the analysis was done with the most widely used approaches in measuring the diversity of species (Kent and Coker, 1992).

Alpha –Diversity

Shannon – Wiener measure of diversity

The Shannon – Wiener index (Shannon and Weaver, 1949) of diversity is the most commonly used to characterize diversity in a community. In this study, the Shannon – Wiener diversity index, species richness and Shannon`s evenness were computed to describe species diversity of macrofungal community type. The Shannon-Wiener diversity index was calculated as follows:-

$$H' = -\sum_{i=1}^{S} pi \ln pi$$

$$p_i = \frac{n_i}{N}$$

Where H' = Shannon – Wiener index of species diversity (calculated with base e logs)
p_i = proportion of total sample belonging to i^{th} species
N = total number of species, n_i= individual number of species i
The Shannon Diversity Index typically falls between 1.5 and 4.0, with lower values indicating lower diversity, and higher values indicating higher diversity.

Shannon's Index of evenness

The evenness component of diversity expresses how individuals are distributed among the species. Among the various indices of evenness, Shannon's evenness was computed as a ratio of observed diversity to maximum diversity (Magurran, 1988).

$$J' = \frac{H'}{H'_{max}} = \frac{\sum_{i=1}^{S} pi \ln pi}{\ln s}$$

Where J' = Equitability (evenness)

where, H' = Shannon–Wiener Diversity Index; and

H'max = lnS where S is the number of species in the sample (Kent and Coker, 1992)

In = log base$_e$.

Beta – diversity

Beta diversity or between-habitat diversity is the measure of the change in species diversity between habitats or communities. It is usually expressed in terms of a similarity index between communities

Sørensen similarity index

$$\mathbf{Ss = \frac{2a}{(2a + b + c)}}$$

Where S_s =Sørensen similarity coefficient;

a = Number of species in one habitat type

b = Number of species in another habitat type

c = Number of species common to both habitat type

This similarity index was used to measure the pattern of species similarity among communities and habitat type identified in both study sites. The similarity values range from 0 to 1 (1 meaning very similar, 0 indicating no similarity).

3.3. Results

Macrofungal species composition and their distribution in different fungal families

Altogether 105 species were identified from both study sites, belonging to 23 families and 49 genera. Out of these, 92 were identified to a species level and the rest 13 were identified to genus level. The total family/genus ratio of 0.48 and genus/species ratio of 0.44 suggested that the presence of moderate family and generic diversity in the collections. The families that contained the most number of species were *Agaricaceae* (49), *Lyophyllaceae* (12), *Tricholomataceae* (9), *Psathyrellaceae* (6) and *Hymenogastraceae* (4), which together accounted for 76.2% of the total surveyed species, where as *Amanitaceae, Cystofilobasidiaceae, Geastraceae, Inocybaceae, Marasmiaceae, Physalacriaceae, Pleurotaceae, Schizophyllaceae* and *Strophariaceae* were each represented by one genus and one species (Table 3-1). The full species list is presented in Appendix 3.

Table 3-1 Total number of family, genera, species and percentage of species collected in this study

No.	Family Name	Number of Genera	Number of species	% of species
1	Agaricaceae	13	49	42.8
2	Amanitaceae	1	1	0.95
3	Auriculariaceae	1	1	0.9
4	Bolbitiaceae	1	2	1.9
5	Cystofilobasidiaceae	1	1	0.95
6	Entolomataceae	2	2	1.9
7	Ganodermataceae	1	2	1.9
8	Geastraceae	1	1	0.95
9	Hydnangiaceae	1	1	0.95
10	Hymenogastraceae	2	4	4.76
11	Inocybaceae	1	1	0.95
12	Lyophyllaceae	2	12	11.43
13	Marasmiaceae	2	2	1.9
14	Mycenaceae	1	1	0.95

15	Physalacriaceae	1	1	0.95
16	Pisolithaceae	1	1	0.95
17	Pleurotaceae	1	1	0.95
18	Polyporaceae	2	2	1.9
19	Psathyrellaceae	5	7	6.66
20	Schizophyllaceae	2	1	0.95
21	Sclerodermataceae	1	2	1.9
22	Strophariaceae	1	1	0.95
23	Tricholomataceae	9	9	9.52

Species composition in Welmera Distict

Seventy five species belonging to 18 families and 37 genera were examined from the three habitat types investigated in Welmera district, (i.e. forest, grazing land and farm land) (Figure 3-6). Among these, higher number of families, genera and species were recorded from Welmera forest (WFO) (17 families, 32 genera, 60 species) followed by grazing land WGR (7 families, 11 genera, 21 species) and WFA was represented with only 4 families, 10 genera and 15 species.

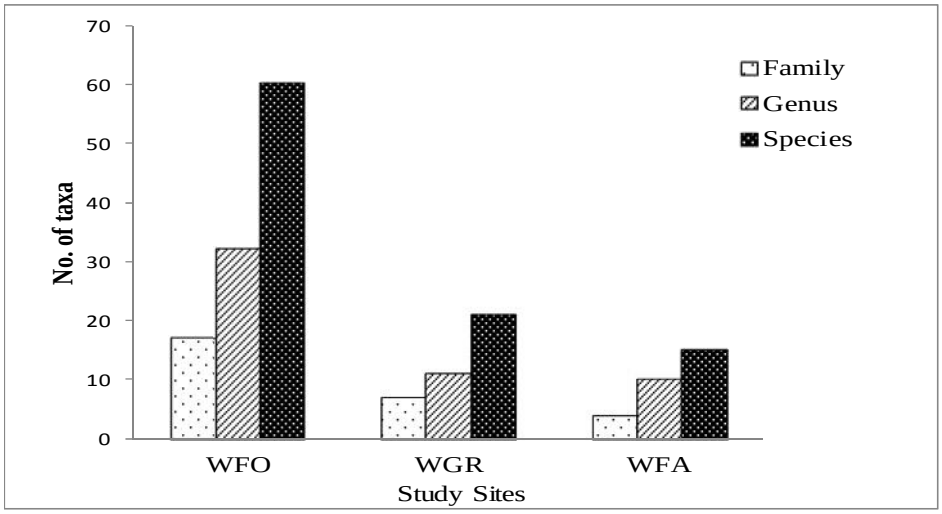

Figure 3-6 Number of families, genera and species in Welmera District

(WFO=Welmera forest, WGR=Welmera grazing land and WFA= Welmera farm land)

Interms of the number of species and genera, family *Agaricaceae* is the species rich family in the three habitats with species number 26, 13 and 9 in WFO, WGR and

WFA respectively followed by *Tricholomataceae, Psathyrellaceae, Marasmiaceae* and *Hymenogastraceae* (Figure 3.7, 3.8 and 3.9). Family *Agaricaceae, Hymenogastraceae* and *Tricholomataceae* were shared by all the three habitats.

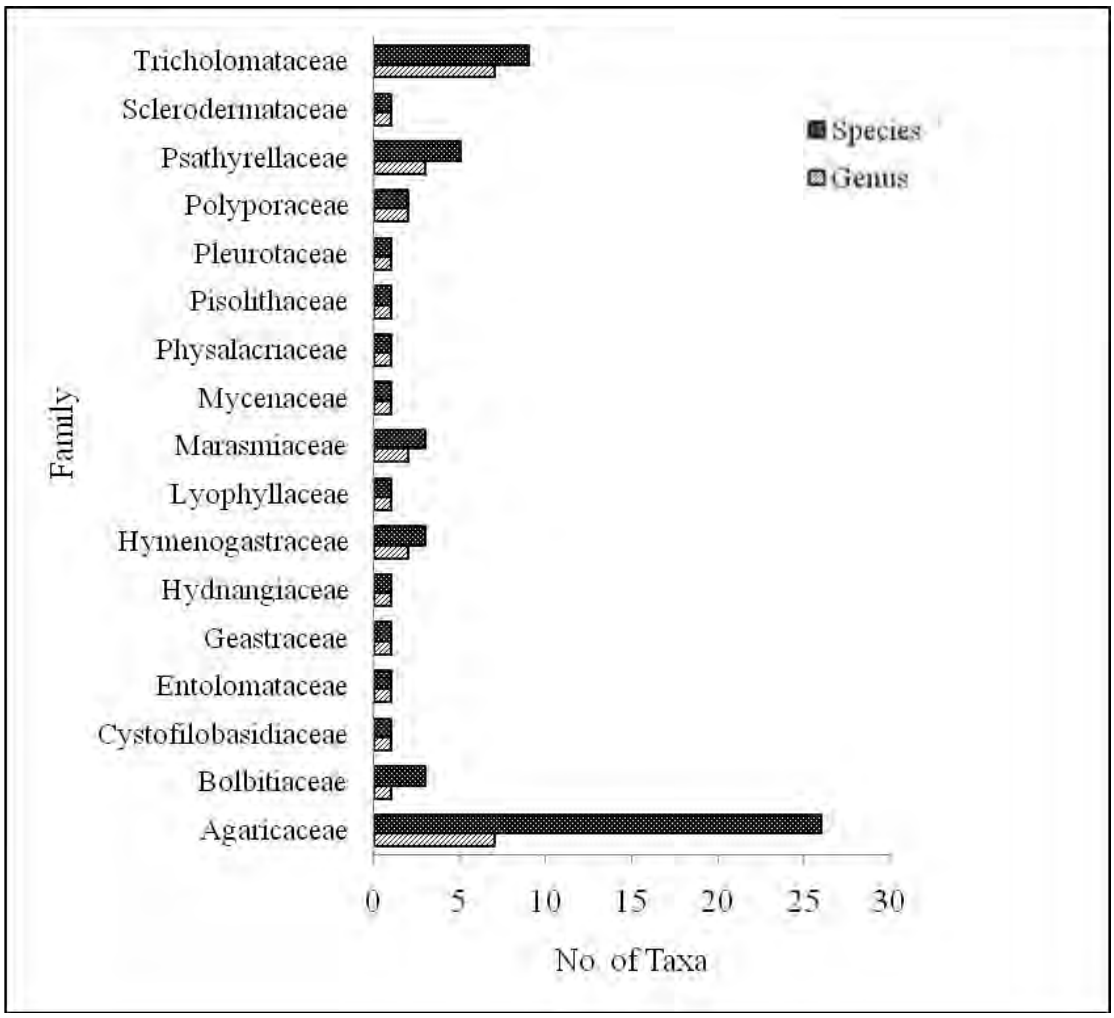

Figure 3-7 Number of species and genera of macrofungi per family in WFO

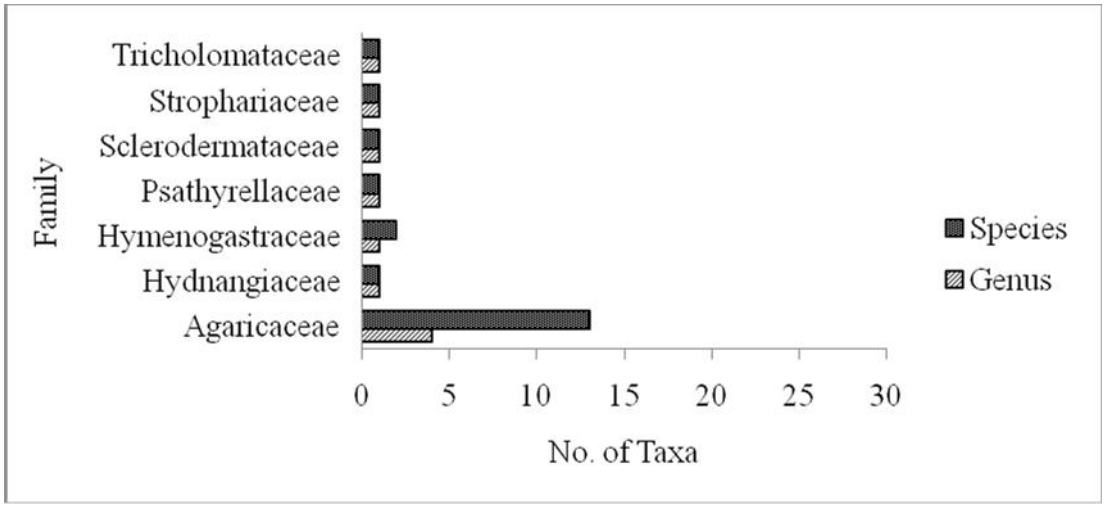

Figure 3-8 Number of species and genera of macrofungi per family in WGR

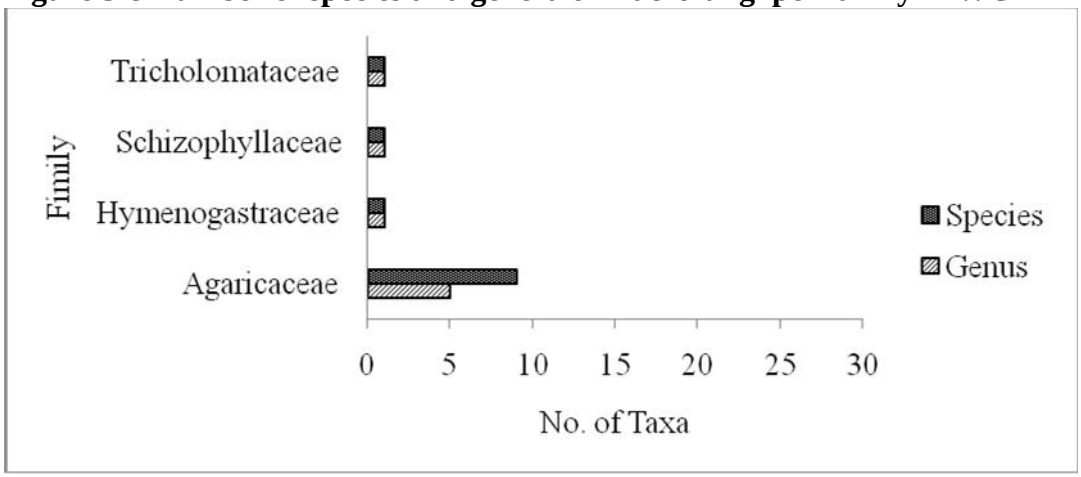

Figure 3-9 Number of species and genera of macrofungi per family in WFA

Overall, there were more species shared between WFO and WGR than between WFO and WFA and between WGA and WFA. The *Agaricus* is the most diverse genus with more than 11 species recorded, followed by *Leucoagaricus* and *Lepiota* with 7 and 5 species respectively. *Agaricus xanthodermus* and *Agaricus* sp1 were found in all habitats.

Species frequency of occurance in Welmera Distict

The frequency of occurrence of species in each habitat of Welmera District is presented in figure 3-10. In WFO most of the species (55%) are occasional while less frequent species made 29%. Only 6% were dominant in WFO. In WGR 19% of the species were occasional whereas less frequent and frequent species made 19% and 28% of the whole species respectively. Very frequent and dominant species made 14% and 19% in WGR. In WFA very frequent and dominant species made each 20% while occasional and rare species made 27% and 1% respectively of the whole species in the area.

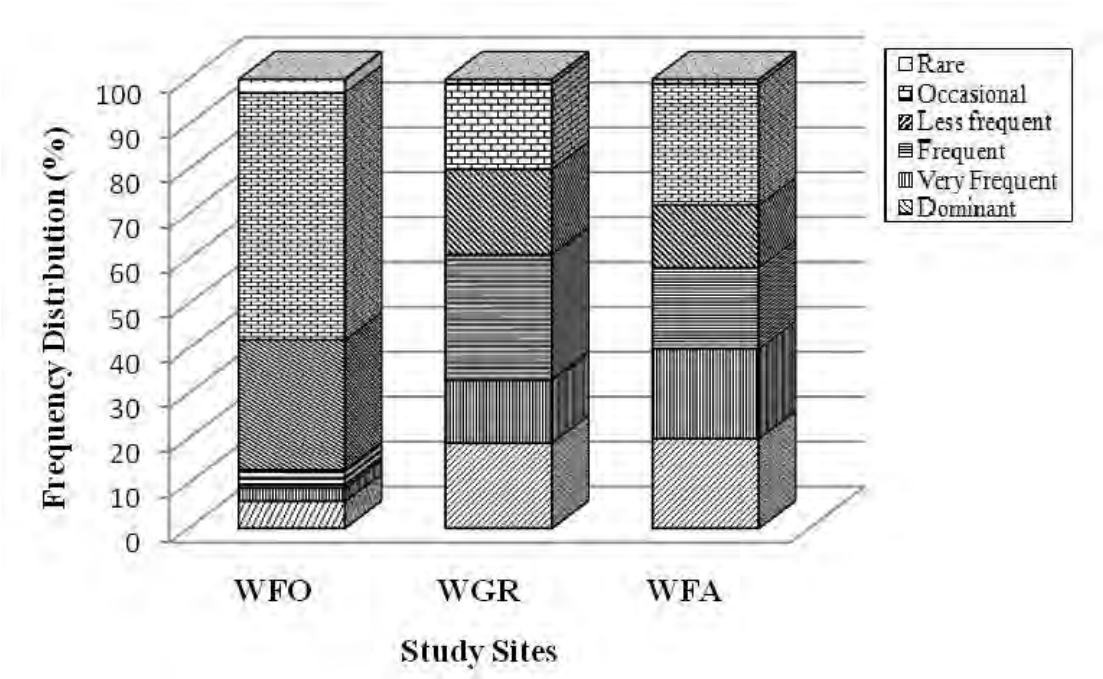

Figure 3-10 Frequency distribution of species of three habitats of Welmera

The distribution of species by trophic groups revealed the dominance of saprobic species in all habitats (Figure 3.11). No mycorrhizal species were recorded from WFA while parasitic species found in lower proportion. There were no termitophlic species collected from all the three habitat types in the district.

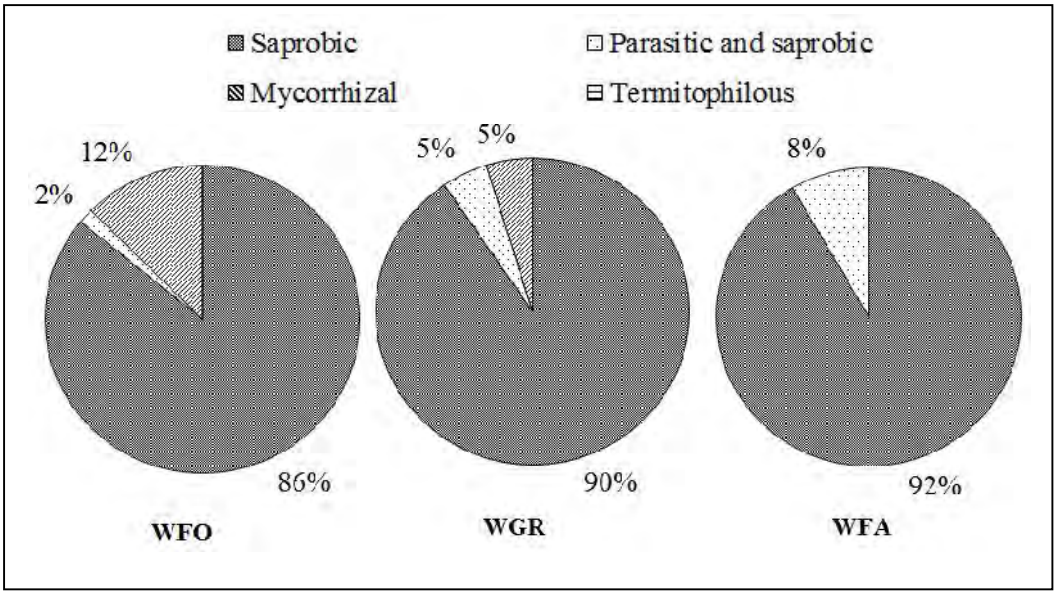

Figure 3-11 Distribution of species by trophic groups in Welmera

Fruiting phenology in Welmera District

Fruiting phenology showed a unimodal pattern each year, with a peak from June to early October (Figure 3-12). The fruiting season is approximately 4- month duration though it varying between years and species type. The total fruitbody recorded over the three years were 556 (203, 172 and 181 in 2012, 2013 and 2014 respectively). The peak number of species occurring in July and August.

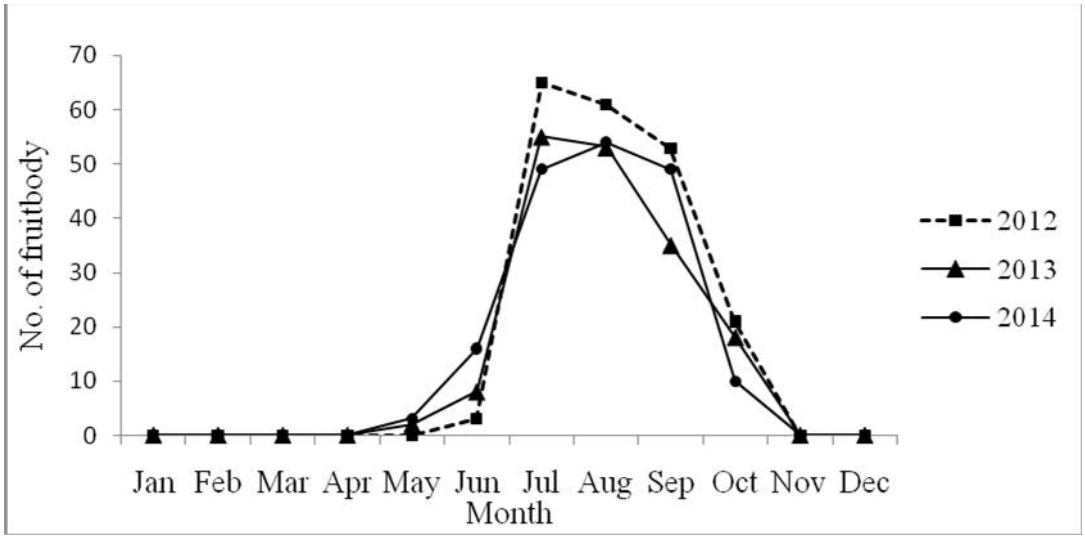

Figure 3-12 Number of fruitbody collected during the survey period in Welmera

The total number of species collected in each month during the study period varied between years. The number of new records decreased throughout the study period, with 43 species in the first study year (2012), 22 additional species in the second year (2013), and only 10 new records in 2014.

Macrofungal species composition in Menge District

A total of 50 species, belonging to 11 families and 23 genera were examined from the three habitats types investigated in Menge District, (i.e. forest, grazing land and farm land) (Figure 3-13). In this study sites the highest number of family, genus and species were recorded from MFO (11 families, 20 genera, 36 species) followed by

MGR (4 families, 9 genera, 21 species) and MFA was represented with only 2 families, 6 genera and 17 species.

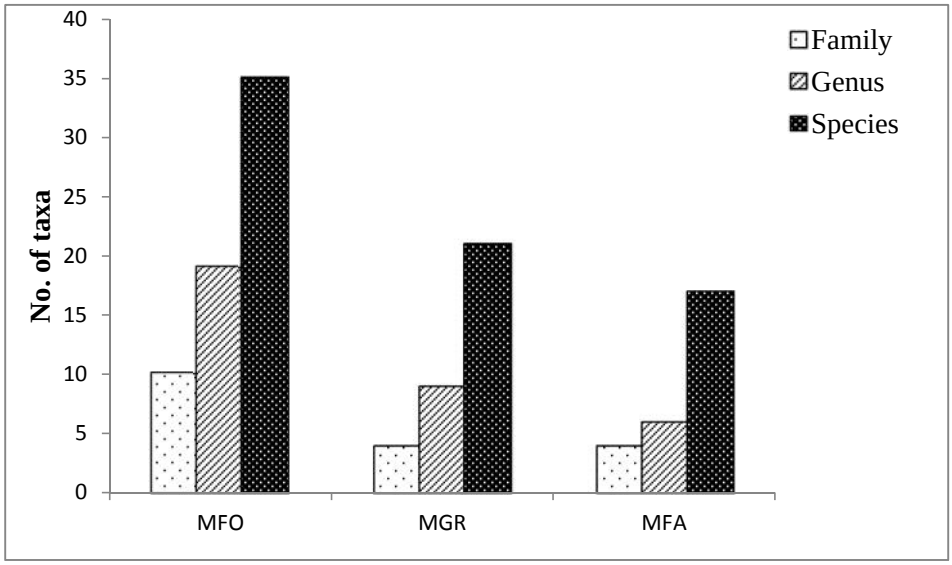

Figure 3-13 Number of families, genera and species in Menge

(MFO=Menge forest, MGR= Menge grazing land and MFA= Menge farm land)

Family Agaricaceae is the richest family in terms of the number of species and genera in all the three habitats with species number 19, 13 and 11 in MFO, MGR and MFA respectively. Family Agaricaceae and Lyophyllaceae were shared by all the three habitats (Figure 3-14, 3-15, 3-16).

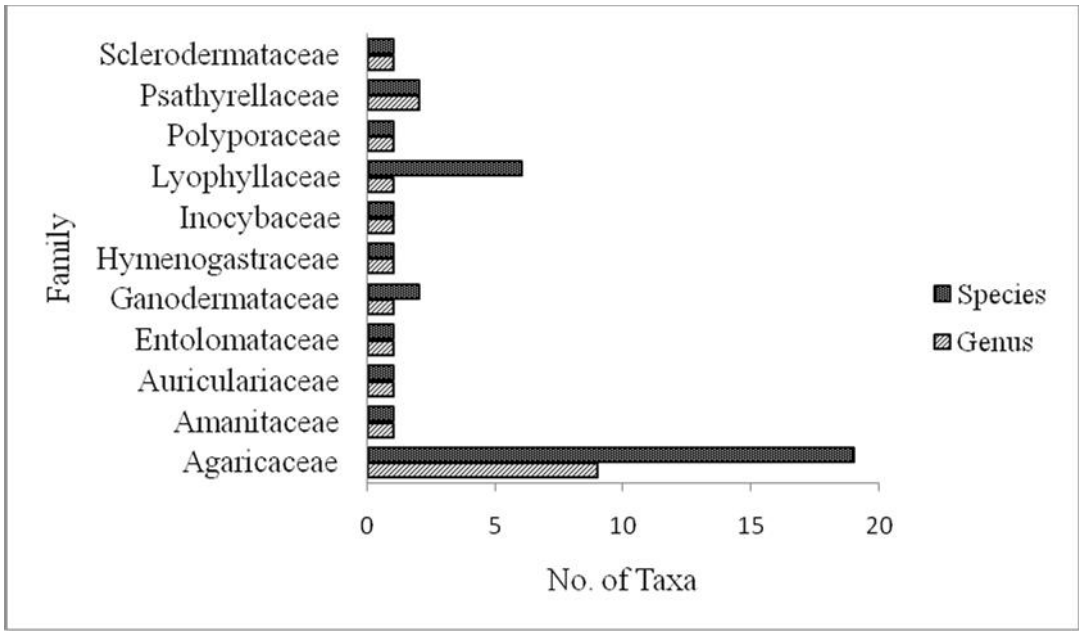

Figure 3-14 Number of species and genera of macrofungi per family in MFO

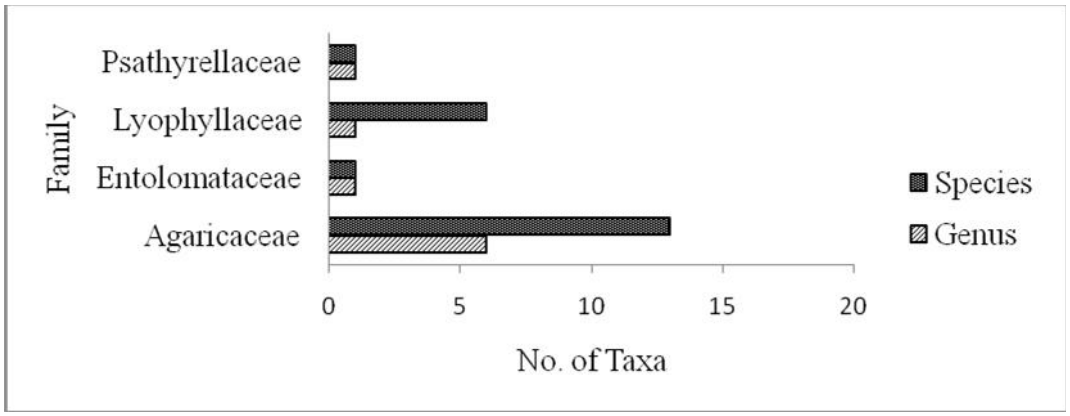

Figure 3-15 Number of species and genera of macrofungi per family in MGR

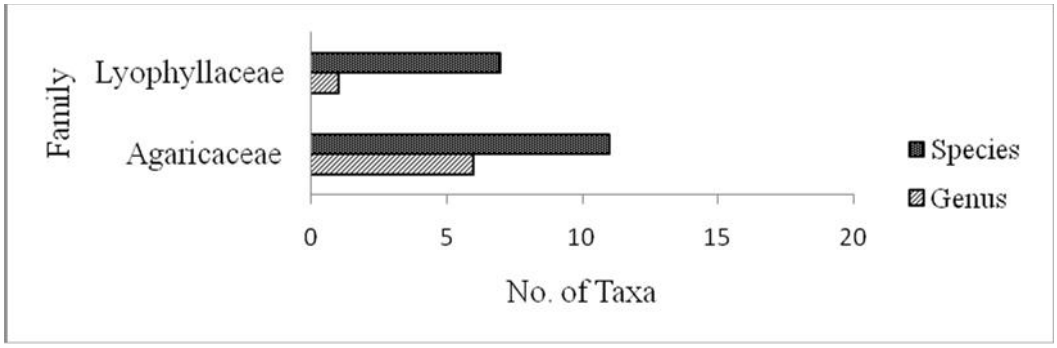

Figure 3-16 Number of species and genera of macrofungi per family in MFA

Species frequency in Menge District

The frequency of occurrence of species in each habitat of Menge district is presented in figure 3-17. In MFO most of the species (68%) were occasional followed by less frequent (23%). Frequent and dominant species made 4% and 2% of the whole species respectively and only 3% were rare. In MGR 54% of the species were occasional. Less frequent and frequent species made each 24% and 17% while very frequent made 3% of the whole species in the area. There is no dominant species in this habitat type. In MFA 33% of the species were occasional followed by less frequent and frequent each with 22% and 20% respectively of the whole species in the area. There is no rare species in MFA.

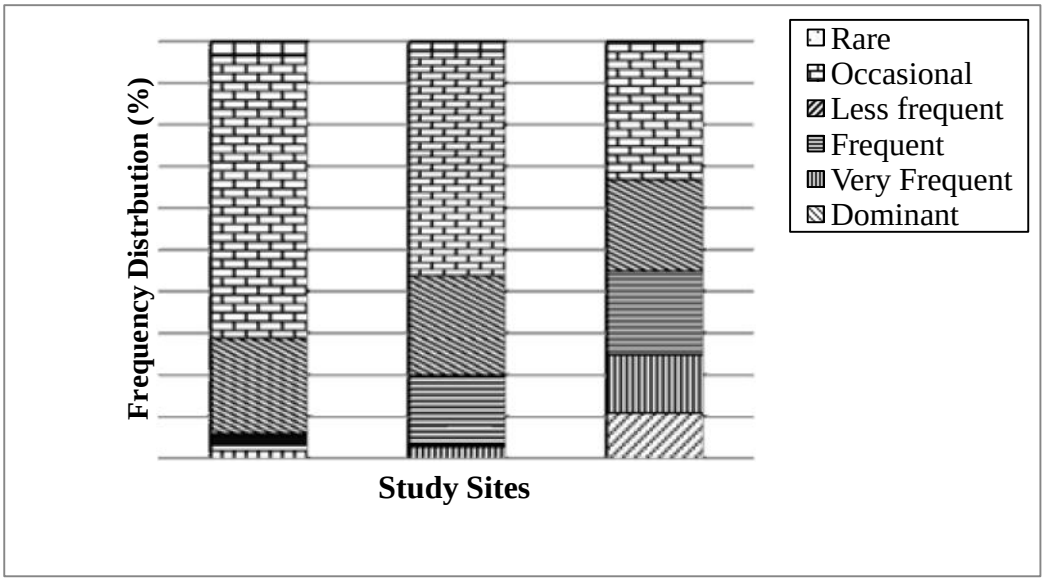

Figure 3-17 Frequency distribution of species of the three habitats in Menge

As it was observed in Welmera District the distribution of species by trophic groups revealed the dominance of saprobic species in all habitat type, 68%, 64% and 59% in MFO, MGR and MFA respectively followed by termitophilous species that account 17 % , 32% and 41% in MFO, MGR and MFA (Figure 3-18). Parasitic species found in lower proportion in MFO (9%) and MGR (4%).

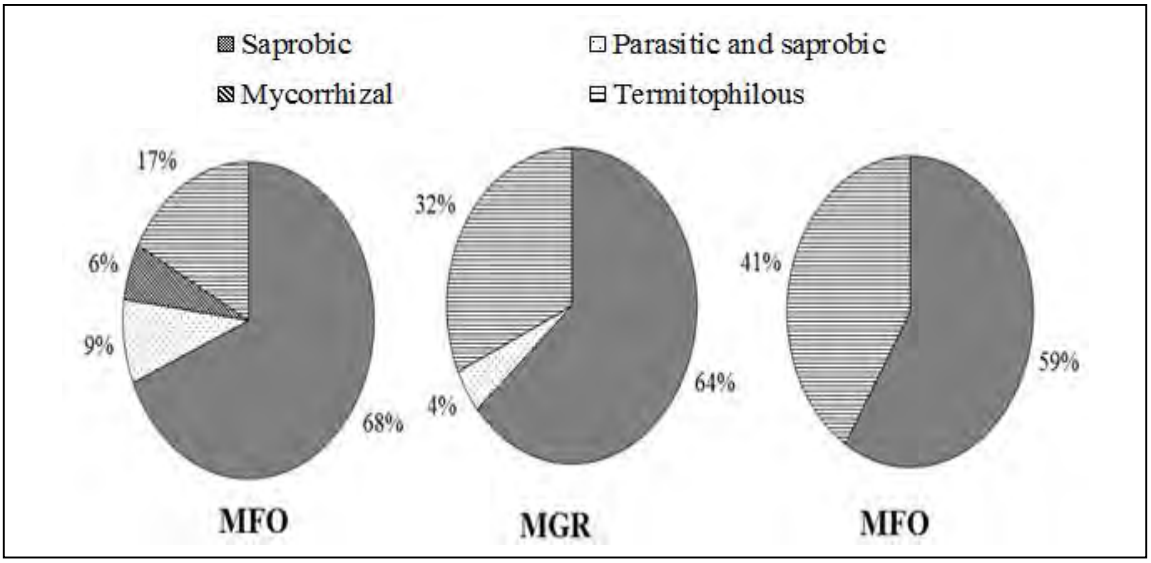

Figure 3-18 Distribution of species by trophic groups of the three habitat type in Menge

Fruiting phenology in Menge distict

During the three years of collection in Menge district fruiting phenology exhibited slightly bimodal pattern. The first wave of mushrooms emerged after a short heavy rain in May and disappeared within 3 weeks. The second wave appeared after a second wave of rain that starts in late June. However, it has shown some decline in June, generally higher number of collections were made from mid July to September (Figure 3-19). The numbers of fruitbody collected over the three-years were comparable. The fruiting season is approximately five months.

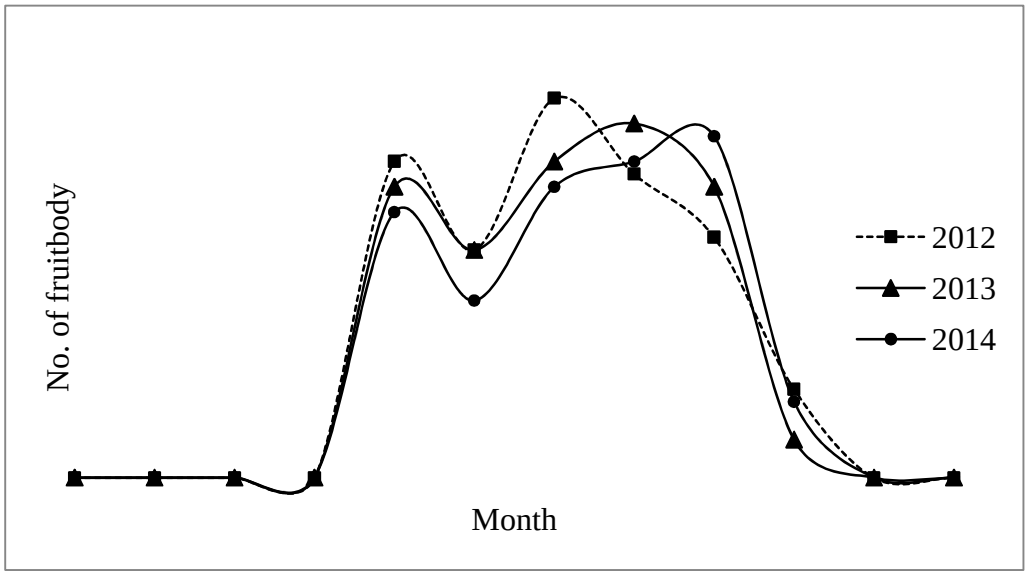

Figure 3-19 Number of fruitbody collected during the survey period in Menge

The number of new recordes during the study period (2012-2014) varied between years. It decreased throughout the study period, with 26 species in the first study year (2012), 15 additional species in the second year (2013), and only 9 new recorded species in 2014. A collection of species in 2012 and 2013 contributed most to the total macrofungal species richness of the study area.

Mean Species Number in each habitat type

Generally, significantly higher (t-value 2.028, $p<0.05$) number of species were collected from Welmera District than in Menge District during the study period. There was more species number per plot in WFO than MFO (t-value 3.8, $p<0.05$). In WFO the mean of 13.3 species were recorded per plot, where as in MFO a mean of 8.25 species were recorded (Table 3-2). The mean species per plot in WGR (8) were significantly different from that of MGR (6.1). However, the mean species per sample of WFA did not show a significant difference as compared to MFA (t-value 2.11, $p=0.054$).

Table 3-2 Paired statistics between same types of habitat from the two sites

Sample	N	Mean	Std. Error Mean	t-value	*p*
WFO	8	13.375	0.924	3.8	0.002
MFO	8	8.25	0.940		
WGR	8	8	0.462	2.19	0.046
MGR	8	6.125	0.718		
WFA	8	5	0.534	-2.11	0.054
MFA	8	6.375	0.375		
Total Welmera	24	8.7917	0.81200	2.028	0.048
Total Menge	24	6.9167	0.44198		

Shanon- Wiener diversity of each habitat type

The results of the α-diversity analysis of macrofungi based on Shanon- Wiener diversity index of each habitat type are presented in (Table 3-3). Calculation of t-test showed a very high significant difference (t-value=3.8, $p<0.05$ (=0.002)) in the Sannon index of Welmera and Menge evidencing a higher α-diversity in Welmera than in Menge. In both cases, the forests have higher diversity than the edges which are used for grazing and farming.

Table 3-3 Diversity Index of macrofungi in the two study area

Habitat	No. of Plots	Richness (S)	Evenness (E)	Shannon (H)
WFO	8	**62**	0.9121	**3.764**
WGR	8	20	0.9409	2.818
WFA	8	26	0.9314	2.582
MFO	8	**44**	0.9445	**3.574**
MGR	8	26	0.9168	2.987
MFA	8	23	0.8717	2.733

Macrofungal community types/Cluster Analysis in the study sites

The results from hierarchical cluster analysis using similarity ratio output showed five different community types (clusters) for the study sites (Figure 3-21).

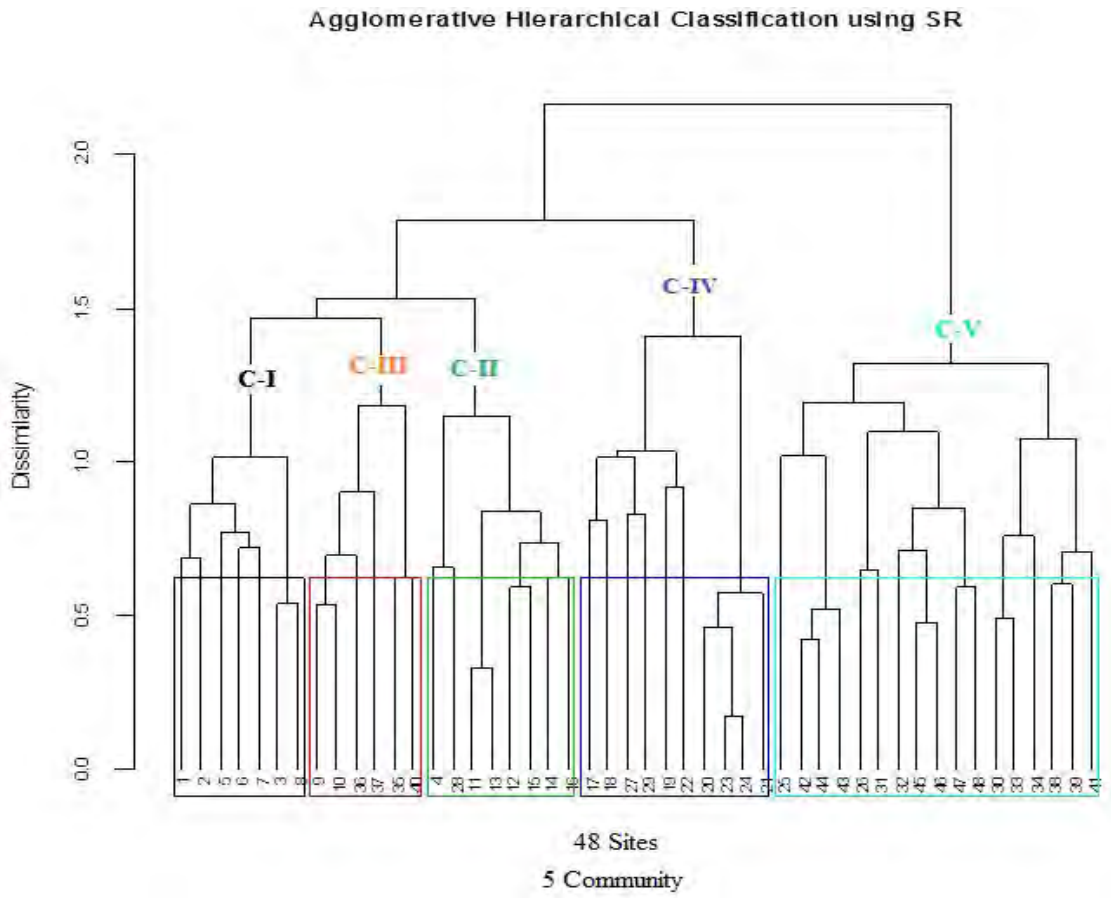

Figure 3-20 Dendrogram obtained from hierarchical cluster analysis

Each community contain the following plots

C-I: - 1, 2, 3, 5, 6, 7 and 8

C-II: - 4, 28, 11, 13, 12, 15, 14 and 16

C-III: - 9, 10, 35, 36, 37 and 40

C-IV: - 17, 18, 27, 29, 19, 22, 20, 23, 24 and 21

C-V: - 25, 42, 44, 43, 26, 31, 32, 45, 46, 47, 48, 30, 33, 34, 38, 39 and 41

The following Table 3-4 shows the Synoptic values of species in each community. Each macrofungal species are different in their value in this summary table. This difference is based on the abundance in the community. The indicator species for each

community type were selected based on the values (highest mean abundance species). The species which are found only in a particular community type was considered as diagnostic species.

Table 3-4 Synoptic table of the communities
(figures in bold relate to the indicator species for each cluster/community)

Species name	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Leucoagaricus vassiljevae	**6.86**	0.00	0.00	0.00	0.29
Agaricus californicus	**4.86**	0.88	2.33	0.50	0.29
Leucoagaricus littoralis	**3.86**	0.00	0.00	0.00	0.00
Leucoagaricus rubrotinctus	**3.43**	0.00	0.00	0.00	0.29
Agaricus caribaeus	**3.86**	0.75	0.00	0.50	0.47
Lepiota acutesquamosa	2.71	1.50	0.00	0.00	0.65
Agaricus xanthodermus	5.71	**7.13**	0.00	1.90	1.24
Psathyrella candolleana	0.00	**4.75**	0.67	0.00	0.88
Pseudoomphalina pachyphylla	0.00	**3.75**	0.00	0.00	0.00
Agaricus bisporiticus	0.00	**3.50**	1.33	**2.40**	2.06
Vascellum curtisii	0.71	2.88	0.00	0.00	0.00
Micropsalliota spp1	0.00	2.88	0.00	0.00	0.00
Agaricus megalosporus	1.00	2.25	2.17	0.00	0.29
Agaricus purpurellus	1.00	2.25	0.00	0.50	0.53
Macrolepiota dolichaula	**5.14**	1.50	**6.00**	0.00	0.59
Agaricus campestris	0.00	**3.40**	**3.50**	0.00	0.00
Laccaria glabripes	1.71	**3.75**	**4.00**	0.00	0.00
Agaricus microvolvatulus	0.71	0.00	**3.67**	0.00	0.29
Micropsalliota globocysti	0.00	2.13	**2.83**	0.00	0.47
Psilocybe ovoideocystidiata	0.86	1.50	**2.67**	0.00	0.00
Termitomyces umkowaanii	0.00	0.00	2.33	0.00	0.00
Psilocybe samuiensis	0.00	0.75	1.50	0.00	0.00
Psilocybe coprophila	0.00	0.00	0.00	**2.50**	0.00
Leucoagaricus viriditinctus	0.00	0.00	0.00	**2.30**	0.82
Schizophyllum commune	0.00	0.00	0.00	**2.20**	0.47
Lepiota cristata	1.00	0.00	0.00	**2.10**	0.29
Termitomyces robustus	0.00	0.00	0.00	0.00	**3.71**
Termitomyces schimperi	0.00	0.00	0.00	0.00	**3.29**
Termitomyces eurrhizus	0.00	0.00	0.00	0.00	**3.59**
Termitomyces aurantiacus	0.00	0.00	1.00	0.00	**3.00**
Termitomyces letestui	0.00	0.00	1.17	0.00	**2.00**
Termitomyces microcarpus	0.00	0.00	0.00	0.00	1.47
Termitomyces clypeatus	0.00	0.00	0.00	0.00	1.00

Description of the five community types:

Community/Cluster I

This community contains lower number of plots 7 (14.6%), however, the richest in the number of species, since it consists of 56 (53.3%) species. All the plots in this community were from WFO (Mnagesha forest). It consists of plots that are located at the highest elevation when compared to other communities in the study area; that is between 2340-2530.

In addition, the following characteristics/dominant species were recorded from this community type: *Leucoagaricus vassiljevae, Agaricus xanthodermus, Agaricus californicus, Macrolepiota dolichaula,* while *Agaricus caribaeus, Lepiota acutesquamosa, Laccaria glabripes* and *Leucoagaricus littorallis* were with lower mean abundance in this community.

Community/ Cluster II

There are 8 (16.6%) plots and 30 (28.6%) species included in this community. This community type is found at the moderate altitudinal gradient i.e., between 2505 - 1440 m a.s.l. All the plots are from WGR (plots 11, 12, 13, 14, 15, 16) except one from WFO (plot 4) and another MFO (plot 28).

The abundant species in this community type were *Agaricus xanthodermus*, *Psathyrella candollean, Pseudoomphalina pachyphylla, Agaricus bisporiticus, Agaricus campestris, Laccaria glabripes.* Moreover, *Agaricus megalosporus*, *Agaricus purpurellus* and *Micropsalliota* sp1 occur with lower abundance.

Community/Cluster III

This community type consisted of 20 (19.04%) species scattered in six (12.5%) plots. The altitudinal range is 2254-1005 m a.s.l. All the plots were from grazing land of

Welmera (WGR plots 9 and 10) and Menge (MGR plots 35, 36, 37 and 40). In addition, the following species were recorded as abundant species in this community type: *Macrolepiota dolichaula*, *Laccaria glabripes*, *Agaricus campestris*, *Agaricus microvolvatulus*, *Micropsalliota globocystis*, *Psilocybe ovoideocystidiata*.

Community/Cluster IV

In this community type, 10 (20.8%) plots with 23 (21.9%) species were included. This community type is located at moderate altitudinal gradient i.e., between 2115-1440 m a.s.l. This community type includes all the plots located at WFA however, it also include two plots from MFO. In addition to the characteristic/dominant species indicated in the table 3-4 above, the following species were recorded frequently from this community type; *Agaricus xanthodermus*, *Clitocybe fragrans,Chlorophyllum hortense* and *Agaricus bisporiticus*.

Community/Cluster V

There are 17 (35.4%) plots and 52 (49.5%) species recorded from this community type. The altitudinal range is 1465-750 ma.s.l. All the plots in this community type were from Menge District (plots 25 and 26 from MFO; 31-34 and 38 from MGR and 41-48 from MFA). This community type consists of plots that are located at the lowest elevation when compared to other communities in the study area. Even though it consists of a higher number of plots, it contains less number of species compared to other community.

Species in the genus *Termitomyces (T. robustus, T. schimperi, T. eurrhizus, T. aurantiacus, T. letestui, T. microcarpus)* are the dominant species in this community type. However, *Agaricus bisporiticus, Chlorophyllum molybdites* and *Micropsalliota*

albosericea. Agaricus xanthodermus, Bovista aestivalis, Leucoagaricus hortensis were recorded with lower abundance.

Table 3-5 shows, the Shannon diversity index, evenness and species richness of each community type. All of the community types have more or less different number of species and evenness. Comparison among the identified macrofungal communities showed the highest value of overall species richness (57), species diversity (Shannon's diversity) (3.67) for community 1, and species evenness (0.933) for community 4 (Table 3-8).

Table 3-5 Altitudinal range, species richness, diversity and evenness values of the five communities

Community type	Altitudinal range (meters)	Species Richness (S)	Shannon's evenness index (E)	Shannon-Wiener Diversity Index (H')
1	2340-2530	57	0.908	3.67
2	2505 - 1440	30	0.918	3.12
3	2254-1005	20	0.915	2.74
4	2115-1440	23	0.933	2.92
5	1465-750	52	0.853	3.37

In addition, overall species similarity analysis of the five macrofungal communities showed more similarity (0.85) between communities two and three, than between any of the others. The least species similarity with any other community was recorded for community one and four (Table 3.6).

Table 3-6 Sorensen's similarity coefficient of the five communities

Community	1	2	3	4	5
1	-				
2	0.17	-			
3	0.14	0.85	-		
4	0.12	0.68	0.41	-	
5	0.14	0.77	0.56	0.56	-

3.4. Discussion

Macrofungal Species diversity in the study sites

The present study was conducted from May–September of 2012–2014 without any prior knowledge or pre-existing information on the diversity, distribution or fruiting patterns of the area. Thus, this study constituted the first relatively long-term systematic, monitoring of agaric mushrooms diversity in some parts of Ethiopia. The study involved three different habitat type in Welmera district (high land) and Menge district (low land) located in central and northwest part of the country respectively. Significantly higher ($p<0.05$) number of species were collected from Welmera district than Menge district during the study period. Results have revealed that the study site in Welmera district is rich in species composition as shown by the presence of 75 species (distributed in 37 genera and 18 families) where as in Menge district 50 species were recorded which are distributed in 23 genera and 11 families.

This higher diversity in Welmera is attributed by WFO (Menagesha suba forest) that contrbute 60 species. In WFO a mean of 13.3 species per sample was recorded, where as in MFO it was 8.25. This result has showed protected forests support more macrofungal species diversity than unprotected forest similar studies in Colombian Amazon forests (Lopez-Quintero *et al.,* 2012), India (Brown *et al.,* 2006), Australia (Sapphire *et al.,* 2002), Ireland (O'Hanlon and Harrington, 2012) has reported similar result. Since fungal diversity is highly associated with the diversity of vascular plant (Sapphire *et al.,* 2002; Garibay-orijel and Cifuentes, 2006) the difference in the vegtation type in two forest investigated where MFO is a Dry Evergreen Afromontane forests which is dominated by *Juniperus procera, Olea europea,Podocarpus falcatus* and *Allophylus abyssinicus*, where as MFO (forests of a lowland type) is characterized

by mixed bamboo-broadleaf deciduous forest dominated by the solid-stemmed bamboo *Oxytenanthera abyssinica* and different species of trees including *Anogeissus leiocarpa, Balanites aegypticus, Boswellia papyrifera, Combretum collinum, Dalbergia melanoxylon, Lannea fruticosa, Lonchocarpus laxiflorus, Pterocarpus lucens, Stereospermum kunthianum* and *Terminalia laxiflora*. This difference in vegtation type could be considered as one of the main factors for the difference in macrofungal species distribution and diversity in the two study sites.

The mean species per sample of WGR (8) were significantly different from that of MGR (6.1) ($p<0.05$). This might be due to less grazing intensity in Welmera than Menge where cattle keeping is the major activity in the latter district. However, the mean species per sample of WFA did not show any significant difference as compared to MFA ($p=0.054$). The mean species per sample of these habitat type in both study sites is the lowest when compared to the other two habitat types. As the same report by O'Hanlon and Harrington (2012) disturbance of the land for farming activity and application of different agro-chemicals (pestside, fertilizer) in this area has a major effect on macrofungal community structure and diversity.

The observed Shannon-Wiener diversity index (H'=3.76) of WFO showed the highest species diversity of the whole habitat investigated followed by Menge forest (MFO) with (H' = 3.57). This value was found to be higher to similar studies in China (Li *et al.,* 2012) evergreen broadleaf montane forests with H'=3.28, bamboo forest H'= 2.37 and mixed bamboo- broadleaf forest (H'= 2.85) and broadleaf forest reported in Taiwan H'=3.43 (Lin *et al.,* 2011). Studies on some forest types in Ireland, Britain, Oregon (USA) and Vancouver Island (Canada) have reported with higher H' that

range from 4.8 - 3.0 (O'Hanlon, 2011; Humphrey *et al.*, 2003; Outerbirdge, 2002). Results also revealed WGR, MGR and MFA are the 3rd , 4th and 5th with H'= 2.8, 2.9, 2.7 respectively while WFA was found to be the last.

Generally, the observed macrofungal diversity of forest in this study is in line with the general pattern of high species richness presence in East African montane forests (Alexander and Selosse, 2009). According to Coetzee (1978), among the Afromontane forest regions, the East African montane have the richest and most diverse flora. The finding shows the pattern that most tropical forests are reservoir of high biodiversity.

Accordingly, the high H' value of WFO indicates rich macrofungal diversity, which might relate to the relatively high elevation extending from 2340 to 2530 m a.s.l., ample temperature and precipitation and low disturbance which may induce different microclimatic conditions. These environmental factors in turn influence growth, and distribution pattern of macrofungal communities in an area.

Similarly WFO has the highest species richness (S= 62) followed by MFO with S = 44, WFA and MGR each with S = 26 while WGR the last with S = 20. This indicate local climatic variations, land use and land cover and habitat disturbances could be among the factors most responsible for variations in species richness and evenness in a given forest and disturbed area surrounding the forests.

In addition, the local communities in Menge district largely depend on wild food, have a greater anthropogenic influence on the forest. As reported in several studies

(Egli *et al.*,2006; Román *et al.*, 2006; Lin *et al.*, 2011), trampling can reduce fruit body production to about 70 % and reduce the mean number of fruiting species per year. Thus trampling of the forest floor associated with wild food collection including mushroom harvesting in MFO could be the reason for the low mushroom richness of the forest in addition to the influence of rainfall, soil, vegtation type and topography (Lin *et al.*, 2011).

Around 95% of the macrofungi documented in this study are new records for Ethiopia. Although there has no checklist of macrofungi of Ethiopia so far, there have been few works on the mycota of the country. The number of macrofungal species published until the present time all together was not more than 20 (Pegler, 1977; Ryvarden and Johansen, 1980), Dawit Abate, 1998; Tuno, 2001; Taddesse Yebo, 2010; Teferi Yenealem *et al*., 2013; Woldegeorgis *et al.*, 2014; Rediet Sitotaw *et al.*, 2015a,b). This number indicates that macrofungi have been overlooked for so long that their diversity and distrbution in the country is almost unknown. Despite these limitations, the current survey is still valuable to assess the macrofungal diversity in the two districts providing a general picture.

Macrofungal species composition of the five community types in the study sites

The highest representation of species from family Agaricaceae in Welmera district (26 species, 34.6%) and Menge district (19 species, 38%) agrees with the observation by Pegler, (1977) that it is one of the species-rich families in east Africa. The dominance of this family could be attributed to their efficient dispersal mechanisms (Go´mez and Williams, 2011) and adaptation to a variety of ecological conditions (Hawksworth, 2001).

Results also show that next to members in family Agaricaceae, species in the family Tricholomataceae and Lyophyllaceae outnumber in Welmera and Menge district respectively when compared to the other species. This observation agrees with similar patterns of the dominance of these families elsewhere in other tropical countries such as India (Pushpa and Purushothama, 2012), China (Li *et al.*, 2012) and Tanzania (Tibuhwa *et al.*, 2011; Tibuhwa, 2012b). The dominance of Lyophyllaceae (with the predominant genus *Termitomyces*) in Menge district could be related partly to the presence of termite in the region, which lives in association with the genus *Termitomyces*. During preliminary survey, farmers in the district have mentioned serious losses of crops by termite and striga which are responsible for about 30-40% (DIA, 2001) annual crop loss in the field and store.

Analysis on macrofungal community composition was done to see if habitats in each region had a distinctive macrofungal assemblage. Differences in species composition observed among the five macrofungal communities identified and this pattern can best be explained by differences in environmental/physical gradients (elevation, climate (especialy microclimate)), biotic responses to these gradients and human-induced environmental disturbances. Similar studies reported that, this environmental variables were recorded as factors that affect patterns of macrofungal distribution (Ratkowsky and Gates, 2008; Gates *et al.*, 2011; O'Hanlon, 2012; O'Hanlon and Harrington, 2012)

Hedberg (1964) stated that elivation affects atmospheric pressure, temperature and moisture in an area which in turn have a directly impact on growth and development of different macrofungal species, and the corresponding patterns of species

distribution or community structure. The role of altitudinal gradients in shaping distribution of flora in East African mountains has also been mentioned by Hamilton (1982). In addition, since most forests are also exposed to human interference principally for food, fuel wood and construction material requirements, disturbances are evident. Such human-induced disturbances are also mentioned to affect local (community) level species diversity.

Community 1 (*Leucoagaricus vassiljevae* - *Agaricus xanthodermus* community) was composed of plots located at higher altitudinal ranges 2340-2530 m a.s.l., (Menagesha suba forest.) and the richest in the number of species, since it consists of 56 (53.3%) species collected in this study. Most species of these genera (*Leucoagaricus* and *Agaricus*) have been described from temperate forests in North America and Europe (Akers and Sundberg, 1997; Vellinga *et al*., 2003; Ratkowsky and Gates, 2005; Geml *et al.,* 2008; Vellinga, 2009). However many studies have reported the general pattern of presence of these genera dominated communities in tropical forests (Liang *et al.,* 2010; Kumari and Upadhyay, 2013; Sysouphanthong *et al.,* 2013).

Additionally, the higher species richness and diversity in community one may be due to the low level of antropogenic disturbance since the area is well protected forest. Similarl findings were reported by Thaung, (2007), Geml *et al.,* (2008), and Lopez-Quintero *et al.* (2012), the absence of ecological pressure due to grazing and fuel wood collection increases the proportion of the macrofungi in most mountain forests. In Menagesha forest, there is relatively less disturbance and human interference, this make the general finding of the trend of increasing in species richness in protected areas (O'Hanlon & Harrington, 2012). Accordingly, few species like *Leucoagaricus*

vassiljevae, Agaricus xanthodermus, Agaricus californicus, Macrolepiota dolichaula, while *Agaricus caribaeus, Lepiota acutesquamosa, Laccaria glabripes* and *Leucoagaricus littorallis* were often observed in this community.

The dominance of *Agaricus campestris, Agaricus bisporiticus, Agaricus microvolvatulus, Agaricus xanthodermus, Laccaria glabripes, Psilocybe samuiensis* and *Psathyrella candolleana* in community two and three (which consist of plotes in the grazing area) indicated ample presence of characteristic species of grazing land in the study areas. *Agaricus campestris* was also mentioned to form the community of grazing land in the north and central highlands of Ethiopia (Dawit Abate, 1999). The other characteristic species of grazing land, i.e. *Psilocybe ovoideocystidiata, Psilocybe samuiensis* which is commonly grown on cow dung was observed in this community type. However, these species have been subjected to anthropogenic pressure due to overgrazing by domestic livestock and expansion of the industry zone and settlement area around this study site in Welmera district. There is also a similar report from Australia (O'Hanlon and Harrington, 2011, 2012) and tropical cloud forest (Morris *et al*., 2009).

The low number of species 23 (21.9%) in community four (consist of plotes from farm land in Welmera District) indicates that the area is highly disturbed. In addition, this community type has the higher evenness and lower diversity indicating few species are dominating the community. The dominance of *Lepiota cristata, Agaricus xanthodermus, Leucoagaricus viriditinctus, Psilocybe coprophila* and *Schizophyllum commune* in community four indicated the presence of characteristic species of farmland which share more or less similar species of grazing land in the study areas. This community type is located at moderate altitudinal gradient i.e., between 2115-

1440 m a.s.l in Welmera where the natural vegetation has removed due to population settlement and extensive agricultural practices (Eshetu Yirdaw, 2002; Dinkissa Beche, 2011) and highly subjected to anthropogenic pressure.

All the plots in community five are from Menge district farming land located at lowest altitudes (1465-750 m) compared to the rest of the communities and were used for cultivation for decades. However, *Termitomyces robustus*, *T. schimperi*, *T. eurrhizus*, *T. aurantiacus*, *T. letestui*, *T. microcarpus*, *Chlorophyllum molybdites* and *Micropsalliota albosericea* were found as a dominant species in this community. Even though it consists of the higher number of plots (17, (35.4%)), it has less number of species when compared to other community type this might be due to high disturbance and human interference, low precipitation and high temperature in the area.

Although different species dominated the identified macrofungal communities in both study sites, various degrees of intermingling of similar species across communities was observed. This can be attributed to an overlap in altitudinal ranges among communities, and the resulting relatively small differences in environmental gradients across adjacent plots forming different communities. According to Lopez-Quintero *et al.,* (2012) and Ohenoja, (1993) minor differences in species composition over macrofungal communities might reflect adaptations of species to different environmental conditions together with efficient dispersal of the respective species forming the communities. It is clearly indicated that species in the genus *Agaricus*, *Leucoagaricus, Termitomyces* and *Macrolepiota* have frequently been observed as dominant in most plots investigated. The similarities between fungal species on these

different ecosystems may reflect the fact that they encompass species with diverse ecological requirements (Richard *et al*. 2005).

Highest numbers of mycorrhizal species were collected from MFO. Studies have shown that number of mycorrhizal species in a given ecosystem dependents on the host plant's age (Dighton *et al.,* 1986; Kernaghan *et al.,* 2003), that actually increase gradually with host plant age and agro-forest stands (Smith *et al*., 2002; Morris *et al*. 2009; Mason *et al*., 1987). As MFO (Menagesha forest) is one of the oldest forests in Africa, with higher plant species diversity (Dinkissa Beche, 2011), it is expected that it support the growth of higher number of mycorrhizal species than the rest of the study habitats.

The distributions of macrofungal species between communities have shown different similarity patterns. The overall Sorensen's similarity coefficient ranges from 0.12 – 0.85. The highest similarity was observed between community two and three (0.85), revealed more species shared between both communities. This could be due to the existence of most plots of this communities are located in similar habitat type (community two comprise plots from grazing land in Welmera where as community three comprise plots from grazing land in Menge district) which shows the existence of distinctive grazing land species have similar adaptation mechanisms and dominate in grazing land of both district. In addition this possibly be due to the relative overlap in altitudinal ranges of the plots forming the two communities.

The least species similarity with any other community was recorded for community one (comprise plots of MFO) this may be as a result of altitudinal gradients that involve different interacting ecological factors which influence growth, development,

diversity and distribution pattern of macrofungi among communities in an area (Lopez-Quintero *et al.,* 2012; Smith *et al*., 2002).

One of the challenges for the precise description of macrofungal communities in a particular site is associated with fruitbody production dependency on weather conditions and the erratic fruiting of different species (Ferris *et al*., 2000). In this study, frequent survey throughout the fruiting season over the three successive years has reflected species richness and the results has shown that this approach is better than shorter and less intensive studies. Finding additional new records in every subsequent sampling year from 2012 to 2014 (42, 22 and 10 respectively in Welmera; 26, 15 and 9 respectively in Menge), is in agreement with other studies that recommended long-term monitoring to assess macrofungal species richness of an area (Vogt *et al*., 1992; Dahlberg, 2001; Smith *et al*., 2002).

Fruiting conditions and phenology

Due to the ephemeral fruiting bodies that can be observed only for a few days each year, phenological data is difficult and time-consuming to obtain. However, results in this study have shown that in most cases, fruiting events of most species were correlated with precipitation events as previously suggested by other authors (Lagana *et al*., 1999; O'Hanlon and Harrington, 2012). In most part of Ethiopia, including the central area, the main rainy season is from June to early October where a large number of fruit body were collected in Welmera District. However, the rainy season is erratic in Menge District, the duration is from May to September. A higher number of sporocarp collections was made from mid-May to July and August to mid-September, however, it has shown some decline in June. Similar studies in the tropical

regions have shown that the beginning of abundant rainfall favoured the diversity and productivity of fruitbodies, with mild temperature (Alexander and Selosse, 2009; Tibuhwa *et al.*, 2011; Go´mez and Williams, 2011).

The response of most of these, especially saprotrophic, fungi to rapid weather changes may be related to their mycelia being found near surface soil/litter, in contrast to mycelia from mycorrhizal fungi, which is usually deeper in the soil or humus layer (Salerni *et al*., 2002). Janík and Mihál (2007) also found that the production of macrofungi was more influenced by the total precipitation than by soil temperature. Similar results have reported by several authors (Jonsson *et al*. 1999; Lagana` *et al*. 1999; Salerni *et al*. 2002), and climate change appears to be having dramatic effects on fungal phenology. Moreover, the physiological status of the nearby plants might also have an impact on this phenomenon (Brundrett *et al*., 1996).

Frequency of occurrence

The infrequent fructification of most fungal species is common (Arnolds 1992; O'Dell *et al*., 1999; Smith *et al*., 2002). Some species fruit in intervals of two or three years which is regulated by biological cycles and the genetic background of species (Ferris *et al*., 2000; O'Dell *et al*., 1999). In this study, the frequency of occurrence of species in each habitat was found to be different. In both forests, occasional and less frequent species made most of the collection i.e. WFO,= 55% and 29% and MFO= 68% and 23% respectively and the distribution of dominant species were very low only 6% in WFO and 2% in MFO indicating that this habitat is a reservoir for more diverse macrofungi than the rest of the habitat. On the other hand, dominant and frequent species made most of the species recorded in grazing and farm land in both

study sites indicating only a limited number of species are unaffected by ecological disturbance and can grow and reproduce in such kind of environment.

In all habitat type, there were few rare species. Local extinction of some rare species might have occurred before mycologists could document their presence in the country. In Ethiopia, there is no 'Red data lists' (rarity, endangerment and distribution) of macrofungi, which means that there are no focal species to aid in conservation and management protocols as in another part of the world particularlly Europe and the America (IUCN, 2015, 2016).

From the total number of macrofungal species found in our study, 88%, 90% and 92% in WFO, WGR and WFA respectively were saprotrophic and the remaining 12 % in WFO and 5% in WGR were mycorrhizal. Parasitic species found in much lower number in all habitat type. There were no termitophlic species recorded in Welmera district. In Menge district similar pattern but in lower proportion with that of Welmera was observed and the result revealed the dominance of saprotrophic species in all habitat type, 68%, 64% and 59% in MFO, MGR and MFA respectively followed by termitophilous species that accoun 17 % , 32% and 41% in MFO, MGR and MFA.

The high percentage of saprotrophic species recorded in our study could be due to saprophytic fungi fruit more regularly from year to year (Villeneuve *et al*., 1989) than that of mycorrhizal fungi. Macrofungal assemblages in forests are likely to be influenced by the vegetation from which the litter is derived (Packham *et al.,* 2002). On the other hand, seasonal differences are least noticeable when the substrate is wood, which can act as a moisture reservoir (Gates and Ratkowsky, 2009).

3.5. Conclusion and Recommendations

It has also been shown that different land use practice in the study areas determine the distinct community of macrofungi. It is clear that substratum properties, tree species composition, elevation and microclimate are the most prominent environmental drivers of macrofungal species composition in the studied region, however, their relative influences differ among functional groups.

For planning macrofungal surveys involving researchers or overseas visitors, the results obtained here suggest that the season to consider for best possible mushroom season are the months May to September in Menge district and from June to October in Welmera district.

Recommendations

The methods used in this study, however, needs to be developed so as to let more rapid investigations which should include the development of practical methods for recording abundance and development of indicator species, for which the current study offers a starting point. Further studies will be needed to facilitate better management of macrofungal diversity across the spectrum of Ethiopian ecosystems.

Thus it is highly recommended for further insight into macrofungal species composition, sporocarp surveys should be combined environmental variables such as tree species composition, soil/litter properties and litter/soil pH together with management history of the surrounding landscape.

Eventhough the full spectrum of macrofungal diversity of the country is far from being described and documented, these initial data on macrofungi occurrence provide

opportunities to forward a stronger conservation plan in different ecosystems, especially forests, in the country.

For the implementation of all of the targets recommended above, training and engagement of adequate number of skilled mycologists in fungal taxonomy and ecology are required. Without strong networks of interaction between mycologists and land managers/Government, there will be little capacity for fungal conservation across the country. Establishing national depositaries is crucial to the documentation and understanding of a country's natural macrofungal resources (both fungarium collection and germplasm repositories) and to making it available for screening for potentially beneficial attributes.

4. Ethnomycological Study of mushrooms used for food and medicine in Menge District, Asossa Zone, Benshangul Gumuz Region, Ethiopia

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Abstract

Menge District has long been inhabited by people who have a long tradition of using wild mushrooms mainly as food, source of income and rarely for medicine. Extensive utilization of wild edible mushrooms (WEM) coupled with an ever-increasing population growth, deforestation and agricultural land expansion threatens WEM utilization and fungal diversity in general in the area. Hence, this study aimed at documenting and analyzing the wild mushroom based ethnomycological knowledge of the people in order to preserve the dwindling indigenous knowledge. Ethnomycological data were collected using semi-structured interviews, focus group discussions, participant observation and walk-in-the-woods methods. Statistical tests were used to compare the indigenous knowledge and practice of wild mushroom among different informant categories. A total of 20 ethnomycologically important wild mushroom species belonging to ten genera and six families were reported of which 15 are reported to be edible in the District. Family *Lyophyllaceae* was represented by the highest number of species (9 species, 45%) followed by *Agaricaseae* (7 species, 35%) and each of the remaining 4 families had single species representation. Significant difference ($P < 0.05$) was observed on the mean number of WEM reported by groups of respondents compared within age, experience and gender parameters. Wild edible mushroom collection habit and practice was significantlly ($P < 0.05$) influenced by gender, age and litracy level. The output of preference ranking exercise indicated *Termitomyces schimperi* was ranked first followed by *T. letestui*, *T. microcarpus* and *T. eurhizus*as the second, third and fourth prefered edible mushrooms respectively. This study shown that Menge District is rich in wild mushroom diversity and associated indigenous knowledge. However, anthropogenic factors together with loss of indiginous knowledge and very poor conservation efforts threaten economically and ecologically important mushrooms survival in the area.

Thus, complementary *in situ* and *ex situ* mushroom conservation strategy for the District is highly recommended.

Keywords: Ethnomycology, Indigenous knowledge, Wild edible mushrooms, Informants, conservation

4.1. Introduction

Mushroom hunting refers the activity of gathering mushrooms in the wild (forests, plantations) and surrounding backyard, farmlands and grasslands (Boa, 2004) typically for eating. Ethnomycology is a study on the outcome of many years of man's interaction and selection of the most useful mushroom present in the immediate environment (Lampman, 2004).

Wild edible mushrooms have been a center of concern among different communities in different part of the world due to their high quality protein that can be produced with greater biological efficiency, rich in fiber, minerals and vitamins (Gregori and Pohleven, 2007). Moreover, the low fat content, with high proportion of polyunsaturated fatty acids relative to total content of fatty acids (Marshall and Nair, 2009) makes them a good source of fat. Besides their use as a food, the ethnomedicinal and ritual use of hallucinogenic mushrooms for divination and curing (Treu and Adamson, 2006; Lampman, 2004) among traditional peoples in various regions around the world is another important aspects of human-fungi interactions.

Despite, the high diversity of wild edible mushroom (WEM) in Africa especially in the tropics, Boa, (2004) briefly reviewed the lack of Ethnomycological reports in many African countries and the availability of only few reports in others. He has also shown in his report that countries of the continent where there are better reports regarding WEM utilization include South Africa, Zambia, Zimbabwe, Nigeria, Congo Democratic Republic Congo, Cameroon, Morocco and Kenya.

Ethiopia is a multiethnic country and is home to around 85 different ethnic groups. Mushroom consumption habit and practice of people in different parts of Ethiopia have not been well documented so far. A growing interest has developed during the last decade in assessing the human-mushrooms interaction among different ethnic groups in Ethiopia. According to a preliminary study by Dawit Abate (1998), indigenous communities in south and southwestern part of Ethiopia have a good habit of hunting and consumption of wild edible mushrooms from nearby forest during the wet season thus considered mycophlic, while the peoples in the north and northeastern parts are regarded as mycophobic. There have been only few specific ethnomycological studies in the country (Tuno, 2001). Tuno has described mushroom utilization by the Mejenger, an ethnic group who resides in the southwestern part, and Teferi Yenealem *et al.,* (2013) and Taddesse Yebo (2010) reported mushroom consumption habits among people in Kaffa zone.

There is a strong emphasis on subsistence uses of wild edible fungi and their importance to rural people in developing countries, although this is an area where there are still significant gaps in information (Boa, 2004).

This study focuses on traditional knowledge of wild useful mushrooms that has, until now, received little attention in Ethiopia. Assessing and documenting indigenous knowledge and practice of wild edible mushroom utilization in Ethiopia will help to serving as primary information for further research in the field of nutrition and mycomedicine, to design people centered natural resource management and biodiversity conservation practices. It is, therefore, imperative to assess and document the knowledge, attitude, practice and the major barriers to mushroom utilization among peoples in the study area. The output of this study will generate awareness for

the encouragement of more research in complex mystery of human-mushroom relationship, and which will systematically draws attention to the variation in mushroom based culture as a basis for comparing and contrasting the state of ethnomycology in different parts of Ethiopia. This scientific investigation on wild mushrooms will be increasingly applied to help to achieve the major development goals, which include poverty alleviation and sustainable use of natural resources.

4.2. Materials and Methods

Detail description of the study area and methods used for collection and identification of macrofungal specimens have been described in chapter three section 3.2.3.

4.2.1. Ethnographic background and socio-economic aspects

Menge District has a total population of 40,240 (20,248 males and 19,992 females) of whom only 1, 101 (2.7 %) are urban inhabitants (PHCE, 2007). Regard to the settlement system of the people, the rural populations living in remote and inaccessible areas follow scattered settlement system, which are about 12 persons per km^2 (PHCE, 2007). Recently, the regional government is taking a pivotal measure to help the communities stay within new and permanent resettlements closer into neighborhoods that could have a prime importance in the provision of adequate social service to the people (BRFSS, 2004).

Majority of the economically active people of Menge District are engaged in subsistence agriculture including farming, hunting and forestry. According to BRFSS, (2004) only 0.5% of the economically active population engaged in the industrial sector and 0.7% involve in service sector. The indigenous people in the region practice shifting hoe cultivation, which is labor intensive. Moreover, labor-intensive

farming tools discourage people to invest their labor in farming individually. In addition, crop diseases and pests are also serious problems in the region contributing to low crop productivity.

According to the study conducted on the termite challenge, the aggregate annual crop loss in the field and store from pests was estimated to be about 30-40% (BRFSS, 2004). This report also indicated people in the region go hungry from four to six months annually. As a result, indigenous people in the region mostly rely on gathering wild foods, hunting and gold mining to compensate low production of crops shifting hoe cultivation (BRFSS, 2004)

Within the Districts, there are various types of religion including Muslim, Orthodox, Protestant, Catholic and traditional believe followers. Muslim population constitutes majority of the population of the study area. With regard to ethnic composition, Berta holds the majority of the population of the District. However, there are also different non- indigenous ethnic groups; Amhara and Oromo which are the second and the third largest ethnic groups respectively in the study area. With regard to languages, Arutana (Berta) language is the most widely spoken languages as a mother tongue. Oromo and Amharic languages are the widely spoken languages as second languages. In addition to this, the Amharic language serves as working language of the study area and the region (BRFSS, 2004).

4.2.2. Informant selection

The ethnomycological survey involved 240 informants (127 male and 113 female) from 10 Kebeles (lowest administrative units in Ethiopia) of the District. Systematic random and purposive sampling methods were employed to select representative

general informants and knowledgeable elderly individuals following the methods described by Martin (1995) and Alexiades (1996). Informants'ages ranged from 15–90 (54 were between 15 and 29 whereas 186 were ≥30 years old). Information for nominations of a participant as key respondents was collected from elderly people and with the help of Kebele administrators. Forty-nine key informants were identified (28 men and 21 women) among the inhabitants. Informed consent was obtained before the start of each interview with general and key informant.

4.2.3. Data collection

The ethnomycological survey was carried out in four different field trips made between May 2012 and September 2014. Ethnomycological data and market survey were made in very close interaction with informants using semi-structured interviews, focus group discussions, participant observation and walk-in-the-woods methods as described in Martin (1995). Interviews were conducted in Amharic, Arutana and Oromo language with the assistant of language translator and run independently for each informant. The questioner is attached at Appendix 2.

Interviews have addressed issues regarding the Socio-demographic profile of the study population. Besides, the knowledge, attitude and practice of human- mushroom interaction which include local names of mushrooms as well as its use (medicine, food, etc.), habitat, seasonality of species, marketability, form of mushrooms used (fresh/dried), methods of preparation for food, preservation (storage), taboos/beliefs related to collection were recorded. In addition information on the source of knowledge, method of indigenous knowledge transfer, the current status/abundance of mushroom, factors affecting the abundance and awareness on commercial cultivation

of mushrooms were also collected. Semi-structured interviews were followed by independent walk-in-the-woods that gave an opportunity for more discussion with key informant and the practical identification of wild edible mushrooms in the natural environment. This method was combined with the participant observation and practice through which reliable information was obtained on the how of collection and preparation.

Additionally, focus group discussions were designed to gain further information on knowledge of the community on mushroom utilization and prove the reliability of the data collected through semi-structured interviews (Alexiades, 1996; Martin, 1995). Fifteen key informants (8 male and 7 female) were participated in a preference ranking exercise in the manner recommended by Martin (1995) to identify the most preferred species for food. Participants for this exercise were selected based on their long years of experience in mushroom collection and utilization. A market survey of wild edible and medicinal mushrooms were made in each major market place in the District and the availability, price, who often involve in purchasing and vending mushrooms was documented and analyzed so as to identify extent of use and income generating potential. The existing threats to wild edible mushrooms and traditional conservation practices were gathered during the interviews. Voucher specimens and colored photos were used during interviews and discussions with key respondents and local field assistants.

4.2.4. Data analysis

Records on informants' backgrounds and wild mushrooms used in Menge were organized for statistical analysis. Traditional knowledge dynamics on use of wild mushrooms by men and women, young to middle aged (23–39 years) and elderly (40–

89 years); literate (completed at least primary education) and illiterate was compared using t-test and One way ANOVA at 95% confidence level between means using SPSS software version 20. Descriptive statistics were also applied to identify the number and percentage of species, genera and families of mushrooms used in the community, preferred habitat for their growth, when and how to collect wild mushroom, indigenous knowledge transfer and conservation practice. Values or scores given by key informants on use-preference of wild edible mushroom were added and ranked to get the output of preference ranking following Martin (1995).

4.3. Results

Table 4-1 Socio-demographic profile of the study population

Variables	Response	Frequency	Percent
Sex	Female	113	47.1
	Male	127	52.9
Age Category	15-29	54	22.5
	30-49	102	42.5
	50-69	57	23.8
	70-90	27	11.3
Marital Status	Single	19	7.9
	Married	221	92.1
Ethnic Group	Berta	195	81.3
	Oromo	14	5.8
	Amhara	31	12.9
Religion	Orthodox Christian	25	10.4
	Muslim	202	84.2
	Protestant Christian	7	2.9
	Catholic Christian	2	0.8
	Traditional believe	4	1.7
Language	Rutana (Berta)	129	53.8
	Arabic	9	3.8
	Rutana and Arabic	66	27.5
	Oromo language	7	2.9
	Amharic	29	12.1
Education level	Illiterate	168	70.0
	Primary school	40	16.7
	Secondary school	26	10.8
	Above secondary	6	2.5
Family Income per Month (in Birr)	200-499	74	30.8
	500-999	104	43.3
	1000-1499	40	16.7
	1500-1999	12	5.0
	2000-2499	7	2.9
	2500-3000	3	1.3

List of ethnomycologically important wild mushrooms and growth habit

Although the diversity of species of macrofungi in Menge District is high, the widely shared body of cultural knowledge is restricted to a small group of not more than 20 species. These species, belonging to ten genera and six families, where majority of them are edible and few are used for medicinal purpose the rest are regarded as

poisonous. Family *Lyophyllaceae* was represented by the highest number of species (9 species, 45%) followed by *Agaricaseae* (7 species, 35%) and each of the remaining 4 families had single species representation (Table 4-2).

Ethnomycological findings of this study showed that almost all the respondents have a very good knowledge about all the species of termitophilous mushrooms; *Termitomyces clypeatus, T. eurhizus, T. letestui, T. microcarpus, T. schimperi, T. robustus, T. striatus, T. umkowaanii* and *T.* sp1 since they are considered to be of excellent quality food by most people. Identification and folk taxonomy of these mushrooms are very easy and consistent across the community (Table 4-2). These mushrooms are extensively searched for and are collected excessively than any other mushroom type, either for consumption as food or for sale.

Generally, 80% of the respondents use mushrooms for food. Only a few respondents recognized their medicinal contribution in the community. Amongst various termitophilous mushrooms *T. microcarpus* and *T. clypeatus* were found to serve as a medicinal mushroom by the local people for constipation and gastric problems in adults and highly recommended for underweight children while the dry powder of *Laetiporus sulphureus* and *Ganoderma* sp. has reported to be used for treatment of common cold (22%) and wound (18%) respectively. The species named by only 40% of interviewees include the following species: *Coprinus* sp.1 and *Auricularia* sp. that are seldom used as food and/or medicine among local inhabitants. Some lepiotoid taxa such as; *Macrolepiota dolichaula, M. procera* and *M. rhacodes* are commonly known as “tsrgunu amigu” and “tsrgunu ashilu” which refers to mushroom with annulus on the stipe and with large umbrella which are considered as edible by few informants (13%).

Table 4 2 List of mushrooms well recognized by peoples in the community, Local names and culinary status and habitat

No	Species/species in the gesus	Family	Vernacular name	Use category			P	Habitat and Substrate
				C	M	IC		
1	*Termitomyces striatus*	Lyophyllaceae	Gultse	+	-	-	2	Farm land, On soil
2	*T. eurhizus*	Lyophyllaceae	Tsergunu	+	-	+	2	Forest, Termite nests
3	*T. schimperi*	Lyophyllaceae	Zoma/Zip alweta	+	-	+	1	Farm land, Termite nests
4	*T. letestui*	Lyophyllaceae	Afifi	+	-	+	1	Farm land, Termite nests
5	*T. umkowaani*	Lyophyllaceae	Abenega	+	-	+	2	Grazing land, Termite nests
6	*T. robustus*	Lyophyllaceae	Gultse	+	-	+	2	Grazing land, Termite nests
7	*T. microcarpus*	Lyophyllaceae	Aburalu	+	+	-	1	Grazing land, Termite nests
8	*T. clypeatus*	Lyophyllaceae	Akukufi	+	+	+	1	Farm land, Termite nests
9	*T.* sp.1	Lyophyllaceae	Angushung	+	-	-	2	Farm land, Termite nests
10	*Psathyrella* sp	Psathyrellaceae	Egnegnero	+	-	-	3	Forest,On soil
11	*Laetiporus sulphureus*	Polyporaceae	Achechereb	+	+	-	3	Forest, On log
12	*Auricularia* sp.	Auriculariaceae	Huntsul	+	-	-	2	Forest, On living tree
13	*Ganoderma* sp.	Ganodermataceae	-	-	+	-	-	Farm land, On dead wood
14	*Coprinus comatus*	Agaricaseae	Egnegnero	+	-	-	3	Farm land, On dead wood
15	*Macrolepiota dolichaula*	Agaricaseae	Tsrgunu amigu/	±	-	-	3	Forest, On leaf litter
16	*M. rhacodes*	Agaricaseae	Tsrgunu ashilu	±	-	-	3	Forest, On leaf litter
17	*Chlorophyllum molybdites*	Agaricaseae	-	-	-	-	4	Grazing land, among grass
18	*Agaricus*	Agaricaseae	Signil tsoro /	-	-	-	4	Forest, On leaf litter
19	*Leucoagaricus*	Agaricaseae	Gel tsoro	-	-	-	4	Forest, On leaf litter
20	*Leucocoprinus*	Agaricaseae	Gel tsoro	-	-	-	4	Forest, On living tree

C = Culinary, M = Medicinal, IC = Income, P = Palatability (1 = Delicious, 2 = Good, 3 = Just edible, 4 = poisonous)

Five non edible species were recorded that are considered toxic by local people. Some species in the genus *Agaricus*, *Chlorophyllum, Leucocoprinus* and *Leucoagaricus* which are growing around animal dung in pastures, are generally considered to be inedible and known by the collective local name “Signil tsoro” and “Gel tsoro” which literally refers to the urine of donkey and dog. All the respondents do not appear to partition this group of macrofungi as finely as termitophilous mushrooms.

According to the respondents, habitat, morphology, color, odor and test are the common parameters used to distinguish edible from non edible mushrooms. Beliefs or ideas about the edibility of wild mushrooms found in this study area are as follows:

i) Mushrooms found on termite mounds and rotting wood are generally collected for consumption.

ii) Mushrooms with mild tast and thick flesh and eaten by rodents, tortoise are treated as safe for consumption.

iii) Mushrooms changes colour to red, yellowish, blackish after touching or cutting are said to be poisonous

iv) Mushrooms which give burning sensation on tongue when tasted raw are considered as unsafe.

v) Mushrooms found on grazing land and near dung are generally inedible.

vi) Most mushrooms with white pilus are poisonous.

Indigenous knowledge of the community

More number of WEM were reported by female respondents, the difference was significant (P <0.05) when the average number of WEM reported by each group was compared (Table 4-2). There was a significant difference (P <0.05) in the number of WEM reported by senior members of the community (>50 years old) and young (15–29 years old) indicating that knowledge increase as the individual gets older. More number of WEM was reported by key informants than general informants and there is a significant difference between the average number of mushrooms listed by these two groups of respondents (P <0.05). There was no significant difference observed in the number of WEM listed by illiterate and literate informant and among respondents with different family income.

Table 4-2 Statistical test of significance on average number of WEM reported by different informant groups in Menge District

Parameteres	Informant groups	N	Average ± SD	t –value	p –value
Gender	Female	113	8.13±2.4	7.8	0.006
	Male	127	7.17±2.9		
Age	15-29 (young member)	54	6.31±2.4	10.568	0.00004*
	30-50 (middle age)	101	7.61±2.7		
	> 50 (senior members)	85	8.36±2.5		
Literacy level	Illiterate	168	7.86±2.4	1.512	0.212
	Primary	40	7.15± 3.2		
	Secondary	26	6.92± 3.1		
	Above secondary	6	7.17±3.6		
Informant category (experience)	Knowledgeable/Key	49	10.59±2.3	18.5	0.0000*
	General informants	191	6.86±2.2		
Family income	Low income (>999)	178	7.72± 2.8	0.89	0.412
	Middle income (1000-2000)	52	7.5±2.45		
	High income (< 2000)	10	6.6±2.54		

*Significant difference (p<0.05); t(0.05) (two tailed), df = 238, N = number of respondents.

Perception, recognition and folk taxonomy

The "total" domain of mushrooms is divided into two smaller groups among the indigenous community in the study area. One group that comprises a small number (about 20 species) of beneficial and morphologically distinct species about which the community have a good ethnoecological knowledge; and a second group that comprises of a large number (all remaining species) which are grouped together and cosidered as potentially toxic, useless or indistinct species about which there is almost no detail knowledge across the respondents (Figure 4-1).

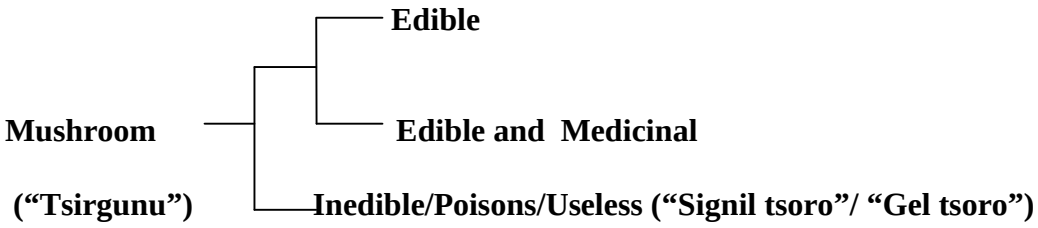

Figure 4-1 Folk division of domain mushrooms into three smaller groups (Arutana language)

All mushrooms are generally known as "Tsergunu" by all the inhabitants in Menge District. Specific naming (folk taxonomy) of mushrooms by the Berta communities was mainly based on the substrates where the mushrooms were actually found, the shape of the sporocarp and growth pattern. For example the local name used for *Termitomyces microcarpus*is "abralu" that means a troop, indicates the typical nature of this mushroom that it occures in dense crowd, numbering often several thousands. The local term "zip alweta" given to *Termitomyces schimperi* meaning "pinus of the earth" indicate the morphology of the immature sporocarp that look a lot like male reproductive organ. The other interesting locale name given to all inedible/poisonouse/useless mushrooms are "Signil tsoro and "Gel tsoro" which

literally means the “urine of donkey and urine of dog” which indicate mushrooms that grow in pastureland where cattles are graizing and regarded as non edible because people belive that they are contaminated with cattle dung.

The local people’s perception on the origin and development of mushrooms could be grouped into four categories. The first is shared by a considereble number (25%) of respondents that believe mushrooms grow only in the wild without being planted or cultivated. The second idea is that it is the soil that produces mushroom. The third is heavy rain with thunder storm are responsible for their origin and development and the fouth idea, which is shared by majority of the native respondents in the community (> 60%), is that mushrooms only grow by Divine will and they only grow by God's will believed that “Mushrooms are a gift from God to human”.

Preference ranking

A preference ranking exercise with purposely-selected 15 key informants on WEM that were used as food was recorded. Termitophilous mushrooms were considered the top, wood inhabiting mushroom (*Coprinus comatus* and *Laetiporus sulphureus*) and lepiotoid mushrooms received an average and least rating respectively in consumer preference as food (Table 4-3). Mushrooms are mainly used for food, nevertheless, three additional use categories in the District were observed. These are use as Medicine, item for sale and as a special gift for respected person.

Table 4-3 Results of simple preference ranking for nine WEM for food

Mushroom Species	Informants labelled R_1 to R_{15}																
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	R_{12}	R_{13}	R_{14}	R_{15}	Total	Rank
Termitomyces striatus	5	4	3	2	3	6	6	4	3	7	3	5	5	6	4	62	6th
T. eurhizus	7	6	5	4	4	9	3	5	7	8	6	7	6	5	6	82	4th
T. schimperi	9	8	9	9	7	8	9	9	6	9	9	8	9	8	8	117	1st
T. letestui	8	9	7	6	6	7	8	8	9	6	7	9	8	9	9	107	2nd
T. umkowaanii	4	5	6	8	8	4	7	7	5	3	5	3	4	5	7	74	5th
T. microcarpus	6	7	8	7	9	5	5	6	8	4	8	6	7	7	5	93	3rd
T. clypeatus	3	3	4	6	5	2	4	1	4	5	4	4	3	2	3	51	7th
Coprinus comatus	2	1	1	3	1	1	1	2	2	2	2	1	2	3	2	23	9th
T. robustus	1	2	2	1	2	3	2	3	1	1	1	2	2	1	1	24	8th

Scores in the table indicate ranks given to WEM based on preference as food. Highest number (9) given for the mushroom which informants thought highly preferred as food, (1) is for the least preferred species.

Wild mushroom collection and utilization practice in Menge District

The main system for obtaining WEM in the community is through direct collection, but in some villages, which are near to the local market, people usually buy mushrooms from market. This study has showen that women in Menge District are highly involved in every stage; collection, marketing and mushroom meal preparetion (Figure 4-2). They deliberetely go for mushroom hunting early in the morning on a regular basis since they use WEM both for income generation and food for their family during rainy season. However, in most cases men collect mushrooms unintentionally when they walk to or from their farm land or during farming activity.

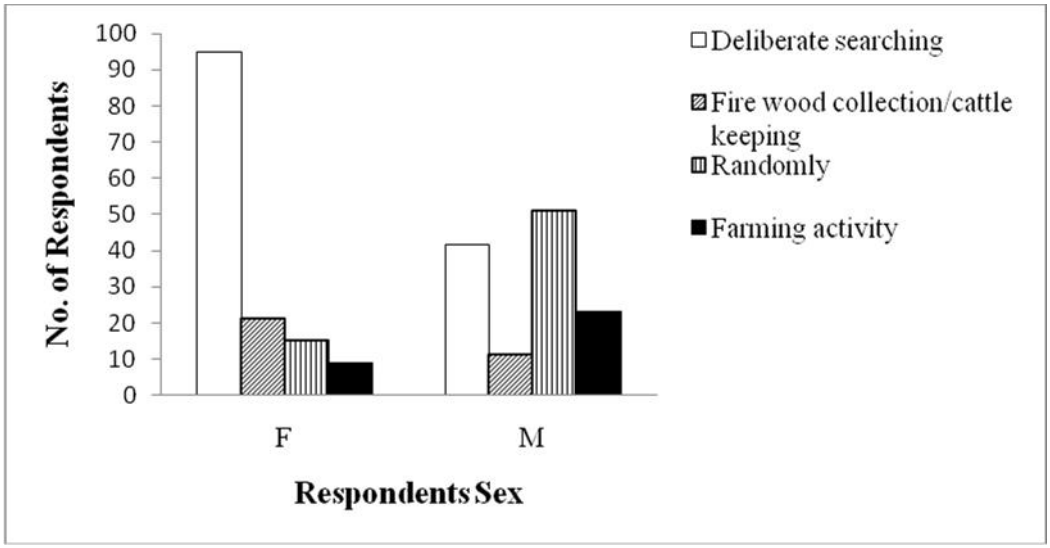

Figure 4-2 Gender difference on collection and utilization practice

Results from this study revealed that there is a relationship between respondent's sex, age, educational status and involvement in WEM collection ($p<0.05$). Women's practice are more energy-efficient which allow them to gather a good amount and a greater variety. Men, in contrast, have a tendency to target some of the most wanted species particularly during scarcity of food. Women also tend to collect in groups and usually with their children, on the other hand men are solitary collectors.

There is also a significant difference in WEM collection practice between senior (> 30 years old) and young (15 – 30 years old) member of the community in this District where elder people (92%) are more involved in WEM collection when compared to younger members (61%) (Table 4-4). In the case of educational status of the respondents, illiterate members of the community actively participate in collection than literate people. However, there is no relationship between family income, distance from forest and involvement in WEM collection.

Table 4-4 Statistical test of significance on percentage of informant groups that involve in WEM collection

Parameters	Informant groups	Involvement in WEM collection?		p
		Yes	No	
Sex	Female	107 (94.7%)	6 (5.3%)	0.000**
	Male	88 (69.3%)	39 (30.7%)	
Age	Young (15- 30)	33 (61.1%)	21 (38.9%)	0.000**
	Senior (> 30)	172 (92.5%)	14 (7.5%)	
Literacy level	Illiterate	156 (92.9%)	12 (7.1%)	0.000**
	Literate	49 (68.1%)	23 (31.9%)	
Informant category	Key	46 (93%)	3 (6.1%)	0.060
	General	159 (83.2%)	32 (16.8%)	
Family income	Low (>999)	152 (85.4%)	26 (14.6%)	0.907
	Middle (1000-2000)	44 (84.6%)	8 (15.4%)	
	High (< 2000)	9 (90%)	1 (10%)	
Distance from forest	< 3 km	110 (86.6%)	17 (13.4%)	0.577
	> 3 km	95 (84.1%)	18 (15.9%)	

***Significant difference (p<0.05); ** t(0.05) (two tailed)**

There is a significant difference in frequency of collection among different informant groups such as gender, age, literacy level and experience while there is no significant difference among respondents with different income and their location from the forest (Table 4-5).

Table 4-5 Statistical test of significance, on how often the different informant groups involve in WEM collection

Parameters	Informant groups	How often do you collect WEM?			p(2-sided)
		Never	Sometimes	Always	
Gender	Female	6 (5.1%)	32 (28.3%)	75 (66.4%)	0.003*
	Male	24 (18.9%)	40 (31.5%)	63 (49.6%)	
Age	Young (15- 30)	18 (33.3%)	14 (25.9%)	22 (40.7%)	0.000**
	Senior (> 30)	12 (6.5%)	58 (31.2%)	116 (62.4%)	
Literacy level	Illiterate	10 (6.0%)	50 (29.8%)	108 (64.3%)	0.000**
	Literate	20 (27.8%)	22 (30.6%)	30 (41.7%)	
Informant category	Key	2 (4.1%)	8 (16.3%)	39 (79.6%)	0.002*
	General	28 (14.7%)	64 (33.5%)	99 (51.8%)	
Family income	Low (>999)	22 (12.4%)	52 (29.2%)	104 (58.4%)	0.960
	Middle (1000-2000)	7 (13.5%)	16 (30.8%)	29 (55.8%)	
	High (< 2000)	1 (10%)	4 (40%)	5 (50%)	
Distance from forest	< 3 km	14 (11%)	46 (36.2%)	67 (52.8%)	0.082
	> 3 km	16 (14.2%)	26 (23%)	71 (62.8%)	

***Significant difference (p<0.05); ** t(0.05) (two tailed)**

Phenology

In Menge District, almost all the respondents know that the mushroom growth season is strictly associated with the rainy season (Table 4-6). Both general and key informants agree that mushrooms fruit from May to September, with July and August being the months with highest abundance for most species, however, according to key informants, the season is slightly variable every year due to the erratic rains in the region. Generally, they cluster the species into two groups: “mushrooms of the dry season”, in which *Laetiporus sulphureus*, *Auricularia* sp and *Ganoderma* sp. are included and “mushrooms of the rainy season”, in which the rest of the species are included.

Table 4-6 Respondent's perception about the phenology of different mushrooms type

		Months										
No.	**Species**	**Jan**	**Feb**	**April**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
1	*Agaricus* sp.						+++++++++					
2	*Auricularia* sp				+++++++++++++++++++++++++++++++++							
3	*Chlorophyllum molybdites*					++++++++++						
4	*Coprinus comatus*						++++++++++++					
5	*Ganoderma* sp.			++								
6	*Laetiporus sulphureus*			+++								
7	*Macrolepiota procera*						++++++++++++					
8	*Psathyrella candolleana*						++++++++++++					
9	*Termitomyces clypeatus*					+++++++						
10	*T. eurhizus*					++++++++++++++						
11	*T. robustus*						++++++++++++					
12	*T. letestui*					++++++++++						
13	*T. microcarpus*						++++++++++					
14	*T. schimperi*					++++++++++++						
15	*T. striatus*						++++++++					
16	*T. umkowaanii*						+++++++					

Ecology

The indiginous knowledge that people have about mushroom ecology varies among individuals in the community. The data obtained during interview reveals that it depends on the activities in which they engage and on their dependence on forest resources. The respondents have recognized three main habitats where the distrbution of mushrooms is high. This consist of mainly agricultural fields/farm land, hill and the mountain (the place where the forest is) and the open areas/plain in most cases where the cattels graze (Figure 4-3). Some respondents also consider home gerden as a good place for mushroom growth.

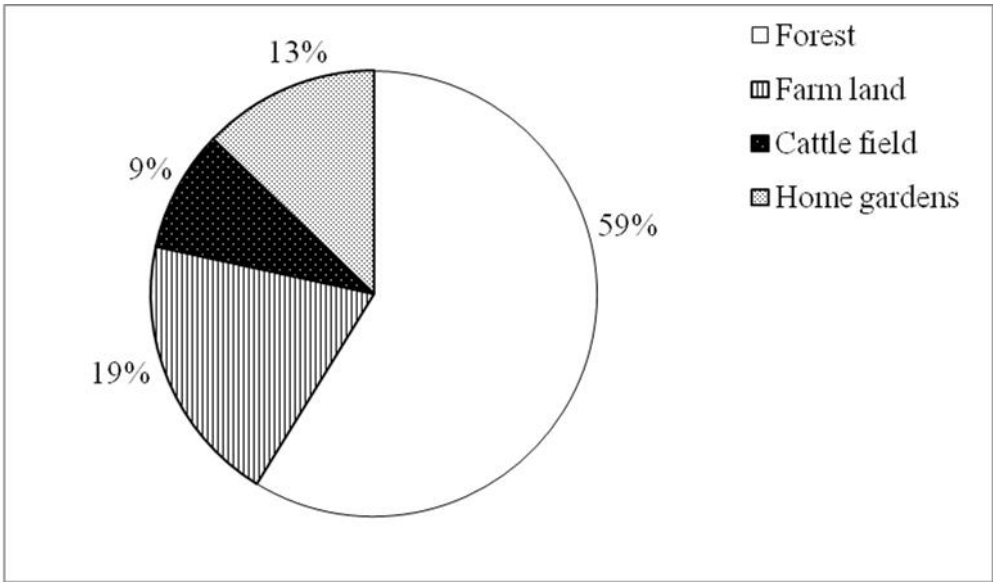

Figure 4-3 Main habitats with high mushroom distribution recognized by respondents

More than half of the respondents (59%) consider the forest to be the principal area for collecting mushrooms followed by farm land, home gardens and cattle field. The indigenous people have an extensive knowledge about the places in which diverse mushroom species grow, especially those which are sold or used regularly (species in the genus *Termitomyces*).

Marketability of WEM

During the rainy season, mushroom become one of the main items in the local market. Majority of the local people (73%) were dedicated themselves either as vendor or a buyer. During market survey in the district, we found that from the total 113 female and 127 male respondent, 68 (58%) women and 32 (25%) men reported that they are involved in selling mushrooms which contrbute a considerable source of income during the rainy season. An adult person spends an average of 3 to 5 hrs to collect wild mushrooms, and walks about 4 to 10 km for round trip.

During each collection trip a person can collect on average of 2 kg of mushroom. Study on different local market in each kebele reveals that price of mushrooms per kg is slightly different depending on the type of mushroom (size, context of sporocarp, test, delicacy and easy digestibility), abundance and distance of the village from major mushroom collection site. However, the price range in majority (57%) of the markets were 10-20 birr (approximately US $ 0.5 - 1) and in some market (26%) was between 20 and 30 Birr (US $ 1 - 1.5) per killo gram (1 dollar = 19.0birr in 2014/15). In some kebeles, 15% of the interviewed people collect mushrooms to sell along the road side with cheap price (5-10 birr per kg). Generally the price for mushroom is higher than most vegtables and lower than meat at markets in the District.

Mushrooms in the market are availible in both fresh and dried forms. According to the respondents, people prefere to buy fresh mushrooms (62%) and the rest (38%) choose the dried mushroom. On many occasions, however, unsold dried mushrooms may remain stored for long time (several months). During this study we found that all of the mushrooms being sold in the local markets belong to one genus; Genus

Termitomyces and *T. schimperi* is the most expensive WEM followed by *T. letestui*,*T. umkowaanii* and *T. eurhizus* as the second, third and fourth respectively.

Indigenous knowledge transfer

The mainsystem of mycological knowledge transfer on grouping and naming (folk taxonomy), identification (differentiation of edible from inedible varieties), habitat and methods of preparation for food and medicine in Menge District was by word of mouth to a family member, commonly old to young people (Figure 4-4). Not a single person, including the key informant, had written documents. The way they share their indigenous knowledge to their children was also found to be similar. The contrbution of other sectors such as school, agriculture experts e.t.c. were found to be insignificant.

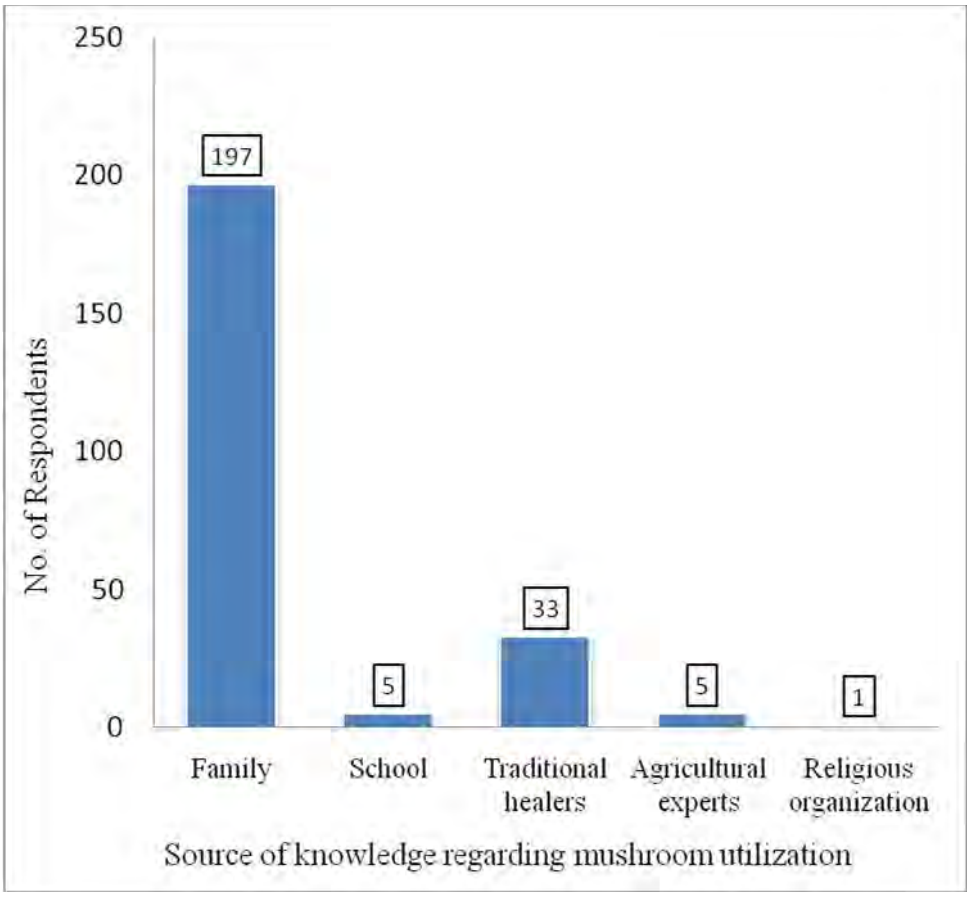

Figure 4-4 Source of Ethnomycological knowledge in the community

Conservation practices

All of the WEM specie sutilized by the community of the District are available from wild sources and home gardens. In addition to the observed poor effort of in-situ conservation, the lack of knowledge on artificial mushroom cultivation put mushrooms under threat due to over harvest and an ever-increasing anthropogenic influence on natural habitat of genetic resources of the area. The major factors affecting wild mushroom wealth of the area as claimed by the respondents includes deforestation, agricultural expansion, urbanization/population pressure and use of agro-chemicals (Figure 4-5).

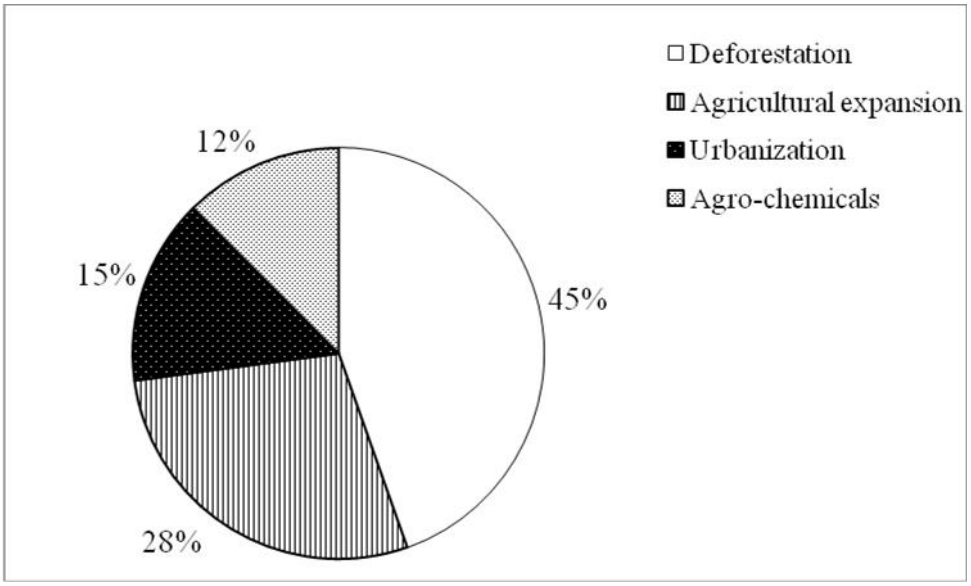

Figure 4-5 Factors affecting wild mushroom wealth of the area as claimed by the respondents.

The respondents > 60 % (mostly elderly people) indicated that some of the mushrooms they used to consume are no longer found in their locality and it is due to human degradation of the ecosystem through farming activities, use of plants for fire wood and construction and annual fire outbreak in the environment.

4.4. Discussion

The people in the community have a very good traditional mycological knowledge in the uses of selected wild edible species and folk taxonomy thus can be considered as mycophilic. Generally, the number of WEM collected and used in the District was found to be higher than reported by Teferi Yenealem *et al.* (2013) and Taddesse Yebo (2010) in Kefa Zone.

Results in this study shows the dominance of species from family Lyophyllaceae and Agaricaceae which could be credited to their widespread distribution and abundance in the study area. There was a report from southwest part of Ethiopia that species in family Lyophyllaceae were the dominant edible mushrooms (Teferi Yenealem *et al.,* 2013; Taddesse Yebo, 2010). The extensive use of species from this family might relate to their appealing taste (as a substitute for meat) and provide better income (Ayodele *et al.,* 2011). This observation is in line with results that have been reported from different parts of Africa and Asia (Aryal and Budathoki, 2012; Osarenkhoe *et al.,* 2014).

Literally more than hundreds of species of macrofungi are expected to exist in the District, however, the widely shared body of traditional knowledge is limited to a small core group of (about 20) species. These species tend to embody one of a few common threads: (1) they are either edible/useful or (2) they are morphologically different and not difficult to recognize, (3) considered poisonous, or (4) they are very abundant. Generally, those species that are inedible/with unknown toxicity and morphologically challenging to distinct are placed together into a huge group of “useless” species. These species do not have a name/linguistic designation, and

generally overlooked. The same type of perception reported from Mexico and Philippines (Garibay-orijel and Cifuentes, 2006; De Leon *et al.,* 2013).

The way they use folk taxonomy especially for species of the genus *Termitomyces* were similar to some level, with those recognized by regions in other part of the world. They almost use distinct name to each edible/useful species. This result is in line with the finding by Tibuhwa from Tanzania (Tibuhwa, 2012a), Nigeria (State and State, 2010) and Mexico (Garibay-orijel and Cifuentes, 2006) who also noted a similar trend of ethnotaxa being comparable to scientific taxa.

More than half of the WEM used in the area (53%) were found to be termitophilous mushrooms that belong to the genus *Termitomyces*. This may possibly relate to the fact that the region is located in one of the termite area and these species are easily accessible in the nearby areas including home gardens and farmland. The finding agrees with the general pattern of dominance of termitophilous species seen in most WEM studies in the tropical regions (Egleé and Stanford, 2011; Aryal and Budathoki, 2012; Garibay-orijel *et al.,* 2012; Tibuhwa, 2012a; Teferi Yenealem *et al.,* 2013; Osarenkhoe *et al.,* 2014).

Farmlands on which maize and sorghum are cultivated were found to be common areas where most of the termite comb and the respective *Termitomyces* species were observed. However, the investigation showed that these habitats are subjected to anthropogenic influences due to the practice of using insecticides to eliminate the termite from the farm. This consequently results in decreasing size of mushroom harvest from such habitat. Misuse of habitats was also observed as the main factor

that decrease wild mushrooms distribution and the amount of harvest (Kik *et al.*, 2013; Teferi Yenealem*et al.,* 2013; Osarenkhoe *et al.,* 2014).

Results also show the availability of WEM in the local market both in fresh and dried form. However, majority of the respondents (62%) prefer to use it in fresh form while (38%) choose to use the dried mushroom. The regular use of freshly harvested WEM was reported to be associated with the perception of getting better food value; test and aroma that they believed could be lost on drying. Medicinal mushrooms were usually dried and preserved for later use when there is a need. Though in some part of the world people prefer the dried form of mushrooms (Garibay-orijel *et al.*, 2012; Tibuhwa, 2012b) many other ethnomycological studies have showed the preferred use of fresh WEM as food (Lampman, 2004). It is due to the fact that the moisture content of most mushrooms fruitbodies are more than 80% and it requires immediate drying before it deteriorate.

The average number of WEM reported by different age groups compared in this investigation showed that indigenous knowledge on the use of WEM is significantly higher ($P < 0.05$) with elderly people than in the younger generation. Furthermore, the observed significant difference ($p < 0.05$) showed knowledge gap between generations and the decline down the age group. The influence of urbanization, modern education and very poor way of sharing indigenous knowledge through word of mouth and mainly restricted along family lines to the younger age group, this observation is common not only with WEM but also with other edible and medicinal products collected from wild (Lahiri *et al.*, 2010; Lulekal *et al.*, 2013).

The young generation has little interest to know and to participate in wild mushroom collection; it might be due to factors associated with 'modernization' and shift to wage labor. Related results were reported for other wild edible and medicinal plants use in Ethiopia (Lulekal *et al.*, 2013) and in other parts of the world (Okhuoya and Akpaja, 2005; Oso, 1985). Furthermore, the absence of any written document about the cultural use of WEM of the area showed that future use potential of indigenous knowledge is at risk.

Experience in collection and utilization was found to be one of the major variables that influence traditional knowledge among peoples in the community. Moreover, the result of this study shows that there is an extremely significant difference ($p = 0.0001$) in traditional knowledge between the key and general informants that might be due to the impact of age-old knowledge and practice in using WEM in the key informants (Lahiri *et al.*, 2010; Miriam *et al.*, 2006). As described in Boa (2004), people with better interaction with wild resource have a better knowledge about the uses of WEM than those with intermittent interaction.

In most developed countries such as UK (Moore, 2001), Sweden (where >50% of the population gathers fungi), Spain and French (de Roma´n and Boa, 2004) interest of the peoples in picking and eating WEM has spread throughout the countries. However, different studies have shown mushroom pickers can create a serious disturbance in the forest (Miriam *et al.*, 2006; Moore, *et al*., 2001; O`Hanlon, 2012). Thus in some regions of these countries some measures have been taken, such as the establishment of legal regulations for the harvest of WEM, the introduction of a license system for mushroom pickers and distribution of leaflets and posters to raise

public awareness about good harvesting practices and the threats of overexploitation (de Roma´n and Boa, 2004).

In contrast, the tradition of collecting and consuming WEM among rural populations in countries such as India is on the decline because of growing urbanization and the associated changes in food habits (Agrahar-Murugkar and Subbulakshmi, 2005). Ethnomycological studies in Africa have shown that knowledge about mushrooms is extensive, though it varies among countries. However, this knowledge does not indicate a high social valuation or a high consumption of fungi (Yorou and De Kesel, 2001; Kuyper, 2002). Even though WEM could be valuable in serving to overcome extreme food shortages in this continent, the amount harvested each year have been limited due to anthropogenic and global climate change which have a greater impact in this region of the world (Buyck and Nzigidahera, 1995; Wong *et al*., 2001; Ayodele *et al*., 2011).

Gender is one of the variables that influence local knowledge distribution. Female informants of the District have reported more WEM on average (8.13±2.4) than male (7.17±2.9) and the difference was statistically significant ($p = 0.006$). As described by Boa, (2004) this knowledge difference can be explained at two levels. The first is a consequence of culturally assigned roles for men and women. The second is derived from division of labor as a result of biological differences between men and women This result showed that women have better knowledge on use of WEM, since those who engage actively in gathering develop a more profound knowledge on the biology, ecology, and phenology of mushrooms and are able to identify them more specifically (Garibay-orijel *et al.,* 2012; Boa, 2004; Montoya and Torres, 2002). This result in the

district could be related to better involvement of women in collection, preparation for food and marketing. In contrast to ethnobotanical and ethnozoological knowledge, women are typically involved in all the processes of wild edible mushroom utilization (Garibay-orijel *et al.,* 2012; Montoya and Torres, 2002).

Survey of major market in the District indicated that all of the mushrooms sold were species from the genus *Termitomyces* and the rest edible and medicinal mushrooms were not available on market places during the study period. This would show that this genus contain species which are highly preferred in terms of their test, size, nutritional benefit and easy of access. Among the species sold in the local market, *Termitomyces schimperi* is the most expensive followed by *T. letestui, T. umkowaanii, T. eurhizus*. The market value of these species (with a price range from 1 USD to 1.5 USD per approximately 1kg of fresh mushroom) showed the potential demand of those marketable WEM by the community and their contribution to additional income for the peoples who are involved in this activity especially women. The same preference of these mushrooms were reported in most east, west and central Africa and south east Asia (Teferi Yenealem *et al.,* 2013; Garibay-orijel *et al.,* 2012; Garibay-orijel and Cifuentes, 2006; Zhang *et al.,* 2014; Lampman, 2004; Tibuhwa, 2012b). However, such marketability could also show that the WEM might be overharvested and as a result are under pressure as they are purposefully collected from wild for economic reasons (Miriam *et al.,* 2006; Boa, 2004).

The preference ranking exercise helped to identify the most-preferred WEM species for consumption. Accordingly, termitophilous mushrooms were considered the most-preferred ones, followed by species of wood-inhabiting mushroom; *Coprinus*

comatus. and *Laetiporus sulphureus* in consumer preference as food in the community. In addition, there is a widespread belief that these mushrooms provide essential nutrients that enhance strength and well-being and the unique flavor and texture of mushrooms is highly valued in the culinary tradition of the community. On the other hand, preference of these species may also be related to their relative frequency and abundance in the area. Ethnomycological study that has done in the southwest part (Teferi Yenealem*et al.*, 2013; Tadese Yabo, 2010) of Ethiopia also reported the consumption of species in the genus *Termitomyces.*

Medicinal use of WEM among the community in Menge district is not as strong as similar studies which have been done in the rest of the world (Boa, 2004; Miriam *et al.*, 2006; Okhuoya and Akpaja, 2005). Most of the inhabitants in Menge District use mushroom mainly as food and rarely for non-food purposes. Only few people (especially key informants) recognized the medicinal contribution of wild mushrooms in the community. Amongst useful various mushrooms *T. microcarpus*, *T. clypeatus* *Laetiporus sulphureus* and *Ganoderma* sp. were found to serve as a medicinal mushroom. *T. microcarpus* used to treat constipation and gastric problems in adults while *T. clypeatus* are usually given to underweight children. On the other hand, the powder of dried *Ganoderma* sp. and *L. sulphureus* is used to speed up wound healing process and to treat common cold respectively. However, medicinal investigation of *L. sulphureus* was reported by Woldegiorgis *et al*. (2015a) from the southwest part of Ethiopia (Kaffa Zone) show its use to relieve stomachache and to expel a woman's retained placenta following delivery. There are also some records about the medicinal property of this mushroom (Anderson *et al.*, 2013; Adhikari *et al.*, 2005). Clinical

investigation of in vivo or animal studies should be carried out on the medicinal use of these mushrooms to validate indigenous practice.

A significant trend of decreasing in WEM distribution and the amount collected in each mushroom season has witnessed by the residents of the community in the past years. This is mainly due to decline in forest coverage, expansion of agricultural land, human population pressure and use of agro-chemicals. The same problem was reported from another part of Ethiopia (Tadese Yabo, 2010; Teferi Yenealem *et al.*, 2013) and many parts of the world especially in the tropical regions (Kik *et al.*, 2013; Ayodele *et al.*, 2011; Tibuhwa, 2012a,b). On the other hand, in developed countries such as Europe, North America, Japan, Korea, and the Russian, not only the tradition of eating wild edible fungi but also a wise use of these resources is much stronger and appears to have resisted the problem experienced elsewhere (Arora and Hepard, 2008; Miriam *et al.*, 2006). As a result, the harvest of wild edible mushroom is increasing as compared to the trend seen in developing countries (Boa, 2004; Miriam *et al.*,2006).

However, only few (<10%) residents in the study area have knowledge about artificial cultivation of mushroom, majority of the people in the district have remarkable interest to learn and to involve in mushroom cultivation. Such interest of the people to adopt mushroom cultivation is in agreement with the investigation made in countries like Tanzania, Cameroon, Nigeria, Japan and China, in which the people strongly accept mushrooms cultivation for their obvious nutritional and medicinal values (Boa, 2004; Garibay-orijel and Cifuentes, 2006; Garibay-orijel *et al.*, 2012; Kik *et al.*, 2013). Moreover, as the United Nations Food and Agriculture Organization (FAO) strongly advise the consumption of mushroom to create a valuable addition to the

nutritional quality of the diet of people in developing countries (Ayodele *et al.*, 2011; Boa, 2004). It also has potential as source of income to support a family especially women thereby contributing to their economic independence (Miriam *et al.*,2006; (Wong *et al*., 2001).

4.5. Conclusion and Recommendation

Technical supports from governmental and non-governmental organizations in the development of effective mushroom utilization in Ethiopia is rare. Disinterest in mushroom research affects negatively the protection of indigenous mushroom knowledge, integration of this knowledge as an input in food security and mycomedicinal studies and establishing a center for diversity data organization and their associated utility in Ethiopia. This perception has affected negatively government funding of mushroom projects and research compared to other food crops in the country.

With Ethiopian rising population together with an increasing demand for food, domestication and cultivation of indigenous varieties of mushroom can be one of the alternatives to contrbute to nutritional security with comparative smallholding and cheap substrates such as agricultural solid wastes, thus leading to reduce environmental pollution and create a job opportunity.

Recommendation

- Thus, it is recommended to establish a center for documentation of traditional knowledge.
- Create awareness among people on cultivation practices by providing professional support and land to establish a year-round production of useful mushrooms. This approach will be helpful to conserve the fast-eroding mushroom genetic resource of the area, to provide a nutritionally better alternative to people`s diet and to provide an additional source of income to the community.
- Conservation actions need to be followed by taking measures for protection of the forest from grazing, human interference, and other anthropogenic influences.

5. Morphological and Molecular Characterization of *Agaricus* Taxa of Central and Northwest Ethiopia

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Abstract

Agaricus is a genus of saprobic fungi that comprises species with considerable ecological, nutritional and medicinal interest. However, knowledge on their diversity and economic importance remain poor in Ethiopia. A taxonomic study of 33 Ethiopian *Agaricus* collection from Menge District and Welmera District which are located in northwest and central Ethiopia respectively was conducted based on morphological and molecular (ITS 1+2 rDNA sequences) characteristics and their phylogenetic relationship with other members of the genus was compared. A data set of 85 ITS sequence of *Aagricus* collection (16 from tropical/subtropical and 36 from temprate sequences retrieved from GenBank) for phylogenetic analysis. In this study, phylogenetic ML, MP and Bayesian analyses revealed relatively similar trees and almost identical clades. More than two-third (27 out of 33) of Ethiopian *Agaricus* sequences examined in this study were distributed amongst four of the eight accepted sections of the subgenus *Agaricus*. None of the Ethiopian sequences in our dataset belongs to section Arvenses, Bivelares, Chitonioides and Spissicaules. The remaining six Ethiopian sequences group together with Africa and/or Asia sequences under four distinct and exclusively tropical clades (Clade I, II, III and IV) with ML/MP/PP branch support 99/100/100, 100/100/100, 83/75/90, 85/75/90 respectively. These tropical clades are not phylogenetically closely linked and this may show species diversification occurs several times in these regions. However, it is still believed that there is a need for more comprehensive studies to include more species from wide range of geographical regions to better understand the biogeography of the genus. From the seventeen species identified to species level; *A. campestris*, *A. cupreobrunneus*, *A.bohusii*, *A. purpurellus*, *A. subsaharianus* and *A. heterocystis*, are known to be edible and are recommended for domestication and cultivation.

***Keywords*:** Macrofungi, *Agaricus*, Menge, Welmera, Phylogeny, Taxonomy

5.1. Introduction

Agaricus L., referred as *Psalliota* in older publications (Cappelli, 1984), is a large and important genus encompassing many edible and poisonous mushrooms. The genus includes the common ("button") mushroom (*Agaricus bisporus*) and the field mushroom (*Agaricus campestris*), the dominant cultivated mushrooms of the West. *Agaricus* species are generally found in pastures among grass and in mixed forests (Bas, 1991). *Agaricus* species are generally distinguished from other members of the family Agaricaceae by their small to large basidiomata, white or brown fleshy pileus, central stipe, presence of annulus, free lamellae that are whitish or pinkish when young and chocolate-brown to dark brown when mature with smooth dark basidiospores (Pegler, 1977; Cappelli, 1984; Singer, 1986; Parra, 2008).

The genus has been considered as one of the most taxonomically difficult genera. Thus, several treatments have been proposed by many mycologists, based on their judgments of how significant each morphological characteristic is in the recognition of species and sections in the genus (Heinemann, 1978; Cappelli, 1984; Kerrigan, 2005; Singer, 1986; Parra 2008, 2013). The current systematic treatments of the genus used four taxonomically important characteristics which has more recently been recognized as a crucial characteristic. These include i) Chemical reactions: Schaeffer‘s cross-reaction (aniline and concentrated nitric acid) and alkali test (either NaOH or KOH), ii) basidiosporocarp colour change when bruised or cut; iii) odour of basidiosporocarp, iv) the structure of the annulus, which can be single or double (Callac *et al*., 2003; Callac and Guinberteau, 2005; Parra 2008, 2013).

However, at the species level and within certain sections, the following traits remain useful for species identification: pileus colour, aspect of on pileus (squamules), base of the stipe (marginate bulbous), spore size, cheilocystidia (catenulate), hyphae and also habit, habitat or the geographic/climatic distribution (Parra, 2013; Karunarathna *et al*., 2016).

According to Bas, (1991) the number of *Agaricus* species worldwide was estimated to be close to 400. Zhao *et al*., (2011) confirmed the existence of 386 species in the genus in their report. From 2011 to the end of 2015, 48 new species have been introduced, including 18 new species from Thailand (Chen *et al*., 2012, 2015, Zhao *et al*., 2012a, b; Karunarathna *et al*., 2014, Thongklang *et al*., 2014), four from China (Li *et al*., 2014, Gui *et al*., 2015), one from Sri Lanka, 16 from Europe (Parra *et al*., 2011, Parra 2013; Parra *et al*., 2014,) and nine new sequestrate species from Australasia (Lebel, 2013). Therefore, the total number of species is presently more than 450, among them 200 are tropical species (Karunarathna *et al*., 2016).

Studies have shown that the genus is monophyletic (Geml *et al.,* 2004) and contain eight recognized sections (Parra, 2008), some of which have been phylogenetically reconstructed, e.g., *A.* sect. *Bivelares* (Kauffman) L.A. Parra and *A.* sect. *Xanthodermatei* Singer (Challen *et al.,* 2003; Kerrigan *et al.*, 2005; Thongklang *et al.,* 2014). The delimitation of these sections originally described from temperate (European and North American) species (Pegler, 1986; Cappelli, 1984; Kerrigan *et al*., 2006; Callac *et al*., 2003; Alberto, 1998; Parra, 2008; Callac and Guinberteau, 2005; Parra 2013) might not be successful to comprise those species originating in tropical (Asia and Africa).

Agaricus species are saprobic and generally humicolous (Angeli *et al*., 2006; Bernarshaw *et al*., 2007) and can be found from sea level up to the vegetation limit in mountainous areas (Cappelli, 1984), and also occurs in some arid areas (Lebel, 2013). Despite their ecological and economic role, their diversity remains poorly known, particularly in tropical areas. Species of this diverse genus are common in tropical regions (Zhao *et al*., 2011; Heinemann, 1980, 1986, 1993; Pegler and Rayner, 1969; Pegler, 1977, 1986; Saini *et al*., 1997; Valenzuela *et al*., 1997; Peterson *et al*., 2000; Natarajan *et al*., 2005).

Information on the diversity of the genus, is still incomplete in Ethiopia. It has been clearly indicated that, in Ethiopia only *A. campestris*, *A. xanthodermus* and *A. xanthodermulus* have been reported (Dawit Abate, 1999; Rediet Sitotaw *et al.*, 2015a). Dawit Abate (1999), who cited *Agaricus campestris* as the dominant mushroom in the grazing areas on the highland (2000–3000 m) plateau in southern (Bale), central (Debreberhan), and northwestern (Gojam) Ethiopia, appearing in a large numbers during the middle of the rainy season (July and August). However, this report did not provide a detailed morphological description or any molecular data of the species. However, recent study by Rediet Sitotaw *et al.* (2015a) have reported that incorporate molecular data together with morphological characteristics to infer phylogenetic relationship of few species in the genus.

Considering that one third of the species in the genus *Agaricus* resulted to be new to science even from the best studied mycological regions (Europe and North America) Kerrigan *et al.* (2005), then it could be possible to expect the number of *Agaricus*

species in subtropical and tropical regions to be much more. Given this research gap in Ethiopia, this study sought to contribute the two important demands in the field of mycology in the country. The first one is collection, documentation and preservation of *Agaricus* species of three different habitats (forest, grazing land and farm area) in two regions (located in northwest and center) of the country. The second one is to provide a morphological, molecular and ecological description. Hence, in this study the DNA sequence of internal transcriber spacer regions (ITS1–5.8S–ITS2) which is the most popular locus for species identification and phylogenetic inference in sequence-based mycological research was used.

5.2. Materials and Methods

5.2.1. Morphological identification

Detail description of the study area and methods used for collection and morphological identification of macrofungal specimens have described in section 3.2.3.

5.2.2. Molecular Characterization

DNA extraction

Molecular characterization component of this research was done at the State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences. Total genomic DNA was extracted using the modified Cetyltrimethylammonium Bromide **(**CTAB) method described by Yao *et al*., (1999), removing the phenol/chloroform step to reduce the use of hazardous chemicals. Approximately 25 mg of each specimen was grounded to a fine powder in liquid nitrogen. The grounded materials were transferred into Eppendrof tube. Pre-warmed extraction buffer (CTAB)

650 µl was added and then incubated at 65°C for 1 hr in a water bath. After removed from the water bath the tubes were centrifuged at 10,000 rpm for 10 minutes at 8°C. Then the supernatant was transferred to a new 1.5 mL Eppendrof tube and an equal volume of chloroform and isoamayl alcohol (24:1v/v) was added and mixed by inverting tubes for 10 minutes. The tubes were centrifuged for 10 minutes at 10,000 rpm for 10 minutes. Then the supernatant was transferred to a new 1.5mL eppendref tube. Again for the second time equal volume of chloroform and isoamayl alcohol (24:1v/v) was added and mixed by inverting tubes for 10 minutes. The tubes were centrifuge for 10 minutes at 10,000 rpm. Upper aqueous layers were removed into clean tubes and 0.1V 3M Sodium acetate (pH 5.2) was added followed by pre-cold 0.7V Isopropanol. This was gently mixed by inverting tubes. The tubes were kept at -20°C for 2 hours.

Then mixture was centrifuged at 13,000 rpm for 20 minutes. The supernatant was discarded and pellets rinsed with 70% alcohol and mixed for 5 minutes. This procedure was repeated two times. After discarding the supernatant, the pellets were dried in a dryer for 20 minutes at room temperature then resuspended in 30µL RNase-free water.

PCR amplification and sequencing

The fungal universal primer pair ITS5 (5` GGAAGTAAAAGTCGTAACAAGG 3`) and ITS4 (5` TCCTCCGCTTATTGATATGC 3`) was used for amplification of the internal transcribed spacer (ITS1+5.8S+ITS2) region (White *et al*., 1990). The PCR reaction mixture was made with the following components: 25 µL of Taq PCR Master Mix (ESTaq MasterMix), 0.5 µL of 10 µM each primer, 1 µL diluted DNA template

and RNase-Free water to bring the total volume to 50 µL. The PCR conditions were as follows: 94°C for 5 min, followed by 35 cycles of 95°C for 1min, 53°C for 1min, 72°C for 1 min and final extension step of 72°C for 10 min on thermocycler (GenAmp PCR System 9700; Version 3.03).

PCR products were sequenced from both directions using the same primers on a capillary sequencer (Applied Biosystems 3730 Analyzer, Foster City, California) by the Beijing Genomics Institute (Beijing, China). Sequence ends were manually edited and assembled using SeqManTM II module in the Lasergene version 6.1 packages (DNAStar Inc., Madison, WI). The sequences were blast in NCBI and related gene sequences for each of the specimens were obtained from GenBank and then, aligned using ClustalW program incorporated in BioEdit v. 7.1.9 (Hall, 2004).

A data set of 85 complete and non-redundant ITS sequences were aligned for phylogenetic analysis to identify the major clades and the distribution of the Ethiopian species among these clades (Table 5-1). This dataset consists of 47 sequences from tropics and subtropics (of which 33 are Ethiopian origin) and 36 temperate samples, which were selected purposefully to insure the representation of these sequences in all presently recognized sections of subgenus *Agaricus. Hymenagaricus ardosiicolor* and *Heinemannomyce splendidissimus* which are found to be closely related to *Agaricus* (Zhao *et al*., 2011) were used as outgroup taxa.

5.2.3. Phylogenetic Analysis

Maximum parsimony (MP) analysis, was performed using PAUP* 4.0b10 (Swofford, 2004), by heuristic searches with unordered characters, random addition of sequences, gaps treated as missing data, and the tree bisection-reconnection (TBR) branch swapping. Maximum likelihood (ML) analysis was performed on Phylogeny.fr platform (http://www.phylogeny.fr/) and the phylogenetic tree was constructed using PhyML version 3.0 (Guindon and Gascuel, 2003). The HKY85 substitution model was selected with an estimated proportion of invariable sites of 0.571 and assuming 4 gamma-distributed rate categories. The gamma shape parameter 0.728 was directly estimated from the data. Reliability for internal branch was assessed using the aLRT test which assesses that the branch being studied provides a significant likelihood gain, in comparison with the null hypothesis that involves collapsing that branch but leaving the rest of the tree topology identical (Anisimova and Gascuel, 2006). The Bootstrap values (BS) were obtained from 1000 replicates.

The phylogeny Bayesian Inference (BI) was also performed with MrBayes 3.1.2 (Ronquist and Heulsenbeck, 2003). One million generations using a GTR+I+G model nucleotide substitution were run for six Markov chains and sampled every 100^{th} generation resulting in 10,000 trees. Those trees sampled prior to searches reaching a split deviation frequency value reaching 0.01 were discarded as the burn-in, and the remaining trees were used to calculate Bayesian posterior probabilities (PP) of the individual clades. Trees were viewed in FigTree v1.4.3 (Chevenet *et al*., 2006).

Table 5-1 ITS sequences of *Agaricus* and out-group species used in the phylogenetic analysis.

No.	Taxa/ Species	Voucher/ strain	Country/ Origin	Location and habitat	GenBank* ITS
1	*Agaricus aridicola*	CA101	France	Oléron Island (In dune)	JF797195
2	*A.* sp1	**RSK151**	Ethiopia	Welmera (Forest)	
3	*A. andrewii*	RWK1917	USA		AF432877
4	*A. arvensis*	CA640	France	Gironde, Villenave d'Ornon (Under Pinus)	JF797194
5	*A. augustus*	CA590	France	Gironde, Labrède (Under Cedrus)	JF797193
6	*A. bisporiticus*	**RSK77**	Ethiopia	Welmera (graizing area)	
7	*A. bisporiticus*	**RSK147**	Ethiopia	Menge (farm land)	
8	*A. bisporiticus*	LD2012111	Thailand		KJ575611
9	*A. bisporiticus*	MRC25	Pakistan		KJ575608
10	*A. benesii*	LAPAG283	Spain	Burgos, Barrio de Cortes (Under Pinus)	JF797179
11	*A. bohusii*	LAPAG531	Czech Repub	Tremosnice near Caslav (Under Carpinus)	JF797180
12	*A. bohusii*	**RSK187**	Ethiopia	Menge (farm area), Welmera (farm area)	
13	*A. bresadolanus*	CA177	France	From GenBank	DQ185570
14	*A.* sp2	**RKS62**	Ethiopia	Welmera (graizing area)	
15	*A.* sp3	**RKS65**	Ethiopia	Welmera (farm area)	
16	*A. bitorquis*	RWK1462	USA	From GenBank	AF432898
17	*A. bitorquis*	BK2	USA		AY484695
18	*A. brunneolus*	CA490	France	Gironde, Blanquefort (Glade of oak)	JF797203
19	*A. brunneolus*	**RSK 255**	Ethiopia	Welmera (graizing area)	
20	*A. bernardii*	ARP173	USA		AF432880
21	*A. campestris*	strain H2	India		KM609406
22	*A. campestris*	**HMAS272461**	Ethiopia	Welmera (graizing area)	**KP229419**
23	*A. campestris*	**HMAS 272462**	Ethiopia	Menge (graizing area)	**KP229420**

24	*A. campestris*	W1H	USA		DQ182533
25	*A. californicus*	RWK 1936	USA		DQ182510
26	*A. californicus*	29-I-1999	USA	campus UC-Berkeley	AF482830
27	*A. californicus*	**RSK103**	Ethiopia	Welmera (Forest)	
28	*A. caribaeus*	**RSK112**	Ethiopia	Welmera (Forest)	
29	*A. caribaeus*	**RSK116**	Ethiopia	Welmera (Forest)	
30	*A. caribaeus*	**RSK80**	Ethiopia	Welmera (Forest)	
31	*A. caribaeus*	F2530	France		JF727856
32	*A. cupreobrunneus*	LAPAG 322	Spain		JQ824136
33	*A. cupreobrunneus*	CA87	France	From GenBank	DQ182532
34	*A. cupreobrunneus*	**RSK197**	Ethiopia	Menge (grazing area)	
35	*A. cupreobrunneus*	**RSK181**	Ethiopia	Menge (farm area)	
36	*A. xanthodermus*	**HMAS272456**	Ethiopia	Welmera (Forest)	**KP229414**
37	*A. xanthodermus*	**HMAS272457**	Ethiopia	Welmera (Forest)	**KP229415**
38	*A. xanthodermus*	**RSK99**	Ethiopia	Menge (Forest)	
39	*A. xanthodermus*	**RSK138**	Ethiopia	Welmera (Forest)	
40	*A. xanthodermus*	CA161	France		AY899272
41	*A. xanthodermus*	CA236	France		DQ185564
42	*A. xanthodermulus*	**HMAS272459**	Ethiopia	Welmera (graizing area)	**KP229416**
43	*A. xanthodermulus*	**HMAS272460**	Ethiopia	Welmera (graizing area)	**KP229417**
44	*A. xanthodermulus*	**RSK96**	Ethiopia	Menge (Forest)	
45	*A. xanthodermulus*	CA174	France		AY899274
46	*A. xanthodermulus*	CA204	France		AY899276
47	*A. xanthosarcus*	**RSK150**	Ethiopia	Welmera (Forest)	
48	*A. xanthosarcus T*	Goossens5415	RDCongo	Panzi, Kivu (Coffee plantation)	JF514523
49	*A. xanthosarcus*	NTS7	Thailand	Chiang Rai, Khun Kone (In forest)	JF514533
50	*A. xanthosarcus*	**RSK 72**	Ethiopia	Welmera (graizing area)	

51	*A. aff. endoxanthus*	ZRL3095	Thailand	Chiang Mai, Mae Taeng (In forest)	JF691554
52	*A. endoxanthus*	LAPAG 225	USA	From GenBank	DQ182511
53	*A. endoxanthus*	**RSK 247**	Ethiopia	Welmera (forest)	
54	*A. subsaharianus*	**RSK19**	Ethiopia	Menge (grazing area)	
55	*A. subsaharianus T*	ADK4732	Burkina-Faso	Ouagadougou (In urban park)	JF440300
56	*A. subsaharianus*	**RSK182**	Ethiopia	Menge (graizing area)	
57	*A. silvaticus*	LAPAG341	Spain	Madrid, Parque del Retiro (Under Cedrus)	JF797178
58	*A. kivuensis*	**RSK36**	Ethiopia	Menge (graizing area)	
59	*A. aff. kivuensis*	Rammeloo5756	Burundi	Mugara (Oil palm plantation)	JF514541
60	*A. microvolvatulus*	Grinling70109	Congo	Brazzaville (In forest edge)	JF514524
61	*A. microvolvatulus*	**RSK63**	Ethiopia	Menge, Welmera (graizing area)	
62	*A. moelleri*	CA31	France		AY899263
63	*A. moelleri*	CA156	France		AY899264
64	*A. langei*	WC784	USA		AY484699
65	*A. langei*	LAPAG141	Spain	Gumiel de Mercado (Grass, oak)	JF797181
66	*A. laskibarii*	LAPAG115	USA		AY943975
67	*A. litoralis*	CA120	France		JN204436
68	*A. litoralis*	CA829	France		JF727867
69	*A. purpurellus*	TRgmb01309	Italy	Gironde, Préchac (In grassland)	KF447903
70	*A. purpurellus*	**RSK83**	Ethiopia	Welmera (graizing area)	
71	*A. purpurellus*	**RSK164**	Ethiopia	Menge (Forest)	
72	*A. parvitigrinus*	CA157	France		AY899266
73	*A. parvitigrinus*	CA158	France		AY899267
74	*A. parvitigrinus*	**RSK 97**	Ethiopia	Welmera(graizing area)	
75	*A. pseudolutosu*	LAPAG77	Spain	Honrubia de la Cuesta (In meadow)	JF727868
76	*A. pseudolutosu*	**RSK 172**	Ethiopia	Welmera (graizing area)	
77	*A. fissuratus*	WC777	Denmark	From GenBank	AY484683
78	*A. gennadii*	CA387	France	Seine-Maritime, (On roadside)	JF797188
79	*A. aff. Impudicus*	LAPAF3	Togo	Ola (on wood litter)	JF797184

80	*A. goossensiae*	ADK2751	Benin	Calavi Campus, Atlantique	JF514519
81	*A. heterocystis*	**RSK52**		Menge (Forest)	
82	*A. heterocystis*	Goossens5066	RD Congo	Panzi, Kivu	JF514522
83	*A. campestroides*	LAPAF2	Togo	Ola-Okpa-Fou	JF727842
84	*H. ardosiicolor*	LAPAF9	Togo	Lomé, University Campus	JF727840
85	*H. splendidissimus*	ecv3586	Thailand		HM488760

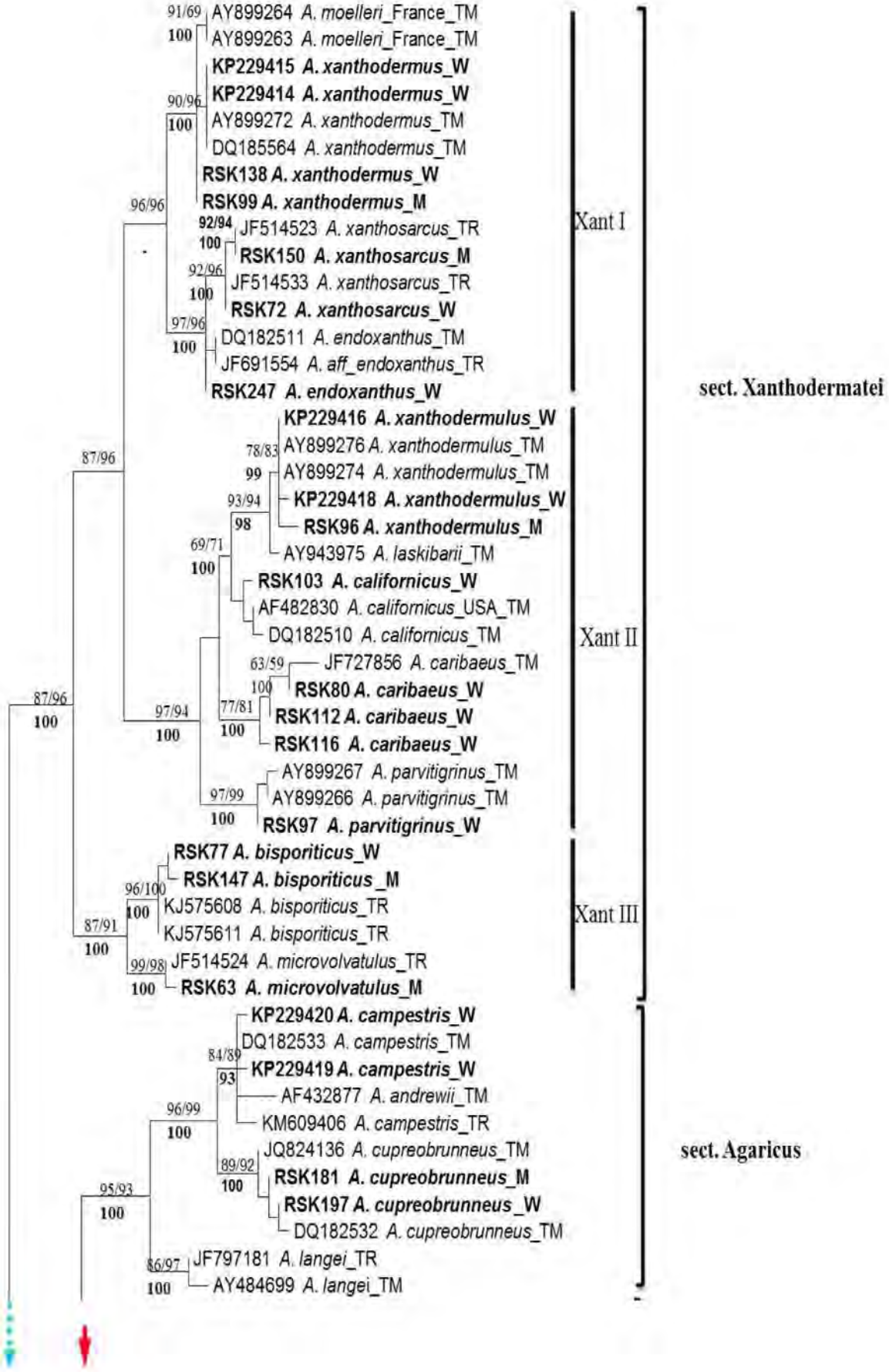
5.3. Results

Amplification of the ITS region of rDNA of all *Agaricus* sp. in this study produced a fragment of approximately 640 – 750 bps. Sequences analysis showed that the ITS1 and ITS2 regions show some differences among species studied, and it could be due to nucleotide insertion/deletions or substitutions, where as the 5.8S rRNA sequence (about 167 bp long) were relatively conserved for all taxa. With in the ITS1 sequence, 113 of the 331 aligned positions (34.1%) varied among the species. Within 5.8S region, five of the 167 aligned positions (3%) and with in the ITS2 sequences, 58 of the 205 aligned positions (32%) varied among the species of *Agaricus*.

The final alignment contained 608 characters, of which 395 characters were constant, 26 variable characters were parsimony-uninformative and the number of parsimony-informative characters were 187. The phylogenetic trees generated by ML, MP and Bayesian methods presented very similar topologies. Most of the major clades were similar and relatively well supported in the ML and Bayesian analyses while the bootstrap support values were sometimes lower.

The 33 *Agaricus* specimen collected were identified in to 21 distinct *Agaricus* species. From these 15 *Agaricus* species were found only in a single study site (ten in Welmera and five in Menge). However, six *Agaricus* species were common to both study sites. Some of these common collections have nearly or entirely redundant sequences and belong to same species, such as RSK 83 and RSK 164 (*A. purpurellus*), RSK77 and RSK 147 (*A. bisporiticus*) or RSK 99 and RSK 138 (*A. xanthodermus*).

In the phylogenetic analysis, few differences were noted in the branching pattern of the positions of samples *A. bohusii* (JF797180), *A. bohusii* (RSK187_W) and *A. langei* (JF797181). The Most Likelihood (ML) tree is shown in Figure 5-1, with bootstrap support values (ML/MP) above 50% and Bayesian posterior probability (PP) values above 90%. In all analyses, the two outgroup taxa cluster at the base of the tree.


AY899264 A. moelleri_France_TM
AY899263 A. moelleri_France_TM
KP229415 A. xanthodermus_W
KP229414 A. xanthodermus_W
AY899272 A. xanthodermus_TM
DQ185564 A. xanthodermus_TM
RSK138 A. xanthodermus_W
RSK99 A. xanthodermus_M
JF514523 A. xanthosarcus_TR
RSK150 A. xanthosarcus_M
JF514533 A. xanthosarcus_TR
RSK72 A. xanthosarcus_W
DQ182511 A. endoxanthus_TM
JF691554 A. aff_endoxanthus_TR
RSK247 A. endoxanthus_W
KP229416 A. xanthodermulus_W
AY899276 A. xanthodermulus_TM
AY899274 A. xanthodermulus_TM
KP229418 A. xanthodermulus_W
RSK96 A. xanthodermulus_M
AY943975 A. laskibarii_TM
RSK103 A. californicus_W
AF482830 A. californicus_USA_TM
DQ182510 A. californicus_TM
JF727856 A. caribaeus_TM
RSK80 A. caribaeus_W
RSK112 A. caribaeus_W
RSK116 A. caribaeus_W
AY899267 A. parvitigrinus_TM
AY899266 A. parvitigrinus_TM
RSK97 A. parvitigrinus_W
RSK77 A. bisporiticus_W
RSK147 A. bisporiticus _M
KJ575608 A. bisporiticus_TR
KJ575611 A. bisporiticus_TR
JF514524 A. microvolvatulus_TR
RSK63 A. microvolvatulus_M
KP229420 A. campestris_W
DQ182533 A. campestris_TM
KP229419 A. campestris_W
AF432877 A. andrewii_TM
KM609406 A. campestris_TR
JQ824136 A. cupreobrunneus_TM
RSK181 A. cupreobrunneus_M
RSK197 A. cupreobrunneus_W
DQ182532 A. cupreobrunneus_TM
JF797181 A. langei_TR
AY484699 A. langei_TM
Xant I
Xant II
Xant III
sect. Xanthodermatei
sect. Agaricus

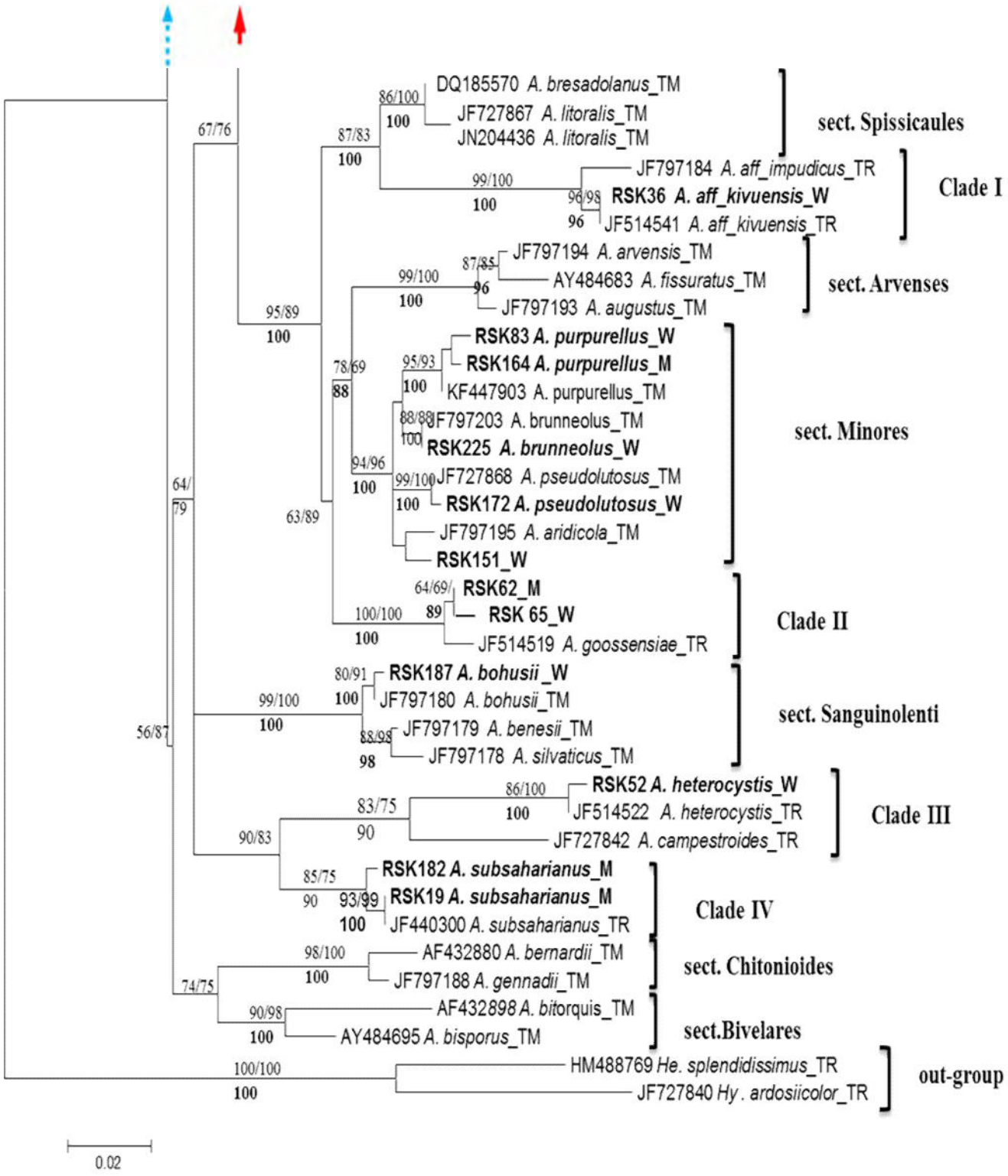


Figure 5-1 Maximum likelihood (ML) phylogram of *Agaricus* sequences
A dataset of 85 ITS1+2 *Agaricus* sequences and two outgroup species of related taxa *Hy. ardosiicolor* and *He. Splendidissimuseas*. Bootstrap support values above 50% and Bayesian posterior probability values above 90% are shown (ML/MP/PP). The species described in this study are in boldface.

Phylogenetic analyses of Ethiopian Agaricus

The phylogenetic analyses place all the *Agaricus* collections into a monophyletic genus *Agaricus* and distributed in different clade (Sections). The two non-*Agaricus* species representing the out-group, made a clade sister to *Agaricus* with 100% supported in all phylogenetic analysis methods in this study.

Most of the major clades were comparable and generally appearing in the same order and well supported with the high ML/MP value and Bayesian Inference (PP). All most all the temperate species were distributed into the major eight previously accepted taxonomic sections of subgenus *Agaricus;* Bivelares, Chitonioides, Xanthodermatei, Sanguinolenti, Agaricus, Spissicaules, Arvenses and Minores. Among these, three of them (Bivelares, Chitonioides and Arvenses) are entirely temperate. In addition to the major sections of subgenus *Agaricus*, the phylogenetic analysis in this study confirmed that there are four additional clades that contain only tropical species (African and Asian). The four tropical clades detected, are well supported in all analyses methods, and numbered as Clade I, II, III and IV with ML/MP/PP branch support 99/100/100, 100/100/100, 83/75/90, 85/75/90 respectively.

Six of the Ethiopian *Agaricus* sequences were placed into these four clades with support value greater than 80% except in clade II RSK 62 and RSK 65 have a lower support value as compared to the rest. In the ML tree, this clade is paraphyletic with clade section Minores. RSK 62 and RSK 65, which are proposed to be new, were grouped together with *A. goossensiae.* Taxon list and their respective support value are provided in Table 5-2.

In the trees shown above, all sections except Sec. Xanthodermatei are monophyletic. In the three trees obtained during the analysis, section Xanthodermatei appeared polyphyly/paraphyletic. It includes three major sub clades which are sister to each other and designated as; Xant I, Xant II and Xant III. However, some minor differences appeared in the supported value of this three clades, as the support value in ML/MP/PP analysis, is 96/96/-, 97/94/100 and 87/91/100 for Xan I, Xant II and Xan III respectively.

More than 80% of *Agaricus* sequences examined in this study (27out of 33) were distributed amongst four of the eight accepted sections of the subgenus *Agaricus* (Table 5-2). The six remaining sequences were placed in the four clades that exclusively contain tropical collections. There were no Ethiopian sequences in our dataset that belong to section Arvenses, Bivelares, Chitonioides and Spissicaules.

Table 5-2 Phylogenetic and geographic distribution of 33 Ethiopian *Agaricus* collections into sections based on temperate species Sections and tropical clades

Sections/Clades	ML/MP/PP branch support	Number of Ethiopian *Agaricus*, Geographic location (District)			Tropical Species	Temprate Species	Identified Ethiopian species
		W-D	M-D	Total			
Sections based on temperate species							
Agaricus	95/93/100	3	1	4	2	5	*A. campestris*, A. cupreobrunneus**
Arvenses	99/100/100	–	-	-	-	3	-
Bivelares	90/98/100	–	–	–	-	2	-
Chitonioides	98/100/100	–	–	–	-	2	-
Minores	94/96/100	4	1	5	-	4	*A. purpurellus*, A. brunneolus*,A. pseudolutosus** *A.* sp1 (RSK151_W)
Sanguinolenti	99/100/100	1	-	1	-	3	*A.bohusii**
Spissicaules	86/100/100	-	-	-	-	3	-
Xanthodermatei	87/96/100	12	5	17	6	16	*A.xanthodermus,A. xanthosarcus, A. endoxanthus,A. xanthodermulus, A. californicus, A. caribaeus, A. parvitigrinus, A. bisporiticus, A. microvolvatulus*
Total			**27**				
Tropical branches and clades of presumed species found in Africa and/or in Asia							
Clade I	99/100/100	1	-	1	2	-	*A. kivuensis*
Clade II	100/100/100	2	-	2	1	-	*A.* sp2 (RSK62_M), *A.* sp3 (RSK65_W)
Clade III	83/75/90	1	-	1	2	-	*A. heterocystis**
Clade IV	85/70/90	-	2	2	1	-	*A. subsaharianus**
Total			**6**				

Tropical Species (Asia, Africa), Temprate Species (America and Europe), W-D= Welmera District, M-D= Menge District

* indicate edible species

Key morphological taxonomic traits in Ethiopian *Agaricus* species

The following general characteristics of the genus was recorded during specimen examination: piluse mostly medium sized to large, with variations in color from white to almost dark color or sometimes with fibrillose or scal of gray, brown and red. Gills are free from the stem, close, whitish to pinkish when young then to chocolate-brown to blackish brown at maturity. Flesh in most cases thick, pure white or whitish. The stem is central, solid, stuffed or hollow and separates easily from the cap. Annulus present, sometimes it was ephemeral. All are saprophytic (living on soil that is rich with dead organic matter, mostly on leaf litter in the forest, open areas like roadsides and grazing land).

Distinctive characteristic such as spore size, specific staining reactions such as KOH and Schäffer's reaction, odor and test were especially noted in fresh specimen for species identification. The distinctive characteristics of each species in each section is provided in the table below (Table 5-3).

Table 5-3 Key morphological traits of Ethiopian *Agaricus* collections

Section	Color change	Odor	Annulus	Context
Agaricus	not changing color, either when bruised or in KOH	pleasant or some times mild	Single/thin, white, short-lived cottony scant, sometimes absent	white or whitish, thick, soft
	Pileus 4- 8 (11) cm. in diam., smooth, surface white to cream-color, slightly ochraceous, with grayish to pale ochraceous fibrils or fibrillose scales, sometimes silky. Stipe 3-8 × 0.5-1 cm or less, tapered toward base (pointed). Spores 5⁻8.5 × 3.5⁻5.5 μ m, avl × avw = 6.5 × 4.5 μ m, Q = 1.4⁻1.5, avQ = 1.45, ellipsoid to ovoid, inamyloid, smooth, thickwalled. ………………….……………………………………….……...*A. campestris*			
	Pileus 4.0-6.0 cm. in diam., medium brown to dark brown, with fibrillose-squamulose. Stipe 2-6 × 1-2.5 cm usually blunt base. Spores. 6-7.5 ×4.5 -5.5. μ m. avl × avw = 6.7 × 5 μ m Q = 1.3⁻1.4, avQ = 1.35, elliptic to subovoid, inamiloid .... *A. cupreobrunneus*			
Minores	stains, and stays yellow (on its own, and with KOH) orange Schäffer's reaction	sweet, sometimes faint	has only one layer single, thin, evanescent white	Small, delicate
	Pileus 4-4.5 cm. in diam; wine red at disc; finely appressed fibrils in small batches radiating outward. Stipe 3.5-7 cm x 0.5 cm with bulbous base. Spores 4.5-5 × 2.5-3.5 μ m, avl × avw = 4.75 × 3, Q = 1.8⁻1.4, avQ = 1.6, narrowly elliptic to obovate.……………………………………………………………...………...*A. purpurellus*			
	Pilus 3-5 cm. in diam., surface covered with fine, purplish fibrillous scales particularly towards the disk. Slightly yellowish on brushing. Stipe 2.5-5 cm x 0.3-0.7 cm cylindrical with a subbulbus base. Spores 5-7 x 3.8-5 , avl × avw = 6 x 4.4 Q = 1.3⁻1.4, avQ = 1.35, short ellipsoid ………………………….*A.* sp 3 RSK151_W			
Sanguinolenti	stains red or red-brown when bruised, no color reaction with KOH	mild	veil white, membranous	Thick, firm ,white
	Cap 5.0-12 cm. in diam., white, the disc sometimes brownish, short loose annulus, often leaving scattered squamules on the lower stipe. Stipe 4.5-13 cm × 1-1.5 cm; nearly cylinderical with bulbous base. Spores 5.3 – 6.5× 3.4 – 4.2 μ m,avl × avw = 5.9 × 3.8, Q = 1.55 - 1.54, avQ = 1.55 , ellipitic (egg-shaped) ………………………………………………………………………………………….…….....*A.bohusii*			
Xanthodermatei	at the base of the stalk staining yellow when cut, turning yellow in KOH, (Scha¨ffer's reaction) -negative	Unpleasant, phenolic or iodine-like	annulus pendant	fleshy

	Pileus 7-12 cm. in diam., white and smooth when young occasionally scaly or cracked with age. Stipe 5-12 cm x 1-2.5 cm; cylindrical with slightly bulbous base.Spores 4-5.5 × 3-4 μ m, avl × avw = 4.5 × 3.5 μ m, Q = 1.3-1.4, avQ = 1.35 ellipsoid. …… *A. xanthodermus*	
	Pileus 5-7 cm. in diam., sometimes squamulate.Stipe 4-8 × 0.5-1 cm, central, cylindrical with slightly bulbous base. spores 5-7 × 4-5.5 μ m, avl × avw = 6.0 × 4.7 μ m, Q = 1.25-1.27, avQ = 1.26 ellipsoid, thick-walled..……..……*A. xanthodermulus*	
	Pileus 6-10 cm. in diam., with dark brown radial squamules, with elongated stip 6-18 cm x 0.7-1.5 cm, cylinderic, with basal bulb, annulus superior double margion, discoloring deep yellow at the base. Spores 4.5 – 6.4 x 3 – 4.5 μ m, avl × avw = 5.4 × 3.7 μ m, Q = 1.5-1.4, avQ = 1.45, ovoid to shortellipsoid ……..…….. *A. endoxanthus*	
	Pileus3-10 cm. in diam., with small repent squamules on a yellowish cream background. Stipe 5.5-12cmx 0.7-2 cm cylinderic, solid, with small basal bulb. Annulus superior , membrainous. Spores 4-6 x 2.5 – 3.5 μ m avl × avw = 5 × 3 μ m, Q = 1.6-1.7, avQ = 1.65, ovoid to elliposoid ……..…….*A. xanthosarcus*	
	Pileus 4 -9(12) cm. in diam., white to silver gray, light brown fibrils at the center. Stipe 3-8(10) cm x 0.5-1(1.5) cm; culinderic, smooth; white. The phenolic odor and yellow-bruising is faint usually barely discernable. Spores 5-7.5 x 4-5.5 µm, avl × avw = 6.3 × 4.7 μ m, Q = 1.25-1.36, avQ = 1.3 elliptical, smooth…..…….*A. californicus*	
	Pileus 5–10 cm. in diam., Surface dull and dry, with fibrillose scales 6–10 × 0.5–0.7, cylinderical, slightly bulbous at the base, smooth on surface, white to light brown, hollo. Annulus superior, membranious. 5.5 - 7.0 x 3.5- 4.0 µm, avl × avw = 6.25 × 3.75 μ m, Q = 1.5-1.7, avQ = 1.6 µm, ellipsoid, smooth,thick walled, ……..…*A. caribaeus*	
	Pileus 4.5–9 cm. in diam., squamose, dense at the disk and more scattered toward the margin on a whitish to gray background, Stipe 3.0–7.0 3 x 0.5–1.2 cm slightly bulbous at the base, exhibits orange discoloration . Spores 4.5–7.3 × 2.9–4.7 μ m, avl × avw = 5.9 × 3.8 μ m, Q = 1.5-1.7, avQ = 1.6 ovoid , bisporic ……..…..*A.bisporiticus*	
	Pileus (7–)10–14 cm. in diam., depressed at the center, dark gray to dark brown at the disk, covered with gray to brown squamules more scattered toward the margin on a white background. Stipe 11.0–12.0 x 0.7–1.5 cm, cylindrical, hollow. Annulus superior, spores 4.5–6 × 2.5–3.8 μ m Q =1.6– 1.7, avQ= 1.65 ellipsoid ……..*A. microvolvatulus*	

Clade I	Pilues 5-10 cm. in diam., surface covered with small brown squamules concentrically arranged on a pale brown background and darker at the center. Stipe 6-10 x 0.5-0.1 cm cylindric , staining ochraceous when cut or brushed. Spores 5-6.5 × 3 – 4.5 μ m, Q = 1.5- 1.65 , avQ=1.57 ellipsoid……..*A. kivuensis*			
Clade II	positive yellow KOH and orange Schäffer's reaction	mild almonds smell	annulus simple, white	fleshy, white or light brown
	Pilus 4-8 cm. in diam., with fine, gray scales towards the disk. Slightly yellowish on brushing. Stipe 2.5-5 × 0.3-0.7 cm cylindrical tapered toward base (pointed). Spores 5-7 × 3.8-5 μ m , avl × avw = 4.4 × 6 , Q = 1.4-1. 5 , avQ=1.45 short ellipsoid ……..*A.* sp2 / RSK62_M.			
	Pilus 3-7(9) cm in diam., brown fibrillous scales on gray background, darker at the center.color reaction not recorded. Stipe 2.5-5 cm × 0.3-0.7 cm cylindrical with a subbulbus base. Spores 4.5-7 × 3.5-6 μ m, Q= 1.5 - 1.8, avQ = 1.65short ellipsoid ……*Agaricus* sp3 /RSK65_M			
Clade III	Pileus 6-10 cm. in diam., brown fibrillous scales. Stipe cylindrical, slightly bulbous, hollow, surface white. Spores 5.5 – 6.0× 3.5 – 4.3 μ m, avl × avw = 5.7 × 3.5, Q = 1.55 - 1.53, avQ = 1.53 , ellipitic, almond odor ……*A. heterocystis*			
Clade IV	Staining yellow or orange KOH reaction positive but usually faint.	pleasant bitter almond to unpleasant like phenol or solvent	Double or complex annulus	
	Pileus 7-12 cm in diam., white covered with brownish squamules. Stipe 4.5-5.8 × 1.5-2 cm. cylindrical to clavate, sub-bulbous, with short rhizomorphs at the base. 4.2 – 6 × 3 – 4.8 μ m, avl × avw = 3.6 × 5.4 μ m, Q = 1.5‑1.4, avQ = 1.45, ovoid to short ellipsoid ……... *A. subsaharianus*			

5.4. Discussion

From the present study, it was observed that there is no major alteration in the classification of the temperate species into eight sections with the addition of tropical/Ethiopian species in the analysis. It has also recognized that a large part of the tropical (Africa and Asia) including Ethiopian samples are clustered together with those of temprate species in four sections of subgenus *Agaricus*; Xanthodermatei, Sanguinolenti, Agaricus, and Minores. Arvenses, Bivelares and Chitonioides do not incorporate tropical including Ethiopian species and are exclusively temprate sections. This result is in aggrement with Zhao *et al*. (2011) that has described the absence of tropical species in sections Bivelares and Chitonioides in their studies that consist of a total of 178 samples, with 89 from Asia, 37 from the Americas, 28 from Europe, 21 from Africa, and 3 from Oceania.

Results also showed that all temperate sections contain North American and Eurasian temperate species, which could indicate the spreading range of numerous temperate species to both continents that possibly dispersed before the whole separation of these continents (Kerrigan *et al*., 2008; Zhao *et al*., 2011; Geml, *et al*., 2004).

In this study, based on rDNA ITS1+2 sequence data, also generally support the presence of separated tropical clades. The presence of distinct tropical and temperate clades could also suggest that species diversification possibly happened separately in tropics (Africa and Asia) like that of the temperate region (Geml, *et al*., 2004). Furthermore, based on the phylogenetic tree shown in Figure 5-1, some of these tropical clades are not phylogenetically closely linked which may show the occurrence of species diversification several times with in these regions (Zhao *et al*.,

2011; Kerrigan *et al*., 2006). The lower and moderate bootstrap supports for some of these clades make the precise relationships unclear. This might give the idea that these major tropical clades ascended within a brief period of evolutionary time, which could make their specific relations difficult to determine (Challen *et al*., 2003; Geml, *et al*., 2004).

The geographical origin of *Agaricus* is unknown, however, there are few proposed assumptions by Callac *et al*. (1993) and Zhao *et al*. (2011), that suggest its pleotropic origin. However, to better underst the biogeography of the genus, there is a need for studies that take account of species from wide range geographical regions.

About two-thirds (27 out of 33) of the Ethiopian *Agaricus* sequences did cluster in the previously recognized sub group of the genus that was established based on temperate species while the rest sequences were distributed in tropical clades, which were designated as Clade I, Clade II, Clade III and Clade IV with branch support range from 80-100%. Recent phylogenetic analyses also revealed that more than one third of the tropical taxa do not cluster in these sections, but form exclusively tropical clades (Chen *et al.*, 2015; Zhao *et al.,* 2011). These tropical clades in this study were found to be equivalent to that of Zhao *et al*. (2011) in that clade I = TR III, clade III and clade IV = TRa while clade II which form paraphyletic with section Minores in this study was grouped under section Minories in Zhao *et al.* (2011). According to a very recent study by Chen *et al*., (2015) reveiled that clade TR I of Zhao *et al*., (2011) is corresponds to a new section Brunneopicti, as it contains *A. brunneopunctatus*, the type species of this section and others with geographical distribution range only from palaeotropics.

This confirmed the need to expand the number of sections in the genus there by investigate their key characteristic and their circumscription to establish exclusively tropical clades that incorporate collection from tropics and subtropics. According to Zhao *et al.* (2011), Zhao *et al.* (2012), Chen *et al.* (2015) and results in this particular study, we can predict new tropical sections will also increase in coming years.

Species in this genus have a limited number of characteristic phenotypic traits. These limited phenotypic characteristics are being affected by environmental factors such as climate, geography, substrate, vegetation type (Largetn and Thiers, 1977; Callac *et al.*, 2003; Zhao *et al.*, 2012). As described by Zhao *et al*., (2012b), geography and climate have had a major impact on the evolution of the genus. However, it is also often difficult to classify the species in climatic/geographic groups (Kerrigan *et al*., 2005). Similar results was obtained by Parra *et al*. (2002) that has reported the geographical range of some temperate species such as *A. bisporus* and *A. bitorquis* extends into tropical areas while, the tropical species *A. subrufescens* and *A. endoxanthus* exists also in Europe and are supposed to have been introduced with plants.

Based on the phylogenetic tree shown in Figure 5-1, the Ethiopian *Agaricus* samples collected from central highlands show higher genotypic characteristics (based on ITS sequences) similarity and distributed well in temperate sections with high branch support value. The result also shows that from a total of 27 collections that were distributed in the those sections, 20 (75%) are from Welmera. This might be as a result of two factors, the first is; in Welmera district, the mean annual temperature range is between 9.5°C and 22.5°C, which is low enough to support this cold loving temprate species (Largeteau *et al*., 2011; Navarro *et al*., 2014). And the second might

be as described in von Breintenbach, (1962) some of the tree seedlings planted in the area (including *Pinus radiata*, *Eucalyptus globules* and some other species) were brought in from abroad (Western US, Australia) by Emperor Zera Yacob (1434-1468) and there could be a chance that some temperate fungal mycelium have been introduced to the area.

In this study, three *Agaricus* specimens were not identified to species level. It was partly due to the absence of type specimens/reference material on *Agaricus* species of Africa and the other reason was encountering immature specimen during collection and inadequate macro-morphological characters. There may be a possibility that these unidentified species might be new to science. Thus, the addition of more tropical taxa and incorporation of different molecular markers (Wiens, 2006) in the analysis will be necessary to clarify the taxonomic status of these collections.

Sections Agaricus, Arvenses, Bivelares, and Sanguinolenti contain edible and commonly consumed species. However, in the remaining four sections, the edibility of most species is generally uncertain for various reasons. There are a number of species that are considered choice by some and toxic by others. This might be simply due to unpleasant odor, test, color or difficulty in identification (Parra, *et al*., 2008; Okhuoya *et al*., 2005).

Species of *A.* sect. Minores are not popular because they are generally small and hard to identify, even for experts. However, it can be noted that intoxication by such species have not been reported and that they generally have a pleasant almond-like odor (Lebel, 2013). *Agaricus brunneolus* (I.E. Lange) Pilát is one of the comparatively large and abundant species of this section which is consumed in

Europe (Cappelli, 2011). In this particular study *A. purpurellus, A. brunneolus, A. pseudolutosus* and *A.* sp1 (RSK151_W) were identified to be in this section and which are regarded as safe for consumption.

Species of the *A.* sect. Xanthodermatei are recognized or well-known to be toxic. Despite this toxicity, some species of this section are eaten and tolerated by some people from Eastern Europe, Russia and Spain (Kerrigan *et al*., 2005, Parra, 2008) without noticeable gastro-intestinal upset. The phenol, that was first detected in *A. xanthodermus,* is the major volatile component and likely responsible for the poisonous symptoms (Wood *et al*., 1998; Parra *et al*., 2011; Thongklang *et al*., 2014). In the medical poison center in Marseille, France, 20% of poisonings by identified mushrooms are due to ingestion of species of *A.* sect. Xanthodermatei (De Haro *et al*., 1999).

Agaricus species are well known for their high nutritional and medicinal values. Edible species such as A. *campestris, A. bisporus, A. cupreobrunneus* and *A.bohusii* are consumed worldwide (De Haro *et al*., 1999; Cappelli, 2011; Zhao *et al*., 2012). However, people in different parts of the world appreciate several wild *Agaricus* species, the condition is different among different communities in Ethiopia. Results in this study as well as studies elsewhere in the country, showed that peoples generally considered species in this genus inedible or not fit for human consumption. This might be due to wrong identification of *Agaricus* species for example species that belong to sect. Xanthodermatei, which comprise members that are known to produce toxic compounds and can cause gastrointestinal distress leading to violent vomiting (Wood *et al.,* 1998; De Haro *et al*., 1999). Moreover, it was observed that species in this section have high frequency distribution in the study area thus it is possible to

point out that people had frequent problem due to misidentification and the toxicity experienced after ingestion is reflected by the local Amharic name for these mushrooms ‘Dem Astef’ meaning causes ‘vomiting of blood’ leading the local community in the central highlands to avoid all the mushrooms growing in the wild.

In addition, local communities in Menge district disregard species in this genus due to the habitat where the mushroom usually grow, which indicate mushrooms that grow in pastureland where cattle graize regarded as not edible because they belive, mushrooms in this habitat are contaminated with cattles` dung.

However, this problem have been solved in most developed countries through increasing awareness and providing field guid books with detail identification keys (Laursen and Taylor, 2008; Miriam *et al.*, 2006; Zhang *et al.*, 2014) which can usually help to distinguish poisonous species from morphologically similar edible mushrooms (like *A. campestris*). For example, in this case species in section Xanthodermatei can be easly identified by their tendency to stain yellow and by the characteristic phenolic or solvent-like odor. Moreover, commercial cultivation of species in this genus has well developed in these regions of the world so that mushroom poisoning due to misidentification is not common.

5.5. Conclusions and Recommendations

Without a proper knowledge of species and their boundaries, it is very difficult to do ecological, applied, or other research. The results of this study have improved our understanding of the taxonomic status of genus *Agaricus* in Ethiopian. Since *Agaricus* is a species-rich genus (more than 400 sp.), the number of *Agaricus* species reported in the country so far was very low. However, being in the tropical region and the

presence of different eco-regions in the country, more number of species are expected to exist.

Recommendations

Based on the result of the current study the following *Agaricus* species *A.campestris, A.bohusii, A. purpurellus, A. subsaharianus* and *A. heterocystis* are highly recommended for domestication and cultivation because of their good nutritional and medicinal value. *Agaricus subsaharianus* and *A. heterocystis* are concedered to be a good candidate for domestication experiment in Ethiopia since these are tropical species and their temperature and moisture requirement for growth can easly be optimized.

This current method of using molecular data and morphological keys for species identification and nomenclature, are adequate for discovering and naming the diversity of fungi in Ethiopia. Based on the present study, more informations are needed on species composition which will allow comparisons of Ethiopian collections with that of temperate and other tropical *Agaricus* species. Further work remains to be done to suggest the economic importance of these species, and their potential for commercial cultivation for food, medicine and other biotechnological applications.

6. Morphological and Molecular Characterization of *Termitomyces* Species of Menge District, Asossa Zone, Northwest Ethiopia

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Abstract

Despite the extensive study on plant and animal biodiversity in Ethiopia, our knowledge of microbial diversity in general and macrofungal diversity in particular is very limited. Thus, as part of the ongoing study on the macrofungal diversity, this is the first report on the morphological and molecular study of the genus *Termitomyces* (Lyophyllaceae) in the country. In this particular report therefore, ten *Termitomyces* species were collected and identified from Menge Districts, Asossa Zone, Benshangul Gumuz region. Identification was based on morphological characteristics and partial LSU rDNA sequences. Phylogenetic analysis of partial LSU rDNA sequences of ten Ethiopian *Termitomyces* species using Distance, Parsimony measurements and Maximum Likelihood presented similar inferred trees that only had minor differences. Based on these phylogenetic analyses of the partial LSU rDNA sequences, seven species of *Termitomyces* identified as *T. aurantiacus*, *T. clypeatus*, *T. eurrhizus*, *T. letestui*, *T. microcarpus*, *T. robustus* and *T. schimperi*. The inferred ML cladogram revealed both Asia and African *Termitomyces* samples in this analysis demonstrated a well-supported monophyletic group with bootstrap value of 99%. Moreover, the monophyletic tree from pure Ethiopian *Termitomyces* collection and a combination of African and Asian *Termitomyces* samples suggested their common origin. However incorporating more samples, more DNA markers and extensive analyses may reveal the true link among the sequences from different region of the world. All the species reported are used for culinary purposes by the native community and few of them such as *T. microcarpus* and *Termitomyces clypeatus* are used as medicinal mushroom by the local people for constipation, gastric problems in adults and highly recommended for underweight children respectively.

***Keywords*:** wild edible macrofungi, Lyophyllaceae *Termitomyces*, LSU, Phylogeny, Taxonomy

6.1. Introduction

Termitomyces R. Heim 1942, belonging to the family Lyophyllaceae, comprises a group of gilled mushrooms called termitophilic which live in association with a particular family of termites *Macrotermitinae (Isoptera*) commonly found in parts of Africa and Asia with warm and humid climate (Aanen *et al.,* 2002; Kirk *et al.,* 2008). Once this group of macrofungi was reported as an independent genus by Heim (1942), a number of species have been described predominantly from tropical areas (Heim, 1952, 1977) mainly Africa (Heim 1942; Pegler 1977; Van der Westhuizen and Eicker 1990; Mossebo *et al.,* 2002; Pegler and Rayner, 1969; Moriss, 1987), South America (Otieno 1964, Alasoadura 1966, Gómez, 1995) and South East Asia (Zhang *et al.,* 1986; Wei *et al* 2003, 2004; Tang *et al.,* 2006).

Cultivation of *Termitomyces* by termites originated in the African rain forests as the main centre and migrated to other geographical regions such as Asia and Madagascar (Aanen *et al*., 2002). However, molecular studies have shown no identical sequences among the *Termitomyces* between Africa and Asia (Frøslev *et al*., 2003), indicating their geographical difference as well as a possible independent evolution.

Their association with termite, pinkish and smooth basidiospores, conspicuous perforatorium on pileus and underground pseudorhiza connected to the comb in the termite nest are some of the major typical characteristic of *Termitomyces* species (Heim, 1977, Frøslev *et al.,* 2003). They are cultivated through transport of spores by eusocial insects (termites) using plant material passing their guts (Frøslev *et al*., 2003).

Altogether, about 60 taxa have been published in this genus, with 81 names containing combinations and autonyms (Kirk *et al.*, 2008). However, the taxonomic statuses of some of them are still doubtful (Wei *et al.*, 2003, 2004; Tang *et al*., 2006). The coverage of *Termitomyces* species in the barcoding databases is still poor. This lack of data will be corrected in the course of time since the number of molecular studies is increasing and more and more data is being deposited each year (Moncalvo *et al.*, 2000; Frøslev *et al*., 2003; Aanen *et al.*, 2002; Oyetayo, 2011).

However, the genus comprises species which are the choice edible mushrooms, the diversity, ecology and distribution of species has not been a focus of taxonomic investigation among mycologists in the country. In most parts of Ethiopia where the Great Rift Valley passes through (Jemaneh Zeleke, *et al*., 2013) and south and west of the country (Taddesse Yebo, 2010; Teferi Yenealem *et al.*, 2013) are some of the locations where these fungi reported. The reports on this genus so far were mainly based on the local use for food and medicine among some communities in rural areas of South and Southwest part of the country (Taddesse Yebo, 2010; Teferi Yenealem *et al.*, 2013; Woldegiorgis *et al*., 2014a; 2014b; 2015)

Any of these reports did not provide a detailed morphological description or any molecular data of the species in the genus, thus the taxonomic status of these species is not clear. This research is the first report on the taxonomic study of *Termitomyces* collections from Menge districts based on morphological diagnosis and molecular data analyzed using LSU rDNA. The results reported here will provide points of reference to facilitate taxonomic, ethnomycological, ecological and economic studies on Ethiopian *Termitomyces* species.

6.2. Materials and Methods

Detail description of the study area and methods used for collection and morphological identification of macrofungal specimens have described in section 3.2.3.

DNA extraction, PCR amplification and Sequencing

Total genomic DNA was extracted using the modified CTAB method described by Yao *et al.,* (1999). The fungal universal primer pair LROR (ACCCGCTGAACTTAAGC), LR5 (TCCTGAGGGAAACTTCG) were used to amplify the nuclear larger subunit RNA (LSU) region (White *et al.,* 1990). The PCR reaction mixture was comprised 25 µl Taq PCR MasterMix, 0.5 µl of 10 µM each primer, 1 µl diluted DNA template, and RNase-Free water to bring the total volume to 50 µl. The PCR conditions were set as follows: 94 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 53 °C for 1 min, 72 °C for 1 min and final extension step of 72 °C for 10 min on a GenAmp PCR System 9700 thermocycler (Vers. 3.03).

PCR products were sequenced from both directions using the same primers on an Applied Biosystems 3730 Analyzer by the Beijing Genomics Institute (Beijing, China). Sequence ends were manually edited and assembled using DNAStar Lasergene SeqManTM II vers. 6.1. Sequences were then aligned using Clustal W algorithm (Thompson *et al.,* 1994) and manually edited with BioEdit vers. 7.1.9 (Hall 1999).

Phylogenetic analysis

Sequences of LSU were obtained from ten *Termitomyces* collections in this study and aligned together with 35 closely related LSU rDNA sequences of *Termitomyces* species and two outgroup taxa (*Lyophyllum semitale* and *Lyophyllum atratum)* which were downloaded from GenBank (Table 6-1). The LSU sequences obtained in this study were submitted to GenBank as KU933604 – 933612 and 933614. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013). The evolutionary history was inferred by using the Maximum Likelihood (ML) method based on the Kimura 2-parameter model (Kimura, 1980). The tree with the highest log likelihood (-1318.5755) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying the Maximum Parsimony method. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 47 nucleotide sequences and 595 positions in the final dataset.

Table 6-1 LSU sequences of *Termitomyces* and out-group species used in the phylogenetic analysis

SPECIES	VOUCHER/ ISOLATE	ORIGIN	GENBANK* LSU	REFERENCES
Termitomyces aurantiacus	tgf82	Tanzania	AY127804	Aanen *et al.*, (2003)
	tgf89	Cameroon	AY232690	Froslev *et al.*, (2003)
	tgf85	Thailand	AY232691	Froslev *et al.*, (2003)
	HMAS273464	Ethiopia	**KU933605**	This study
	HMAS273466	Ethiopia	**KU933608**	This study
T. clypeatus	JMleg.MUID	Asia	AF261398	Moncalvo *et al.*, (2002)
	tgf93	Burundi	AY127803	Aanen *et al.*, (2002)
	PG	Malaysia	HM036342	Tan *et al.*, (2010)
	HMAS273462	Ethiopia	**KU933604**	This Study
T. cylindricus	JM/leg.R.S	-	AF042585	Moncalvo *et al.*, (2000)
T. aff. entolomoides	tgf10	Malaysia	AY232692	Froslev *et al.*, (2003)
T. entolomoides	tgf103	Africa	AY232693	Froslev *et al.*, (2003)
T. eurhizus	tgf101	Burundi	AY232694	Froslev *et al.*, (2003)
	HMAS273459	Ethiopia	**KU933607**	This study
	HMAS273458	Ethiopia	**KU933609**	This study
T. globulus	tgf11	Cameroon	AY232695	Froslev *et al.*, (2003)
T. heimii	JM/leg.S	Asia	AF042586	Moncalvo *et al.*, (2000)
	tgf9	Malaysia	AY232696	Froslev *et al.*, (2003)
	TB	Malaysia	HM036345	Tan *et al.*, (2010)
T. letestui	tgf16	Zimbabwe	AY127800	Aanen *et al.*, (2002)
	tgf5	Cameroon	AY232699	Froslev *et al.*, (2003)
	tgf83	Denmark	AY232698	Froslev *et al.*, (2003)
	HMAS273763	Ethiopia	**KU933606**	This study
T. mammiformis	tgf100	Burundi	AY232701	Froslev *et al.*, (2003)
	tgf102	Burundi	AY232700	Froslev *et al.*, (2003)
	tgf92	Burundi	AY232703	Froslev *et al.*, (2003)
T. medius	dka138	Cameroon	AY127796	Aanen *et al.*, (2003)
	tgf70	Cameroon	AY232704	Froslev *et al.*, (2003)
	tgf7	Cameroon	AY232705	Froslev *et al.*, (2003)
T. microcarpus	tgf	Tanzania	AY127799	Froslev *et al.*, (2003)
	tgf88	Tanzania	AY232708	Froslev *et al.*, (2003)
	tgf86	Zimbabwe	AY232706	Froslev *et al.*, (2003)
	HMAS273461	Ethiopia	**KU933610**	This study
T. robustus	tgf95	Burundi	AY127797	Aanen *et al.*, (2003)
	tgf72	Tanzania	AY232710	Froslev *et al.*, (2003)
	tgf81	Tanzania	AY232709	Froslev *et al.*, (2003)
	HMAS273465	Ethiopia	**KU933611**	This study
	HMAS273456	Ethiopia	**KU933612**	This study
T. schimperi	tgf18	Zimbabwe	AY232712	Froslev *et al.*, (2003)
	HMAS273460	Ethiopia	**KU933614**	This study
T. singidensis	tgf74	Tanzania	AY232713	Froslev *et al.*, (2003)
T. striatus	tgf99	Burundi	AY232714	Froslev *et al.*, (2003)
T. subhyalinus	–	-	AF223174	Moncalvo *et al.*, (2002)
T. titanicus	tgf96	Burundi	AY232715	Froslev *et al.*, (2003)
	tgf94	Burundi	AY127801	Aanen *et al.*, (2003)
Lyophyllum semitale	HC85/13	USA	AF042581	Moncalvo *et al.*, (2000)
Lyophyllum atratum	CBS 709.87	Switzerland	AF223210	Moncalvo *et al.*, (2002)

*** Sequences produced in this study in bold.**

6.3. Results and Discussion

Phylogenetic analyses

Phylogenetic analysis of partial LSU rDNA sequences of 10 Ethiopian *Termitomyces* collections using Distance, Parsimony measurements and Maximum Likelihood presented similarly inferred trees that only had minor differences. Based on these phylogenetic analyses of the partial LSU rDNA sequences, the *Termitomyces* collection were identified as *T. aurantiacus, T. clypeatus, T. eurrhizus, T. letestui, T. microcarpus, T. robustus* and *T. schimperi.* The inferred ML cladogram shown (Figure 6-1) all the *Termitomyces* samples in our analysis demonstrated a well-supported monophyletic group with bootstrap value of 99%. This result was corresponding with the previous phylogenetic studies on this genus (Aanen *et al.,* 2002; Rouland-Lefevre *et al.,* 2002; Froslev *et al.,* 2003). Moreover, the monophyletic tree from Ethiopian *Termitomyces* collection and a combination of African and Asian *Termitomyces* samples may suggest their common origin.

Results of LSU sequence alignment showed that the two sequences of *T. eurhizus* (KU933607, KU933609) which were collected from different habitat have identical sequences and similar results were observed with *T. robustus* (KU933611, KU933612). *Termitomyces microcarpus* (KU933610) and *T. clypeatus* (KU933604) show low LSU sequence similarity (bootstrap support value of 89 and 85 respectively for the clade) with both African and/or Asian collections.

Except *T. robustus* and *T. schimperi* the rest five species of *Termitomyces* were also commonly found in the other region of Ethiopia (Taddesse Yebo, 2010; Teferi Yenealem*et al.,* 2013; Woldegiorgis, *et al.,* 2014; 2015a; 2015b) particularly in southwest part of the country. During the present study, it was documented that all *Termitomyces* species are in use for culinary purposes during the rainy season by the

native community in Menge District but only few of them (*T. microcarpus* and *T. clypeatus*) are known for treatment of constipation and gastric problems in adults and undernourished /underweight children.

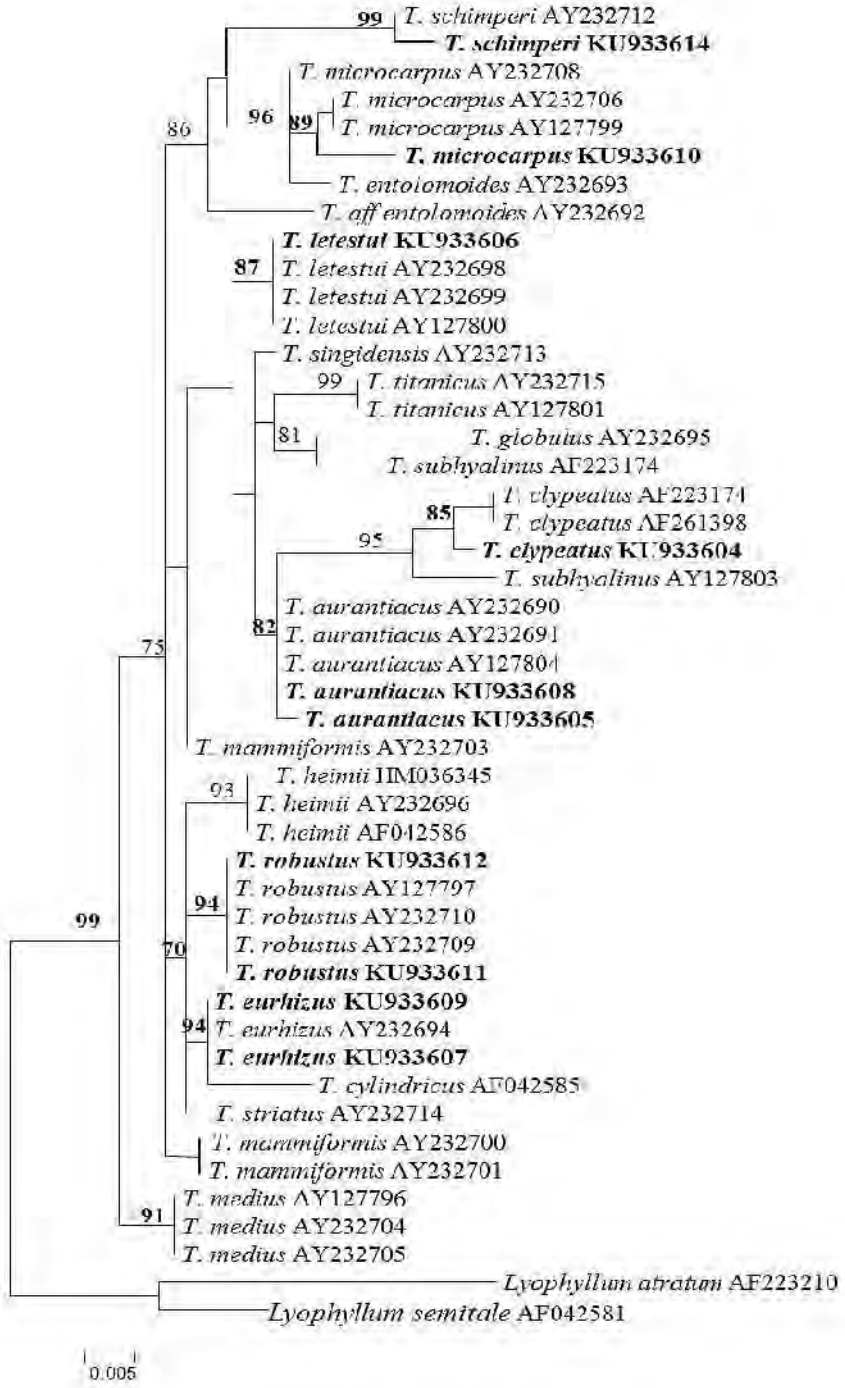

Figure 6-1 ML tree based on nrDNA LSU region sequences of *Termitomyces*
The species in bold were sequenced in this study. The percentage of trees in which the associated taxa clustered together is shown above the branches. Evolutionary analyses was done with MEGA6.

Table 6-2 Habitat, Geographical locations and Local names of *Termitomyces* specimen examined from Menge District

Voucher	Taxa	Habitat	Geographical coordinates	Altitude (m.a.s.l)	Use	Vernacular Name
HMAS273464	*T. aurantiacus*	FA	10° 19' 51"E 34° 42' 33"N	1238 m	E	Abenega
HMAS273466	*T. aurantiacus*	GR	10° 19' 48"E 34° 42' 32"N	1174 m	E	Abenega
HMAS273462	*T. clypeatus*	FA	10° 18' 50"E 34° 42' 33"N	1238 m	E & M	Akukufi
HMAS273458	*T. eurrhizus*	GR	10° 19' 48"E 34° 42' 31"N	1174 m	E	Tsergunu
HMAS273459	*T. eurrhizus*	FA	10° 20' 41"E 34° 48',04"N	1174 m	E	Tsergunu
HMAS273763	*T. letestui*	FA	10° 20' 41"E 34° 48' 04"N	1174 m	E	Afifi
HMAS273461	*T. microcarpus*	FA	10° 17' 50"E 34° 44' 00"N	1174 m	E & M	Aburalu
HMAS273465	*T. robustus*	FA	10° 19' 47"E 34° 51' 10"N	1135 m	E	Gultse
HMAS273456	*T. robustus*	GR	10° 20' 10"E 34° 44' 35"N	1278 m	E	Gultse
HMAS273460	*T. schimperi*	FO	10° 14' 05'E 34° 48' 18"N	1332 m	E	Zoma

FO= Forest, FA= Farm area, GR= grazing land; In Vernacular name, quoted names are in Berta (widely spoken language in the study area). E= Edible, M= Medicinal

Taxonomic Description of Termitomyces Collected from Menge District

Detail morphological description and illustration of each species provided below.

***Termitomyces aurantiacus* (R. Heim)** R. Heim in Termites et Champignons (Paris): 56 (1977). (Figure 6-2 (A1- 4))

Pileus 6–10 cm in diam., conical–applanate, with a small and pointed perforatorium; surface bright reddish/orange to brown and darker at the centre, glabrous. Margin radially striate and splitting, slightly viscid when moist. Lamellae free, 3–6 mm wide, white and crowded, with few lamellulae. Stipe 6–7 × 1.5–2.0 cm, central; surface greyish white to brownish, smooth, solid; pseudorrhiza white, up to 15 cm, cylindrical but tapering downward the base, sometimes slightly swollen before tapering. Context white, thick and firm. Basidiospores deposit pinkish cream. Taste mild. Odor mushroomy.

Basidiospores 5–7.5 × 3.5–4.5 µm, average length (avl) × average width (avw) = 6.25 × 4 µm, Q (avl/avw)= 1.4 –1.6, avQ =1.5 ovoid to ellipsoid, thin-walled and subhyaline. Basidia 16–20 × 5–7.5 µm, clavate, bearing four sterigmata, hyaline and thin-walled. Lamella margin heterogeneous. Cheilocystidia 22–40 × 10–25 µm, clavate, thin-walled and hyaline. Hymenophoral trama subregular, thin-walled and hyaline hyphae, 4–15 µm diam. Clamp connection absent.

Remark: According to Heim (1985), *T. aurantiacus* can easily be distinguished from other species of the genus by its cylindrical pseudorrhiza and bright reddish to orange pileus and its firm texture (Pegler and Vanhaecke 1994) which is in line with our observation. However, in this study small squamules cited by He, (1985) on the surface of the stipe was not observed. This may be due to the nature of the ephemeral remains of partial veil that cannot be observed in mature specimen of *T. aurantiacus*. It is a well- known edible species among peoples in the community.

Termitomyces clypeatus R. Heim, Bull. Jard. bot. État Brux. 21: 207 (1951) (Figure 6-2. (B1- 4))

Pileus 4–6 cm in diam., conical turns applanate when mature with strongly spiniform to acutely pointed umbo and with unevenly lobed margin; first brownish fading to ash-brown, lighter towards the margin, smooth, silky and viscid when wet, otherwise dry; context of pileus white, thin. Lamellae free, crowded, white to pinkish. Stipe 5–11 × 0.5–1 cm, central, solid, whitish, cylindrical and with a slightly bulbous base, with tapering pseudorrhiza. Annulus absent. Basidiospores deposit pinkish cream. Taste pleasant. Odour mild, weak.

Basidiospores 5.5–7.0 × 3.5–4.5 µm, avl x avw = 6.25 x 4 µm, Q = 1.57 – 1.55, avQ = 1.56, broadly ellipsoidal, hyaline and smooth. Basidia 20–25 × 7–9 µm, clavate, with four sterigmata. Cheilocystidia 18–25 × 8–18 µm, pyriform, hyaline with a faintly thickened wall. Hymenophoral trama regular, hyaline. Clamp connection absent.

Remark: *Termitomyces clypeatus* is one of the smallest species in the genus. It partially resembles *T. tylerianus* in fruit body dimensions, but it differs in exhibiting a spiniform to acute umbo with a silky and greyish brown pileal surface and stipe with long black pseudorrhiza (Pegler, 1977). It is edible and used as medicine among the local people to treat problems related to gastric and constipation in adults and to treat underweight children. The health benefit of this species also indicated on reports from West Africa and Asia (Anderson *et al.*, 2013; Adhikari *et al.*, 2005; Boa, 2004; Okhuoya and Akpaja, 2005).

Termitomyces eurrhizus (Berk.) R. Heim[as 'eurhizus'], Arch. Mus. Hist. Nat. Paris, ser. 6 18: 140 (1942). (Figure 6-2 (C1-4))

Pileus 5–22 cm in diam., conico-campanulate to nearly plane with a broadly ambonate perforatorium; surface greyish brown over the perforatorium becoming usually lighter near the margin. Margin slightly incurved, splitting at maturity. Lamellae sub-free to adnexed, whitish cream to light pink, moderately crowded with few lamellulae. Stipe 5–20 × 1–4 cm above ground, central, solid, cylindrical, surface whitish, with elongated pseudorrhiza, up to 20-25 cm. Context white, fleshy, thin-walled-hyphae, 3–5 µm diam. Basidiospores deposit brownish pink. Taste and odor mild and pleasant.

Basidiospores 6.0–8.5 × 4.0–5.5 µm, avl × avw = 7.5 × 5.0 µm, Q = 1.3–1.4, avQ = 1.35, smooth, ellipsoid, thin-walled, hyaline, inamyloid. Basidia 20–25 × 6–10 µm, clavate, with four sterigmata. Cheilocystidia pyriform or rarely obovoid, 20–40 × 9–25 µm, hyaline with a slightly thickened wall. Hymenophoral trama regular, hyphae of 3-5 µm in diam., hyaline. Clamp connection absent.

Remarks: The special character of *T. eurrhizus* is the long, thick pseudorrhiza, which is black below the ground level. The structure of this species has been dealt with considerable detail by Petch (1913) who accepted two distinct forms; with persistent annulus and with out annulus commenting that the two forms were identical in size, shape, structure of pileus and gills, size and colour of spores. No persistent annulus was clearly observed in all of our *T. eurrhizus* collections in this study. However, it is quite clear that the two forms simply represent the same species. As described in Pegler, (1977) and Wei *et al.,* (2004) the presence or absence of a ring is a more or less an accidental phenomenon brought about by a difference in the point of dehiscence of the universal veil. *T.eurrhizus* is a common species at grassland, edges of forests and cultivated fields in the study area. It is esteemed as delicacy by the indigenous people but not common at the local market.

***Termitomyces letestui* (Pat.)**R. Heim, Arch. Mus. Hist. Nat. Paris, ser. 6 18: 109 (1942). (Figure 6-2 (D1-4))

Pileus 12–20 cm in diam., convex, with mammillate perforatorium, surface cream to light brown becoming dark brown or rust brown towards the center, with squamulose at the disk. Margin incurved, splitting radially at maturity. Lamellae free, cream to pinkish, thin, crowded with numerous lamellulae up to 12mm broad. Stipe 8–15 × 1–3.5 cm, central, cylindrical, surface whitish, prolonged below in to tapering

pseudorrhiza. Context white, firm, up to 23-25mm thick. Annulus white to whitish, membranous, superior and pendant. Basidiospores deposit pinkish cream. Taste mild and Odor strong and pleasant.

Basidiospores 5.5–8.5 × 4.0–5.5 µm, avl × avw = 7 × 4.7 µm, Q = 1.37 –1.5, avQ =1.4 obovoid to ellipsoid, hyaline, thin-walled. Basidia 20–25 × 5 –7 µm, clavate, bearing four sterigmata. Cheilocystidia 20–45 × 9–22 µm, mostly broadly clavate but sometimes cylinderic, with a fairly thick wall, hyaline. Subhymenial layer fairly broad, 7– 10 µm wide. Hymenophoral trama sub regular to vaguely bilateral, 2–6 µm diam., hyaline, with thin-walled hyphae. Clamp connection absent.

Remarks: It is recognized mainly by its large and fleshy pileus with a characteristic mammillate perforatorium (Heim 1945; Pegler 1977) and by the sheathing annulus. *Termitomyces letestui* is wide spread throughout Menge district and represent one of the largest species. It is one of the first mushrooms to appear at the beginning of the rainy season (mid of June). It is edible and highly prized due to its good test which is true in elsewere in Africa and Asia (Tibuhwa, 2012a; Tang, *et al*., 2006; Rammeloo and Walleyn, 1993). It is offered for sale in local markets and on roadsides at quite an expensive price.

Termitomyces microcarpus (Berk. & Broome) R. Heim, Arch. Mus. Hist. Nat. Paris, ser. 6 18: 128 (1942). (Figure 6-2 (E1-4))

Pileus 1.5–2.5(3) cm in diam., convex then expanding to broad convex and almost applanate, often umbonate, upper surface whitish to cream, darkening at the center, dry. Margin incurved or straight. Lamellae free, thin, white, 1-2 mm wide, moderately crowded with lamellulae. Stipe 2–4(–5) × 0.1–0.3 cm, central, solid, cylindric,

sometimes with small bulbose base, surface whitish, smooth, lacking pseudorrhiza. Context white, thin. Annulus absent. Basidiospores deposit cream. Taste mild. Odor mild, sometimes odourless.

Basidiospores 6.0–8.5 × 3.5–4.5 µm, avl × avw = 7.5 × 4.15 µm, Q = 1.5–1.8, avQ = 1.65, ovoid to ellipsoid, hyaline, with usually one refractive guttule, inamyloid, thin-walled. Basidia 20–25 × 7–9 µm, clavate, with four sterigmata. Cheilocystidia rare on the lamella edge, 15–40 × 9–16 µm, pyriform to cylindric, slightly thickened wall. Hymenophoral trama regular, hyaline hyphae. Clamp connection absent.

Remarks: Distinctive characteristics of this *Termitomyces* species is its small size, occurrence in dense troops and the absence of pseudorrhiza, however, its association with termite, the presence of pinkish spore and other micromorphological characters that it shares with other species in the genus lead mycologists to put it under this genus (Heim,1952, 1977; Pegler, 1977). From the African region, Frøslev *et al.* (2003) have documented the pileus diameter of *T. microcarpus* being ≤ 2 cm, while Pegler and Vanhaecke (1994) from Southeast Asia and Tibuhwa (2012b) from Tanzania, considered the pileus diameter to be < 3 cm. The specimen in our study showed an average pileus diameter 2.25 cm; which is < 3 and an average stipe length of 3. 5 cm (range 2 –5 cm). It is assumed that geographical difference, environmental conditions and species of termite involved in cultivation have a major influence on the dimension of the mushrooms.

In contrast to previous researches (Pegler, 1977; Pegler and Vanhaecke 1994), *T. microcarpus* from Menge has cream colour spore deposit instead of the pink spore deposit. It is common and abundant around the farming field and semi-opening bamboo forest in Menge District from the end of June to August. It is edible and well-

liked for its test and flavor and medicinal benefit, however, due to its small size people do not prefer it since it takes much time to collect even for one meal.

Termitomyces robustus (Beeli) R. Heim, Bull. Jard. bot. État Brux. 21: 210 (1951). (Figure 6-2(F1-4))

Pileus 7-11 cm diameter, at first conical then expanding to convex, pointed to perforatorium, surface grey. Lamellae free, crowded 5-6 mm wide, ivory/creamy. Stipe 6-8 × 3-7 cm thick, white, solid, central, cylindrical slightly tapering downwars. Context white, firm, up to 12mm thick. Annulus absent in mature specimens. Basidiospores deposit pinkish-cream. Taste and Odor mild.

Basidiospore 4-8×3-4.5 µm, avl × avw = 6 × 3.7 µm, Q = 1.3–1.7, avQ =1.5 ovoid to ellipsoid, thin-walled, inamyloid, hyaline. Basidia 18–25 × 6 –8 µm, clavate, bearing four sterigmata. Cheilocystidia 25–40 × 10–20 µm, clavate, with a fairly thick wall, hyaline. Hymenophoral trama ambiguously bilateral, 3–6 µm diam., hyaline, with thin-walled hyphae. Clamp connection absent.

Remarks: *Termitomyces robustus* is readily recognized by its large, tough basidiomata with brown cap surface conical umbo and a swollen stipe and blackish psudorrhiza (Pegler and Vanhaecke 1994); Tibuhwa *et al*., 2010; De Kesel, 2011; Tibuhwa, 2012b). The fruiting body appear after good rains from mid-July until the end of September. It is edible and tasty.


A1
A2
A3
10 µm
A4
5 µm
B1
B2
B3
10 µm
B4
5 µm
C1
C2
C3
10 µm
C4
5 µm
D1
D2
10 µm
D3
D4
10 µm
E1
E2
E3
10 µm
E4
5 µm

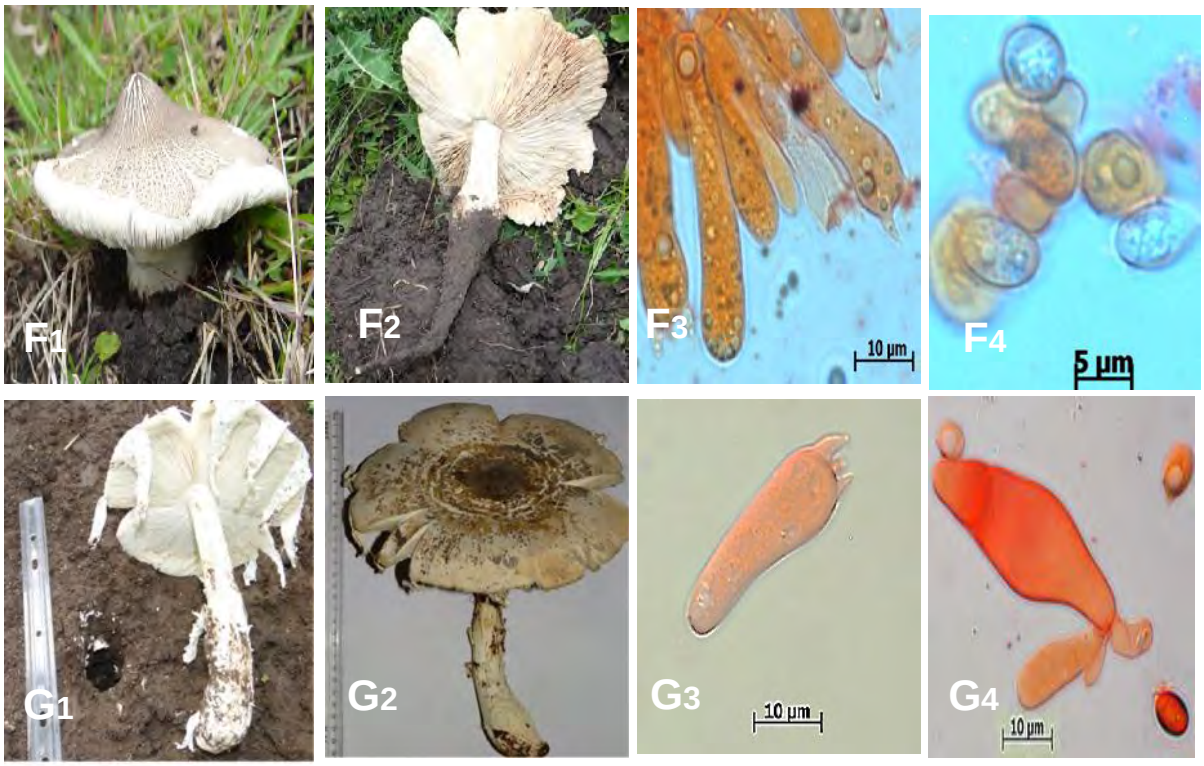

Figure 6-2 Macro and micromorphological characters of *Termitomyces* species collected from Menge District

A1-A4: *Termitomyces aurantiacus,* B1-B4: *T. clypeatus,* C1-C4: *T. eurrhizus*,D1-D4: *T. letestui,* E1-E4: *T. microcarpus,* F1-F4: *T. robustus*, G1-G4: *T. schimperi*.
1 and 2 represent- Basidiomata, 3- Basidia and Cheilocystida, 4- Basidospores.

Termitomyces schimperi (Pat.) R. Heim, Arch. Mus. Hist. Nat. Paris, ser. 6 18: 114 (1942) (Figure 6-2 (G1-4))

Pileus 18-40 cm in diam., convex to flat, lacking umbo, covered with thick large persistent scales concentric and forming plate-like covering at disc, surface white but stained light brown to reddish-brown by the soil of the mound. Margin entire, splitting radially, with persistent, long and membranous partial veil. Lamellae free to adnexed, up to 10 mm wide, whitish to cream, moderately crowded. Stipe 30–40 × 3–4 cm, cylindric, swollen towards the base then tapering to a long pseudorrhiza. context thick, white. Annulus absent. Basidiospores deposit cream to pinkish. Taste mild. Odor pleasant.

Basidiospore 5-9 × 4-5.5 µm, avl × avw = 7 × 4.7 µm, Q = 1.25–1.6, avQ =1.4 ovoid to ellipsoid, thick-walled, inamyloid, hyaline. Basidia 18–30 × 5–8.5 µm, clavate, bearing four sterigmata. Cheilocystidia 35–55 × 15–20 µm, clavate, with thick wall, hyaline. Hymenophoral trama bilateral, 4–6 µm diam., hyaline, with thick-walled hyphae. Clamp connection absent.

Remark: *Termitomyces schimperi* is well known for its large robust fruiting body, usually the cap may reach about 40 cm. in diam (Pegler, 1977; Pegler and Piearce, 1980; Singer, 1986). It is easily recognized by its rough scaly cap and stem, the brown scales of the cup usually in the form of radiating concentric rings unlike many other *Termitomyces* species it lacks umbo. Usually found on top of termite hills and appear after heavy rains from July to mid September which is the main rainy season in the study area.

6.4. Conclusion and Recommendation

Termitomyces is a palaeotropical genus and many more new species of *Termitomyces* are likely to be reported from this part of the world. Being in tropics, there is a high possibility of finding new *Termitomyces* species to the world and new records to the country this underscores the need for further study of the genus.

As *Termitomyces* are obligate symbionts with termites, *ex-situ* cultivation is challenging. However, suitable *in-situ* conservations might be maintained in termite gardens based on the experience of expanding the natural cultivation of *Termitomyces*. Particular plans to preserve small pockets rich in *Termitomyces* on cultivated lands (e.g. coffee and wood land) remain essential.

Attention should be given by harvesters' during collection of *Termitomyces* in their natural habitats for commercial benefits and during application of pesticide on farm field since this antropogenic factors could destroy the location for growth and dissemination of *Termitomyces*. Thus, habitat preservation, sustainable use and research focused on *Termitomyces* will be highly rewarding in developing countries like Africa and Asia since these mushrooms are source of alternate protein and energy to the local community especially during winter.

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Appendix

Appendix 1 : Macro and micromorphological observation checklist

Specimen number: _____Exam date:_____

Scientific name:_____ Collector: Collecting date:_____

Determined author and data:_____

Collecting place: _____Habit:_____

Basidiocarp: solitary _____group_____

Pileus: size (diam)_____

Shape: convex ____conical, ____ hemispherical, ___ plane, ___ flabellate, ___ egg-shaped, __ infundibuliform ____

Surface: color_____dry, __ wet, __ moist, __ viscid, __ lucidus, __ dull ___

Smooth___silky__, powdery__, the thickness of the center of pileus_____cm

Margin: plane___, decurved___, snubby___, reflexed__ pendulous involute crenate

Lamellae: decurrent__ adnexed__ adnate__ notched; distant__ subdistant__ close __ crowded__ color:___breadth:____mm; edge: entire___ serrate___ serrulate eroded __ wavy___ crisped crenate

Stipe: size: _____base width: ____enlarged___ not enlarged

Shape: central___ excentric___ lateral___; equal___ tapering upwards___ tapering downwards____ clavate___

Consistency: spongioplasm____ solid___ stuffed___ fistulose___

Surface: reticulate___ scabrous__ glandular-dotted __longitudinally-striate__ glabrous

color___: ring: apical ___ basal ___ central___ double____

Volva: scaly zoned____ bractial___ cuppy____ degraded

Context: up to____ cm at disk, color: _____hyphae diam (µm):

Taste and Odor: _____

Basidiospore: shape: globose__ elliptical__ oblong__ ovate__ reniform__ starlike __

Q=_____µm, avQ_____µm

Basidia: shape: clavate (short__ long__) cylindrical (short__ long__),bearing four sterigmata

Hymenial cystidia:

Hymenophoral trama: regular_____, with hyphae_____um diam.

Subhymenial layer_____µm thick, hyphae narrow,_____interwowen

Pileipellis a repent cutis_____,of thin-walled, uninflates hyphae,_____umdiam.

Cheilocystidia: shape: filiform cylindrical___ cylindro-clavate__ pyriform__ obclavate __

color:wall(thick thin) free hyphae end: absent__ present __

color:wall(thick thin) free hyphae end: absent___ present____

Appendix 2 : Semi-structured interview questions employed during the research in Menge District

Questionnaire on General knowledge, attitude, and practice towards wild edibe and medicinal mushrooms

I/ Informant's data/ profile

Name/code of respondent _____

1. Name of the Kebele _____
2. Woreda_____ Zone_____
3. Age _____
4. Sex_____ Occupation_____
5. Ethnic group_____
6. Local language_____
7. Educational status:
   Illiterate ☐ Primary school ☐ Secondary ☐ Above secondary ☐

II/ **General knowledge about wild edible mushroom**

What forest products do you collect from the forest(s)?

_____

8. Who collect mushroom for the family?

   _____

   Women ☐ men ☐ children ☐ elderly people ☐ others ☐
9. Importance of mushroom collection for the community
   For food ☐ Health benefit ☐

   For religious purpose ☐

   Entertainment ☐

   Others (please specify) _____
10. Where is the location for mushroom collection?

Termite mounds	☐	Under big trees	☐
On log or wood	☐	Swampy areas	☐
Farmed areas	☐	Garden old	☐
Dry raised lands	☐	Cattle field	☐

Others (please specify)_____

11. When do people go for searching mushroom?
    By deliberately searching ☐ when collecting fire wood ☐
    By chance or randomly ☐ Birds sound ☐
    Observing a muddy soil ☐ when keeping cattle ☐

    Any other (please specify) _____

12. From where you got the knowledge regarding mushroom?
    From your family☐ School Local ☐ medicinal expert ☐ Religious bodies Agricultural experts☐ NGOs☐ any other ☐
    source_____
13. Are you involved in collection and consumption of wild mushroom from forest? ☐Yes ☐ No
14. If your answer is **Yes**, how do you differentiate the edible /medicinal from poisons one?

    _____

15. Can you list name of mushrooms you know by local language

    _____ _____ _____
    _____ _____ _____

16. Would you indicate the abundance period of wild edible mushroom in your locality?
17. List which mushroom is available in which part of the year (period within a year)

    _____ _____
    _____ _____
    _____ _____

18. Which mushroom type/s is /are/ the most abundant?

    _____

19. How is the mushroom prepared for food? Explain the local recipes or procedures

    _____
    _____

20. Do you preserve mushroom for future use? Yes ☐ No ☐
21. If your answer is **Yes** how do you preserve?

    _____

22. Are mushroom used for medicine? Yes ☐ No ☐
23. If yes, which mushroom for what disease?

    _____

    For human☐ animal ☐ plants ☐ any other
24. Who frequently collect medicinal mushroom and use it?
    Women ☐ men ☐ elderly people ☐ others ☐
25. Are mushroom sold in the local markets? Yes ☐ No ☐
26. If yes, in what form? fresh ☐ and/or dried ☐
27. Estimate the price of the mushroom per kg_____
28. Which mushroom type/s is (are) the most expensive? _____
29. Why this mushroom is most preferred one? Due to its:

    Its delicacy ☐ Meaty flavour ☐
    Medicinal value ☐ Easley digestible ☐
    Good light smell ☐

30. How do people transport the mushroom to local market?

_____

31. Did any mushroom poising occur in the area?

_____

Because of which mushroom type_____

32. What is the current status of wild edible mushrooms distribution in your locality?

Decreasing ☐ Increasing ☐

33. If decreasing, what do you think will be the main reason(s)

_____

Deforestation ☐ Population pressure ☐

Habitat destriction ☐ Agro chemicals (herbicides, fungicides) ☐

Urbanization ☐

Others (please specify)_____

34. Are there wild mushrooms which totally disappeared at present?

Yes ☐ No ☐

III/ attitude towards wild edible mushroom

35. Do you know that mushroom could be cultivated artificially?

_____

36. Do you want to adopt mushroom cultivation in your locality? Yes ☐ No ☐

If your answer is **Yes** which type of mushroom do you prefer to cultivate?

_____

37. Are there any folklore and traditional beliefs in your community/tribe concerning mushrooms appearance?

_____

38. Which social groups buy mushrooms more frequently?

Educated people ☐ People from rural area ☐ people from urban area ☐

Others (please specify) _____

Why do you think the reason?

_____

39. What factors responsible for mushroom development

Rain and soil ☐ Heavy thunder rain ☐ Soil type coupled with heavy rains ☐

Appendix 3 : List of Macrofungal species collected from Welmera and Menge District

No.	Macrofungi taxa	Family	Life mode*
1	*Agaricus bisporiticus* Nawaz, Callac, Thongklang & Khalid	Agaricaceae	S
2	*Agaricus litoralis* (Wakef. & A. Pearson) Pilát	Agaricaceae	S
3	*Agaricus microvolvatulus* Heinem.	Agaricaceae	S
4	*Agaricus aff. kivuensis* Heinem. & Gooss.-Font.	Agaricaceae	S
5	*Agaricus bohusii* Bon. **	Agaricaceae	S
6	*Agaricus californicus* Peck.	Agaricaceae	S
7	*Agaricus campestris L.: Fr.***	Agaricaceae	S
8	*Agaricus caribaeus Pegler*	Agaricaceae	S
9	*Agaricus endoxanthus* Berk. & Broome	Agaricaceae	S
10	*Agaricus heterocystis* Heinem. & Gooss.-Font. **	Agaricaceae	S
11	*Agaricus laskibarii* L.A. Parra & P. Arrill.	Agaricaceae	S
12	*Agaricus litoralis* (Wakef. & A. Pearson) Pilát	Agaricaceae	S
13	*Agaricus microvolvatulus* Heinem.	Agaricaceae	S
14	*Agaricus parvitigrinus* Guinb. & Callac	Agaricaceae	S
15	*Agaricus purpurellus* F.H. Møller	Agaricaceae	S
16	*Agaricus* sp1	Agaricaceae	S
17	*Agaricus* sp2	Agaricaceae	S
18	*Agaricus* sp3	Agaricaceae	S
19	*Agaricus subsaharianus* L.A. Parra, Hama & De Kesel **	Agaricaceae	S
20	*Agaricus xanthodermulus* Callac & Guinb.	Agaricaceae	S
21	*Agaricus xanthodermus Gen.*	Agaricaceae	S
22	*Agaricus xanthosarcus* Heinem. & Gooss.-Font.	Agaricaceae	S
23	*Amanita phalloides* (Fr.) Link	Amanitaceae	M
24	*Auricularia* sp1.	Auriculariaceae	S
25	*Bovista aestivalis* (Bonord.) Demoulin	Agaricaceae	S
26	*Callistosporium* sp1	Tricholomataceae	S
27	*Calvatia fenzlii* (Reichardt) Kawam.	Agaricaceae	S
28	*Calvatia fragilis* (Reichardt) Kawam.	Agaricaceae	S
29	*Chlorophyllum globosum* (Mossebo) Vellinga	Agaricaceae	S
30	*Chlorophyllum hortense* (Murill) Vellinga	Agaricaceae	S
31	*Chlorophyllum molybdites* (G. Mey.) Massee	Agaricaceae	S
32	*Chlorophyllum nothorachodes*Vellinga & Lepp	Agaricaceae	S
33	*Clarkeinda trachodes* (Berk.) Singer	Agaricaceae	S
34	*Clitocybe carolinensis* H.E. Bigelow & Hesler	Tricholomataceae	S
35	*Clitocybe fragrans* (With.) P. Kumm.	Tricholomataceae	S
36	*Clitopilus prunulus* (Scop.) P. Kumm.	Tricholomataceae	S
37	*Conocybe aurea* (Jul. Schäff.) Hongo	Bolbitiaceae	S

38	*Conocybe* sp1	Bolbitiaceae	S
39	*Coprinellus disseminatus* (Pers.) J.E. Lange	Psathyrellaceae	S
40	*Coprinus comatus* (O.F. Müll.) Pers.	Agaricaceae	S
41	*Cystofilobasidium* sp1	Cystofilobasidiaceae	S
42	*Entoloma henricii* E. Horak & Aeberh.	Entolomataceae	S
43	*Galerina autumnalis (*Peck) A.H. Sm. & Singer	Hymenogastraceae	S
44	***Ganoderma neojaponicum* Imazeki ****	Ganodermataceae	P & S
45	*Ganoderma* sp1	Ganodermataceae	P & S
46	*Geastrum fimbriatum* Fr.	Geastraceae	S
47	*Hohenbuehelia petalodes* (Bull.) Schulzer	Pleurotaceae	S
48	*Hymenagaricus ardosiicolor* (Heinem.) Heinem.	Agaricaceae	S
49	*Hymenopellis gigaspora*(Cooke & Massee) R.H. Petersen	Physalacriaceae	S
50	*Inocybe* sp1	Inocybaceae	M
51	*Laccaria glabripes* McNabb	Hydnangiaceae	M
52	***Laetiporus sulphureus* (Bull.) Murrill ****	Polyporaceae	P & S
53	*Lepiota acutesquamosa* (Weinm.) P. Kumm.	Agaricaceae	P & S
54	*Lepiota cf. fuscovinacea* F.H. Møller & J.E. Lange	Agaricaceae	S
55	*Lepiota cristata (Bolton) P. Kumm*	Agaricaceae	S
56	*Lepiota forquignonii* Quél.	Agaricaceae	S
57	*Lepista sordida* (Schumach.) Singer	Tricholomataceae	S
58	*Leucoagaricus crystallifer*T.K.A. Kumar & Manim	Agaricaceae	S
59	*Leucoagaricus hortensis* (Murrill) Pegler	Agaricaceae	S
60	*Leucoagaricus littoralis* (Menier) Bon & Boiffard	Agaricaceae	S
61	*Leucoagaricus vassiljevae* E.F. Malysheva, T.Yu. Svetasheva & E.M. Bulakh	Agaricaceae	S
62	*Leucoagaricus viriditinctus* (Berk. & Broome) J.F. Liang, Zhu L. Yang & J. Xu	Agaricaceae	S
63	*Leucocoprinus cepistipes* (Sowerby) Pat.	Agaricaceae	S
64	*Leucocoprinus cretaceus* (Bull.) Locq	Agaricaceae	S
65	*Lyophyllum decastes* (Fr.) Singer	Lyophyllaceae	S
66	*Macrolepiota dolichaula(Berk. & Broome) Pegler & R.W. Rayner* **	Agaricaceae	S
67	*Macrolepiota excoriata* (Schaeff.) M.M. Moser **	Agaricaceae	S
67	*Macrolepiota procera(Scop.) Singer*	Agaricaceae	S
68	*Marasmius confertus* Berk. & Broome	Marasmiaceae	S
69	*Micropsalliota albosericea* Heinem. & Leelavathy	Agaricaceae	S
70	*Micropsalliota globocystis* Heinem	Agaricaceae	S
71	*Mycena chlorophos* (Berk. & M.A. Curtis) Sacc.	Mycenaceae	S
72	*Mycena pura* (Pers.) P. Kumm.	Mycenaceae	S

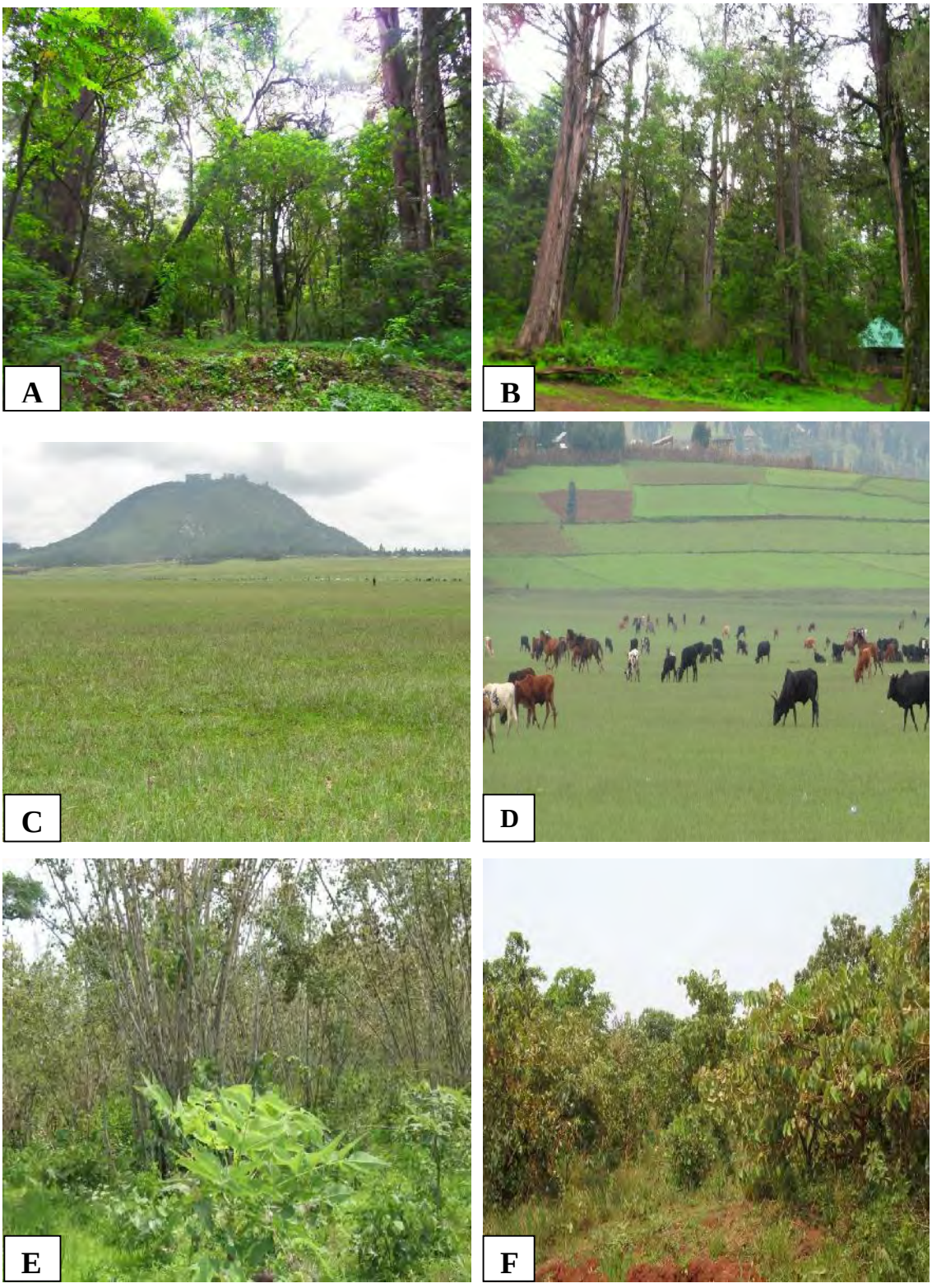
73	*Mycena* sp1	Mycenaceae	S
74	*Omphalina chionophila* Lamoure	Tricholomataceae	S
75	*Omphalotus japonicus* (Kawam.) Kirchm. & O.K. Mill.	Omphalotaceae	S
76	*Omphalotus olearius* (DC.) Singer	Omphalotaceae	S
77	*Panaeolus foenisecii* (Pers.) J. Schröt.	Psathyrellaceae	S
78	*Panaeolus papilionaceus* (Bull.) Fr	Psathyrellaceae	S
79	*Panaeolus sphinctrinus* (Fr.) Quél.	Psathyrellaceae	S
80	*Pisolithus microcarpus* (Cooke & Massee) G. Cunn.	Psathyrellaceae	M
81	*Polyporus* sp1	Polyporaceae	S
82	*Psathyrella candolleana* (Fr.) Maire	Psathyrellaceae	S
83	*Pseudoomphalina pachyphylla (*Fr.) Knudsen	Tricholomataceae	S
84	*Psilocybe coprophila* (Bull.) P. Kumm.	Hymenogastraceae	S
85	*Psilocybe ovoideocystidiata* Guzmán & Gaines	Hymenogastraceae	S
86	*Psilocybe samuiensis* Guzmán, Bandala & J.W. Allen	Hymenogastraceae	S
87	*Schizophyllum commune* Fr.	Schizophyllaceae	P & S
88	*Scleroderma* sp1	Sclerodermataceae	S
89	*Scleroderma aurantium* (L.) Pers.	Sclerodermataceae	S
90	*Stropharia rugosoannulata* Farl. ex Murrill	Strophariaceae	S
91	***Termitomyces letestui* R. Heim, Arch. Mus. Hist. ****	Lyophyllaceae	T
92	***Termitomyces aurantiacus* R. Heim ****	Lyophyllaceae	T
93	***Termitomyces clypeatus* R. Heim ****	Lyophyllaceae	T
94	***Termitomyces eurhizus* (Berk.) R. Heim ****	Lyophyllaceae	T
95	***Termitomyces letestui* (Pat.) R. Heim ****	Lyophyllaceae	T
96	***Termitomyces microcarpus* (Berk. & Broome) R. Heim ****	Lyophyllaceae	T
97	***Termitomyces robustus* (Beeli) R. Heim ****	Lyophyllaceae	T
98	***Termitomyces schimperi* (Pat.) R. Heim ****	Lyophyllaceae	T
99	***Termitomyces* sp1****	Lyophyllaceae	T
100	***Termitomyces striatus* (Beeli) R. Heim ****	Lyophyllaceae	T
101	***Termitomyces umkowaanii* (Cooke & Massee) D.A. Reid ****	Lyophyllaceae	T
102	*Tricholoma mongolicum* S. Imai	Tricholomataceae	M
103	*Tricholosporum porphyrophyllum* (S. Imai) Guzmán	Tricholomataceae	S
104	*Vascellum curtisii* (Berk.) Kreisel	Agaricaceae	S
105	*Vascellum pratense* (Pers.: Pers.) Kreisel	Agaricaceae	S

** Growth form S=Saprobic, M= Mycorrhizal, P= Parasitic, T= Termitophillus*
*** Edible species*
N:B- Species in Bold are used as food and/or traditional medicine in the study area

Appendix 4 : Diversity Index of macrofungi in each plot in study areas

Plots	Richness (S)	Evenness (E)	Shannon (H)	Plots	Richness (S)	Evenness (E)	Shannon (H)
		WFO				**MFO**	
1	21	0.968	**2.948**	**25**	7	0.949	1.848
2	16	0.962	**2.668**	**26**	9	0.983	**2.159**
3	14	0.970	**2.560**	**27**	6	0.983	1.762
4	12	0.977	2.428	**28**	13	0.988	**2.535**
5	12	0.979	2.432	**29**	6	0.976	1.750
6	17	0.975	**2.763**	**30**	5	0.949	1.527
7	12	0.970	2.411	**31**	13	0.979	**2.511**
8	9	0.982	2.157	**32**	9	0.962	2.115
		WGR				**MGR**	
9	19	0.975	**2.246**	**33**	4	0.983	1.363
10	6	0.982	1.760	**34**	5	0.959	1.543
11	8	0.981	**2.041**	**35**	6	0.938	1.681
12	8	0.981	**2.039**	**36**	7	0.974	1.896
13	9	0.987	**2.170**	**37**	3	0.982	1.079
14	6	0.972	1.742	**38**	5	0.833	1.341
15	9	0.975	**2.144**	**39**	7	0.948	1.846
16	9	0.970	**2.132**	**40**	12	0.927	2.304
		WFA				MFA	
17	4	0.968	1.342	**41**	13	0.952	**2.442**
18	5	0.982	1.581	**42**	5	0.970	1.562
19	5	0.985	1.585	**43**	8	0.946	1.967
20	6	0.980	1.757	**44**	7	0.940	1.830
21	3	0.941	1.034	**45**	5	0.947	1.525
22	5	0.961	1.546	**46**	7	0.973	1.895
23	6	0.981	1.757	**47**	13	0.941	**2.415**
24	7	0.988	1.923	**48**	10	0.913	**2.103**
				Mean	**8.6**	**0.9647**	**1.501**

Appendix 5 : Photographs illustrating study sites and field activities



A and B= Menagesha suba forest; C and D = grazing land and farm land in welmera; E and F= Menge forest; G-J= specimen collection at different site

Appendix 6 : Photographs illustrating some common mushrooms collected

Agaricus campestris L.: Fr.

Agaricus heterocystis Heinem. & Gooss.-

Agaricus sp.2

Agaricus bohusii Bon.

Agaricus xanthodermus Gen.

Macrolepiota procera (Scop.)

Chlorophyllum globosum (Mossebo) Vellinga

Laccaria glabripes McNabb

Lepiota acutesquamosa (Weinm.) P.

Leucoagaricus hortensis (Murrill)

Scleroderma aurantium (L.) Pers.

Vascellum curtisii (Berk.) Kreisel

.

Appendix 7 : Photographs illustrating Ethnomycological data collection and wild edible mushrooms sold in open markets in Menge District

A= Interview at open market at Belmegua kebele; **B** and **C** = Open market at Kudiyu kebele; **D** = walk in the woods with key informant; **F** and **G** = group discussion at different kebeles in Menge district

Appendix 8 : Photographs illustrating some aspects of the laboratory work

A, B and **C** =Morphological and molecular work at IM, CAS

D= Conselting Fungarium specimen at Jodrell Laboratory, Royal Botanic Gardens, Kew, UK