Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.4, No.21, 2014

A comprehensive study on Agaricus-like mushrooms from Mwalimu JK Nyerere Mlimani Campus, Tanzania

Donatha Damian Tibuhwa (Corresponding author)
Department of Molecular Biology and Biotechnology, University of Dar es Salaam,
P.O. Box 35179, Dar es Salaam, Tanzania
Tel: +255 22 241 0223 E-mail: dtibuhwa@udsm.ac.tz

Zuhura Ndoika Mwanga
Department of Molecular Biology and Biotechnology, University of Dar es Salaam,
P.O. Box 35179, Dar es Salaam, Tanzania.
Tel: +255 22 241 0223 E-mail: mwanga.zuhura@gmail.com

The Department of Molecular Biology and Biotechnology University of Dar es Salaam, is acknowledged for providing venue and facilities during the study

Abstract

A 3 years survey was conducted from 2011 to 2014 during which 133 Agaricus-like mushrooms from different places in primary forests, fields and gardens of the University of Dar es Salaam, Mwalimu JK Nyerere Mlimani Campus were collected. Agaricus-like mushrooms are morphologically characterized by medium to large size basidiocarp on the central stalk that separates easily from the cap, free gills, presence of veil and chocolate brown basidiospores in mature specimens. Characterizing them using both macro-micromorphological features and molecular markers (ITS sequences), they were revealed to be 12 species belonging to two distinct genera *Agaricus* L. and *Hymenagaricus* H. The species *Agaricus xanthodermus* and one un-described were suspected poisonous, edibility of 3 species were known while the edibility of the rest were unknown. Based on the result finding, one *Hymenagaricus* and two *Agaricus* species are also proposed as novel species for scientific descriptions based on International Code of Nomenclature.

Keywords: *Agaricus*, *Hymenagaricus*, Mushroom, University of Dar es Salaam

1. Introduction

Mushrooms play significant roles as an integral part of the forest ecosystem including nutrient recycling. They export nutrients such as Ca, Fe, K, Mn, N, P, Zn from the wood that are returned to the forest floor by insects and animals ingesting them, or by the senescence of fruit body (Gates and Ratkowsky 2009, Tibuhwa et al., 2011). Forest flourishing through mycorrhizal associations and their fruitbodies have enormous use for the general welfare of human life. Mushrooms are excellent food source, very rich in nutrients such as 25-30% protein content. Edible mushrooms provide a wide range of minerals and vitamins although the total nutrient contents vary significantly among species. It is amicable that a diverse fungal population contributes to a diverse diet for wildlife and humans. The jelly-like fruit bodies have been shown to contain various bio-compounds that have anti-tumor, antiviral, antibacterial and anti-parasitic effects making it a choice food (Chang and Miles, 2004; Masalu et al., 2010; Tibuhwa, 2012; Tibuhwa et al., 2012). Generally Agaricus-like mushrooms are fleshy, gilled mushrooms with well-determined stipe and cap. The stipe elevates the mushroom above object/substrate on which the mushroom grows. The partial veil protects the developing gills and later forms a ring or annulus on the talk (Kuo, 2007).

In Tanzania there are limited studies on mushrooms and on the Agaricus-like mushrooms in particular. For example, to the best knowledge of this study, few studies so far which described or reported on *Agaricus* species in the country include (Härkönen et al., 1995, 2003, Tibuhwa et al., 2011, 2011 a & b). There is no thoroughly study on the genus *Agaricus* or Agaricus- like mushrooms and even the few studies mentioned above reported them based on morphological characters, which might be subtle and difficult in demarcating the closely resembling taxa (Tibuhwa et al., 2012). This study used both micro-macromorphological and molecular markers which helped in delineating the closely related species. Furthermore, the study used DNA sequences of ITS region in determining phylogenetic position of Tanzanian Agaricus-like mushrooms in relation to other similar

taxa from other parts of the world.

2. Materials and Methods

2.1. *Study sites and sample collection*

A three years survey from 2011 conducted during the short rain of September –November and Long rains of March–June every year yielded a total of 133 collections of Agaricus-like mushrooms. Mushroom samples were collected from big unintentionally conserved natural trees forming huge thicket bushes of the University of Dar es Salaam Mwalimu JK Nyerere Mlimani Campus (Figure 1). The campus is situated on the western side of the city of Dar es Salaam 6°48' South, 39°17' East (−6.8000, 39.2833), on the observation hill, 13 km from the city center. The area occupies 1625 acres out of which buildings occupy 20% of the total area and roads while the remaining 80% constitutes a uniquely complex ecosystem which supports a wide range of organisms including Macrofungi as noted by Tibuhwa (2011). A remarkable feature of the studied area is its enormous orography, geological, floristic diversity as well as different land use units (natural trees, planted trees and gardens) that give rise to its macrofungi diversity described in Tibuhwa (2011a).

2.2. *Macro-morphological characters*

Mushrooms were collected, field photos taken and all morphological characters important for demarcating the taxa observed and recorded. Among the recorded macromorphological characters included colour of the basidiocarp, presence of veil, colour change on bruising, surface smoothness and cap margin, and spore print. All the characters were mostly observed in both young and mature fruitbodies. The samples were then packed in plastic bag carried in bucket to the Molecular Biology and Biotechnology Laboratory, of the University of Dar es Salaam for further analysis.

2.3. *Micro-morphological characters*

Basidiospores measurements were made on a compound microscope and statistical average value of 20 basidiospores and basidia recorded as explained in Tibuhwa et al., (2008). The value were indicated as: (min) min – SD – AV – max – SD (max) Q, in which min = lowest value recorded for the measured specimen, max = highest value, AV = arithmetic mean and the SD standard deviation; Q the ratio length/width. Spore shapes were described according to Bas (1969). Photos of the observed feature were taken direct using the inbuilt Camera of the digital microscope at a magnification of 1000 for basidiospores and x 400 for basidia and suprapellis.

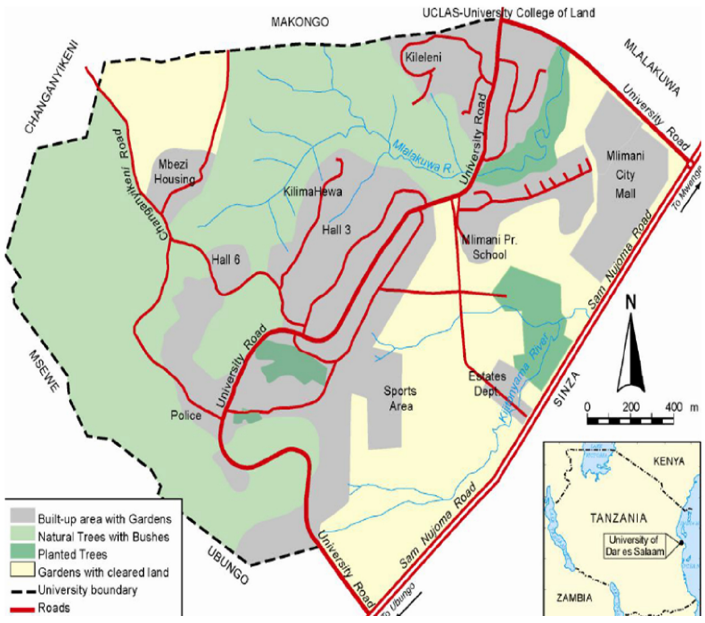

Figure 1: University of Dar es Salaam Mwalimu JK Nyerere Campus showing study sites (After Tibuhwa, 2011a&b)

2.4. Molecular study

2.4.1 *DNA Extraction and PCR amplification*

Genomic DNA was isolated from fruit body using the protocol that was developed by the Department of Molecular Biology and Biotechnology of University of Dar es Salaam with some modification illustrated by Graham et al. (1994). The DNA was extracted from fruit bodies dried in silica gel using the CTAB method. Amplification of 5.8S rRNA gene for assessing ITS length variation was done using primer ITS 1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) as described by (White et al., 1990). PCR amplification products were electrophoretically separated on 1.5% agarose gel prepared in 1X TAE then stained in ethidium bromide (5 mg/ml). The gel was then run for at 90 V for 45 min followed by patterns visualization on UV transilluminator, and photographing using digital camera. The genomic DNA was amplified using ITS 4 (TCCTCCGCTTATTGATATGC) and ITS 5 (GGAAGTAAAAGTCGTAACAAGG) primers. The generated sequences of the species were compared to those available in the GenBank database at: (http://www.ncbi.nlm.nih.gov/BLAST/) using the BLASTN search as detailed in Tibuhwa et al. (2012).

2.4.2 *Phylogenetic analyses*

The generated sequences in this study which were later used in phylogenetic analysis were submitted to gene Bank and their accession number, together with those obtained from the GenBank are summarised in Table 1. The sequences were aligned using ClustalW Olgorith (Higgins et al., 1990) of Mega Align 4.03 followed by manual editing. One sequence of non Agaricus-like (*Aspergillus niger*) was included in sequence analysis to serve as an out-group. A maximum likelihood (ML) tree was constructed using Kimura 2-parameter model. Bootstrap analyses with 1000 replicates were used to evaluate the stability of clade (Kimura, 1980).

Table 1: Taxa included in the phylogenetic analysis

No	Species	Voucher	Collection no.	ITS- Accession #
1	*Agaricus subsaharianus*	ZNM1	Zuhura 7.2013	KM360157
2	*Agaricus porphyrocephalus*	Z NM21	Zuhura 21.2013	KM360156
3	*Hymenagaricus* species nov.	ZNM A	Zuhura A.2013	MB809981
5	*Agaricus arvensis*	ZNM 15	Zuhura 15.2013	KM1360158
6	*Agaricus species nov.*	*ZNM 18*	Zuhura 18. 2014	MB809983
7	*Agaricus fuscofibrillosus*	ZNM7	Zuhura 7.2013	KM1360155
8	*Agaricus campestris*	ZNM 19	Zuhura 19. 2014	KM1360161
9	*Hymenagaricus ordosiicolor*	ZNM 4	Zuhura A. 2014	KM1360160
10	*Agaricus subsaharianus*	ADK 4733	Hama, O. et al.	JF440301
11	*Agaricus porphyrocephalus*	CA 856	Møller	JF797202
12	*Hymenagaricus* species nov.	CA 801	Heinemman	JF727859
13	*Agaricus arvensis*	ADK 2564	Schaeff	JF514518
14	*Agaricus* species nov.	ZNM 18	Zuhura	MB809982
15	*Agaricus fiscofibrillosusfis*	CA800	Møller	JF727862
16	*Agaricus campestris*	CA 819	Møller	JF727860
17	*Hymenagaricus ordosiicolor*	LAPAF9	Heinemann	JF727840
18	*Hymenagaricus* species nov.	CA801	Heinemann	JF727859.1

3. **Results and Discussion**

3.1. Scientific classification

The phylogenetic analysis revealed the studied Agaricus-like mushroom to belong to two distinct genera: *Agaricus* L and *Hyemenagaricus* H (Figure 2). Mushrooms in these two genera superficially looks alike by ranging from medium to large size basidiocarp on the central stalk that separate easily from the cap, free gills,

presence of veil and chocolate brown basidiospores. They both belong to Kingdom: Fungi; Division: Basidiomycota; Subdivision: Homobasidiomycete; Class: Agaricomycotina Order: Agaricales and same Family: Agaricaceae. Members in this genus were previously classified in one genus until 1981 when Heinemann described it as a new genus in Agaricaceae. In this study, 133 collections were made and after characterization using both macro-micromorphology and molecular markers (ITS) they were found to belong to twelve species in two distinct genera. Out of twelve species two belonged to *Hymenagaricus* genus while ten were *Agaricus* species. They were both found growing on different habitat including decaying organic mater, emerging from garden soil, and some growing on, or surrounding the termite mounds (Figure 3). Some fruited out immediately after one week of rain while others fruited later after 3-4 weeks of continuous rain. Likewise, the frequencies of their occurrence differed markedly with *Agaricus subsahrianus* leading having 38 times of encounter (Figure 4) while the proposed new species *Agaricus* species nov. (ZNM 18) had the least frequency of encounter (4 times).

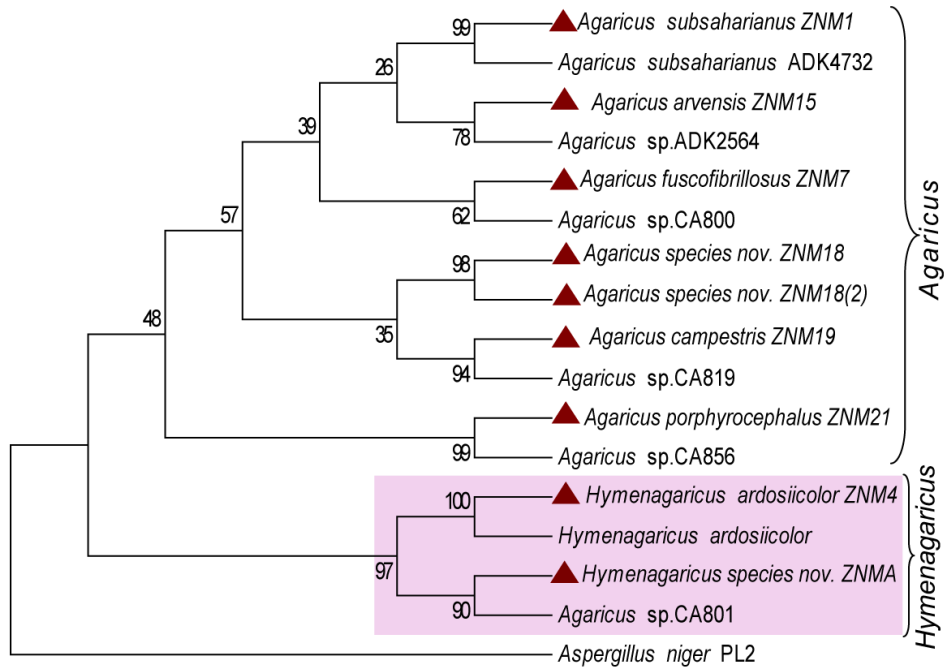

Figure 2: Phylogenetic tree by Neighbour-joining revealing two distinct clade representing the two genera *Hymenagaricus* and *Agaricus*. (Bootstrap value 1000).

3.2. Taxonomic remarks on the examined taxa:

3.2.1. *Agaricus subsahrianus* L.A. Parra, Hama & De Kesel (ADK 4732)

Comment: This is a large sized mushroom with its Cap measuring 9 to 22 centimeters broad. It looks white-cream in colour interrupted by brown scales that are evenly distributed over the surface but concentrating to blackening at the broad umbo. In young stages the scales are soft which give it a wooly appearance (Figure 3 a). Initially the basidiocarp looks hemispherical, which later flattens to applanate. It has relatively smooth margin, crowded gills that are initially pink, and then red-brown and finally dark brown attached free to the stipe. The flesh is white and turns slightly reddish on bruising, stipe is centrally positioned and clavate in shape having conspicuous thick superior white ring that in young stages completely cover the hymenium. Viewed from below, on a closed-cap specimen, the twin-layered ring has a well-developed 'cogwheel' pattern around the stipe (Figure 3 b). It is the most commonly collected species with the highest frequencies of occurrence (Figure 4). It fruit out even in unexpected rains of July. It actually fruit out one week following each heavy rains regardless of the season. Edibility: It is not edible and has been found to posses some insect side compounds (Nyigo et al., 2005).

3.2.2. *Agaricus arvensis* Schaeff. ex Secr. s. (ADK 2564)

Comment: This is a larger sized 3 to 10 centimeters diameter mushroom with straight margin. Initially it looks

hemispherical which flatten with age. Surface looks whitish or pale yellowish to cream when young, covered with fine pale brown scales which are down pressed concentrated toward the center. Gills: At first pink then turn dark brown in age, generally free, crowded and continuous. Stipe: White and centrally positioned, equal shape or slightly bulbous toward the base with a conspicuous ring that is the superiorly positioned, measuring 2 - 4 x 1 cm (See figure 3b). The mushroom is edible and delicious.

Figure 3: Some studied Agaricus-like mushrooms in the field: (a&b) *A. subsahrianus* (c&d) *A. trisulphuratus* (e&f) *A. arvensis* (g&h) *A. augustus* (i&j) *A. campestris* (k&l) *Hymenagaricus ardosiicolor* (m&n) *A. xanthodermus* (o&p) *A. fiscofibrillosusfis.*

3.2.3. *Agaricus campestris* L. Ex. Fr.

Comment: This is a beautiful white *Agaricus* mushroom species, young gills looks bright pink which later darkens to chocolate brown to completely black with age. The stipe is white with short-lived thin ring and woolly mass at the base. The mushroom is also known to be edible.

3.2.4. *Agaricus trisulphuratus* Berk.

Comment: Is a small sized (2-4 cm broad) mushroom which is distinctively bright orange with sticky scales when young and scaly bright orange stipe but less scales with dull-brown lamellae at maturity. While, its scales fall or stick to fingers when disturbed (Figure 3 c&d) its unique bright orange makes it unmistakably identified among other *Agaricus* species in the field. Its edibility is not well known.

3.2. 5. *Hymenagaricus ardosiicolor* (Heinem.) Heinem.

Comment: A medium sized mushroom 5-6 centimeters, surface white convex covered with brown scales which increases toward the center. Margin incurved, the stipe is central slightly bulbous with conspicuous thick superior white ring which in young stages completely cover the hymenium which when opened expose beautiful pink crowded free attached gills (Figure 3 l). Its edibility is not known, however, its small size, fragile nature and not growing in large numbers contributes to it not being the choice for many mushroom eaters.

3.2.6. *Agaricus porphyrocephalus* Mö11 (CA 856).

Comment: This is a small sized mushroom with basidiocarp ranging from 2-6.5 centimeters broad. The mushroom looks hemispherical when young then expanding to broadly convex at maturity. Surface covered with dark to purplish brown fibres and scales which increase towards the center. Gills are closely spaced, free attached to the stipe, covered with a thin white partial veil when in the button stage. Stipe looks cream which turn brown on bruising, equal shaped with central ring which is somehow persistent compare to other *Agaricus porphyrocephalus*. In the blast search its sequence did not have any closest match (Figure 3 i&j). Its edibility is not well known.

3.2.7. *Hymenagaricus* species nov.

Comment: This small sized *Hymenagaricus* species is uniquely distinguished by the presence of small woolly scales covering the whole fruit body including the stipe, that are easily removed on touch and the conspicuous brown matted center at the broad umbo. It is a new proposed species to be described in a separate study following International Nomenclatural Rules. Its edibility is not known, however its small size, fragile nature and not growing in large numbers contribute largely to not being favored by mushroom hunters.

3.2. 8. *Agaricus xanthodermus*

Comment: This is a medium sized mushroom with whitish or pale yellowish to cream colour surface. It has straight margin, initially hemispherical and flattening at maturity. The stipe is centrally positioned equal shaped or slightly bulbous toward the base. It is the most is notable poisonous *Agaricus* species due to its yellow-staining character.

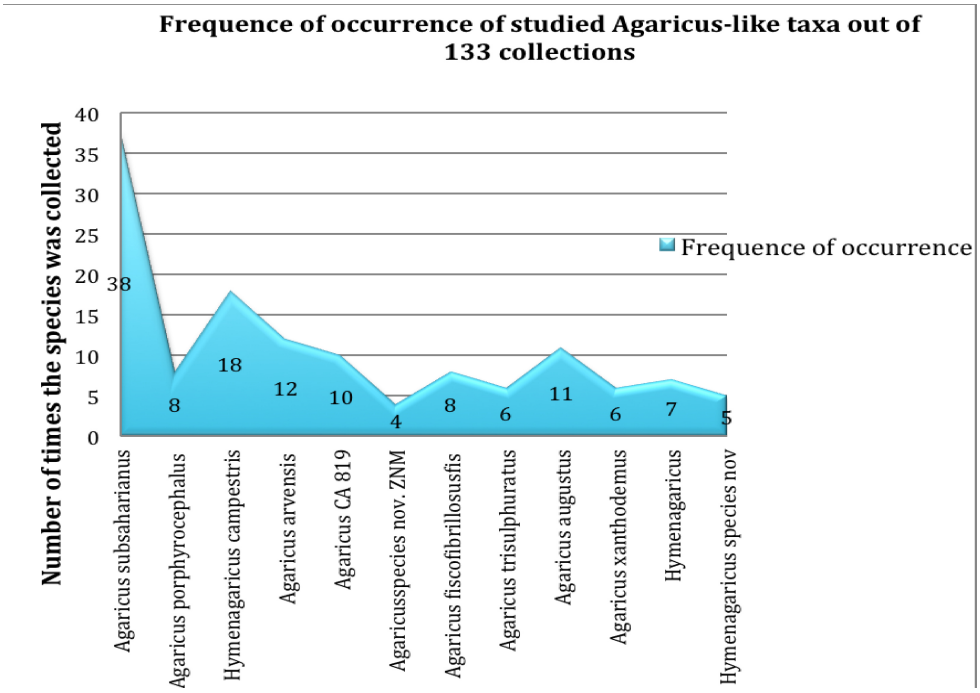

Figure 4: Frequency of occurrence showing *Agaricus subsaharianus* having highest frequency of occurrence

3.2.9. *Agaricus* species nov. (ZNM 18)

Comment: This is a new proposed *Agaricus* species to be described in a separate study. It is uniquely distinguished by the presences of conspicuous brown to reddish scales which cracks exposing the white cap make this species resemble the two other *Agaricus* mushrooms *Agaricus bernadii* and *Agaricus augustus*. It differs from the former by having a short and thick stem usually less than the diameter of the cap, while with the later lacking of large pendulous white ring on the stipe with small woolly scales below the ring and having no strong smell of bitter almonds demarcate it. Its edibility is not well known although it has a very good mushroom smell.

3.2.10. *Agaricus fiscofibrillosusfis* Møll (CA800)

Comment: This is among large sized *Agaricus* species with basidiocarp measuring up to 16 cm centimeters and the surface covered with brown scales which increase and become dense at the center. It appears hemispherical on early stages, then convex. This specie is uniquely noted in the field by its large size and surface interrupted with silk fibrillose scale at surface. The flesh stain quick to reddish as when bruised. Its edibility was not known. (Figure 3 o&p).

3.2.11. *Agaricus campestris* L. Ex. Fr. (CA 819)

Comment: This is a beautiful white Agaricus mushroom species, young gills looks bright pink which later darkens to chocolate brown to completely black with age. The stipe is white with short lived thin ring and woolly mass at the base. It is generally medium sized measuring up to 7 centimeters broad without conspicuous scales. In young stages, it looks hemispherical expanding to convex at maturity with incurved margin. Gills are generally overcrowded, pink in young specimen then turn dark brown with age. It is edible and well liked (Figure 3 o&p).

3.2.12. *Agaricus augustus* Fr.

Comment: This is a large sized *Agaricus* mushroom with expanded convex shape to flat in mature specimens. It has a typical dry surface densely covered with concentrically arranged brown-coloured scales on a white to yellow background. Gills are bright chocolate in mature specimen while in young specimen looks pale pink. The stipe is central with a large pendulous ring; the surface of the stem is smooth above the ring and has small woolly scales below (Figure 3 g&h). It is known to be edible from the literature although the questioned people within the campus were not aware of its edibility.

3.3 Two distinct genera based on molecular phylogenetic analysis

Genetically, phylogenetic analysis using ITS data set, from the maximum likelihood, two major clades of genera *Agaricus* and *Hymenagaricus* were retrieved (Figure 2). Identification of *Hymenagaricus* genus has a well-supported bootstrap value 97, having four species out of which three belongs to *Hymenagaricus* and one unidentified *Agaricus* species Agaricus (CA 801) which probably belongs to this genus too. The *Hymenagaricus* species are similar with members in the Agaricus clade by sharing some morphological feature such as pink gills which turn dark brown at maturity, presence of rings and gills with free attachment Heinemann (1981). This result concur with the finding obtained in, a preliminary analyses of nLSU datasets by Zhao et al. (2010). In their analysis, they included numerous species of *Agaricus* and allied genera of Micropsalliota plus several taxa of Heinemannomyces and *Hymenagaricus epipastus*, the latter species consistently formed a sister clade to Micropsalliota clade, with Bayesian support of 69%, while the two formed a sister clade to Agaricus clade (Zhao et al. 2010). In this study it interesting to note that (see figure 4), the clade that circumscribed the genus *Hymenagaricus* comprise of four taxa and is highly supported (97) while the rest constitute Agaricus species clade with less support value of 48 since the species in this genus are so diverse and represent an independent line of evolution (Young, 1998). This observation concurs with the recent study by Zhao et al., (2010) who also noted that *Agaricus* is a species-rich genus in the tropics as well as in temperate regions. In their study they found the genus *Agaricus* to comprise three distinct clades with one-third of tropical species belonging to the classical sections based on temperate species; two-thirds of tropical species, (from Americas and those from Africa and/or Asia) and one clades of classical sections contain American and African+Asian species along with temperate species. This fact is also reflected in this study (Figure 2), whereby the Tanzanian taxa did not form a

monophyletic clade but rather distributed into three clades but with very low support value.

Conclusion

Agaricus-like mushroom found at Mwalimu JK Nyerere Mlimani Campus belongs to two distinct genera. Although the studied area is relatively small out of several indigenous forests throughout the country, yet the study came up with three novel species proposed for scientific descriptions. This study thus recommends further study to explore the diversity and richness of the studied taxa in unstudied parts.

Acknowledgements

The Department of Molecular Biology and Biotechnology University of Dar es Salaam is acknowledged for providing venue and facilities during the study.

References

Bas, C. (1969). Morphology and subdivision of Amanita and a monograph on its section Lepidella. Persoonia 5, 285-579.

Chang, S.T. & Miles, P.G. (2004). Mushrooms, Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact, CRC Press. Mycologist. 6, 64-65. http://dx.doi.org/10.1201/9780203492086

Fan, L., A. Soccol, A. Pandey & Soccol C. (2007). Effect of nutritional and environmental conditions on the production of exo-polysaccharide of *Agaricus brasiliensis* by submerged fermentation and its antitumor activity. Food Sci. Technol., 40, 30-35.

Gates, G.M., Ratkowsky D.A., & Grove, S.J. (2005). A comparison of macrofungi in young silvicultural regeneration and mature forest at the Warra LTER site in the southern forests of Tasmania. Tasforests 16, 127-152.

Graham, G.C., Mayer, P. & Henry, R.J. (1994). BioTechniques 16, 48-50. www.pelagiaresearchlibrary.com.

Härkönen, M., Saarimäki, T. & Mwasumbwi, L. (1995). Edible mushrooms of Tanzania. Karstenia 35, suppl.,p. 92p.

Härkönen, M., Niemelä, T. & Mwasumbwi, L. (2003). Tanzanian Mushrooms: Edible, Harmful and Other Fungi. Norrlinia 200p.

Heinemann, P. (1981). *Hymenagaricus* Heinem. gen. nov. (Agaricaceae) Bulletin du Jardin botanique national de Belgique / Bulletin van de National Plantentuin van België. 51, 465-466.

Higgins, D.G., Bleasby, A.J., & Fuchs, A. (1990). CLUSTAL W: improved software for multiple sequence alignment. CABIOS 8, 189-191.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16, 111-120. http://dx.doi.org/10.1007/BF01731581.

Kuo, M. (2007). The genus Agaricus, Retrieved from the Mushrooms.Expert.Com Web site http://www.mushroomexpert.com/agaricus.html.

Masalu, R., Hosea, K.M.M., Meyer, M., Lyantagaye S.L. & Ndimba B. (2014). Glucose Regulated Protein 78 (GRP 78) as a Cytoprotection against Apoptosis in Small Cell Lung Carcinoma. Current Trends in Biotechnology and Pharmacy 8, 185-191.

Nyigo, V., Mdachi, S. J., Joseph, Nkunya, M.M., C.C. & Waziri, A. (2005). Chemical constitutes and cytotoxicity of some Tanzania wild mushrooms. Tanzania Journal of Science 31, 1-4

Zhao, R., et al. (2010). A monograph of Micropsalliota in Northern Thailand based on morphological and molecular data. Fungal Diversity 45, 33-79. http://dx.doi.org/10.1007/s13225-010-0050-4.

Tibuhwa, D.D., Buyck, B., Kivaisi, A.K., & Tibell, L. (2008). *Cantharellus fistulosus* sp. nov. from Tanzania. Cryptogamie Mycologie 29, 129-135.

Tibuhwa, D.D. (2011). Diversity of macrofungi at the University of Dar es Salaam Mlimani Main Campus in Tanzania. International Journal of Biodiversity and Conservation 3: 540-550.

Tibuhwa, D.D., Nyawira, M., Masiga, C.W., Mugoya, C., & Muchai, M. (2011). An inventory of Macro-fungi and their Diversity in the Serengeti-Masai Mara ecosystem, Tanzania and Kenya. Journal of Biological Sciences 11, 399-410. http://dx.doi.org/10.3923/jbs.2011.399.410

Tibuhwa, D.D. (2011). Substrate specificity and Phenology of Macrofungi Community at the University of Dar es Salaam Main Campus, Tanzania. Journal of Applied Biological Sciences 46, 3173– 3184.

Tibuhwa, D.D. (2012). Folk taxonomy and use of mushrooms in the communities around Ngorongoro and Serengeti National Park. Tanzania Journal of Ethnobiology and Ethnomedicine 8, 8-36. doi:10.1186/1746-4269-8-36. http://dx.doi.org/10.1186/1746-4269-8-36.

Tibuhwa D.D, Savić S, Tibell L. & Kivaisi A.K. (2012). Afrocantharellus gen. nov. is part of a rich diversity of African Cantharellaceae. IMA Fungus 3, 25–39. http://dx.doi.org/10.5598/imafungus.2012.03.01.04.

Tibuhwa, D.D. (2013). Wild mushroom- an underutilized resource for healthy food and income generation: experience from Tanzania rural areas. Journal of Ethnobiology and Ethnomedicine 9, 2-14. http://dx.doi.org/10.1186/1746-4269-9-49.

Ge, Z.W, Chen, C.M. & Yang, Z.L. (2008). A new species of the genus Hymenagaricus (Basodiomycota) from Taiwan and its phylogenetic position inferred from ITS and nLSU sequences. Cryptogamie Mycologie 29, 259–265

White, T.J., Bruns, T.D., LEE, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols, a guide to methods and applications, pp. 315-322. San Diego, California: Academic Press.