ORIGINAL RESEARCH ARTICLE

Phylogenetic analysis and abundance of culturable Fungi from Tropical glaciers; Lewis Glacier, Mt. Kenya

Isaac Okello[1], *Kevin Mbogo*[1], *Josiah Kuja*[2]

[1]*Department of Biochemistry, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya*
[2]*Department of Botany, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya*

Corresponding author email: obonyoisaac001@gmail.com

Abstract

Equatorial and tropical glaciers of Africa are mainly found on mountains summit. Due to climatic change, the receding glaciers exposes cryoconites which are ideal spots for microbial growth. Industrially important fungi are among the psychrophilic microorganism inhabiting cryoconites. In this study, the characterization and determination of the abundant fungal taxa in the tropical glaciers; Lewis glaciers Mount Kenya was done. Randomly selected cryoconite holes were mapped out across the study area by grid method, fifty samples were collected in different reagent bottles. Each sample was then serially diluted and cultured in Sabouraud Dextrose Agar (SDA) mixed with a broad-spectrum antibiotic to inhibit bacterial growth. The culturable pure strains that successfully grew were twenty-three. The pure isolates were first morphologically characterized Genomic DNA was extracted using CTAB method followed by DNA quality and yield assessments using agarose gel electrophoresis and nanodrop. For the molecular identification of the isolated fungi at the species level, the extracted fungal DNA was amplified by PCR using specific internal transcribed spacer primer (ITS1/ITS4). The amplified products were sequenced, and the sequence data were trimmed using BioEdit. The trimmed sequences were then characterized and there was a total of hundred fungi species; most of which had a range of 80-100% similarity with other fungi when subjected to BLASTn. For evolutionary relationship studies, fungi species that had a percentage similarity ranging between 95-100% were downloaded from NCBI and saved in MEGA X for further diversity analysis. Nine filamentous fungal species were identified as *Bjerkandera adusta, Coprinellus micaceus, Penicillium chrysogenum*, *Polyporales sp., Schizophyllum commune, Trametes hirsuta, Trametes polyzona, Trametes versicolor* and *uncultured fungi clone.* The results showed that *T. polyzona* was the most abundant fungus revealed from all the locations. It was also noticed that our isolates were of two phyla identified as: Basidiomycota and Ascomycota. Most of the fungi belonged to Basidiomycota.

Keywords: Ascomycota, basidiomycota, cryoconite, sanger sequencing, tropical glaciers.

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1.0 Introduction

Glacier forefield chronosequences, which previously contain substrate after glacier retreat make ideal niche for studying primary microbial colonization and succession in a natural environment. On the glacier surfaces, microorganisms colonize the cryoconite holes which are ice-cold habitats (Zumsteg et al., 2012).The occurrence of microbes in the cryoconite holes is characterized by the cryoconite granules comprising organic and inorganic particles. Organic particles are products of active biological activities occurring in the glacial niche which leads to glacial melting and subsequently soil formation (Takeuchi et al., 2001).

Microorganisms aid in the soil development process, biogeochemical cycling and also facilitate vegetation establishment, marking the beginning of secondary succession (Nemergut et al., 2013). Fungi and bacteria are among the first colonizers of soil, and they mainly facilitate the formation of fertile soils that subsequently aid to sustain the growth and development of several complex and simple vegetation communities (Fierer et al., 2010).

With the increasing interest in the causes of the accelerating glacier recession (Barry & Elith, 2006) and the possibility of changes in global geochemical patterns, there has been a rise in the necessity to understand the fundamentals of microbial composition and their succession dynamics in their ecological zone. Due to the current improvements in high-throughput sequencing, the microbial communities in various primary successional environments can now be examined and studied in-depth for both fungi and bacteria (Blaalid et al., 2012).

Mt. Kenya is among the tropical zones with glacial environments ecosystems in Africa. It has Lewis glacier (a tropical glacier) which is structured into two sections at 4,819 m and 4,670 m above sea level with slope gradients of 17.4^{o} and 19.8^{o} respectively. This glacier is similarly characterized by rich cryoconite materials and surface dust particles. Like any other cryoconite material based on the description by (Stibal, Šabacká, and Žárský, 2012), the cryoconite materials in the Lewis glacier have complex extracellular polymers and various microbial communities. A study on alpine and polar glaciers by Takeuchi et al., 2011 also reveal cryoconites as micro-biogeochemical reactors that are highly exposed to deglaciation as a result of active biological activities.

The gradients of the two sections are exposed to high sunlight intensity within the tropics thus the accelerated recession. For last two decades, Lewis glacier has been under close monitoring. Prinz, Nicholson, and Käser, 2016 reports that the quantity of Lewis glacier area change is averagely 15-50m annually with the highest change and mass loss witnessed in 2011/2012. No recent survey on the rate of glacial recession and mass balance has been done on Lewis's glacier. However, based on the effects of global warming on glacial environment as documented by McKay *et. al.*, 2022, the cryosphere of global glaciers has a negative shift causing loss of unique ice-associated ecosystems.

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The objective of this study was to determine the abundance of various fungal taxa and phylogenetic relationship of fungal community from cryoconites samples of Lewis glacier in Mt. Kenya.

2.0 Material and methods

2.1 Study site

The fungi communities under study were collected from Lewis glacier, Mt. Kenya (situated around latitude 0° 9′ 30′′S - 0° 9′ 15′′S, longitude 37° 18′ 45′′E - 37° 19′ 0′′E, in Nyeri County, Kenya. It has an elevation above 4,819 m and 4,670 m above sea level.

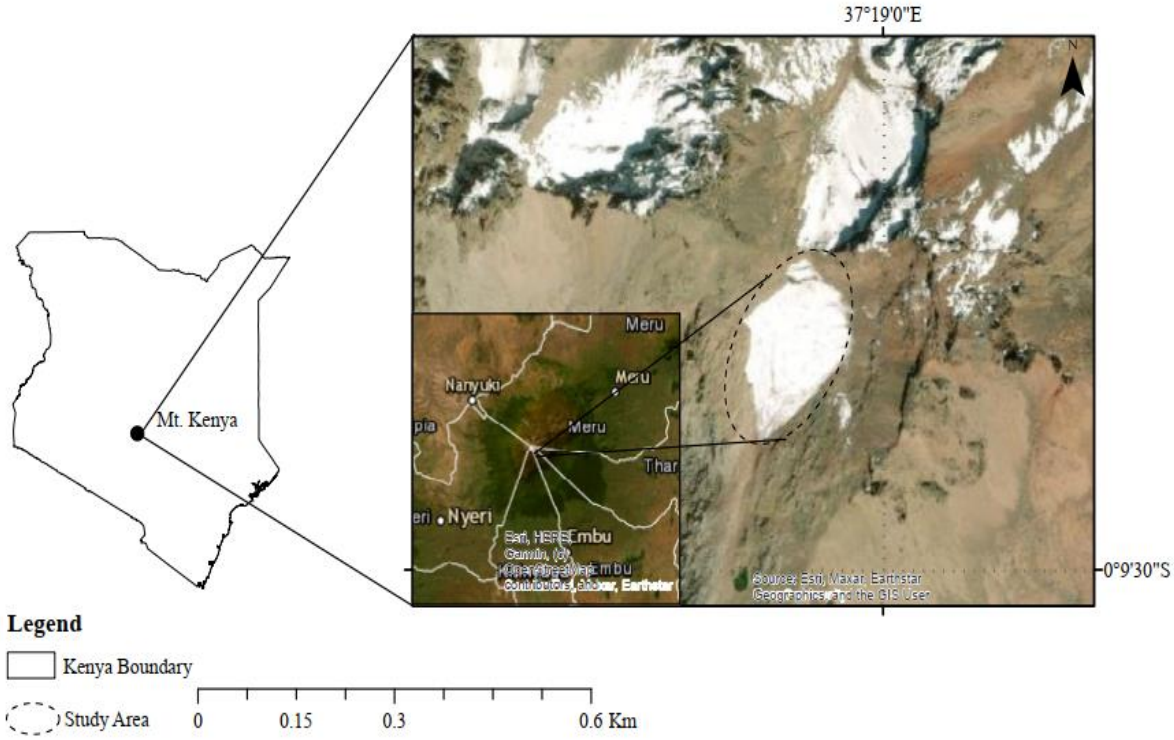

Figure 1. A map showing Lewis glacier on Mt. Kenya (ArcGIS v.10.8)

2.1.1 Sample collection

Grid sampling method was used to collect the cryoconite debris at an interval of 4m apart within the Lewis glacier. The scooping of the cryoconite debris was done using a sterilized spatula and put into 15ml tubes then kept in the icebox for preservation as they get transported for lab analysis.

2.1.2 Dilution of debris

The debris from each sample was serially diluted using the two-fold method and the resultant solution was used for culturing.

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2.1.3 Fungal culture

The debris solution obtained after serial dilution (200 µl) from each tube was spread onto different plates of Sabouraud Dextrose Agar (SDA) media with ingredients 2% Dextrose, 2% Agar, 1% enzymatic digest of casein. Despite the media having a low pH that inhibits growth of some bacteria, it was supplemented with a broad-spectrum antibiotic (Ciprofloxacin) to completely inhibit bacterial growth.

Incubation was then done for 2 weeks and frequently monitored for any growth to enable further incubation in a set of temperature of 4^{o}C for 2 weeks. Plates with no growth were incubated at 22^{o}C and monitored at regular intervals for microbial growth. Any noted cultures proliferation was recorded and appropriately sub cultured for pure strains.

In all the culturing procedures and setting of the control plates (two plates of Malt Extract Agar and two plates of Potato Dextran Agar), decontamination was ensured by carrying out the activities in the laminar flow hood.

2.1.4 Isolation of fungal cultures

A distinct colony of the fungal growth on each plate was inoculated in a new plate of prepared Sabouraud Dextrose media supplemented with a broad-spectrum antibiotic (Ciprofloxacin). The incubation temperatures were then set at 4°C and 15°C. Incubation was done for 1 week at 4^{o}C and frequently monitored for any growth. Plates with no growth were subsequently incubated in temperature adjustment to 15^{o}C. Pure isolates were then characterized.

2.1.5 Morphological characterization

The morphological characterization of the pure fungal isolates was done macroscopically. Image analysis method by Vanhoutte et al., 1995 was applied with the focus on the color, texture and density of the hyphae (filaments). To ensure accurate details and color resolutions, a hand lens of magnification x20 was used.

2.2 Molecular characterization

2.2.1 DNA extraction

CTAB (cetyltrimethylammonium bromide) extraction method was used to extract genomic DNA. In brief, freshly scrapped fungi mycelia were first grounded in mortar and the wet homogenized materials then transferred into well-labeled 1.5 ml tubes. Cold 700 µl extraction buffer (SDS 10%) was added.

Subsequently, there was resuspension of the cells in 0.8 ml of pre-warmed CTAB extraction buffer at 60°C.The CTAB buffer which is a mixture of; 2% CTAB (hexadecyltrimethylammonium bromide), 100mM Tris-HCl of pH.8, 20mM of EDTA, 1.4 M NaCl, 0.2% β-mercaptoethanol (added before use) and 0.1 mg/ml Proteinase K (added before use). This mixture was incubated for 1 hour at 60°C in a microtube.

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The microtube was inverted from time to time to gently mix the contents. After an hour, 0.8 ml of chloroform/isoamyl alcohol (24:1) solution was added then gently mixed for about 2 minutes. All this was done in the fume hood to prevent contamination.

The mixture was then spined for 10 minutes at a maximum speed of 14000rpm at a temperature of 4°C. The resultant aqueous phase (usually on top of the white interface layer) was instantly transferred into a sterilized microtube, and the rest were discarded. 1 µL RNase (DNase-free) was added then incubation of the mixture followed at 37°C for 30 minutes.

Gentle mixing was then done by inverting the microtube to ensure complete mixing. The resultant precipitate was later stored overnight at room temperature to allow the formation of the "DNA jellyfish". Spinning then followed for 15 minutes at 14000rpm at 4°C to pellet the DNA. The obtained supernatant was then carefully removed and the washing of the pellet with cold ethanol was done twice

Spinning was again done for 15 minutes at a maximum speed at 4°C, the obtained supernatant was removed and the pellet dried by leaving the tube to open at room temperature. The pellet was then resuspended in nuclease free water and stored at -20°C. To confirm the quality and quantity of the DNA extracted, nanodrop and gel electrophoresis analysis were done, respectively.

2.2.2. PCR amplification of the ITS regions

General universal primers for fungi were used in the amplification of the ITS region of the fungal isolates The primer sequence composition was as follows: forward: ITS1-F 5'CTT GGT CAT TTA GAG GAA GTA A3' and (reverse) ITS4-R 5'CAG GAG ACT TGT ACA CGG TCC AG3') (White et al., 1990).

The conventional PCR parameters were used during amplification: activation was done at 94^{o}C for 5 minutes, then denaturation process followed at a temperature of 94^{o}C for a minute, this was then followed by annealing at a temperature of 48^{o}C for a minute too, thereafter, there was extension process at a temperature of 72^{o}C for 2minutes and finally an elongation at a temperature of 72^{o}C for 10 minutes. Amplification was performed using a thermal cycler (TC 9639 AND T5000 Thermal Cycler, Benchmark Scientific). The presence of PCR products was then confirmed using 1% agarose in 1 x TAE buffer. A gene ruler of 1 kb was used. Visualization was done beneath a beam of ultraviolet rays after a staining process using the intercalating agent; RedSafe. The PCR products were then preserved at -20°C for further analysis.

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2.2.3 Sequencing and phylogenetic analysis

The sequencing process of the purified PCR products was performed by a commercial service provider, Macrogen, Seoul, Korea, using Sanger sequencing technique.

The sequences were trimmed and edited using Bioedit then Multiple Sequence Alignment performed using MEGA X(Kumar et al., 2018). The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model(Saitou & Nei, 1987). The tree with the highest log likelihood (-7409.89) was inferred.

The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with a superior log-likelihood value. The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. This analysis involved 32 nucleotide sequences.

Codon positions included were 1st+2nd+3rd+Noncoding. There was a total of 747 positions in the final dataset. Evolutionary analyses were conducted in MEGA X(Kumar et al., 2018)

3.0 Results

3.1 Morphological features

Twenty-three pure isolates were macroscopically analyzed to reveal their morphological features. Most of the fungus displayed an alternate white and off-white colors, high density and varied filamentous features making them look woolly with a velvety like texture. The observed distinct features from each colony were clustered and their similarity and dissimilarity levels were shown by the dendrogram and heatmap respectively.

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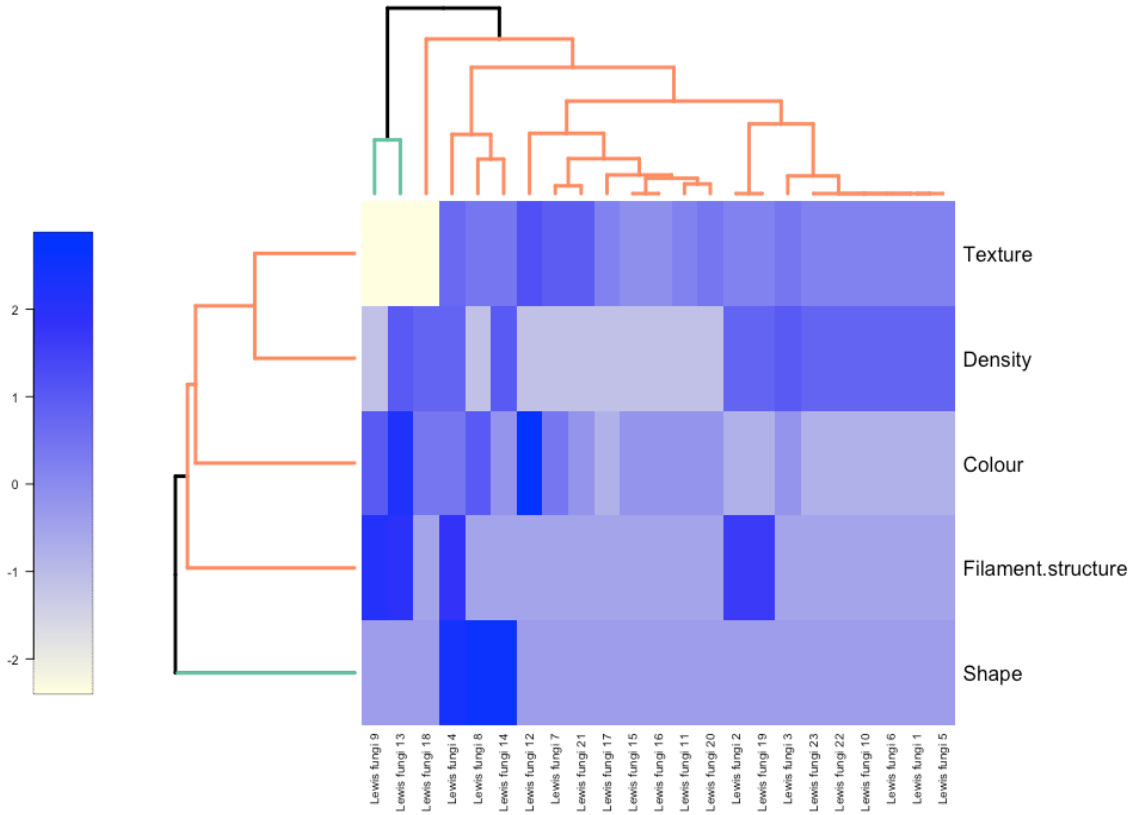

Figure 2. Heatmap showing the morphological features of each isolate from Lewis glacier.

The branch lengths of the cluster dendrogram show the similarity level. Clustering among samples was based on a distance matrix to compute their dissimilarity.

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3.1.1 Association of 18S rRNA gene sequences of the isolates with the GenBank sequences

A total of 18 isolates of 18S rRNA gene sequences prefixed (Lewis fungi) which were used in the downstream analysis were individually compared to known Genbank sequences. The Blastn comparison analysis indicated sequence similarities ranging from 83% to 100% with the known sequences.

Table 1. A table showing the Blast-n results of the isolates18S rRNA gene sequences from Lewis glacier; their closest relatives, sources, and accession number.

Sample ID	NCBI closest relative	Source	% of closeness	Accession No.
Lewis fungi 1	*Trametes polyzona*	N/A	99.84	JN164980.1
Lewis fungi 2	*Trametes polyzona*	N/A	100	JN164980.1
Lewis fungi 4	*Tricholoma robustum*	Base of dead kolanut stump	98.91	MT644927.1
Lewis fungi 5	*Trametes polyzona*	Fruiting body	96.51	KP013053.1
Lewis fungi 6	*Trametes polyzona*	N/A	90.58	OL685335.1
Lewis fungi 8	*Penicillium Chrysogenum*	N/A	99.61	KY218674.1
Lewis fungi 9	*Bjerkandera adusta*	Washed-organic particles	99.57	KC176354.1
Lewis fungi 10	*Trametes polyzona*	N/A	100	LT629240.1
Lewis fungi 11	*Tricholoma robustum*	Dead mango stem log	97.53	MF037416.1
Lewis fungi 12	*Coprinellus micaceus*	Root of Agathis australis	98.47	MN218798.1
Lewis fungi 13	*Schizophyllum commune*	On bark/wood of beech trunk	99.75	MH307932.1
Lewis fungi 14	Uncultured fungi	Washed organic particles	94.32	KC176335.1
Lewis fungi 15	*Polyporales sp.*	Surface of fern frond	83.15	KU747897.1
Lewis fungi 16	*Polyporales sp.*	Respiratory sample	95.38	JQ312191.1
Lewis fungi 18	*Trametes sanguinea*	Fruiting-body	95.69	KP012989.1
Lewis fungi 19	*Trametes versicolor*	N/A	95.16	FJ608587.1
Lewis fungi 22	*Trametes polyzona*	N/A	99.84	LT629240.1
Lewis fungi 23	*Trametes hirsuta*	Soil	95.17	CP019375.1

3.1.2. Relative abundance of the fungi based on their taxa; Phylum, Genus and Species

Quantitatively the results indicated that 94.10% of the isolates belonged to Basidiomycota while 5.90% were from Ascomycota.

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The fungus abundance analysis based on their genus indicated a descending trend as follows; *Trametes (52.90%), Tricholoma* and *Polyporales* at 11.80% while *Bjerkandera, Coprinellus*, *Schizophyllum* and *Penicillium* each at 5.9%.

In terms of the species, *T. polyzona* was the most abundant at 35.30%, *T. robustum* and *Polyporales* species were both relatively abundant at 11.80%. The least abundant species were *B. adusta*, *C. micaceus*, *P. Chrysogenum*, *S. commune, T. hirsuta, T. sanguinea* and *T. versicolor,* each at 5.90%.

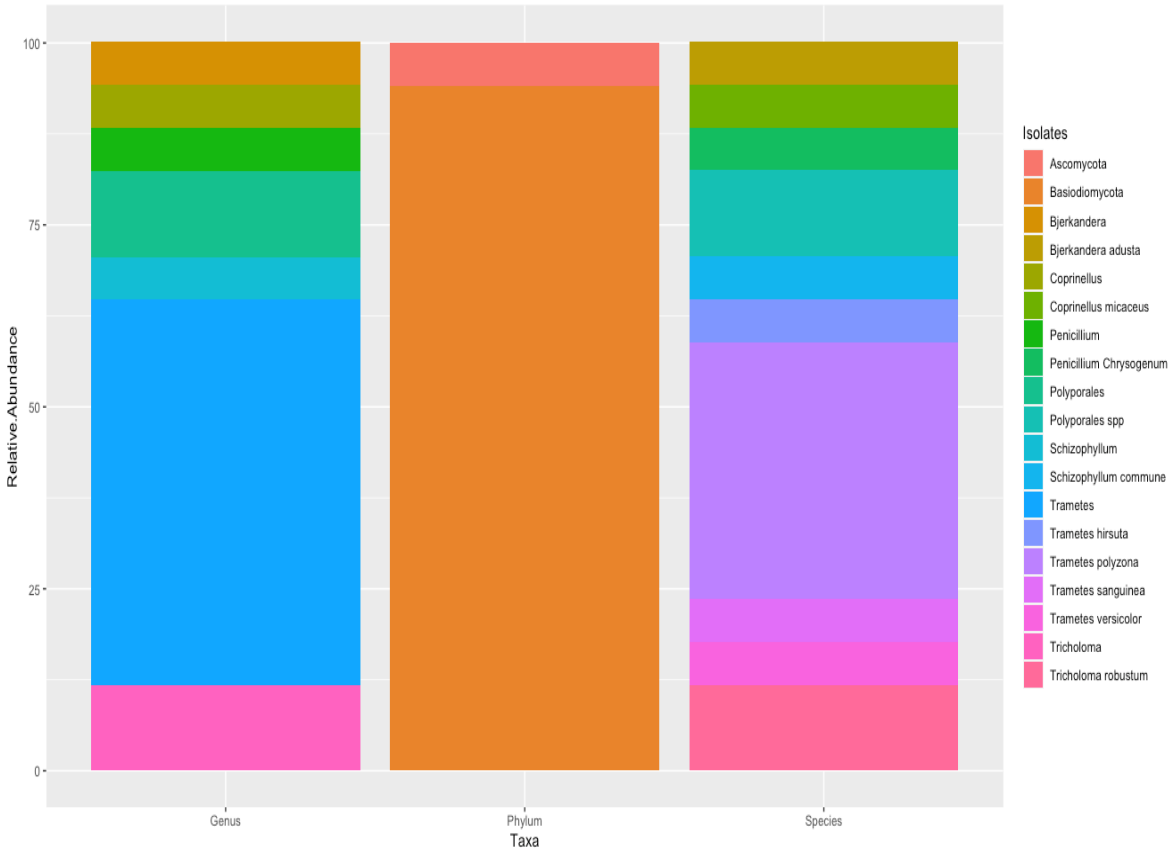

Figure 3. A graph showing the taxonomic relative abundance of the isolates from Lewis Glacier

3.2 Phylogenetic analysis

Evolutionary relationships between partial 18S rRNA gene sequences of the isolates and some of their blast-n relatives were done. *Methanoculleus thermophiles* (AB065297) was used to root the tree. *Methanoculleus thermophiles* is a thermophilic bacterium thus makes a perfect outgroup for an evolutionary study amongst psychrophilic fungi.

The analysis involved 32 nucleotide sequences with an inclusion of the 1st+2nd+3rd+Noncoding as the codon positions. There was a total of 747 positions in the final dataset.

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The percentage of relatedness between the associated taxa are shown next to the branches of the tree (Figure 5). The phylogenetic tree was further analyzed to clades. The resultant clades clustered the isolated fungi to their blast-n relatives in terms of species (Figure 5). This further led to two major taxonomic clustering where the major phyla were Ascomycota and Basidiomycota which relates to the fungal phyla percentage abundance.

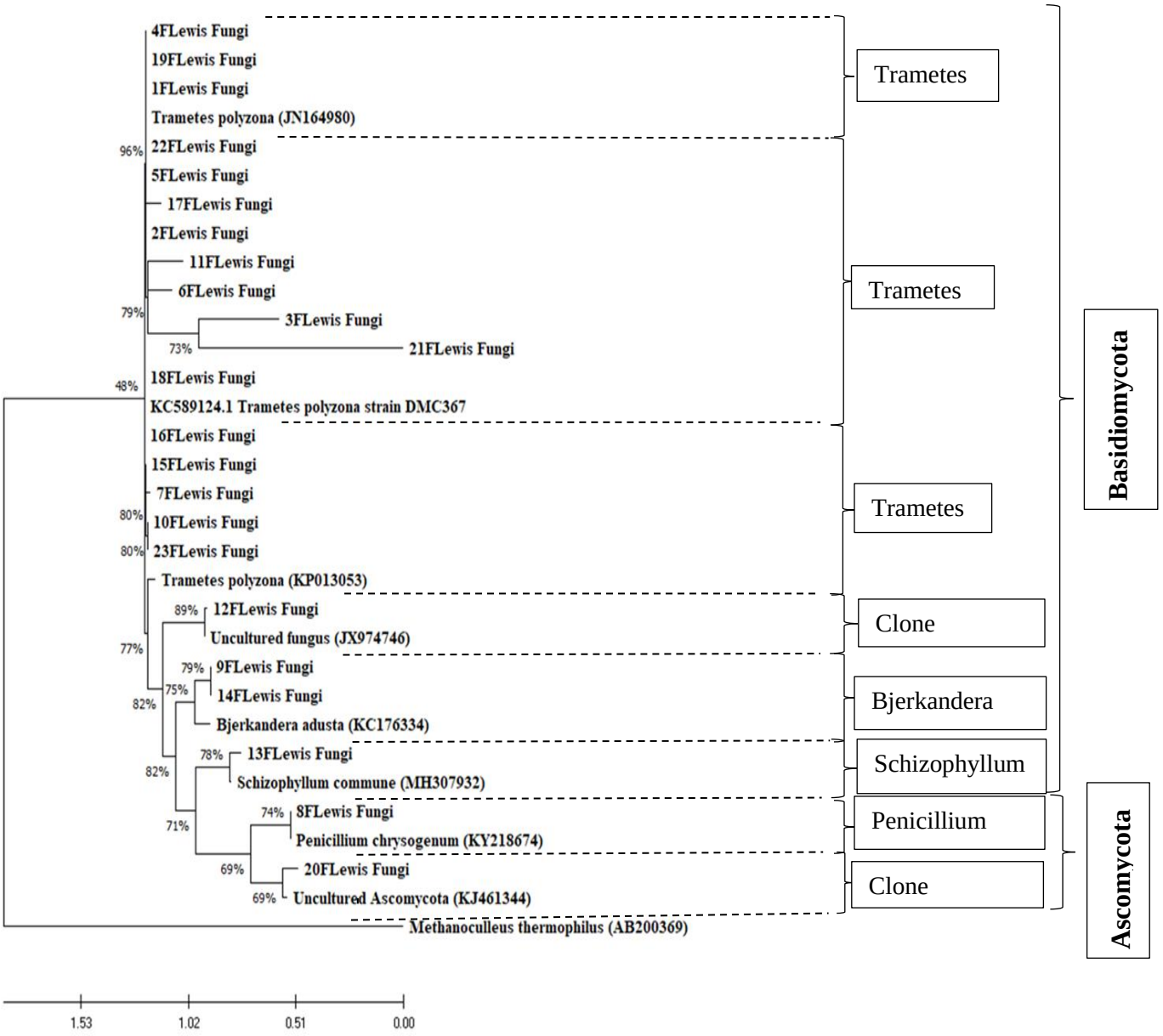

Figure 4. Evolutionary relationships between partial 18S rRNA gene sequences of the isolates and some selected known fungal species. M. thermophiles (AB065297) was used to root the tree.

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4.0 Discussion

4.1 Morphology of the fungal isolates

In this study, a total of twenty-three filamentous fungi were recovered from fifty samples collected from the Lewis glacier. The low number of the detected fungal strains was suspected to be due to very slow growing rate of psychrophilic fungi as noted by dos Santos, Meyer, and Sette, 2020. The culture-dependent approach may have also been a set back because it supports growth of a fraction of the microorganisms, causing a hindrance of the entire diversity detection (Cometto et al., 2022).

Morphologically, most of these fungal strains were white in color, high density hypha with velvety texture. A study by Spribille et al., 2020, reported similar characteristics exhibited by lichen forming and lichenicolous non-mutualistic or lichen-associated fungi. Lichens have symbiotic relationship with cyanobacteria. Most microbial diversity studies in glacial environments such as alpine and antarctic glaciers have shown that the lichens and cyanobacteria have a positive relationship (Almela et al., 2022). Cyanobacteria which are photosynthetic, efficiently trap the harmful solar radiation. Beneath, are the lichenised fungi which are obligate symbionts. The cyanobacteria (algae) are phototrophs and primary producers, while the fungi and bacteria are consumers (Mezzasoma et al., 2022). Raggio et. al., 2012 also indicates that lichens utilize their relationship with *cyanobacterium* species to fix atmospheric nitrogen (N), thus allowing them to colonize nutrient poor areas like glaciers. Possibilities of nitrogen cycling done by the Lichen-Cyanobacteria photo-symbiosis besides other bacterial species in the Lewis glacier was revealed in the study by Josiah et al., 2018. Lewis glacier recession due to climatic change is also confirmed by the presence of these Lichens since they are bioindicators of environmental change in such ecosystems as reported by Robison *et. al*., 2022.

4.2 Abundance of fungal phylum in Lewis glacier

The current study revealed a higher prevalence of *Basidiomycota* (94.1%) and *Ascomycota* (5.9%). Contrary, other studies on glacial ecosystems such as Werenskiold glacier, Antarctica and Tibetan plateau (China), revealed a higher prevalence of *Ascomycota* than *Basidiomycota* (Borzęcka et al., 2022;dos Santos et al., 2020;Ma et al., 2022). A study on divergent assemblage patterns of microbial communities along glacier forefield chronosequences by Jiang et al., 2018 also reports higher *Ascomycota* prevalence than *Basidiomycota*.

Basidiomycota dominance over *Ascomycota* in the Lewis glacier can be related to their higher growth rate and gluttonous nature as suggested by Edwards et al., 2013. Elsewhere, it was reported that *Basidiomycota* mainly dominate old soils while *Ascomycota* mainly dominate young soils (Hassan et al., 2016). This indicates that the organic and inorganic matter in the cryoconites of Lewis glacier are mostly from the old soils. The *Ascomycetes* in Lewis glacier are likely to be originally transported to the cryoconite in windblown plant litter or soil. A study by Dresch et al., 2019 on soil developmental stages of a receding glacier also revealed that Basidiomycota are

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generalist fungi and *Ascomycota* are specialist fungi. With the scarcity of nutrients and extreme environmental conditions, generalists have advantage to survive and grow on mineralized organic matter from fixed atmospheric Nitrogen and Carbon cycle (Glacier et al., 2010).

4.3 Abundance of fungal genus in Lewis glacier

Further molecular analyses based on 18S rRNA gene sequences indicated a genus dominance of *Trametes* (52.90%) and relatively low densities of other genus; *Tricholoma* (11.8%), *Penicillium* (5.9%), *Bjerkandera* (5.9%), *Coprinellus* (5.9%), *Schizophyllum* (5.9%), Uncultured fungi (5.9%) and *Polyporales sp.* (11.8%). *Trametes* are in Basidiomycota, Polyporaceae. They are mostly found in forest ecosystems in temperate and tropical areas(Hardin et al., 2017). They are wood decomposers leaving behind a white substance thus known as white rot fungi. They are frequently found on numerous genera of hardwoods growing in forest ecosystems and are believed to be not host specific in the tropics. (Aires, 2018). Their decomposing ability is due to the secondary metabolites (lignocelluloses and laccases) they produce during the extreme conditions (Hassan et al., 2016).

The study also revealed an average abundance of *Tricholoma.* They are ubiquitous ectomycorrhizal basidiomycetes, having symbiotic relationships with most plant species (Grubisha et al., 2012). The species under Tricholoma in this study was *Tricholoma robustum;* a symbiont of conifers producing matsutake-like mushrooms (Murata & Babasaki, 2005). The genus with least abundance as per the study were *Penicillium, Bjerkandera, Coprinellus, Schizophyllum*, Uncultured fungi and *Polyporales sp. Penicillium* belongs to Ascomycota and are found in unique and diverse range of habitats. This makes them the most versatile 'mycofactories' producing different bioactive compounds (Yadav et al., 2017).The blast search revealed *P. chrysogenum* which is a species under this phylum. A study done in the glacial ice of Antarctica by de Menezes et al.,2020 also revealed similar low densities of *P. chrysogenum*. It is also reported by Brunner, Goren, and Schlumpf, 2014 that *P. chrysogenum* utilize pollen grains as its main source of carbohydrates to enable it exude organic acids such as oxalate. It again indicates that the Ascomycota in the Lewis glacier are windblown agents thus their low density as compared to Basidiomycota.

Other genera that were observed are *Polyporales, Bjerkandera, Coprinellus* and *Schizophyllum. Bjerkandera, Polyporales* and *Schizophyllum* are white-rot polypore basidiomycete fungi usually growing on dead broadleaved wood or on woody plant hosts(Pristas et al., 2022). In this study, these genera had species; *B. adusta*, *Polyporales sp* and *S. commune.* From the blast search, the sources of these species were washed-organic particles, surface of fern frond and bark/wood of beech trunks respectively. Kochkina, Ivanushkina, and Ozerskaya, 2021 reveals that besides being wood destroying fungi, *Bjerkandera adusta* and *Schizophyllum commune* also remain viable in natural cryoprotectants. Moreover, *B. adusta* morphologically has pileate, effused-reflexed to resupinate basidiomata(Pristas et al., 2022). These features enable their anchorage on washed organic particles despite the glacial slump as a result of recession. *Polyporales sp.* are a cryptic species with the *Trametes sp.*; specifically, *T. hirsuta*. They exhibit similar symbiotic patterns with

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their host organisms(Shabaev et al., 2022). Based on their sources from blast search, it is conceivable that they are both aero aquatic fungi since they are endophytes of the healthy leaves or root tissues of higher plants or rhizosphere (Edwards et al., 2013).

The dominant genus in this study was *Trametes* (52.9%) with varied abundance of respective species; *T. polyzona (35.3%), T. versicolor (5.90%), T. hirsuta (5.90%)* and *T. Sanguinea (5.90%).* This genus is composed of both white and brown rot fungi (Welti et al., 2012).Carlson, Justo, and Hibbett, 2014 describes them as white, trimitic hyphal systems with smooth non-dextrinoid and non-amyloid spores; congruent characteristics with most morphological features of isolates in this study that suited most *Trametes* species. *T. polyzona, T. versicolor* and *T. hirsuta* are reported to be psychrophilic novel producers of laccase enzyme by (Wiśniewska, Twarda-Clapa, and Białkowska, 2021 & Chairin et al., 2013). This is an indication that they are cold adapted fungi and the environmental stress due to extremely low temperatures enables them to produce such important metabolites. Elsewhere, *T. polyzona*, is revealed to produce both ligninolytic and cellulolytic enzymes, making them the most efficient wood decomposers including hardwood and woody biomass(Acheampong et al., 2021). Their abundance in the Lewis glacier indicates high degradation of woody plants, a bioindicator of environmental change and microbial community succession.

4.4 Phylogeny of the fungal isolates

Phylogenetic tree of the Lewis glacier species belonging to Ascomycota and Basidiomycota were obtained by a Maximum likelihood method. This was via determining the evolutionary relationships between the Internal Transcribed Spacer (ITS) region of 18S rRNA gene sequences of the isolates and some selected known fungal species. The analysis of this species revealed a monophyletic relationship with most of the isolated fungus except those in second *Trametes* clade (Lewis glacier fungi 3 and 27). There were also less evolutionary events within the members of Basidiomycota with majority being polychotomous. The three genus clades; *Clone, Bjerkandera* and *Schizophyllum* had a disparity from the *Trametes* clade which had a dominant polychotomy.
The point of divergence began at the clone clade where a common node from which some members of *Basidiomycota* and *Ascomycota* originate. Until the point of divergence, they had a percentage similarity of 71%; a threshold that can confirm that Basidiomycota and Ascomycota are sister phyla.

5.0 Conclusion

In our study, fungi existence in Lewis glacier was studied for the first time. Eighteen fungal isolates were isolated and identified through 18S rRNA sequencing. Majority of the fungal isolates belonged to the phylum Basidiomycota, genus *Trametes*. The abundant species was *T. polyzona*. Most of the species revealed under this study have also been noted to secrete metabolites of industrial and economic importance. Therefore, this study recommends further work to be done in the future to isolate and identify psychrophilic fungus for cryo-proteins and enzymes extraction.

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6.0 Acknowledgments

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7.0 Conflicts of Interest

There was no conflict of interest in the study.

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