Diversity of fungi in soils of different degrees of degradation in Panama and Germany

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1 Abstract

The main goal of this work is to contribute to the existing knowledge of soil micro-fungi in Panama and Germany. Studies about soil degradation and its influents in the soil fungi diversity have not been investigated as extensively in these countries. This is an extensive and challenging topic to examine since there is an immense phenotypic and genetic diversity in the soil fungal community and relating this community together with factors of soil degradation is an extensive task. For this reason, the present thesis studies the species identified in the study areas, in other words, the soil fungal diversity in relation to environmental factors in the Taunus Mountain range in Frankfurt, Germany, and in the Majagua valley in Chiriquí, Panama. Two complementary objectives were achieved, the first was the development of a theoretical irrigation model for degraded soils. The second was the development of a mobile application to facilitate laboratory work in the cultivation of soil micro-fungi.

The design of the methodology was based on identifying the species and relating the diversity found to soil factors. Soil samples were taken in both countries: the Taunus Mountain range was sampled eight times from January to November 2012 and the Majagua valley was sampled on three occasions between February and July 2012. In both studies, the areas included three different vegetation types (forest, grassland, and bare soil). Samples were separated for two purposes: the assessment of fungal diversity by molecular and morphological methods and soil characterization.

Soil samples used in the methodology of pyrosequencing were related to global climatic factors. Morphological identification was achieved with identification keys.

Micro-fungi were cultivated in different media until obtaining pure cultures. Molecular identification was performed by getting the DNA sequences using the ITS1 and ITS4 primers and comparing the sequences with other reference sequences from GenBank. This was done considering the BLAST algorithm, which considered sequences that matched 98 % or more of maximum identity as reliable identifications.

Soil characterization was carried out to measure the soil's Physico-chemical properties; those abiotic factors were compaction, temperature, pH, moisture, and soil composition.

Species richness was calculated in each study area with the estimators Chao, Jackknife, and Bootstrap. Furthermore, the species accumulation curves were performed to observe the species discovery rate and estimate sample completeness. Estimate linear regression models correlated the influence between the soil factors (temperature, moisture, pH, soil compaction, and soil composition) and the species richness. In the same way, an analysis of ecological distance was undertaken based on the similarity in the species composition, compared across samples, and correlated with soil factors, using non-metric multidimensional scaling (NMDs).

Study of abundance showed differences between the bare soil abundances and the forest abundances in Germany and Panama; the grasslands in both countries work as transitional areas in the fungi abundance. The key stone species in Germany were *Penicillium daleae*, and *Pochonia bulbillosa,* whereas in Panama were *Purpureocillium lilacinum* and *Trichoderma harzianum*. Based on Pareto

analysis, a theoretical irrigation model was developed to counteract the degradation effects on the abundance of micro-fungi in the soil.

Applications for mobile devices dealing with the cultivation of soil micro fungi were sought. Due to the small number of existing applications, a new App called *Soil-Fungi-Cultures (SFC)* was developed to facilitate data collection of cultivated soil micro fungi. *App Inventor* was the program used to design, program, test, and publish the application developed. The developed application was compared with other applications used in identifying bacteria cultures. The results showed that the new application needed more time to capture the records because it saves more information, the navigation flow was acceptable, the number of clicks was high, but it is due to the usefulness in data capture, and finally, the users rated it as a good application with an eight out of ten rating.

Pyrosequencing resulted in 204 Operational Taxonomic Units (OTUs) considering the two study areas (the Taunus Mountain range and the Majagua valley). The Pyrosequencing database was used to contribute to the most important study of fungal diversity globally based on OTUs, which surpasses any study of molecular and taxonomic diversity previously conducted. The principal result in this study was that the climatic factor is the best predictor of fungal richness and community composition on a global scale. However, the part of the research that focused on the local scale, that is to say, on the correlation patterns between the distribution of fungal species and abiotic factors, showed that the soil properties and degradation levels were not associated with fungal richness, diversity or soil composition in the study areas in Germany or Panama. The above confirms that there are exceptions to the way relationships between soil factors with fungal diversity are established at the local level.

In the case of soil samples used for morphological identification, 71 fungal species were obtained, 47 from Germany, and 32 from Panama. It is worth highlighting that 10 of the total species found in Panama are new reports for this country: five species belonging to the Eurotiales order (*Aspergillus roseoglobulosus, A. tamarii, Penicillium janczewskii, P. miczynskii, Talaromyces verruculosus*), four species of Hypocreales order (*Mariannaea elegans, Purpureocillium lilacinum, Trichoderma harzianum, T. spirale*), and one species of Mucorales order (*Mucor moelleri*).

In Panama, soil fungal diversity was composed of four orders, dominated by Eurotiales with 53 % and Hypocreales with 38 % of total isolates. In comparison, eight orders formed the fungal diversity in Germany, where Eurotiales with 38 % and Hypocreales with 22 % of total isolates were the most abundant orders. An analysis of diversity was performed. Shannon diversity index showed values of 3.36 and 3.15 in Germany and Panama, respectively. However, in the correlation between diversity and soil factors, our results do not show evident effects of soil degradation on communities of soil fungi from Germany and Panama.

An important aspect found in this work was that the use of different methodologies to identify the soil fungi species resulted in the observation that not all methods identify the same fungi. For this reason, the methods are not mutually exclusive but complementary.

Finally, while the contribution of this research was small on the global level and Germany contributed slightly to expand knowledge about soil fungi, it was specifically relevant in Panama, where this study was a pioneer.

2 Zusammenfassung

Das Hauptziel dieser Arbeit ist es, zum Wissen über Bodenmikropilze in Panama und Deutschland beizutragen. Bisher wurden in diesem Bereich noch keine umfassenden Studien zur Bodendegradation und deren Einfluss auf die Bodenpilzvielfalt erstellt. Hierbei handelt es sich um ein komplexes Forschungsfeld, da es eine immense phänotypische und genetische Vielfalt in der Bodenpilzgemeinschaft gibt und diese Gemeinschaft mit Faktoren der Bodendegradation in Beziehung steht. Aus diesem Grund untersucht diese Arbeit die (in den Untersuchungsgebieten) im Taunus (Hessen) und im Majagual in Chiriquí Panama identifizierten Bodenpilzarten in Bezug auf Umweltfaktoren. Zwei komplementäre Ziele wurden erreicht: Das erste war die Entwicklung eines theoretischen Bewässerungsmodells für degradierte Böden. Das zweite war die Entwicklung einer Applikation für mobile Endgeräte zur Erleichterung der Laborarbeit bei der Kultivierung von Mikropilzen im Boden.

Der Aufbau der Methodik basierte auf der Identifizierung der Arten und der Zuordnung der gefundenen Vielfalt zu Bodenfaktoren. Bodenproben wurden in beiden Ländern entnommen, achtmal im Taunus im Zeitraum von Januar bis November 2012 und im Majagual zweimal im Februar und einmal im Juli 2012. In beiden Studien wurden Gebiete mit drei verschiedenen Vegetationstypen (Wald Grünland und kahler Boden) untersucht. Die Proben wurden in Bezug auf zwei Faktoren analysiert: Die Bewertung der Pilzvielfalt durch molekulare und morphologische Methoden sowie die Charakterisierung des Bodens.

Bodenproben, die in der Methodik der Pyrosequenzierung verwendet wurden, wurden mit globalen Klimafaktoren in Beziehung gesetzt. Die morphologische

Identifizierung erfolgte mit Identifikationsschlüsseln, Mikropilze wurden in verschiedenen Medien kultiviert, bis Reinkulturen erhalten wurden. Zur molekularen Identifizierung dienten die mit den Primern ITS1 und ITS4 amplifizierten DNA-Sequenzen. Diese Sequenzen wurden durch den BLAST-Algorithmus mit Referenzsequenzen von GenBank verglichen, wobei eine Übereinstimmung von 98 % oder mehr gewährleistet sein muss, um als zuverlässige Identifikation betrachtet werden zu können. Die Bodencharakterisierung wurde durchgeführt, um die physikalisch-chemischen Eigen-schaften des Bodens zu messen, wobei die abiotischen Faktoren Verdichtung, Temperatur, pH-Wert, Feuchtigkeit und Zusammensetzung des Bodens waren.

Der Artenreichtum wurde im jeweiligen Untersuchungsgebiet mit den Schätzmethoden Chao, Jackknife und Bootstrap berechnet. Außerdem wurden Artenakkumulationskurven erstellt, um die Entdeckungsrate der Arten festzustellen und die Vollständigkeit der Proben abzuschätzen. Die geschätzten linearen Regressionsmodelle stellten eine Korrelation zwischen dem Einfluss der Bodenfaktoren (Temperatur, Feuchtigkeit, pH-Wert, Bodenverdichtung und Boden-zusammensetzung) und dem Artenreichtum dar. Auf die gleiche Weise wurde eine Analyse der ökologischen Distanz durchgeführt, die auf der Ähnlichkeit der Artzusammensetzung basiert. Dies diente dem Vergleich der Proben und der Korrelation mit Bodenfaktoren. Hierbei sei angemerkt, dass eine nichtmetrische mehrdimensionale Skalierung (NMD) verwendet wurde.

Die Untersuchung der Abundanz zeigte Unterschiede zwischen der Abundanz auf nackten Böden und der Abundanz in Wäldern in Deutschland und Panama; das Grasland in beiden Ländern fungiert als Übergangsbereich für die

Pilzabundanz. Die wichtigsten Schlüsselarten in Deutschland waren *Penicillium daleae* und *Pochonia bulbillosa*, in Panama *Purpureocillium lilacinum* und *Trichoderma harzianum*. Auf der Grundlage der Pareto-Analyse wurde ein theoretisches Bewässerungsmodell entwickelt, um den degradierenden Auswirkungen auf die Häufigkeit von Mikropilzen im Boden entgegenzuwirken.

Es wurde nach Anwendungen für mobile Endgeräte gesucht, die sich mit der Kultivierung von Bodenmikropilzen befassen. Aufgrund der geringen Anzahl bestehender Applikationen wurde eine neue App namens *Soil-Fungi-Cultures (SFC)* entwickelt, um die Datenerfassung von kultivierten Bodenmikropilzen zu erleichtern. *App Inventor* war das Programm, das zum Entwerfen, Programmieren, Testen und Veröffentlichen der entwickelten Anwendung verwendet wurde. Die entwickelte Applikation wurde mit einer anderen, für Bakterienkulturen verwendeten Anwendung verglichen. Die Ergebnisse zeigten, dass die Applikation *SFC* mehr Zeit für die Erfassung der Datensätze benötigte, weil sie mehr Informationen speichert. Ferner wurde festgestellt, dass der Navigationsfluss gut und die Anzahl der Klicks hoch war, was aber auf die Nützlichkeit bei der Datenerfassung zurückzuführen ist. Aufgrund dessen bewerteten die Nutzer die Anwendung mit acht von zehn Punkten als gut.

Bodenproben, die mit der Methodik der Pyrosequenzierung untersucht wurden, ergaben 204 taxonomische Betriebseinheiten (OTUs) unter Berücksichtigung der beiden Untersuchungsgebiete (Taunus und Majagual). Die Pyrosequenzierungs-datenbank wurde verwendet, um einen Beitrag zur weltweit wichtigsten Untersuchung der Pilzdiversität auf der Grundlage von OTUs zu leisten, die alle zuvor durchgeführten Untersuchungen zur molekularen und taxonomischen Diversität übertrifft. Das Hauptergebnis dieser Studie war, dass global betrachtet

der Klimafaktor am besten geeignet ist, um den Pilzreichtum und die Zusammensetzung der Pilzgemeinschaft vorherzusagen. Der auf die lokale Ebene konzentrierte Forschungsteil in den Studiengebieten in Deutschland oder Panama in Bezug auf die Korrelationsmuster zwischen der Verbreitung von Pilzarten und abiotischen Faktoren, zeigte jedoch, dass die Bodeneigenschaften und der Zersetzungsgrad nicht mit Pilzreichtum, -vielfalt oder -zusammensetzung in Böden zusammenhing. Dies bestätigt, dass es Ausnahmen gibt bei der Bestimmung der Beziehungen zwischen Bodenfaktoren und Pilzvielfalt auf lokaler Ebene.

Die morphologische Identifizierung der Bodenproben ergab 71 Pilzarten, davon 47 aus Deutschland und 32 aus Panama. Von den in Panama identifizierten Arten wurden 10 davon zum ersten Mal registriert. Fünf Arten gehören zur Ordnung Eurotiales (*Aspergillus roseoglobulosus, A. tamarii, Penicillium janczewskii, P. miczynskii, Talaromyces verruculosus*), vier Arten zur Ordnung Hypocreales (*Mariannaea elegans, Purpureocillium lilacinum, Trichoderma harzianum* und *T. spirale*) und eine Art zur Ordnung Mucorales (*Mucor moelleri*).

Die Vielfalt der Bodenpilze setzte sich bei den Proben aus Panama aus vier Ordnungen zusammen, die von Eurotiales mit 53 % und Hypocreales mit 38 % der Gesamtisolate dominiert wurden. Im Gegensatz dazu gehörten die Proben aus Deutschland zu acht Ordnungen, wobei die meisten den Eurotiales mit 38 % und den Hypocreales mit 22 % der Gesamtisolate zugeordnet werden konnten. Die Analyse der Diversität mit Hilfe des Shannon Diversity Index lieferte in Deutschland Werte von 3,36 und in Panama Werte von 3,15. In der Korrelation zwischen Diversität und Bodenfaktoren zeigen unsere Ergebnisse jedoch keine

offensichtlichen Auswirkungen der Bodendegradation auf Gemeinschaften von Bodenpilzen aus Deutschland und Panama.

Ein wichtiger Aspekt dieser Arbeit war, dass die Verwendung unterschiedlicher Methoden zur Identifizierung der Bodenpilzarten zu der Beobachtung führte, dass nicht alle Methoden dieselben Pilze identifizieren; die Methoden schließen sich aber nicht gegenseitig aus, sondern ergänzen sich.

Schließlich trug diese Studie zur Pilzvielfalt auf globaler Ebene bei. In Deutschland erweiterte sie das Wissen über Bodenpilze, war jedoch speziell in Panama relevant, da diese Studie ein Pionier war.

3 Introduction

Preface

The fungal community is an important constituent of the soil ecosystem, playing central roles in biotic and abiotic interactions, cycling processes, and decomposition (Pompéia *et al.* 2013). The immense phenotypic and genetic diversity of soil fungal communities makes them one of the most difficult communities to study (Kirk *et al.* 2004). Soil fungi diversity is modified by environmental conditions. In particular, the soil degradation process modifies the biological, chemical, and physical status of the soil with consequences in the composition of fungal communities (Ferrol *et al.* 2004, Requena *et al.* 2001). Soil degradation is an important factor around the world; however, the effects of degradation on soil fungi diversity have not been investigated as extensively as those on vascular plants and vertebrates. The majority of human activities result in soil degradation, which affects the services provided by soil biodiversity (Turbé *et al.* 2010). Population fluctuations of fungi increase over short and long term to a different extent depending on degradation intensity (Eickhorst *et al.* 2010). All changes in the soil system affect the fungal community. Based on the aforementioned, the present study is focused on the characterization of fungal diversity in soils in Germany and Panama with different degrees of degradation, i.e., in the Taunus mountain range in Germany as well as in the Majagua Valley in Panama. The soils in the studied areas were characterized concerning their level of degradation based on pH, compactation, soil composition, and their fungal species content. Correlation patterns between the distribution of fungal species and abiotic factors were also studied. Finally, a discussion on how the levels of soil degradation might influence fungal diversity is provided.

3.1 Soil

Soil ecosystems present complex relationships between abiotic and biotic factors. Soil is defined as the loose top layer of the earth's surface, consisting of rock and mineral particles mixed with decayed organic matter, capable of retaining water, providing nutrients for plants, and supporting a wide range of biotic communities (Bot and Benites 2005). Soils act as a reservoir and source for many biochemical components, like silicates, carbonates, sulfates, chlorides, and nitrates, that can be beneficial or damaging for life, depending on the concentrations (Lal 2013).

Soil genesis begins by a slow process involving physical disintegration from the weathering of bedrock and chemical decomposition from dead organisms in the earth's solid crust (Buol *et al*. 2011). Temperature changes dissociate bedrock, and erosive forces of water, wind and living organisms produce physical stress that breaks the rock. This material is at a rate of a few millimeters per millennium (Hall and Andre 2001). Concurrently the chemical process of weathering sets upon newly exposed rock surfaces where fungi play an essential role in the production of humus. Thereby, organic matter is added to the soil, participating in regulating carbon fluxes between the biosphere and lithosphere, making it able to support life (Zhu and Miller 2003). As soil formation continues, horizontal zones differentiate, separate, and diversify.

Soils are composed of various minerals, organic matter, water, air, and soil organisms, including bacteria and fungi (Coleman *et al.* 2004). Soil biota participates directly or indirectly in soil aggregation and leads to soil structure (Siddikya *et al*. 2012). The soil structure is essential for different processes like nutrient cycling, carbon storage, water and gas exchange, and resistance to

erosion (Coleman *et al.* 2004). Soil aggregates are clumps of soil particles that are held together by organic material and moist clay. Inside organic material, a fungal community influences soil aggregation at various scales (micro and macro) by different mechanisms like humidity retention and nutrient disposition (Hunt and Gilkes 1992). Soil depends on different physical factors like topography, climate, parent material, organisms present, and time. Most soils present layers called horizons. The first layer is the topsoil, or A horizon, the most fertile horizon because it contains organic material. The next layer is the B horizon or subsoil, characterized by an accumulation of soluble or suspended organic material, clay, iron, or aluminum. Usually, this layer is less fertile than the A horizon because the water has leached the finer particles down into the profile. The next layer is the parent material or horizon C (Hunt and Gilkes 1992). Most biological processes occur in the topsoil, where organisms add nutrients to the soil, improving soil structure and helping to stabilize the soil against erosion.

There are different kinds of soil because its constitution changes according to the region with different biological, physical, and chemical attributes (Lal 2013). Soil classification is based on the specific proprieties of soils, like composition in every horizon (amount of mineral and organic matter), physical and chemical characteristics, texture, color, and level of degradation by human activity (IUSS Grupo de Trabajo WRB 2007). Degradation can be vertical and sometimes horizontal (Brandy and Weil 2007). Soil degradation processes in forests are mostly due to a temporary or permanent deterioration in the density or structure of vegetation cover (Mithal *et al.* 2011), which produces higher temperatures that affect the structure of fungal assemblages fungal biomass, and reproduction (Geraldes *et al.* 2012). With about 625 million hectares of the earth's surface,

tropical rain forests cover a relatively small area (Achard *et al.* 2002). The tropical forest can be found in 85 countries worldwide, including Panama, which has over 30 % of its original old-growth forests still intact, with soils necessary for water storage, carbon balance, and plant survival (Stephen *et al.* 1996, Griscom and Ashton 2011). The turnover of organic matter in soils is faster in tropical than in temperate soils because of heavy rainfalls and high temperatures that produce higher moisture and faster decomposition (Six *et al.* 2002). Soils of tropical forests are regulators, transformers, buffers, and water and nutrient filtration systems and provide nutrient cycling services to the biosphere by their microorganisms and soil fauna (Wall *et al.* 2012). However, rainforests are very fragile habitats because, in many tropical regions, extensive areas are degraded or lost by human activity, with dramatic consequences for biodiversity (Morris 2010). Deforestation, climate change, invasive species, and land-use change are the principal factors for biodiversity loss in tropical forests. When used for agriculture, soils of tropical forests often have a poor physical structure, low pH, high salt concentrations, and low phosphorus values, resulting in degraded soils being poor in nutrients (Griscom and Ashton 2011).

Temperate forests grow between the subtropics and the polar region. They present a wide range of temperatures, which are correlated with distinctive seasons. In these forests, humidity and precipitation values can be high, with the highest biomass levels and many different soil types found in temperate regions (Six *et al.* 2002). Most mycological studies in temperate forests focused on ectomycorrhizal fungi and root-associated communities. However, communities of soil fungi involve more species, which are related to changes in the soil environment (Wubet *et al.* 2012). Fungi are essential in temperate forests, specifically for the long-term

stabilization of organic matter. However, this function can be influenced or limited by the degradation of the soil. Both temperate and tropical forests need soil fungi to ensure concentrations of organic matter, which is necessary for the ecosystem for the physical, chemical, and biological functioning (Six *et al.* 2002).

The process of soil degradation is a significant environmental problem all around the world. By degradation, the soil deteriorates because of human activity and loses its nutrients together with its organic matter (Eswaran *et al.* 2001). Understanding the degradation processes is critical for the development of management recommendations for ecosystems (Ashton *et al.* 2001). Most human activities result in soil degradation, which affects the services provided by soil biodiversity (Turbé *et al.* 2010), including fungi that depend on organic matter (Bouckaert *et al.* 2013, Rohilla and Salar 2012). Organic matter in soil is composed of about 85 percent of dead matter, i.e., remains of soil animals, bacteria, fungi, other microorganisms, roots (with about 10 % of them living), and other underground organs of plants (Wall *et al.* 2012). Roots interact with fungi in the rhizosphere, with fungi mostly saprotrophic. The organic matter content, the diversity, and activity of soil organisms are influenced by soil structure, moisture-holding capacity, and nutrient availability. Fresh organic matter stimulates the activity of fungi because they can degrade this material. Fungi are heterotrophic and obtain energy from the degradation of organic matter, so the amount of organic matter influences the abundance and diversity of fungi. It is estimated that there are between 5,000 to one million fungi per gram of soil, with the amount being closely related to abiotic and biotic factors (Anthony and Franzluebbers 2003).

3.2 Diversity of soil fungi

Soil fungi diversity can be studied at different scales: global, macro and micro-ecological. On the global scale, the climatic factors latitude and biogeographical patterns play an important role in predicting fungal diversity. However, it has not been tested until the study of global diversity and geography of soil fungi (Tedersoo *et al.* 2014). It found that these factors can be used to predict fungal diversity with some exceptions to this model. At the local level (micro-scale), it is assumed that the diversity of soil fungi is influenced by different factors, as occurs with bacterial diversity. Research the fungal diversity is necessary to consider different aspects and previous works. The literature is mentioned that different factors influence the diversity of soil fungi. First, fungal species must be able to disperse to an environment withstanding environmental stresses (Lavorel and Grigulis 2012). After that, those species must survive to different abiotic factors in the environment. Finally, the species that compound the community must successfully compete between species for resources (Craine *et al.* 2009, Suding *et al.* 2005). Based on the above mentioned, to study the fungal diversity of the soil at a global level, this work contributed with two local studies from two countries, Panama and Germany. In the case of Panama, these studies are scarce, and the information is not abundant. However, some studies have been done (Farrow 1954, Guzman and Piepenbring 2011 Mangan *et al.* 2004, Piepenbring 2000, 2006, 2007)). A global scale, it is the first time that Panama was included in a study of global fungi diversity with biogeographic factors (Tedersoo *et al.* 2014). In the case of Germany, the second study area, the knowledge on soil fungi is in development, although initiatives in biodiversity exploratory focused on soil fungal diversity granted by the German Science

Foundation (DFG) and numerous studies based on isolation of soil fungi, molecular analyses including DNA-extraction and PCR/DGGE (denaturing gradient gel electrophoresis) analyses (Eickhorst *et al.* 2010). The studies that include morphological and molecular data in correlation with soil environmental conditions are not extensive (Christ *et al.* 2010). The reason is that there are problems associated with studying fungal diversity in soil, i.e., it is difficult to isolate all the fungi from a particular soil representative of one area, and due to a lack of taxonomic knowledge, it is difficult to identify all the species (Kirk *et al.* 2004). However, our knowledge and understanding of soil fungi diversity have changed in the last decades due to advances in molecular technology (Christ *et al.* 2010).

3.3 Systematic groups of soil fungi

Research in Panama showed a great diversity of soil fungi, which includes new records for listings of species in Panama (Rosas *et al.* 2019). In this context, to illustrate the systematic groups of soil fungi found in Panama, below is the list of identified orders with their respective characteristics.

3.3.1 Ascomycota

Ascomycota is the largest phylum of the kingdom Fungi, with over 64 000 known species (Bennett and Turgeon 2016). Species classified in this phylum are characterized by a sac-like structure, the ascus, which contains spores. Many species of ascomycetes are frequently observed in their asexual stage (Kirk *et al.* 2008).

Species of ascomycetes are nowadays identified and classified based on morphological or physiological similarities and molecular analyses of DNA sequences (Ravichandra 2013).

Ascomycota orders

3.3.1.1 Capnodiales

The Capnodiales include pathogens of animals (including humans) and plants (endophytes and epiphytes) (Crous *et al.* 2009). Many species of Capnodiales are saprobes, which can recycle organic components in the ecosystem. Their habitats are tropical and sub-tropical regions as well as the temperate regions of Europe and North America (Bose 2013). Capnodiales include the families Antennulariellaceae, Capnodiaceae, Davidiellaceae, Dissoconiaceae, Metacapnodiaceae, Mycosphaerellaceae, Piedraiaceae, Schizothyriaceae, and Teratosphaeriaceae (Lumbsch and Huhndorf 2010). Species of Capnodiaceae are sooty molds; they are classified as saprotrophic fungi. Their main ecological niche is the surface of plants, and they usually grow in association with other saprotrophic fungi (Bose 2013). Species of Capnodiales colonize the aerial system of plants. However, it is also possible to find them in the soil when the leaf has fallen and start the decomposition (Chomnunti *et al.* 2011). The principal characteristic of species in this order are darkly pigmented cells in the superficial, often well-developed mycelium, with many variations in form, present erect branches in some cases, sometimes with mucous coating. Usually, the asci are small, ovoid or saccate, fissitunicate. Ascopores are hyaline to brown, septate, sometimes muriform, rarely ornamented, and lack a sheath (Kirk *et al.* 2008).

3.3.1.2 Eurotiales

Species of Eurotiales are mostly saprotrophic, they are widespread and abundant fungi, and they can live on fruits forming moulds (Mayamor and Poeaim 2014). Species of Eurotiales are filamentous fungi producing specific asexual spores, which are essential for the taxonomical classification; some species have specific structures for reproduction or protection (Guarro *et al.* 1999). Many species of this order are important for humans because they produce drugs or other chemicals, invade food, and are used to ferment substances; other species can be pathogens of humans, plants, and animals (Deshmukh and Rai 2005). Species of Eurotiales are distributed in different environments, it is possible to find them in air, fresh water, and they are common soil organisms, some of them with nearly cosmopolitan distribution (Henkel *et al.* 2006). The Eurotiales include four families: Aspergillaceae, Elaphomycetaceae, Thermoascaceae, and Trichocomaceae, with 49 genera and approximately 928 species (Kirk *et al.* 2008). The best known genera are Penicillium and Aspergillus. These genera include hundreds of species that are important by the production of antibiotics as well as mycotoxins. Species of Penicillium and Aspergillus develop conidia in basipetal chains, with conidiogenous cells called phialides, and the cells bearing the phialides termed metulae (Carlile *et al.* 2001). Aspergillus spp. has numerous chains of conidia arising from a vesicle Penicillium spp. have branching conidiophores and chains of conidia, carried by phialides, that are supported by metulae which arise from ramuli, that are carried by the rami and finally the stipe of the conidiophore. Taxonomically, the Eurotiales are a complicated order because the species in this order are distinguished by subtle and frequently

variable differences in the details of asexual sporulation, and more studies are necessary to classify all the species (Frisvad and Samson 2004).

3.3.1.3 Hypocreales

Species of Hypocreales are important fungi, especially in their asexual forms. They include fungi of economic importance ranging from virulent plant pathogens to effective biological control agents, other produce antibiotics or mycotoxins (Chaverri *et al.* 2002). Species of this order influence agriculture and other human activities; for this reason, it is crucial to study the systematics of the Hypocreales, particularly the generic concepts (Rossman 1996). Species of Hypocreales are saprobes or parasites of plants, often fungicolous or lichenicolous, rarely coprophilous (Kirk *et al.* 2008). As a taxonomic unit, the Hypocreales might seem to be relatively small. However, a literature review shows that this order is an order of fungi consisting of seven families that include Bionectriaceae, Clavicipitaceae, Hypocreaceae, Nectriaceae, and Ophiocordycipitaceae, with 237 genera and 2647 species (Rogerson 1970, Kirk *et al.* 2008). Species of Hypocreales are easily recognized in their sexual form by their brightly colored fructifications (yellow, orange, or red), and many asexual forms produce elongated conidiophores and green conidia. Asci cylindrical, thin-walled, ascospores are usually septate, conidia occasionally muriform, sometimes elongated and fragmented (Kirk *et al.* 2008). Hypocreales are common in all types of moist forests, and many species have a worldwide distribution (Chaverri *et al*. 2003). In the last twenty years, progress toward understanding the systematics of the Hypocreales has been made primarily through descriptive accounts of

species in combination with the identification by molecular analysis. However, researchers still have a long way to exhaustively understand the Hypocreales (Rossman 1996, Quandt *et al.* 2014).

3.3.1.4 Onygenales

The Onygenales are an assemblage of fungi commonly found on hooves, hair, feathers, skin, and horn, i.e., substrates composed of keratin, form part of the integument of birds, reptiles, and mammals (Doveri *et al.* 2012). Species of Onygenales can digest keratin, which is a complex protein that most fungi cannot digest. For this reason, they become dominant organisms in environments where keratin is available (Sharma *et al.* 2013). Keratin is an abundant substrate in soils due to regularly shed integument by a large number of vertebrates. Species of this order can also be capable of causing human diseases, ranging from superficial skin infections to fatal invasions of internal organs (Sharma *et al.* 2013, Doveri *et al.* 2012). Species of Onygenales are cosmopolitan, they are distributed worldwide, and their habitat is wherever there is keratin (Kirk *et al.* 2008). The Onygenales include four families: Ajellomycetaceae, Arthrodermataceae, Gymnoascaceae, and Onygenaceae, 52 genera and 271 species. Species of this order are characterized by the branches of an often tree-like conidiophore disarticulating into thallic-arthric conidia (Currah 1985, Kirk *et al*. 2008). Such thallic ontogeny is a decisive factor for distinguishing species of this order from species of Eurotiales (Doveri *et al.* 2012). The identification by morphological and molecular characteristics in Onygenales is difficult (Scott *et al.* 1993).

3.3.1.5 Pleosporales

Species of Pleosporales play an essential role in agricultural soil owing to their participation in the assimilation of nitrate and the degradation of plant litter (Gorfer *et al.* 2011, Ortiz-Bermúdez *et al.* 2007). Members of Pleosporales have saprobic, coprophilous, or parasitic lifestyles on dead leaves or stems in terrestrial or aquatic environments, and they also can be endophytes or epiphytes (Zhang *et al.* 2012). Sexual forms of Pleosporales are recognized by prominent pseudoparaphyses and bitunicate asci (Zhang *et al*. 2009). Species of Pleosporales are widely distributed in diverse environments on woody, herbaceous debris and the overwintered stems of non-woody plants (Ortiz-Bermúdez *et al.* 2007). Many species of this order are parasites of herbaceous plants showing some degree of host-specificity, with some species growing on dicots. In contrast, others are found only on monocots, including grasses and sedges. Species of other genera are almost always found on the dung of herbivorous animals, and some can survive in this habitat for a long time while others last only a few days (Zhang *et al.* 2009). The Pleosporales are the most significant order in the class Dothideomycetes, with 23 families, 332 genera, and more than 4700 species (Kirk *et al.* 2008). Species of Pleosporales are morphologically diverse, but in their sexual stage, they are characterized by ascospores that are hyaline to heavily pigmented, mostly brown, multi-septate, with germ slits, and thin or thick-walled, sometimes muriform, often with a gelatinous sheath (Zhang *et al.* 2012).

3.3.1.6 Xylariales

Species of Xylariales are mostly endophytes and develop sporogenous structures on the surface of deadwood. Isolates of Xylariales are used for their capability to produce antibiotics and oligosaccharides from various natural polysaccharide sources. Endophytic species in the grass have been used in agriculture and industry (Tomita 2003, Davis *et al*. 2003). The Xylariales contain six accepted families (Amphisphaeriaceae, Clypeosphaeriaceae, Diatrypaceae, Graphostromataceae, Hyponectriaceae, and Xylariaceae) as well as members of the Apiosporaceae, and over 92 genera and 795 species (Smith *et al.* 2006). The distribution of the order Xylariales is cosmopolitan; its most remarkable diversity is found in the tropical and subtropical regions (Sir *et al.* 2012). Species of the order Xylariales are characterized by the production of well-developed stromata containing perithecia, eight-spored unitunicate asci with apical apparatus, and mostly pigmented, simples ascospores. In the asexual state, species of Xylariales are usually hyphomycetous, with holoblastic conidial production (Whalley 1996).

3.3.2 Basidiomycota

Species of Basidiomycota are unicellular or multicellular, sexual or asexual, and they find in almost all terrestrial ecosystems, also in marine and freshwater habitats (Kohlmeyer and Kohlmeyer 1979, Hibbett and Thorn 2001). This phylum contains about 30 000 described species, which is 37 % of the described species of true Fungi (Kirk *et al.* 2001) and includes yeasts (Fell *et al.* 2001). Many species of Basidiomycota obtain nutrition by degrading dead organic matter, including

wood and leaf litter; for this reason, Basidiomycota species play a significant role in the carbon cycle in the soil (Thorn *et al.* 1996).

Basidiomycota orders

3.3.2.1 Tremellales

Species of Tremellales have considerable industrial and medical importance because they are ubiquitous in the environment and serve as model organisms for fungal pathogenesis; some are opportunistic human pathogens of global importance (Rodríguez *et al.* 2010). Species of Tremellales are dominant in wet soils covered by vegetation (trees, forbs, and grass), with relatively low pH. In these soils, species of this order can be distinguished from other fungi by their degradation of many C compounds, lignin products, lack nitrate, raffinose, and some species can degrade urea (Satyanarayana and Kunze 2009). Species of the Tremellales are characterized by the production of arthroconidia and ballistoconidia with adjoining walls that are usually oblique but vary from transverse to longitudinal (Satyanarayana and Kunze 2009). The dolipore and saccate parenthesome complex in the dikaryotic hyphae is typical for Tremellales (Boekhout *et al.* 1991, Wells 1994). The Tremellales consist of nine families: Carcinomycetaceae, Cuniculitremaceae, Hyaloriaceae, Phragmoxenidiaceae, Rhynchogastremataceae, Sirobasidiaceae, Tetragoniomycetaceae, Tremellaceae, and Trichosporonaceae, including 38 genera and 341 species (Kirk *et al.* 2008). In the past, the classification of the Tremellales was difficult (Bandoni 1963). However, currently, studies indicate that it is possible to complement morphological identification with molecular identification using the ITS1, ITS2,

and the 5.8 rDNA regions. More specifically, for the order Tremellales, the D1-D2 region can complement the molecular identification (Fell *et al.* 2000).

3.3.2.2 Sporidiobolales

Species of Sporidiobolales can be found in soil of dense forests with a low anthropogenic influence (Libkind *et al.* 2003). Species of this order are mostly associated mainly with the phylloplane of terrestrial plants. They are also present in aquatic environments, including marine systems. Some species of red yeasts are opportunistic human pathogens (Libkind *et al.* 2005). The concept of the Sporidiobolales is consistent between morphological and phylogenetic analyses (Sampaio *et al.* 2003). The classification of species is sometimes difficult in the case of parasitic fungi because they are commonly described based on infection symptoms on their hosts and the morphology of the probasidia even though there is variance in teliospore germination patterns. Furthermore, many species do not germinate on artificial media (Yurkov *et al.* 2012). Many asexual stages of species of Sporidiobolales develop pink colored cultures, asexual stages proliferating by budding and sometimes by ballistoconidia which are hyaline, thin-walled, and passively discharged (Sampaio *et al*. 2003). The Sporidiobolales include one family, Sporidiobolaceae with seven genera and 83 species. This order is cosmopolitan, some species are psychrophiles occurring at high latitudes and altitudes (Cannon and Kirk 2007).

3.3.3 Zygomycota

The Zygomycota is a heterogenous assemblage of Fungi characterized by zygomycete hyphae that can be coenocytic because they lack cross walls or septa. Sporangiospores have a walled with internal cleavage of the sporangial cytoplasm; sporangia are formed at the ends of specialized hyphae called sporangiophores (Domsch *et al.* 2007). Phylogenetic studies consider that Zygomycota can be para- or polyphyletic (Bruns *et al.* 1992; Tanabe *et al.* 2000, 2004). Zygomycota species are common in terrestrial and aquatic ecosystems; however, Zygomycota in the soil ecosystem takes part in the carbon cycle as decomposers of organic matter (Hajek 1999).

Zygomycota orders

3.3.3.1 Mortierellales

The Mortierellales are one of the most oversized basal fungal orders, including one family, six genera, and 93 described species (Kirk *et al.* 2008). The possibility to find new species in this order is high, i.e., recently, one new species was described from the infection of an animal (Wagner *et al.* 2013). There also is one potential additional genus that was described recently (Hoffmann *et al.* 2011). It is important because many species of this order can be used in biotechnological applications. Many species of Mortierellales can convert organic compounds; in fact, some species can produce polyunsaturated fatty acids; for this reason, they are interesting organisms for biotransformations (Wagner *et al.* 2013). Species of Mortierellales are widespread in the temperate zone, where they are almost cosmopolitan. It is common to find species of this order in soil on decaying organic

matter (Nagy *et al.* 2011). Mortierellales are characterized by white to light-grey colored colonies, with coenocytic mycelium that is septate in old cultures. Asexual spores are produced in sporangia and are passively released. The sporangiophores can be widened at the base and variously branched (Hoffmann *et al.* 2011). The morphological identification is based on asexual features, leading to the traditional classification. Nonetheless, there is still an unknown percentage of undescribed species in the order Mortierellales, which might be solved with the help of phylogenetic analyses (Nagy *et al.* 2011).

3.3.3.2 Mucorales

Species of Mucorales are known as bioindicators in soil, showing correlations with the physical and chemical properties of the soil, like soil moisture, soil temperature, levels of lead, K, N Mg, and pH. These aspects of the soil change due to destruction of the vegetation cover, the introduction of harmful materials to the soil environment, and differences in cover vegetation by seasonal changes, generally causing a decrease of species diversity in Mucorales populations (Sheila 1996). Species of Mucorales have biodegradation and fermentation properties, and several beneficial species are used in the production of diverse organic acids, traditional East Asian soybean-based foods, vitamins, and foodstuffs (O´Donell *et al.* 2001). Some species of Mucorales are responsible for opportunistic infections in humans and other animals (Machouart *et al.* 2006). Members of the Mucorales are ubiquitous, being found in food, decaying vegetation, organic debris, dung, air, and soil (Morace and Borghi 2012). Mucorales are among the most common microorganisms in soil because they are

among the fastest growing fungi and the first to colonize and sporulate on diverse terrestrial substrates rich in simple carbohydrates (O´Donell *et al.* 2001). Species of Mucorales are fungi that belong to the Zygomycetes, distinguished from the Ascomycota and Basidiomycota by their coenocytic hyphae and their sexual reproduction by zygospores (Machouart *et al.* 2006). The Mucorales contains 13 families, 54 genera, and 63 species (O´Donnell *et al.* 2001), and they are characterized by aseptate (coenocytic) hyaline hyphae, sexual reproduction with the formation of zygospores, and asexual reproduction with nonmotile sporangiospores (Morace and Borghi 2012). The identification of species of Mucorales is difficult because a discrepancy in the determination of genera between morphological and sequence-based methods has been found. Based on the above, both approaches are needed (Machouart *et al.* 2006).

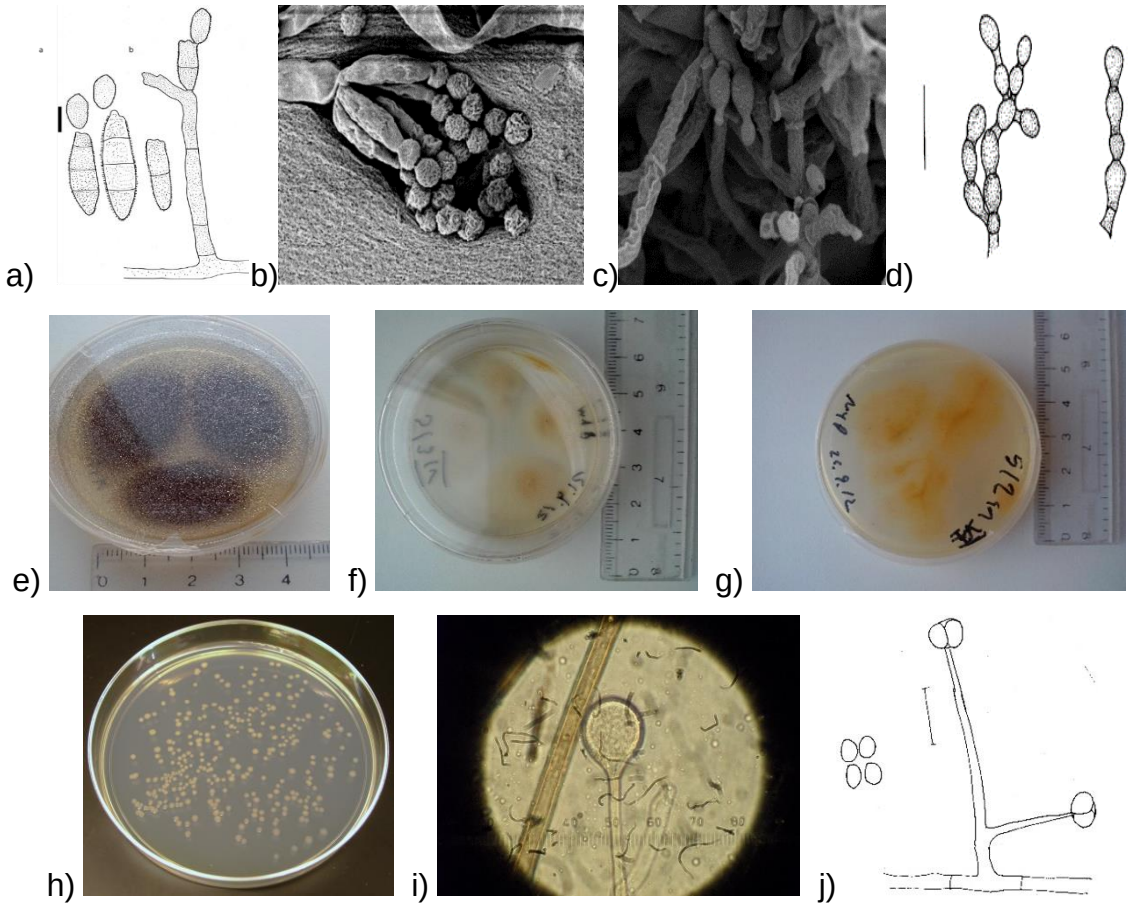


Fig. 1. Orders. Conidia and ramoconidia drawing of *Cladosporium ossifragi*, Order Capnodiales. a. Phialides with conidia belonging to *Penicillium verruculosum*, Order Eurotiales. b. Phialides of *Trichoderma koningii*, Order Hypocreales. c. Drawing of pyriform conidia belonging to *Chrysosporium merdarium*, Order Onygenales. d. Culture belonging to *Alternaria alternata*, Order Pleosporales. e. Culture belonging to *Pestalotiopsis* sp., Order Xylariales. f. Colonies of *Cryptococcus terreus*, Order Tremellales. g. Culture belonging to *Rhodotorula mucilaginosa*, Order Sporidiobolales. h. Sporangiophore with sporangium of *Zygorhynchus moelleri*, Order Mucorales. i. Sporangiophores with sporangia belonging to *Mortierella verticilata.* Order Mortierellales. j.

3.4 Molecular biology for the analysis of fungal communities

The analysis of soil fungal diversity has been historically based on characteristics of organisms, using their morphology, biochemistry, and physiology (Martínez *et al.* 2004). Nowadays, information obtained by nucleotide sequences of DNA and RNA molecules is also used to determine and classify organisms. Actually, DNA sequence information is playing an important role in mycology, i.e. by the recent notion of fungal barcoding (Raja *et al.* 2017). The concept of barcoding proposes one option to explore biological diversity, the classification by analysis of one or several segments of sequences from the same section of every genome, which can be read and annotated. These standard segments can be used as an unique identifier for “barcoding”, a molecular and bioinformatics tool to identify biological species (Hebert *et al.* 2003). In the past, cytochrome c oxidase subunit 1 (CO1), a marker from a mitochondrial gene was used for barcoding (Casiraghi *et al.* 2009). However, in mycology actually, more markers are used, the most popular locus for species identification being the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. This marker is used for subgeneric phylogenetic inference in sequence-based mycological research (Nilsson *et al.* 2008).

The ITS helps to identify fungal species by showing differences between species. This DNA can be extracted from any part or fragment of the organism and in any stage of its cycle of life, asexual or sexual (Gilmore *et al.* 2009). The ITS region (ITS1-5.8S-ITS2) is the genetic marker most frequently used to be sequenced to identify fungi, systematics, phylogeny, strain identification at species level and, sometimes at sub-species level (Begerow *et al.* 2010, Seifert 2009). Nevertheless, this marker is not suited for universal DNA barcoding of fungi because it can present intraspecific variability (Smith *et al.* 2007).

The ITS region is the most used region for DNA-based mycological studies because it gives more advantages than disadvantages. The ITS region is a multi-copy, tripartite region with roughly 550 base pairs that combine the advantages of resolution at various scales, i.e., the ITS1 is rapidly evolving, 5.8S is very conserved, and the ITS2 is moderately rapid to rapid. The ITS region allows to readily obtain a product whose variability typically reflects synapomorphies at the species level (Nilsson *et al.* 2008).

For the ITS region, numerous sequences are available in GenBank, because the DNA barcoding data are meant to be easily and widely accessed, based on a proper sequence submission procedure available for GenBank (Casiraghi *et al.* 2009). With 172 000 full-length fungal ITS sequences in GenBank (Raja *et al.* 2017), the ITS region presents the largest number of identified fungal species (Begerow *et al.* 2010). There are other frequently sequenced regions (SSU, LSU, Beta-tubulin, RPB2, EF1a, CO1) but the numbers of identified species by those regions are lower in comparison with ITS. However, they can be combined with other regions to optimize the identification (Begerow *et al.* 2010).

3.5 Soil degradation and fungal communities

Soil ecosystems present complex relationships between abiotic and biotic factors. Soil is denominated as the top cover of the planet Earth, consisting of different particles integrated of minerals and decayed organic matter organized in different soil horizons, which contain water, air, nutrients, and food for biotic communities (Bot and Benites 2005, FAO and ITPS 2015). Soil ecosystems have different problems, disturbance in the soil can be produced by agriculture, deforestation, the addition of chemicals, organic fertilizers, habitat degradation, slash and burn agriculture. All these factors can affect soil biodiversity by reducing species numbers and evenness (Hawksworth 2001). The process of soil degradation is an important environmental problem all around the world. By degradation, the soil deteriorates because of human activity and loses its nutrients together with its organic matter (Eswaran *et al.* 2001).

Degradation can be vertical and sometimes horizontal (Brandy and Weil 2007). Soil degradation processes in forests are mostly due to a temporary or permanent deterioration in the density or structure of vegetation cover (Mithal *et al.* 2011), which produces higher temperatures that affect the structure of fungal assemblages, fungal biomass, and reproduction (Geraldes *et al.* 2012). In summary, anthropogenic activities and global change affect the diversity of soil fungi; these activities often produce soil degradation, causing alterations in abiotic soil factors like temperature, moisture, and pH values (Kirk *et al.* 2004). Degradation and pollution have similar effects on the fungal community, reducing the diversity of fungal species (Tsui and Hyde 2004, Ferrari *et al.* 2011, Lentendu *et al.* 2011, Chandrashekar and Kaveriappa, 1989, 1994).

In this context, fungal diversity can be influenced by different factors. The ability of communities of soil fungi to recover after a disturbance like soil degradation is possible. However, the dynamics of fungal populations in soil are not completely known.

On the other hand, it should not be forgotten that soil fungi are involved in several biogeochemical cycles and they are vital for the continuing cycling of organic compounds (nutrients) and for driving above-ground ecosystems by contributing to plant nutrition, plant health, soil structure, and soil fertility (Dodd *et al.* 2000, Kirk *et al.* 2004, Wall and Virginia 1999). Fungal communities can enhance to different degrees soil aggregation, contributing to the structure of the soil (Rillig and Mummey 2006).

Disturbance of the community of soil fungi can be measured by ecological parameters that include effects on species-abundance distribution patterns, which show the effect on the relationships between distribution and number of species (De Ascoli *et al.* 2005, Persiani and Maggi 2013). Soil degradation has direct or indirect effects on the fungal community, abiotic factors are altered with the degradation process, and variability in moisture and temperature influences the number of species in the fungal community. These disturbances reduce the number of organisms causing unsaturated habitats that other species can colonize in a species succession. If the soil recovers from this degradation, diversity can increase over time. However, degradation effects on soil microfungi have not been investigated as extensively as for another organism (Persiani and Maggi 2013). It is important to understand the effect of human disturbance on fungal diversity in the tropics and temperate forests to conserve renewable resources effectively (Hawksworth 2001). Understanding the degradation

processes is critical for the development of management recommendations for ecosystems (Ashton *et al.* 2001). Therefore, in the context of the present investigation, fungal diversity in a tropical and temperate forest is analyzed.

3.6 Theoretical irrigation models with biofertilizers

Theoretical irrigation models are mathematical tools used to simulate and predict the behavior of water in an irrigation system. These models are based on physical and mathematical principles and are used to optimize the use of water and fertilizers in agriculture (Arriaga Sevilla 2015). Irrigation models with the use of biofertilizers are an increasingly popular option in sustainable agriculture. These models combine the use of efficient irrigation technologies with the use of biofertilizers, which include the use of soil fungi (Santos Torres 2006). Irrigation models that use biofertilizers can also reduce the need for chemical fertilizers, which can have a positive impact on the environment (Irizar Garza *et al.* 2003).

In summary, irrigation models that use biofertilizers are a sustainable and cost-effective option for modern agriculture. These models can improve soil quality, increase crop production, and reduce the environmental impact of agriculture.

3.7 App prototype development Soil Fungi Cultures

Mobile applications are innovative computer programs designed to run on mobile devices such as smartphones and tablets to provide computer service to the end user using the device (Arun and Kamalasab 2017). Currently, Apps are developed in most fields; they are an innovative technological tool since specific

Apps are created for each need and thus able to facilitate the work carried out by people or companies (Cuello and Vitone 2013). There are different programs for building an App. However, they all focus on one of the two main areas of App development: visual design and technical operation (Adiono *et al.* 2019). The App inventor program integrates all development areas in an easy and didactic way for people who are not experts in programming (Logan 2020). Mobile scientific applications are on the rise. However, many disciplines need the development of new Apps to facilitate the work of scientific users in their respective areas (Cuello and Vitone 2013, Inukollu *et al.* 2014). In biology, there is a large number of particular areas of study that need tools to make work easier; an example of this is the study of soil microfungi (Srivastava *et al.* 2011, Tedersoo *et al.* 2014, Singh *et al.* 2015, Rosas-Medina y Piepenbring 2018). This area of research is limited. Nevertheless, it is associated with great difficulties, especially in the cultivation of soil microfungi in the laboratory. The main problem is the control of the records of the growth of colonies of microfungi in the soil. Over time, normally the plate fills up with colonies, becoming indistinguishable and contaminating the cultures. Usually, when the culture is in a single Petri dish, it is easy to keep track, but if there are several samples, it becomes a problem. The growth of cultures at the same time in several Petri dishes does not allow keeping a record of all the colonies that develop. The dominant colonies cover the Petri dish completely, thus losing all the information of the other colonies (Rosas-Medina *et al.* 2019). Based on the above, the principal aim of this App is to solve this problem by developing a tool that allows keeping a photographic record and a database on the characteristics of the cultures. The App will use the tools of portable phones to facilitate and speed up the data collection of cultures in Petri dishes.

The main justification for the development of this App is that scientific Apps are relevant for enhancing accessibility for scientists and students, providing portability in the field or laboratory, and assisting with data collection or species identification. This can contribute to the expansion of the general database of mycology. Additionally, the innovation of a tool that facilitates scientific work is a scientific advancement, especially with the integration of automation and artificial intelligence in the future.

4 General objective

The objective of this thesis is to characterize fungal diversity in soils in Germany and Panama with different degrees of degradation.

4.1 Specific objectives

1. Identification of fungal diversity in soils at a site in the Taunus mountain range, in Frankfurt, Germany, and at a site in the Majagua valley, Chiriquí in Panama.
2. Characterization of the soils and determination of their levels of degradation based on pH, compactation, temperature, moisture, and soil composition.
3. Determine patterns of distribution of species in the microfungal community and their correlation with biotic and abiotic factors.
4. Contribution to the knowledge of the geographic distribution and ecology of soil fungi.
5. Development of a theoretical model of irrigation for degraded soils.
6. Programming, development, and evaluation of an application that facilitates the counting and registration of microfungal cultures.

5. Material and methods

5.1 Areas of study

The first location was within a temperate forest in the Taunus mountain range north-west of Frankfurt in Hesse, Germany. The area includes three different vegetation types, defined as forest, grassland, and bare soil, showing a degradation gradient. In each of these different sites, soil samples were collected. The geographical position data were taken by GPS to determine the coordinates and altitudes for each selected zone (50° 08´280´´N, 008°16´055´´ E, ca. 360-380 m a.s.l.). This first location was sampled eight times during the period from January to November 2012. The second location was within a tropical, semideciduous forest in the Majagua valley in the province Chiriquí in Panama. Within this, similar vegetation types like forest, grassland, and bare soil were found. The geographical position data were taken by GPS to determine the coordinates and altitudes for each selected zone (08° 29´ 335´´ N, 82° 25´ 594´´ E, ca. 120 m a.s.l.). This location was sampled on three occasions during February and July 2012.

5.2 Soil collection

Soil samples were collected from the upper surface of the A1 horizon. First, the organic litter was removed from the surface and after that, a plastic (PVC) tube was introduced into the first five centimeters of the soil. By doing this, core samples of five centimeters diameter and five centimeters of depth were obtained. two core samples were collected at each site (forest, grassland, and bare soil), where the exact points were selected randomly.

The three cores were kept in plastic bags and mixed until a total sample for each site was obtained, following the protocol described by Carrasco *et al.* (2002). These total samples were separated into three parts for two different aspects: assessment of fungal diversity by molecular and morphological methods and soil characterization. Molecular and morphological assessments were performed based on the isolation of fungal cultures based on the method of powdered soil samples (Rosas *et al.* 2019). Besides, following instructions by L. Tedersoo, soil samples for molecular 454 pyrosequencing were collected in an area of approximately 0.25 ha, where ectomycorrhizal trees dominate. Twenty trees were selected randomly with a distance of at last eight meters. The relative basal areas of all trees were estimated to determine their contribution to the basal area of all trees. Below each tree, two soil samples were collected, following the same method. The plastic tubes were used to obtain the core samples (5 cm diam. and 5 cm deep) from opposite directions and at 0.5 m from the trunk. A mix was made composed of the 40 core samples. This mixed sample was crushed to obtain fine soil particles, which were removed into another bag (Tedersoo *et al.* 2010). These samples were sent to molecular analyses by the 454 pyrosequencing technique. The abiotic factors that characterize soil were compactation BD(Mg/m^3), temperature, pH, moisture, and soil composition (by sedimentation), which were measured.

5.3 Isolation of fungi from the soil samples

The isolation techniques involve the separation of fungal propagules from the soil and the initiation of growth on an isolation medium (Agnihothrudu 1961, Zuñiga 2004). Soil samples were shaken to obtain fine soil particles. After that, the cultivation of soil microfungi from the soil particles of each sample was done as described in detail in Rosas-Medina and Piepenbring (2018). In order to observe the diversity of fungi, it is important to separate the propagules and maintain them separated during colony maturation (Routien 1957, Meyling 2007). All samples were labeled with data of the site, date, incubation temperature, and sample number. Once the fungi grew in the petri dishes, all the colonies were isolated in new petri dishes with MEA media. When the colony grew only slowly, and it was difficult to obtain a sample, a plastic film was used on the surface on the medium to obtain a maximum amount of fungal cells. The isolated cultures were incubated at 25 °C for 2 to 25 days in an incubation chamber until colonies developed. In order to observe the fungal growth on different culture media, all the observed morphospecies of fungi were picked and cultivated separately on malt extract agar (MEA), malt yeast peptone agar (MYP), and potato dextrose agar (PDA) media. For each morphospecies, two portions of the culture were stored on cryo-culture in a 1.5 ml eppendorf cup with 500 µl distilled water at -80 °C (Homolka 2007). All the cultures were subcultured as many times as necessary to keep the isolates axenic.

5.4 Morphological identification

Morphological identification of fungi was achieved by using different keys, first the compendium of soil fungi (Domsch *et al.* 2007), and for more detailed determination Samson *et al.* (2006), Stolk and Samson (1983), Booth (1971), Hesseltine and Ellis (1964), Samuels (2011), Martínez (2003), and Chaverri *et al.* (2003). For every plate, the colony was studied macroscopically with the aid of a stereo microscope, observing features like gross morphology, color, texture, growth speed, in some cases odor, as well as shape of colonies, i. e. rings, circles or other forms, and the color of the bottom side (Mueller *et al.* 2004). Microscopical structures were observed in small parts of the fresh culture on a microscope slide covered by cover glass treated with potassium hydroxide (KOH) and stained with lactophenol with cotton blue or phloxin to observe hyphae, conidiophores, conidia, the arrangement of spores, and other different structures (Baxter and Van der Linde 1999).

For some species, electron microscopic pictures were taken with a Hitachi S 4500 scanning electron microscope with 5 kV, processed with Digital Image Processing System 2.5, and saved in TIF format. The processing of the samples started with a small sample from a pure culture mounted on an adhesive carbon layer fixed on mini aluminum tubes and sputtered with gold for 60 sec in a Sputter Coater.

5.5 Molecular identification

5.5.1 DNA extraction

Approximately 0.25-0.5 g of pure fungal cultures were taken from the Petri dish previously cultivated. The fresh material was transferred to an Eppendorf tube and conserved at -8 °C. DNA extraction from cultures was conducted according to the protocol of DNA isolation with CTAB (Talbot 2001), modified by Weisenborn (2012). The homogenization of fungal material was done with a sterile metal ball inside an Eppendorf tube at 30 hertz for 30 s.

After the homogenization, 150 µl of 3.5 M NaCl solution was added and vortexed, later 500 µl of CTAB lysis buffer was added, immediately 20 µl RNAse was added and incubated for 5 min at room temperature. Subsequently, 10 µl 2-Mercaptoethanol was added and incubated for 30 min at 65 °C while the tubes were inverted every 10 min to ensure adequate mixing. After this time, 500 µl ChIoroform/Isoamyl-Alcohol were added and the mix was transferred to a shaking platform for 20 min at 60 r.p.m. In the next step, the extract was centrifugated at 9650 g for 20 min at 4 °C. After this procedure, the content of the tube separated itself into two layers from which the aqueous layer was removed, whereas care had to be taken that the denatured proteins and debris at the interface were not removed. Subsequently, 500 µl of Isopropanol was added, the sample was incubated at 8 °C for one hour, and then it was centrifuged at 13000 r.p.m. for 10 min at 4 °C to obtain a pellet. This pellet was washed in 800 µl of 70 % ethanol and centrifuged twice. Next, the pellet was dried for 5-10 min at room temperature and resuspended in Tris buffer (10 mM, pH 7.2). Finally, the amount of DNA was determined in the nanodrop, and the sample was stored at 4 °C.

5.5.2 Polymerase chain reaction (PCR)

The PCR reaction for every sample was performed in a volume of 50 µl with the master mix Kapa HiFi/Phusion, the reaction volume contained 10 µl Buffer, 25 mM MgCl, 1 µl dNTPs, 1 µl primer (ITS1 and ITS4, Sigma-Aldrich, Steinheim, Germany), 0.5 µl DNA polymerase, 34.5 µl ddH^2O and 2 µl with 20 ng of DNA. The amplification was done with a PEQSTAR 2X GRADIENT Thermal Cycler (PEQLAB, Erlangen, Germany). For the PCR process, the primers ITS1 and ITS4 (White *et al.* 1990) targeting the fungal ribosomal DNA (rDNA) were utilized, as this region is one of the most commonly used in phylogenetic studies and barcoding approaches to distinguish fungal species. Separation of DNA fragments was done in TRIS-Borat-EDTA (TBE) electrophoresis gels containing 1.5 % agarose and 400 ml TBE buffer. Electrophoresis was realized in a Maxi ExW gel chamber with 120 V and 200 mA. Pictures of gels were taken using UV transiluminador. The DNA purification was done with the Cycle pure kit by VWR after that the samples were sent to the laboratory of the Biodiversity and Climate Center (BiK-F) for sequencing.

5.5.3 Comparison of sequences

The assembling and preparation of the sequences were conducted with the software CodonCode Aligner version 8.1. (CodonCode Corporation, www.codoncode.com). Sequences were aligned in MEGA and the original sequences were compared with other reference sequences from GenBank (Seifert 2009). Results were obtained from the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov). According to the data in

GenBank and the BLAST algorithm, strains that match with 98 % or more of maximum identity were considered as reliable identifications, while sequences with less than 98 % identity were subjected to critical morphological analysis (Kõljalg *et al.* 2005).

The principal reference for the selection of the currently valid names was Mycobank (http://es.mycobank.org). The identified names were compared with Index Fungorum and Mycobank, but in cases of doubt Mycobank names were given priority.

5.6 Analysis of the soil samples

5.6.1 Analysis of soil characteristics

The soil samples were analyzed to measure the following soil characteristics: temperature, compactation, pH, and moisture. The temperature was measured by a thermometer in all soil sampling areas. Before disturbing the soil sampling area with the core tube, the thermometer was introduced in 15 cm depth of the soil and after 15 minutes, the temperature was recorded. The pH for all samplings zones was determined with litmus paper in a soluble extraction of the soil samples. Moisture changes in the soil during the rainy and the dry season is important to measure for the water balance. A small metal container was weighed (4 cm height by 6 cm diameter approximately), then a soil sample was put inside, and the metal container was weighed one more time to obtain the value of the wet weight (this process was repeated for all samples). Later the samples were dried in an oven at 105 °C for 48 hours and the samples were weighted to obtain the dry weight. After that with the next equation, the moisture value was obtained:

W = [g H_2O/ g dry soil] x 100 (Roa 2012). Gravimetric (moisture content) is the relationship between the weight of water (grams) and the weight of dry soil (grams) (Roa 2012).

Compactation refers to the ratio of the mass to the bulk or macroscopic volume of soil particles plus pore spaces in a sample. Based on the calculations above, the data of moisture were used for the compactation calculation. Approximately 45 cm^2 of the soil layer was tested. A steel dolly was placed on top of the soil and the soil core was removed carefully (the process was repeated for all the sampling areas). After that, the samples were placed in an oven at 105 °C for 48 hours to obtain the moisture value, the radius of the cylindrical steel core was taken to complete the data for the compactation calculation (Bernier 2000), and the next equation was used:

$$\text{Bulk density } (g/cm^3) = \text{Mass of dry soil } (g) / \text{Volume of core } (cm^3)$$

5.6.2 Analysis of soil composition

The next analysis was the determination of the composition of soil by the method of sedimentation. The analysis by sedimentation is a process by which the solid material is transported in a water column, and this material is deposited at the bottom of the plastic container. Stokes' law indicates that solid particles settle more rapidly when their diameter is greater, their specific weight is higher compared to the liquid, and when the viscosity of the liquid is lower. This sedimentation technique refers to the process of precipitation of the small solid particles in a water column by gravity (Cano 2011). 150 g of soil was put in a

glass container with 100 ml of water, after that, the sample was heated on a hot plate at ca. 90-100 °C, then 50 ml of hydrogen peroxide H_2O_2 were added.

During 5 minutes, the solute was shaken until the organic matter was burned, then 150 ml of water were added, and this mix was left standing still for one hour until all particles settled and the sediment layers built up. At that time, the measurements were done with a rule for clay, sand, and silt. Determination of clay, sand, and silt percentage was done with the next equations:

Sand: cm of sand*100/8.8 cm (total cm of sediment) = Sand percentage

Clay: cm of clay*100/8.8 cm (total cm of sediment) = Clay percentage

Silt: cm of silt*100/8.8 cm (total cm of sediment) = Silt percentage

When the percentages were obtained the soil was classified with the texture triangle (Fig. 2 Triangle of soil composition).

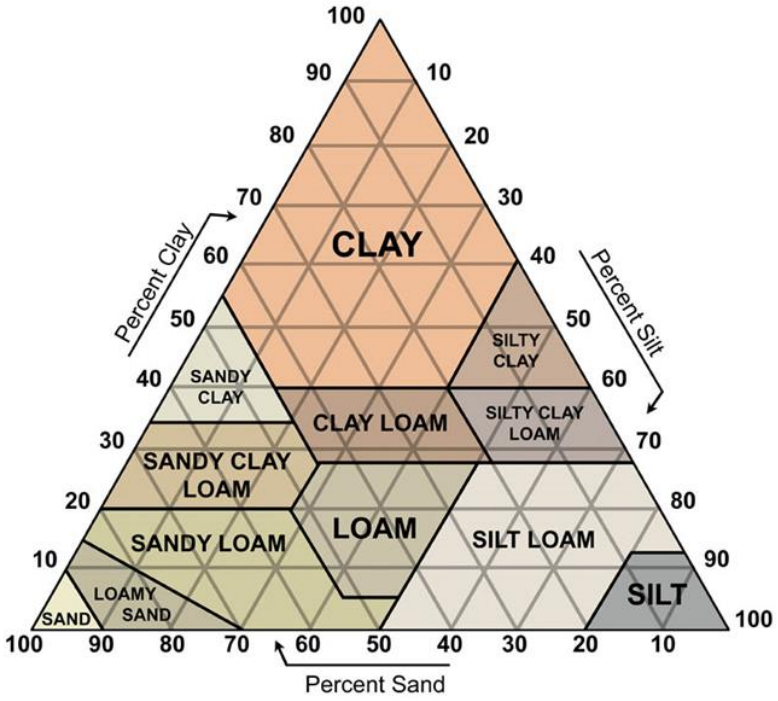


Fig. 2 Triangle of soil composition of the US Department of Agriculture (USDA) (taken from Cano 2011)

5.7 Statistical analysis

The statistical analyses were performed in R version 3.1.1. (32-bit application with Rstudio). After the classification of the fungal colonies, the species richness was calculated to know the number of species found in each study area with ESTIMATES 8.2 (Colwell 2004). Species accumulation curves were done to study the rate of species discovery with sampling effort and evaluate sample completeness. Also, the richness estimators Chao 2, Jackknife 1, and Bootstrap (Kindt and Coe 2005) were used to estimate the possible total species richness in the areas. There are several methods to estimate the richness of species. However, those mentioned above are the most commonly used, like no parametric methods.

Chao 2 estimator was used because it is based on incidence of the species and takes into account the distribution of species between samples, for this reason, it is recommended for small samples (Chao 2005, Colwell and Coddington 1994, Leitner and Turner 2001, Moreno 2001). Jack 1 and Bootstrap are methods based on resampling: the first one is based on the number of species that occur only in one sample, and the second works best when there are rare species in communities (Bautista-Hernández *et al.* 2013, Palmer 1990), however, used together can be complemented (González–Oreja *et al*. 2010). Analysis of diversity was carried out with the most common diversity indices, Fisher alpha diversity index, Shannon diversity index, and Simpson diversity index. Fisher alpha diversity index assumes that the abundance of species follows the log series distribution (Carmona 2013). Shannon diversity index is an equity index which expresses how uniform the species are represented (focus in the abundance) considering all sampled species, and Simpson diversity index is a

dominance index which indicates the probability that two individuals are drawn at random from a sample corresponding to the same species (Villarreal *et al.* 2004). The aforementioned diversity indexes use the values of richness, but the mathematical operations with these values are organized in different ways. Linear regression models were done to estimate if the soil factors influence the species richness. Each soil factor: temperature, moisture, pH, soil compaction, and soil composition (percentage of sand, lime and clay) was correlated with the species richness in each study area to see what kind of influences or relationship they have.

Across samples differences in soil fungi community composition was studied, the data set was built based on the species matrix, which shows the presence or absence of species. This index reflects only two values: 0 and 1, where zero indicates that the species was not observed, and one means that the species was observed.

The other data set was based on the environmental matrix which contains two types of variables: quantitative variables that reference the soil factors values in numbers and categorical variables that reference the soil treatment, which means bare soil, grassland, and forest. With this data set, the analysis of ecological distance was done, based on the similarity in the species composition, compared across samples, and correlated with soil factors, using non-metric multidimensional scaling (NMDs). The analysis mentioned is a non-constrained ordination technique that uses information from the species distance matrix, where the distance of samples in the ordination represents ecological similarity in species composition (e.g. samples far apart are less similar than samples

closer to each other; Kindt and Coe 2005). One complementary analysis was done with the abundance.

First, the abundance was recorded over time in Germany and Panama by morphological identification; with this record, one ANOVA test was applied in the different soils of Germany and Panama, with the complement Tukey test being carried out. Also, the same test was done by order in both countries. Pareto analysis of species abundance was done on the different types of soils in Germany and Panama. Pareto analysis was complemented with the beta and gamma diversity to find the keystone species, the nested species, and the succession species. Finally, one T-test was done with the abundance obtained in both methodologies.

5.8 Theoretical irrigation model

The theoretical irrigation model was constructed as follows; first, the objective of the model was identified, which in this project is to establish the species of soil fungi that should be present as biofertilizers in the irrigation system (Castellanos-González *et al.* 2020). Secondly, relevant variables were selected, such as the amount of water used and the proportion of soil fungi in the biofertilizer (Irizar Garza *et al*. 2003). The third step was the choice of the model, the Model-Driven Development flowchart model was chosen (Pons *et al*. 2010), considering the scheduled cultivation systems (Servin Palestina *et al.* 2018).

5.9 App development

The first step was creating a Google account to access App inventor. Once in the application, App design was made in the user interface part and in the server part as follows. Six activities were programmed in the App Inventor block system; the introduction, work area, general database capture, new data, update, delete and photograph.

The introduction has the user interface part with a logo that contains a camera on a Petri dish showing a *Penicilium*. On the server, this introduction lasts a second, and then the work area activity appears; the opening logo will be the same logo of the App in the App store.

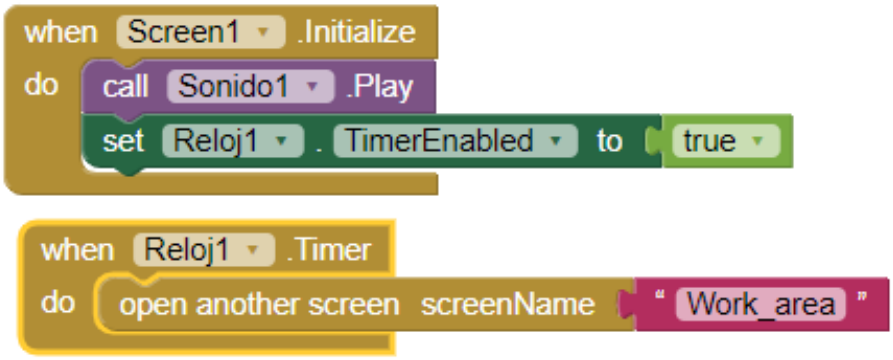


Fig. 3 Programming of the introduction

The next activity is the work area; in the user interface, two buttons were placed (Picture and Database). On the server side, they were programmed so that the Picture-button sends the user directly to the photo activity, and the other Database-button goes to the data capture. The above was done because, once the data is captured, the user will likely want more photos of the same dish previously recorded over time.

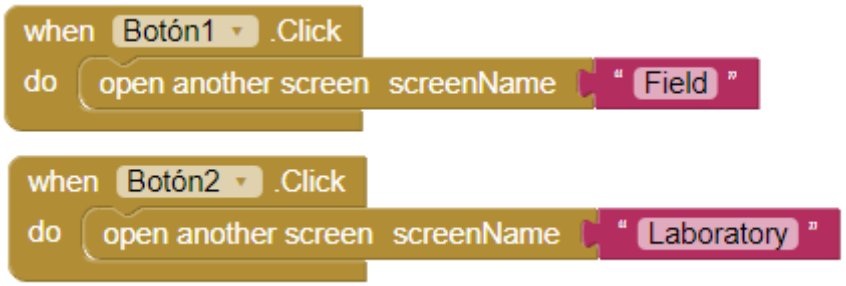


Fig. 4 Work area programming

The database activity (Fig. 5) contains five buttons on the user interface; New, Update, Delete, Display, Back, and Export. These buttons were programmed to each perform different functions. The New-button was programmed to send to another activity where the data is captured. The Update-button was programmed to open another activity where the captured data can be modified. The Delete-button opens another activity where the captured data can be deleted. The Back-button returns to the previous activity. The Export-button sends the data to the memory of the phone or electronic device in an excel format. The database was built with six viewers with the following variables: Petri dish number, culture medium, storage temperature, inoculations days, number of colonies, and note. The database is filled utilizing the buttons that send to the mentioned activities.

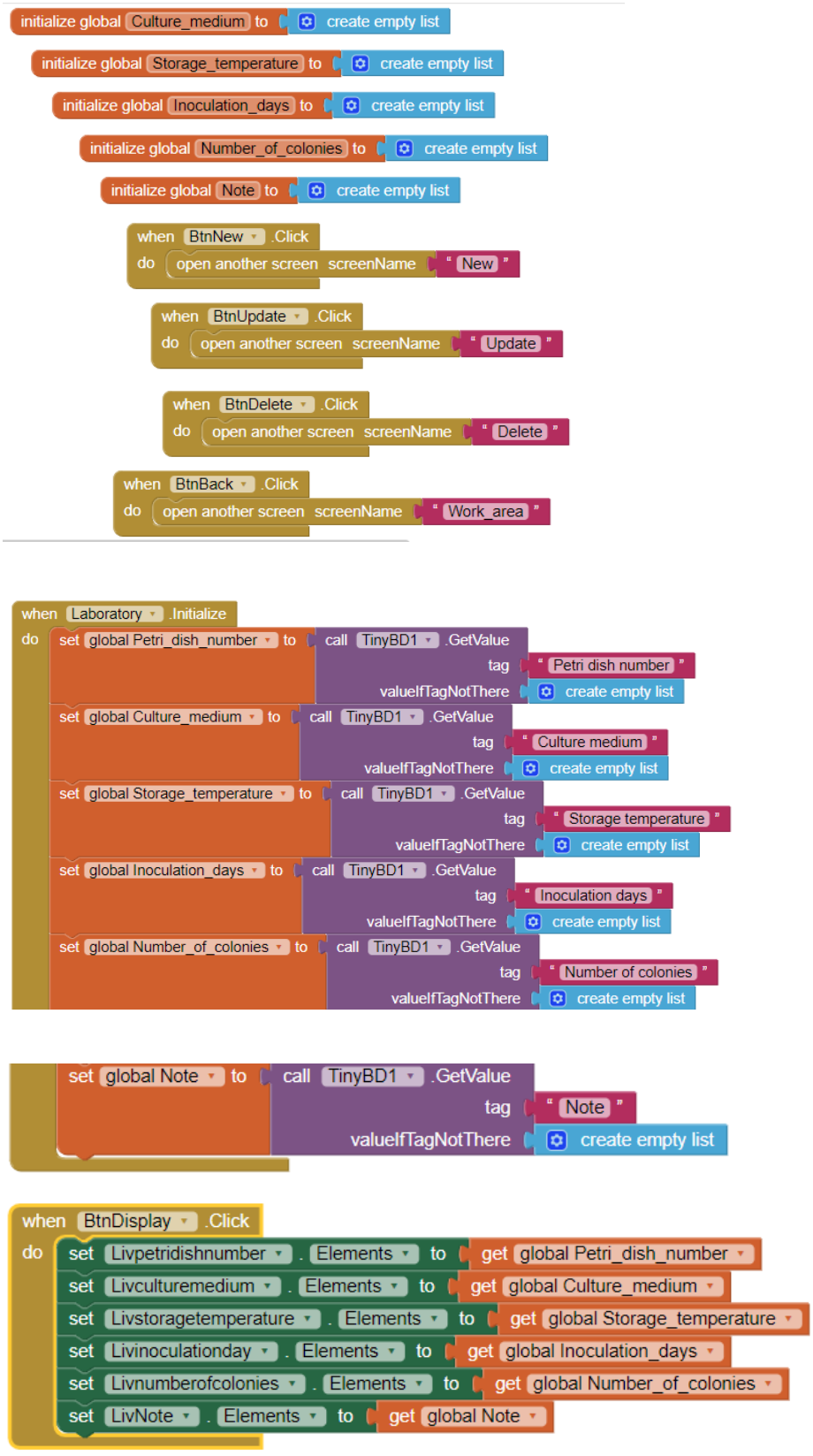


Fig. 5 Database activity programming

The photo activity (Fig. 6) contains nine buttons in the user interface, of which one is a dropdown, a camva, a text field, and a slider.

The first four buttons placed at the top are Image, Photo, Color, and Back. The Image-button sends the user to the device's image base so it can be imported into the photo activity. The Photo-button activates the camera function on the device so it can take a photo directly in the photo activity. The Color-button displays a menu with nine colors to choose from white, black, yellow, red, blue, green, orange, gray, and pink. The Back-button returns to the previous activity. The Draw-button displays a menu where you can choose what type of mark can be put on the photo, and these include point (which marks a line if it moves), filled circle, ring, and text. The grade bar increases or decreases the size of what is placed on top of the photo. The text field allows the user to write a text placed by selecting text in the drawing menu and pressing on the screen where the user wants to put it. The camva is the space where the photo appears, and the image taken can be worked on. At the bottom are two buttons; the Delete-button, which deletes everything worked on the photo, and the Save-button, which saves the edited image.

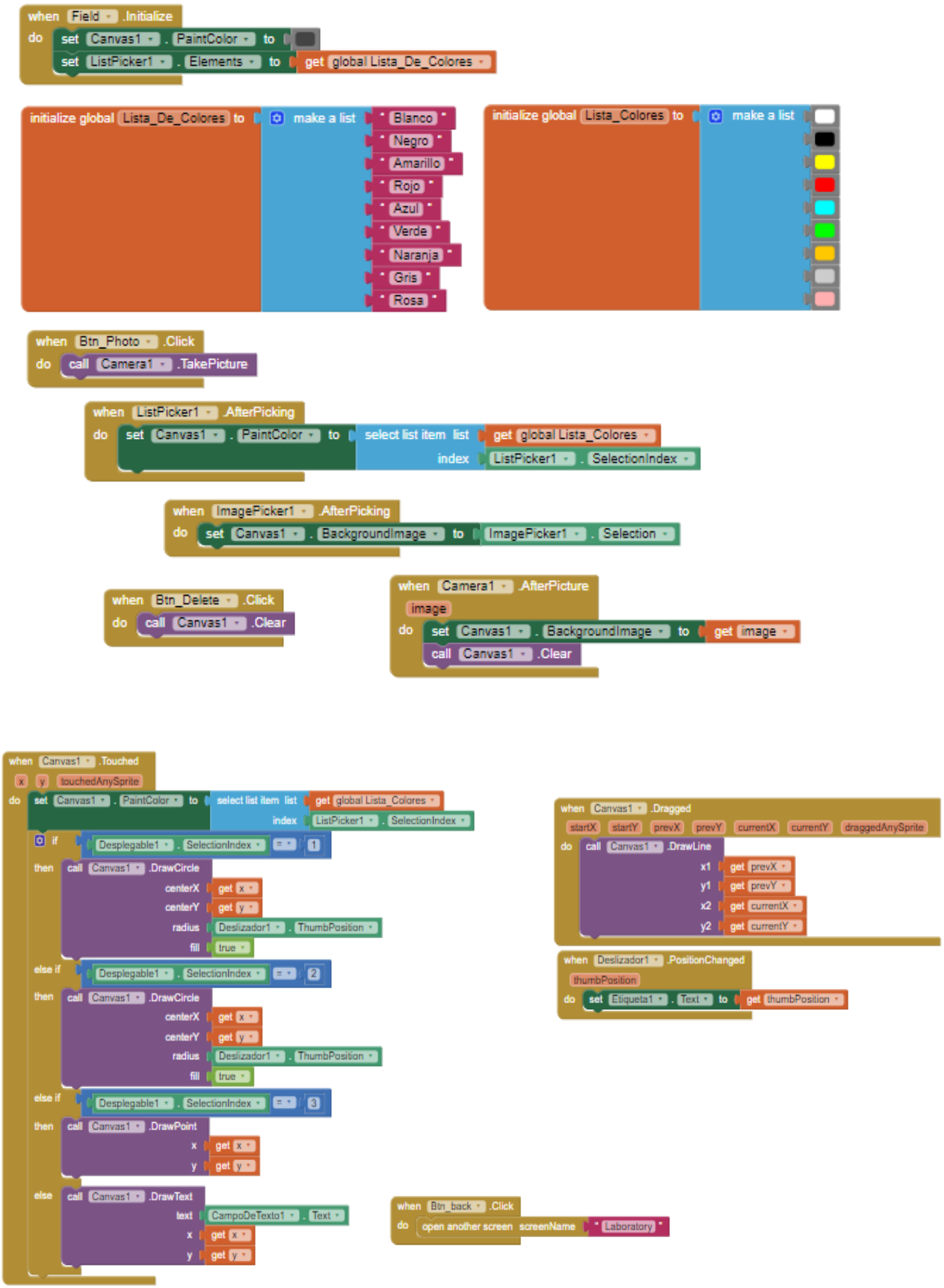


Fig. 6 Photo activity programming

Finally, once the SFC App was developed, it was compared with the most similar App in the Google Store called Colony Counter (CC), which is used for counting bacterial colonies. The following factors were considered: Capture time in seconds, Flow navigation divided into; database data transfer function of the camera and without hiccups, the number of clicks, and rating of the App.

6 Results

6.1 Species identified by pyrosequencing

The following results are the species identified in Germany and Panama through the pyrosequencing method. These results contribute to the global work carried out by Tedersoo *et al.* (2014).

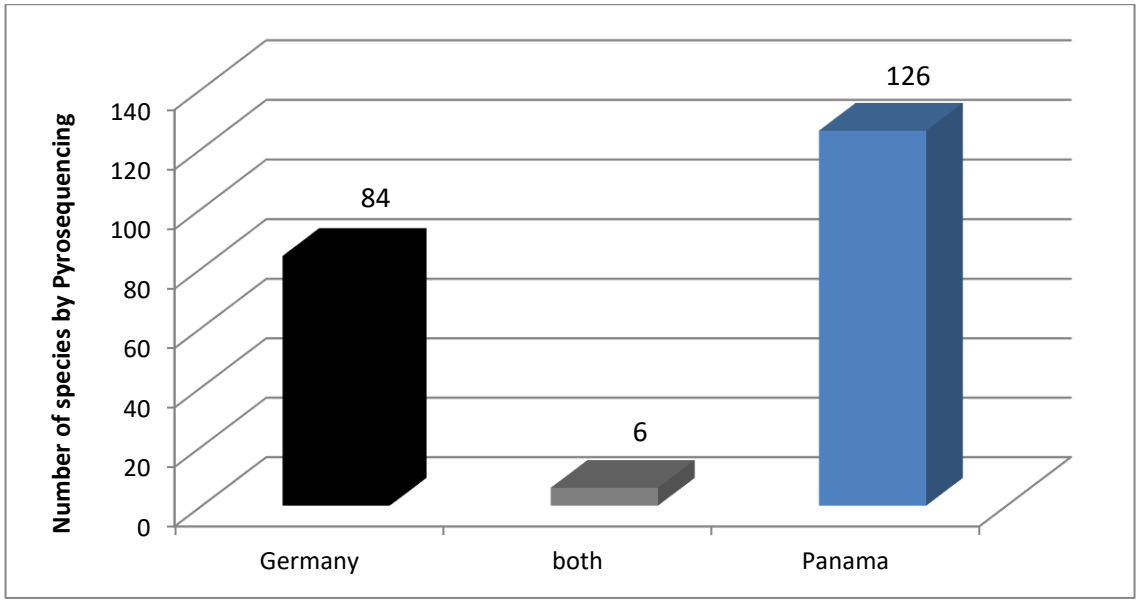


Fig. 7 Number of species by Pyrosequencing

The total number of species found were 204 species in both countries with the pyrosequencing technique. The number of species that were found in both areas were six, the species are: (*Cryptococcus podzolicus*, *Mortierella kuhlmanii*, *Mortierella minutissima, Russula nigricans, Trichosporon gamsii, Trichosporon porosum*) most of these species are cosmopolitan. It is worth mentioning that the numbers of species identified by pyrosequencing are more than the number of species identified by the molecular and morphological way, in Germany about double (47 vs 84 species), but in Panama, the difference is significant (32 vs 126

species). However, there are many individuals, who are not identified by pyrosequencing technique, instead are classified until genera or uncultured fungi.

6.2 Percentage of orders by pyrosequencing

Based on the pyrosequencing identification, the species were grouped in their corresponding orders to which they belong, obtaining the following results.

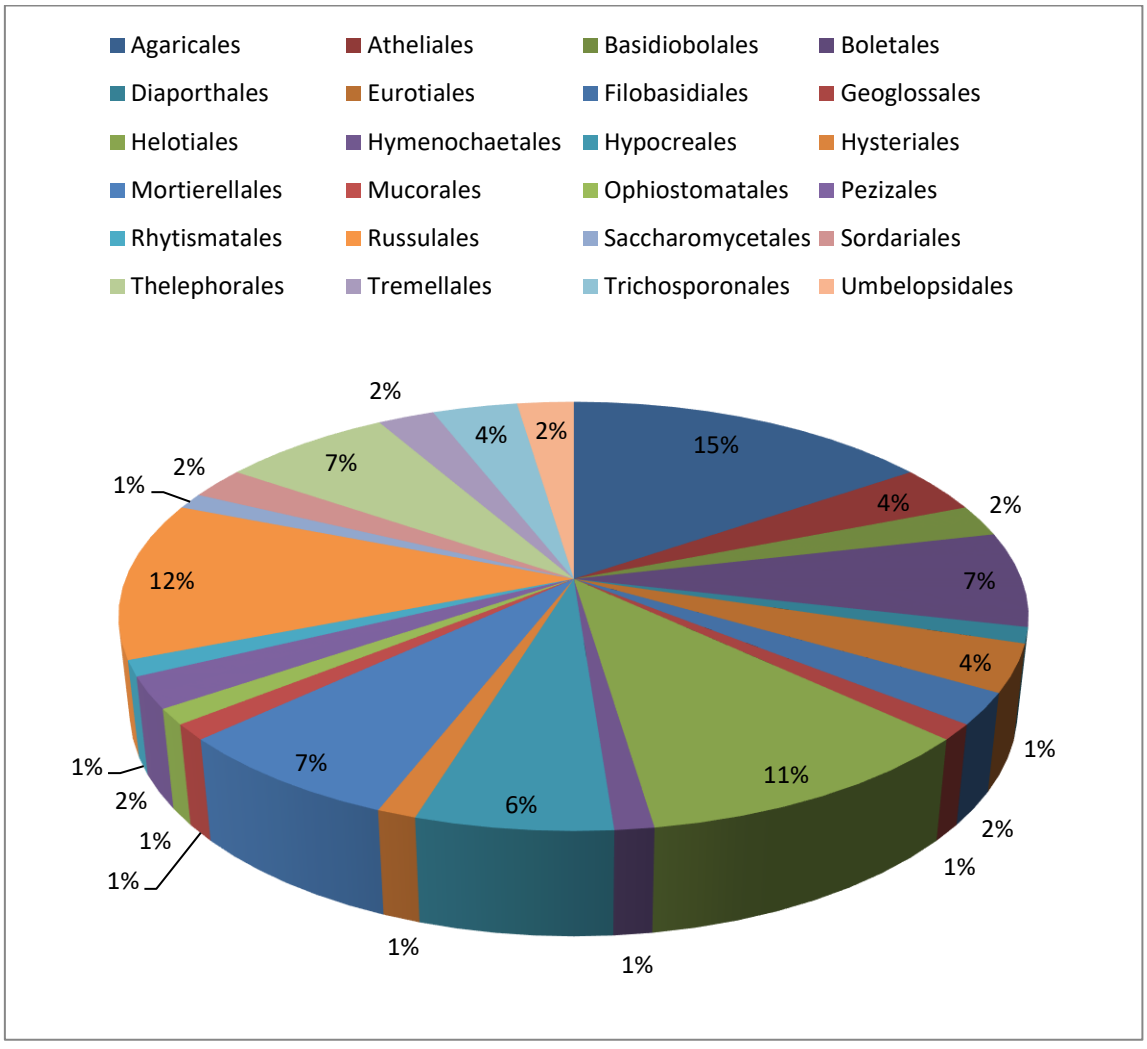


Fig. 8 The number of percentages in different orders in Germany by pyrosequencing

Germany shows in the pyrosequencing test one dominance by Basidiomycota (Agaricales, Atheliales, Boletales, Filobasidiales, Hymenochaetales, Russulales, Thelephorales, Tremellales, Trichosporonales) with around 55 % of species, while Ascomycota (Diaporthales, Eurotiales, Geoglossales, Helotiales, Hypocreales, Hysteriales, Ophiostomatales, Pezizales, Rhytismatales, Saccharomycetales, Sordariales) has 32 % of species, and the last position is Zygomycota (Basidiobolales, Mortierellales, Mucorales, Umbelopsidales) with just 13 %.

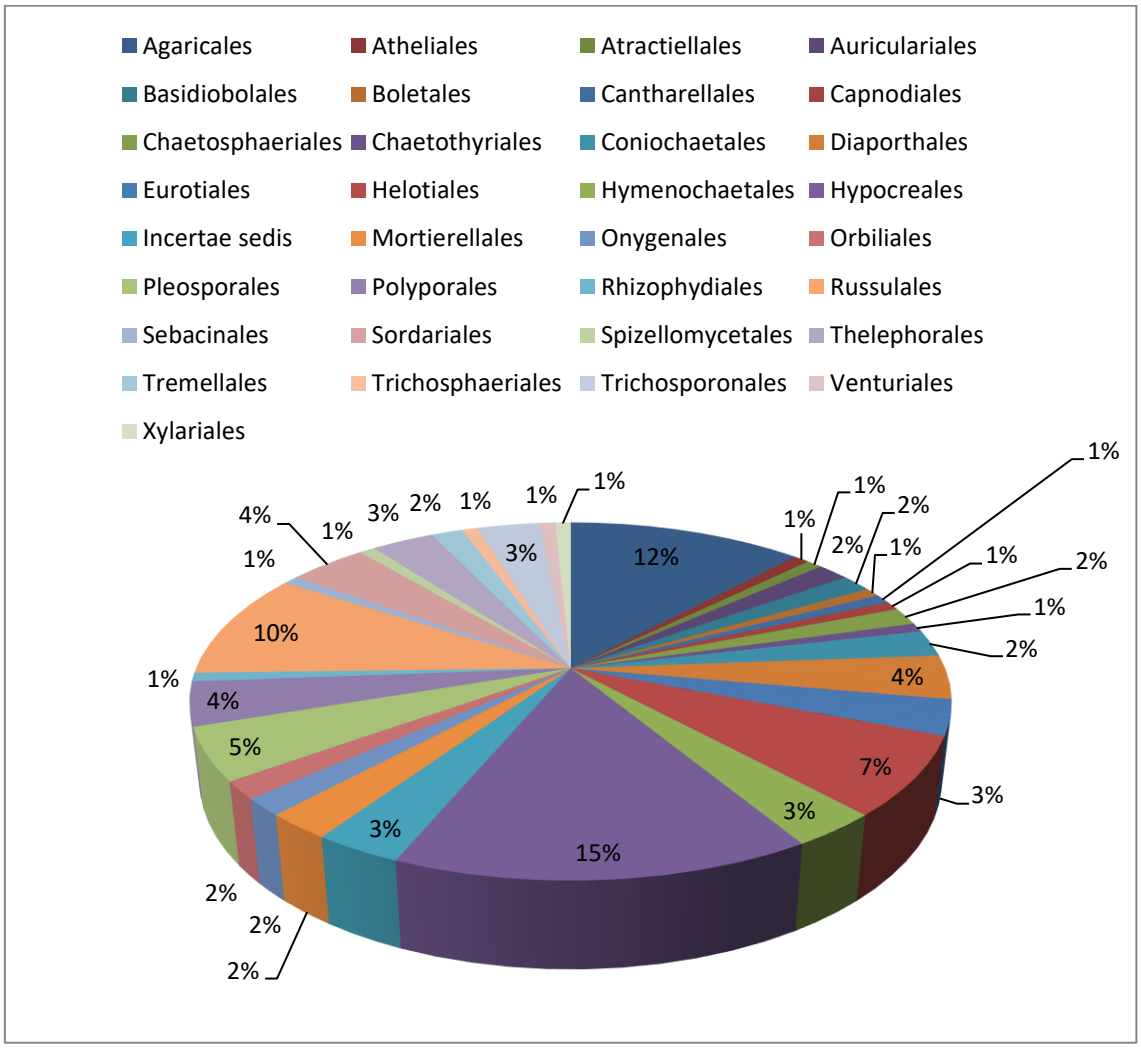


Fig. 9 The number of percentages in different orders in Panama by pyrosequencing

Numbers of species by pyrosequencing in Panama are dominant by Ascomycota 48 % (Capnodiales, Chaetosphaeriales, Chaetothyriales, Coniochaetales, Diaporthales, Eurotiales, Helotiales, Hypocreales, Onygenales, Orbiliales, Pleosporales, Sordariales, Venturiales, Xylariales) and Basidiomycota with 42% (Agaricales, Atheliales, Atractiellales, Auriculariales, Boletales, Cantharellales, Hymenochaetales, Polyporales, Russulales, Sebacinales, Thelephorales, Tremellales, Trichosphaeriales, Trichosporonales) while Chytridiomycota (Rhizophydiales and Spizellomycetales) has 2 % and Zygomycota (Basidiobolales and Mortierellales) with 4 %, and finally the pyrosequencing shows 3 % in Incertae sedis.

6.3 Number of isolates for morphological identification

The following results show the species identified in Germany and Panama through the morphological identification method. A total of 764 isolates of soil fungi were obtained from the soils of Germany and Panama. The number of isolates and species in both countries is shown in Figures 10 and 11. Mentioning that the number of isolates per month was the next; in Germany, the first sampling yielded 47 isolates, the second 107, the third had 88, the fourth had 77, the fifth had 60, the sixth had 64, the seventh had 55 and the eighth had 35. In Panama, the first sampling yielded 106 isolates, in the second 60, and the third was 65.

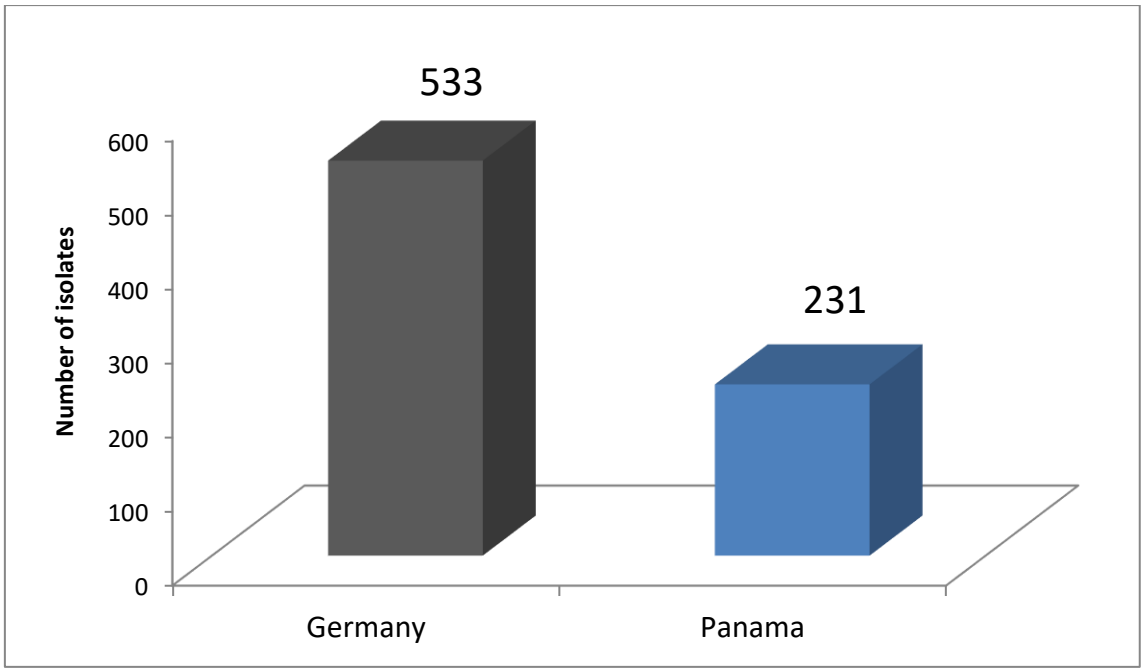


Fig. 10 Number of fungal isolated from soils in Germany and Panama

Within this study, major differences were found among the soil fungi species. The lower number of fungi isolated from Panama compared to those from Germany is caused by the lower number of samples from Panama (three samplings versus eight samplings). However, Panama showed a high number of isolates with just these three samplings. These results suggest the greater richness of species in Panama's tropical areas than in German areas.

6.4 Number of species by morphological identification

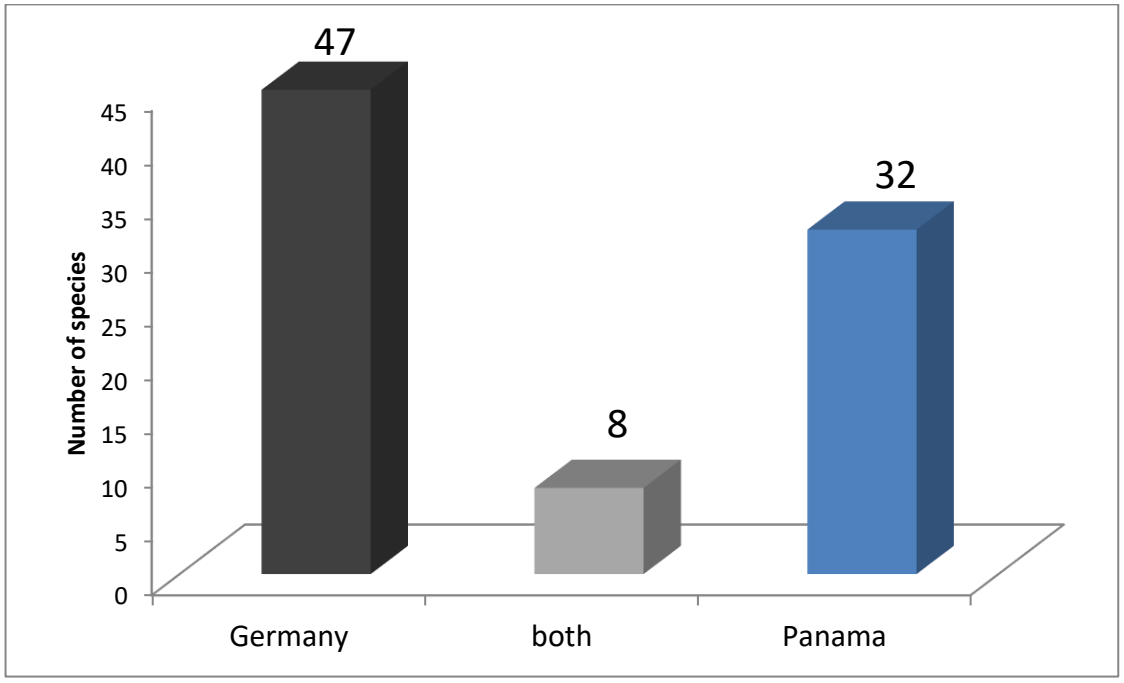


Fig. 11 Number of species isolated from soils in Germany and Panama

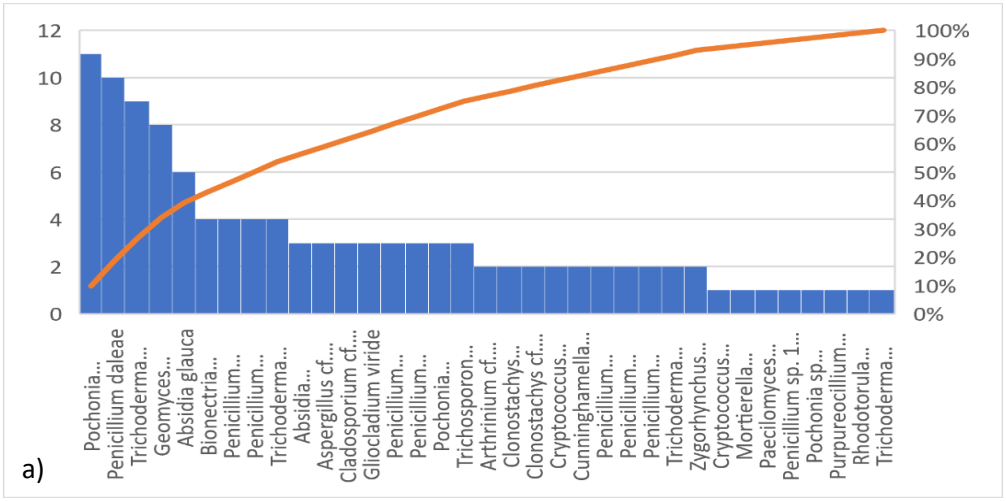
In total, 71 species were identified. In Germany, the first sampling yielded seven species, in the second 28, the third 19, the fourth 13, the fifth 19, the sixth 16, the seventh 28 and the eighth 26. In Panama, the first sampling yielded 24 species, in the second 18 and the third 25. Considering that the number of samples in Panama was three and eight in Germany, the richness of soil fungi found in Panama was proportionally higher.

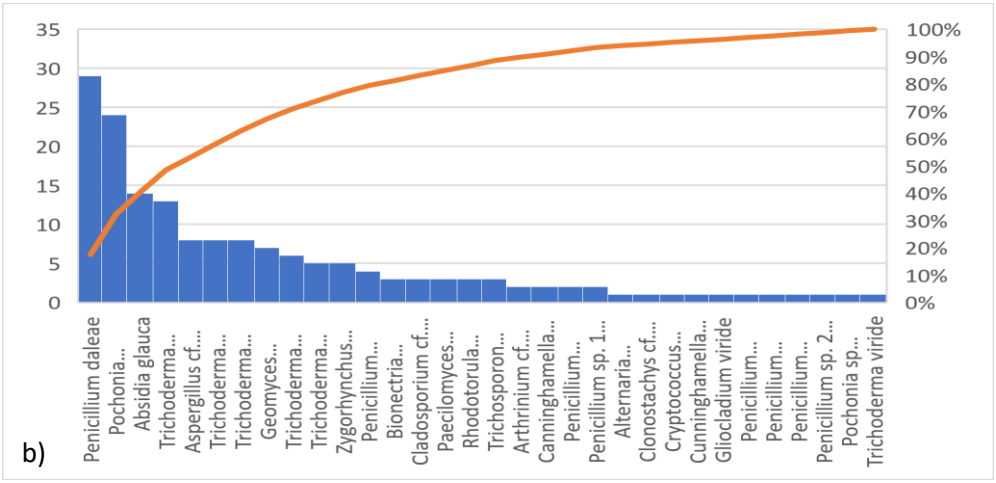
Eight species were found in both countries, the species are: *Absidia cylindrospora Bionectria ochroleuca, Penicillium citrinum, Purpureocillium lilacinum, Penicillium simplicissimum Trichoderma harzianum, Trichoderma koningi, Mucor moelleri.* Most of these species are known to be cosmopolitan (Tedersoo *et al.* 2014).

Table 1: Most abundant species isolated from Germany and Panama

Taunus-Germany	N -Isolates	% Total
Pochonia bulbillosa	81	10.6 %
Penicillium daleae	74	9.6 %
Majagua-Panama		
Trichoderma harzianum	27	3.5 %
Penicillium simplicissimum	22	2.8 %

The two most abundant species in each area represent a different percentage of the total number of isolates. Around 20 % of the Taunus-Germany isolates are represented by the two most abundant species, while in the Majagua-Panama isolates, the two most abundant species represent just 6 % (see table 1). Presumably, the abundance of species is more balanced in the Panama sites than in the German sites. However, the dominance of fungi in culture could be an inadequate representation of the species distribution in fungal communities because this dominance can be artificially caused by the ability or non-ability of fungi to grow on in artificial media, under different habitat conditions.





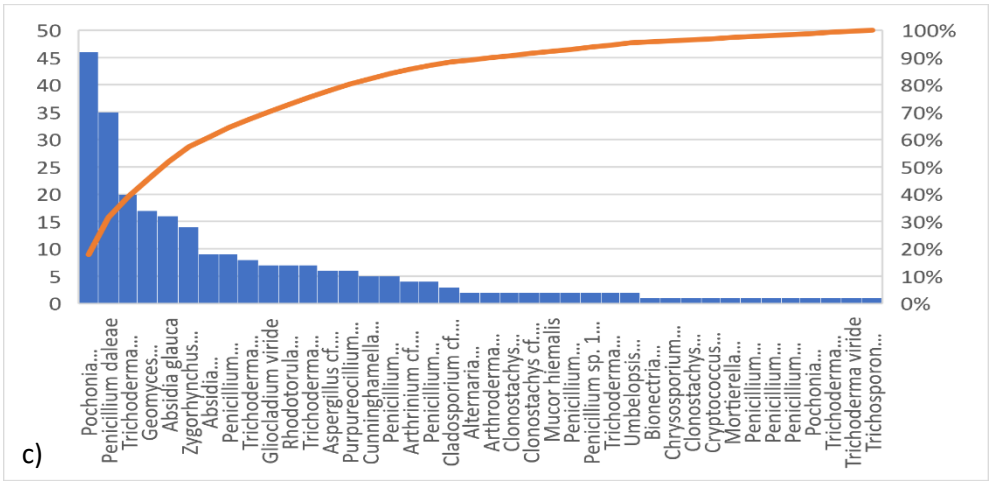


Fig. 12 Pareto analysis of the abundance of species on the different types of soils in Germany. a) Bare soil, b) Grassland, c) Forest, blue is the frequency and orange is the accumulated percentage

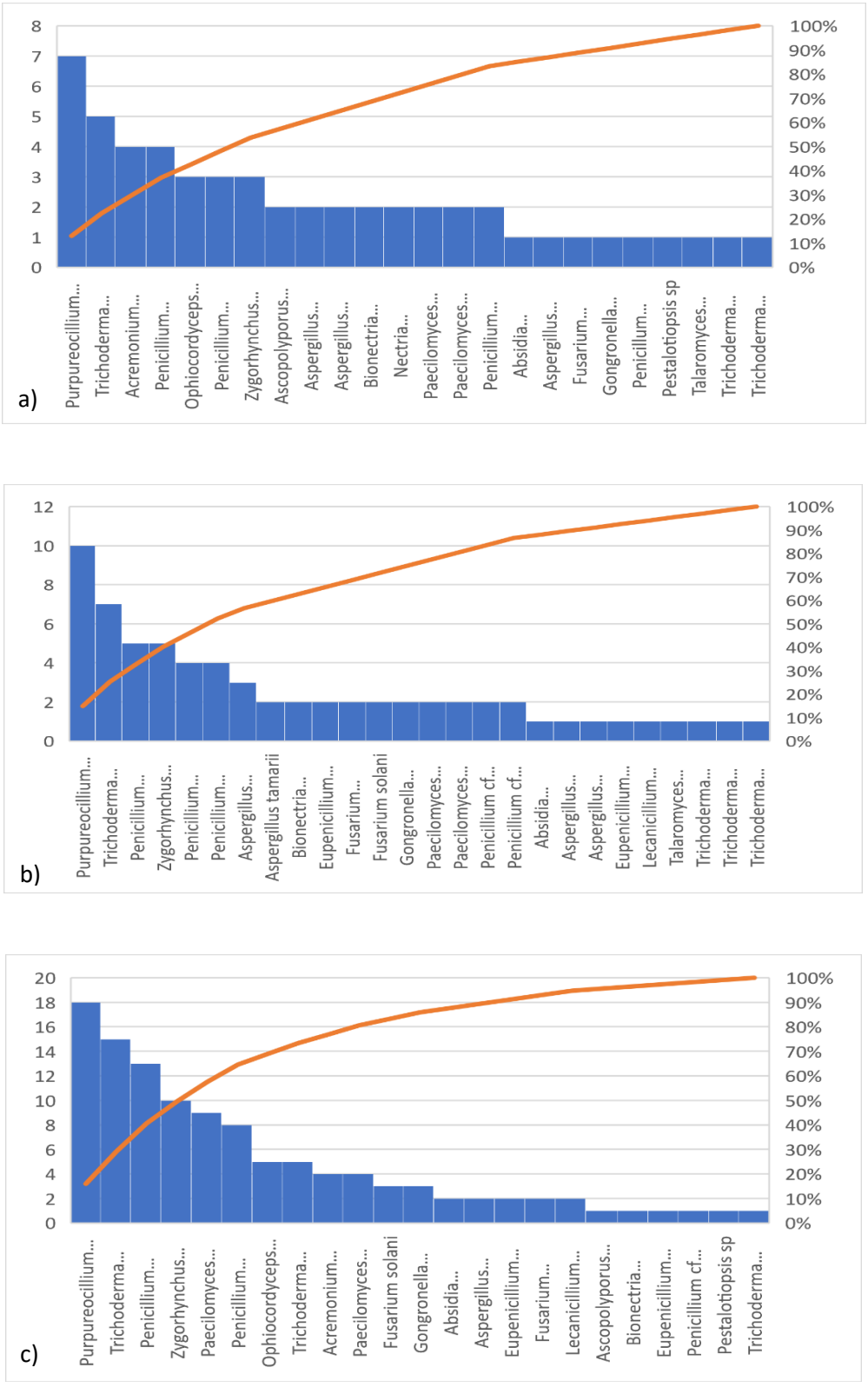


Fig. 13 Pareto analysis of the abundance of species on the different types of soils in Panama. a) Bare soil, b) Grassland, c) Forest, blue is the frequency and orange is the accumulated percentage

The Pareto analysis in Germany (Fig. 12) shows that, in the three soils, are two dominant species (*Pochonia bulbillosa* and *Penicillium daleae*). These species increase as the vegetation likewise. There is a second group of species that is found in all soils. Its abundance is higher than the rest of the species (*Absidia glauca, Trichoderma hamatum, Geomyces pannorum, Penicillium simplicissimum, Trichoderma asperellum*). The third group comprises species whose abundance is minor (from one to five individuals) but present in all soils and finally, the fourth group where the abundance and presence differ in each soil.

The Pareto analysis in the soils of Panama (Fig. 13) showed the dominance of two species (*Purpureocillium lilacinum* and *Penicillium daleae*). Like in Germany, these species increase as degradation decreases. The second group with the highest abundance comprises three species (*Penicillium citrinum, Penicillium simplicissimum, Zygorhynchus moelleri Vuill*). The third group includes species with minimal abundance (from one to three individuals). Ending with the fourth group where the species present contain one to two individuals and their presence varies in each soil.

6.5 Percentage of orders by morphology identification

Based on the morphological identification, the species were grouped in their corresponding orders to which they belong, obtaining the following results.

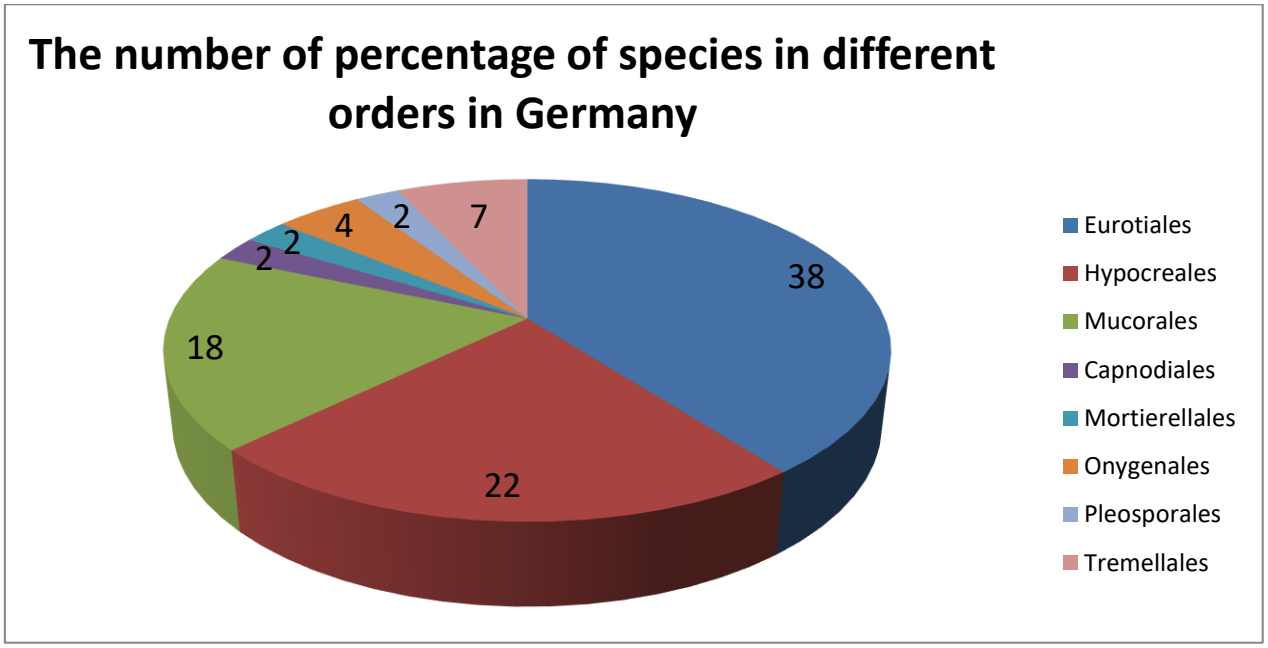


Fig. 14 Classification by orders in Germany. Adapted from Rosas *et al.* (2019)

Grouping the orders of figure 8, it was found that around 70 % of the species isolated from the German sites were Ascomycota, 20 % were Zygomycota, and only 7 % were Basidiomycota (represented just for Tremellales). This dominance by Ascomycota is common in soil fungi community in the forest.

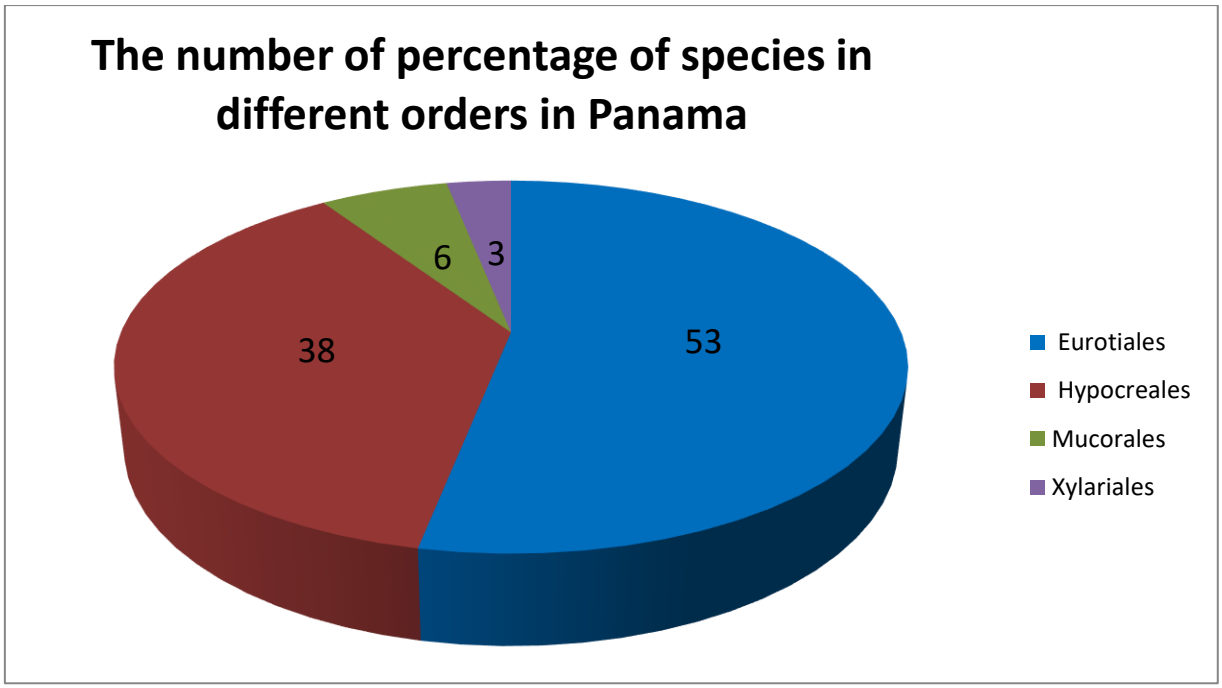


Fig. 15 Classification by orders in Panama. Adapted from Rosas *et al.* (2019)

Grouping the orders of figure 9, it was found that around 94 % of the species isolated from the Panamanian sites were Ascomycota and 6 % were Zygomycota, while no Basidiomycota were isolated. Therefore, the proportion of Ascomycota in relation to the other phyla of fungi is much higher. Within the Ascomycota the orders Eurotiales and Hypocreales represent the highest percentage of species.

6.6 Diversity indices applied to morphologically identified samples

The diversity indices were calculated to quantify biodiversity and the results, in general, show a high diversity (greater than level three on the shannon index) in both countries.

Shannon index was plotted because this index explains the evenness and the abundance of the species present. Results show a high diversity in both countries.

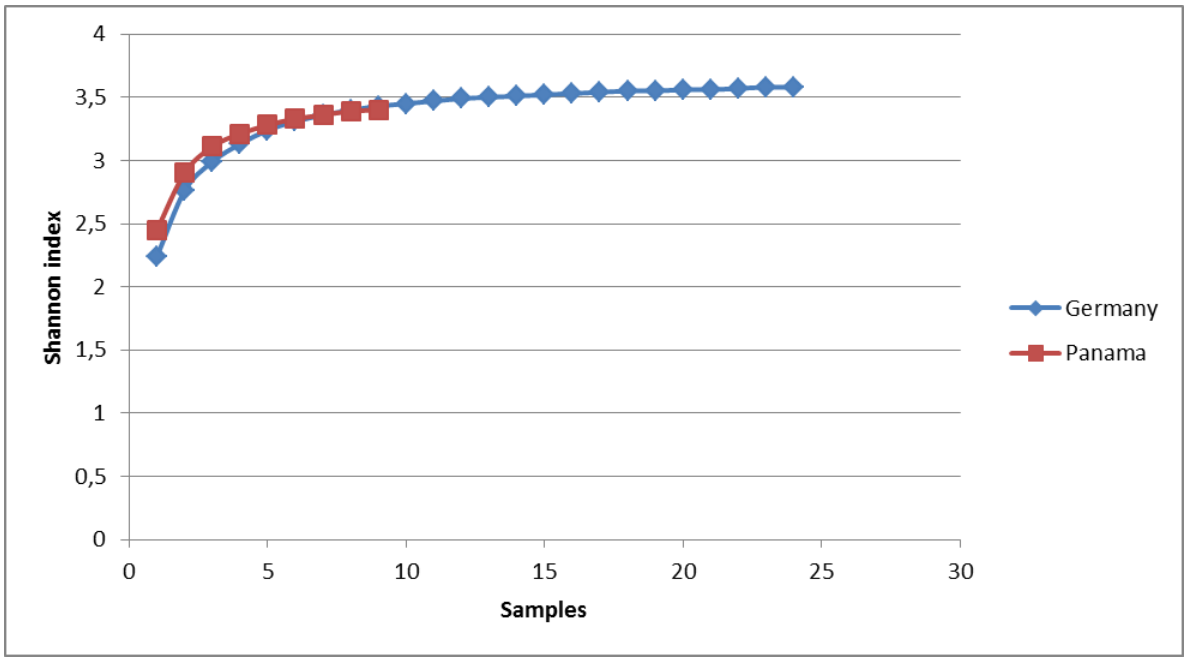


Fig. 16 Species rarefaction curves showing the increase in Shannon diversity with sampling effort for both Germany and Panama

Figure 10 shows the accumulation of species across the sampling, with similar diversity values in both sites. Forest in Germany and Panama are different for the environmental factors, as previously mentioned before the number of sampling was different at each site; however, both sites have high diversity. Shannon index is used to measure the specific biodiversity where the values fewer than two are considered to be low in biodiversity, but the upper three are high in biodiversity. Regardless of the area and environmental factors, the fungal soil communities have a high diversity.

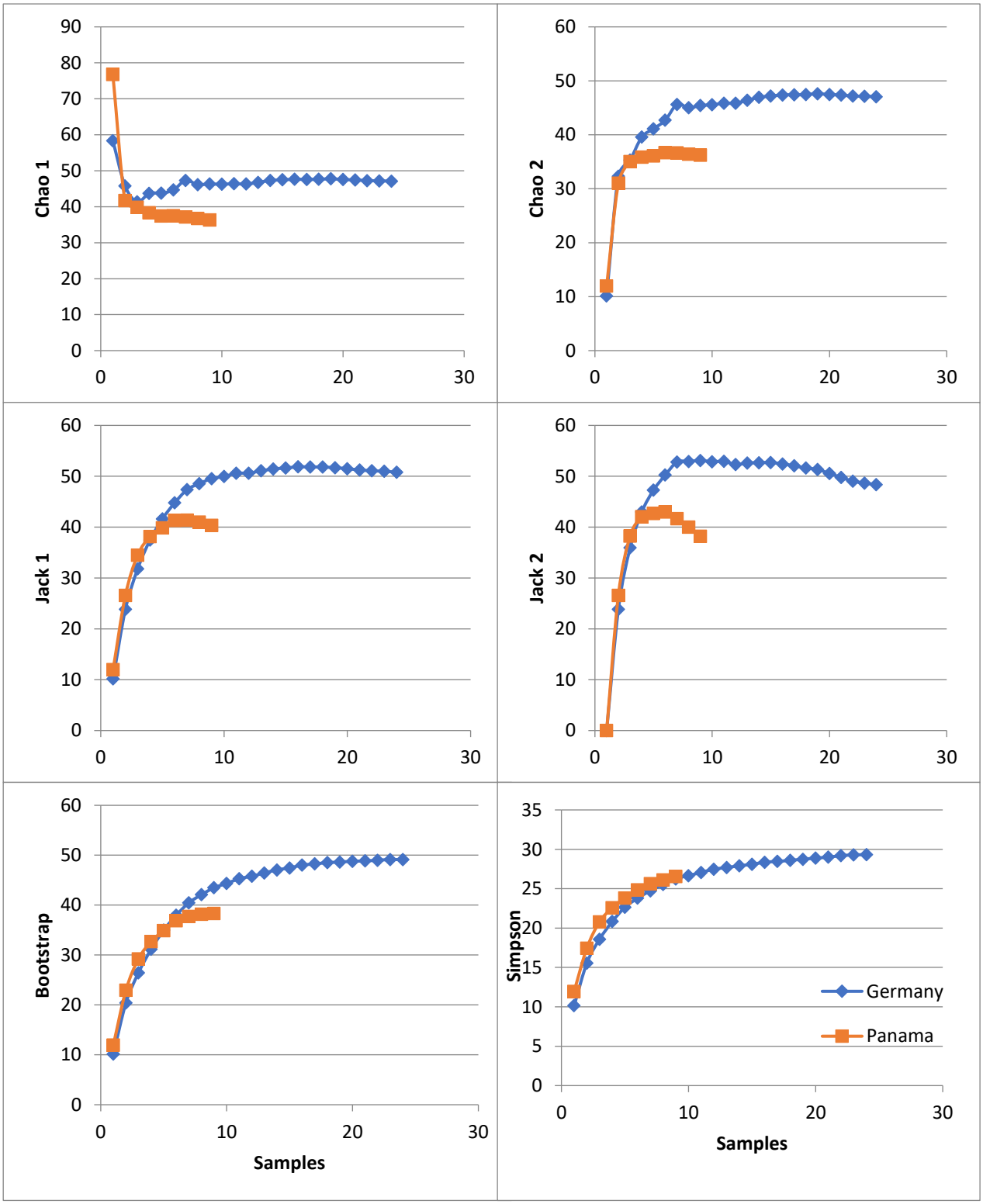


Fig. 17 Rarefaction Curves using each of the measures of richness and diversity in Germany and Panama

Diversity indices give a summary of richness and evenness in a single statistic, highlighting that there are different ways to combine both measures resulting in different diversity indices.

The result of the diversity indices is shown in Fig. 17. Chao1 and 2 show the more significant differences between the species observed and the species expected. Jack 1 shows that the expected species in Germany are around 50 and 40 for Panama, while Jack 2 shows some expected species more in both countries, the differences are minimal. Bootstrap estimate the expected species similar to a jack 1 and 2, while in the Simpson index was observed more similarities with the richness species data, also, when increasing the number of species show the species distribution in proportions. Result with this index show high diversity like the Shannon index. The indices result confirm that high diversity and representative samples were obtained in both countries.

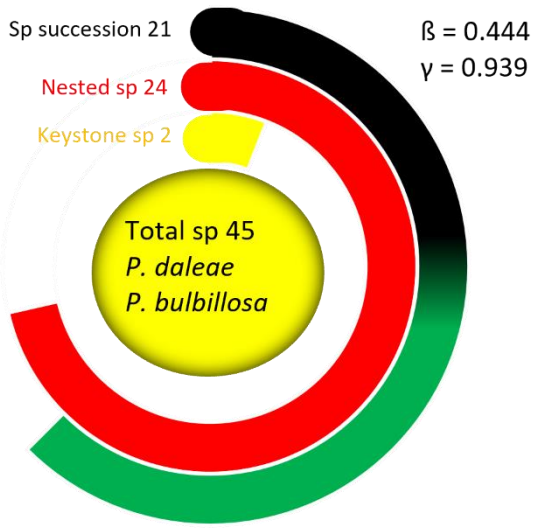


Fig. 18 Beta and gamma diversity in Germany

The beta diversity marks the dissimilarities between the populations and ranges the accumulation of species when adding the areas, as observed in the Pareto analysis, there are two dominant species in the three soils studied. Likewise, there are 24 species nested in the three soils while there is a succession of 21 species through degraded soils.

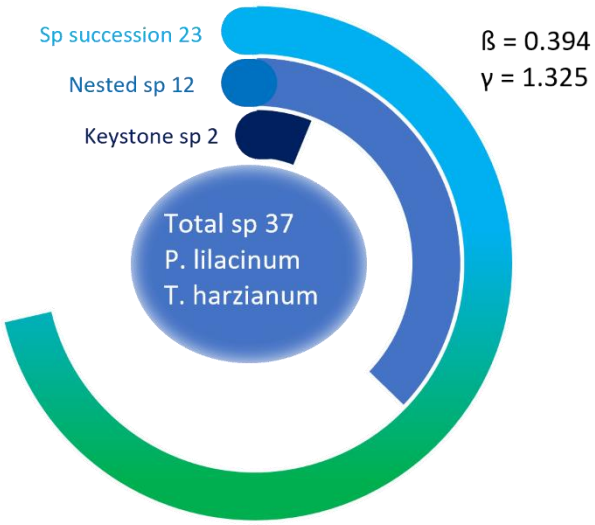


Fig. 19 Beta and gamma diversity in Panama

The beta diversity marks a dissimilarity of 39 % between the populations, and the gamma diversity shows that in all the areas added there are 1.3 populations in the three areas. Two dominant species are shown with 12 nested, and the number of species in succession or by loss adds up to 23 species through the studied soils.

6.7 Analysis of abundance of species in different types of soils

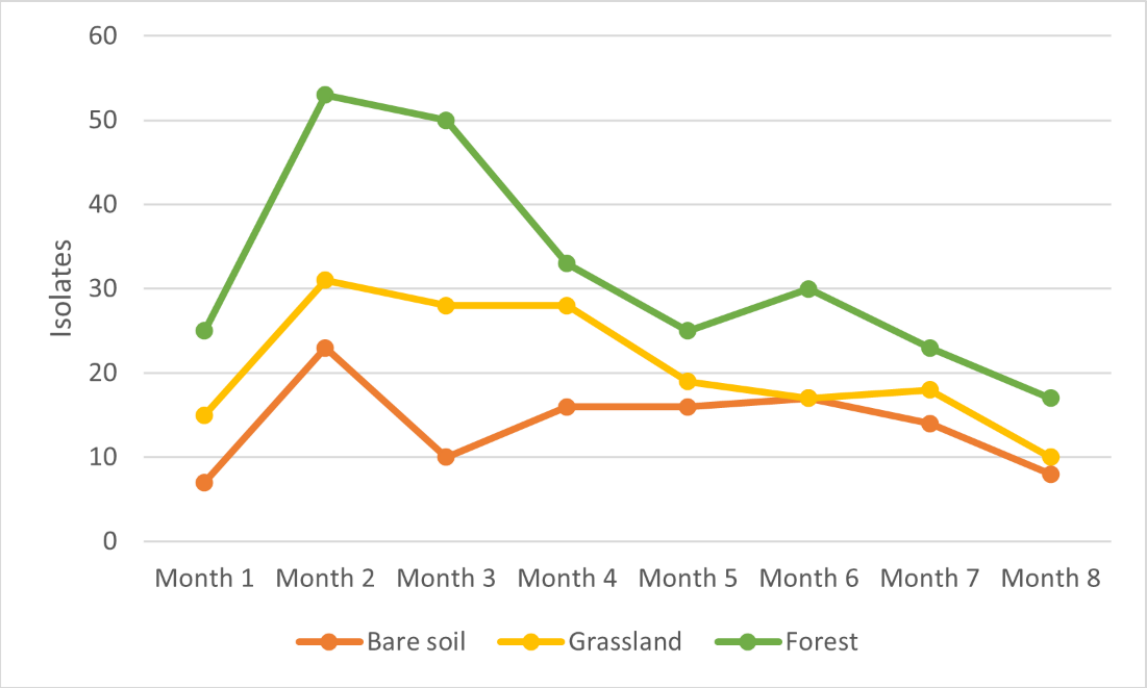


Fig. 20 Recorded abundance over time in Germany by morphological identification

Degradation of soil has an effect directly on the abundance. The maximum number of individuals present in a bare soil sample is 23 in the second month, while the first and last have seven and eight, respectively. In the forest, the number of individuals in each sample is twice the amount of bare soil. The samples from grassland show a transition in the number of individuals closer to the bare soil.

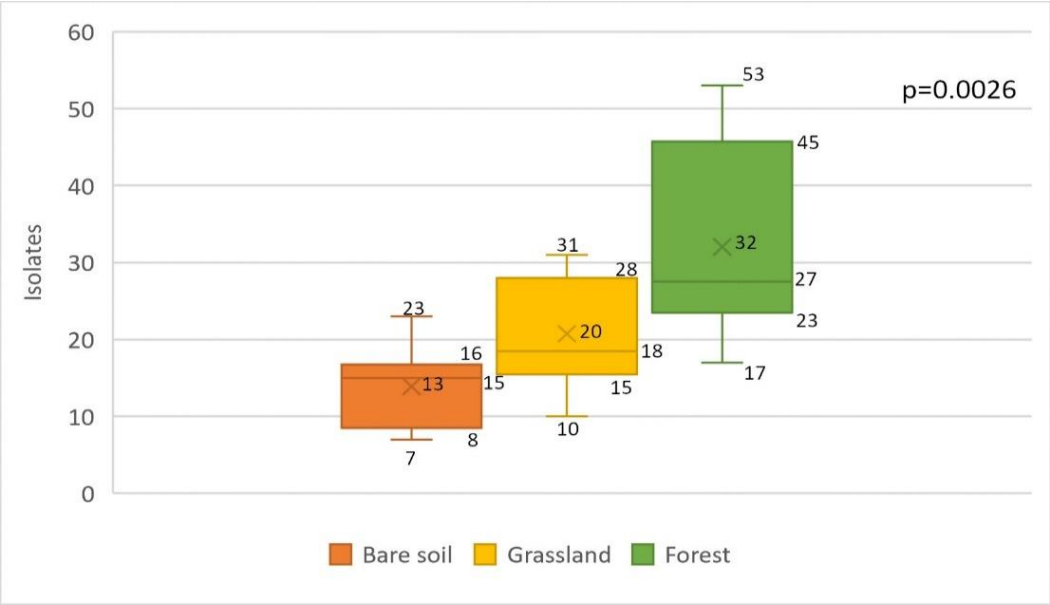


Fig. 21 Abundance in each of the soils in Germany

The ANOVA test applied to the abundance present in the different soils of Germany showed statistically significant differences, for which a Tukey test was carried out. The main difference was found between the bare soil and the forest soil. Grassland soils did not have statistically significant differences from bare and forest soils. Grassland works as a transition gradient.

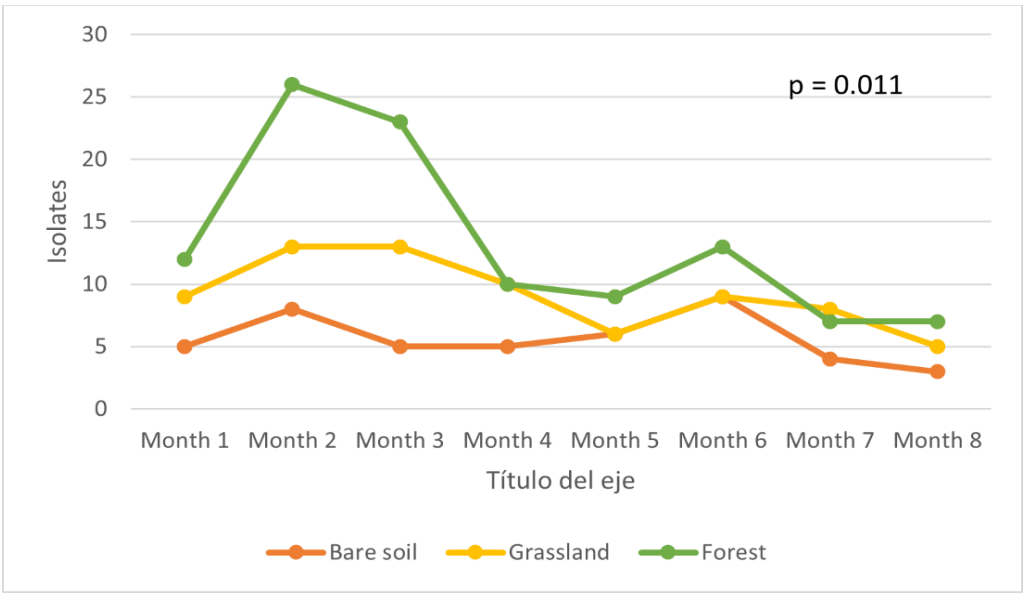


Fig. 22 Recorded Hypocreales abundance over time in Germany

Hypocreales order showed a significant difference in the abundance present in the different types of soil in Germany. The variation in abundance on bare soil was from three individuals to nine, maintaining a mean of five, while on grassland it remained between five to thirteen. However, on the forest soil, the variation in May was from seven to twenty-three.

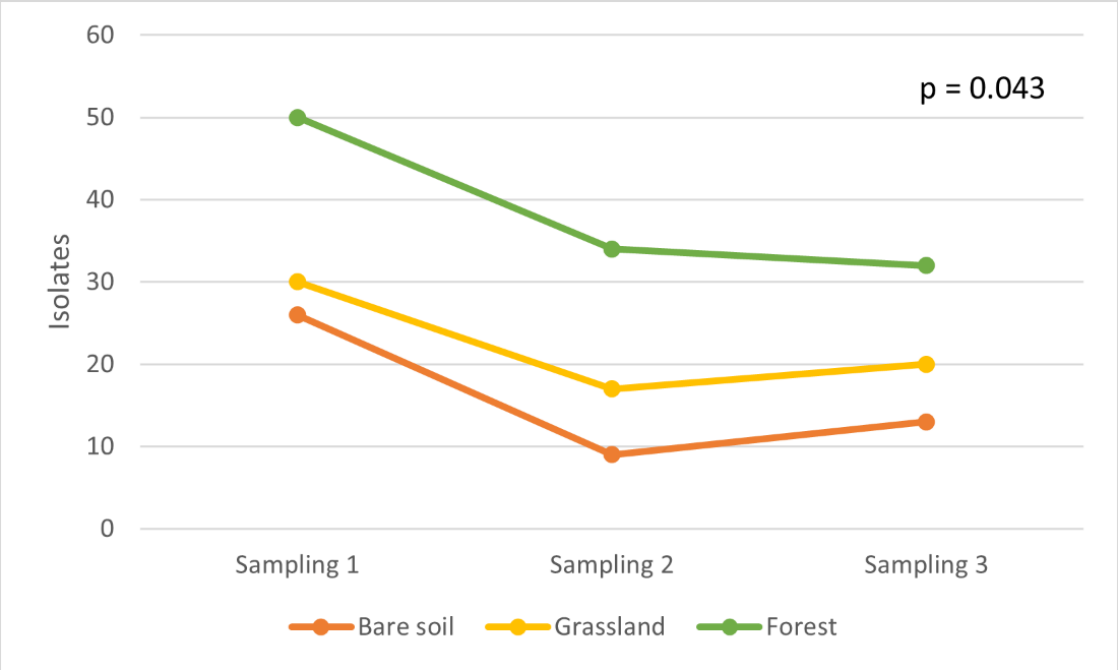


Fig. 23 Recorded abundance over time in Panama by morphological identification

Panama has only three samples; however, degradation has a statistically significant effect on abundance (based on the ANOVA test). Tukey's test showed that the main difference is between the bare soil and the forest soil. The grassland did not present statistically significant differences with any of the other two soils, assuming this soil is a transition between the abundances of the soils.

6.8 Comparison of methodologies

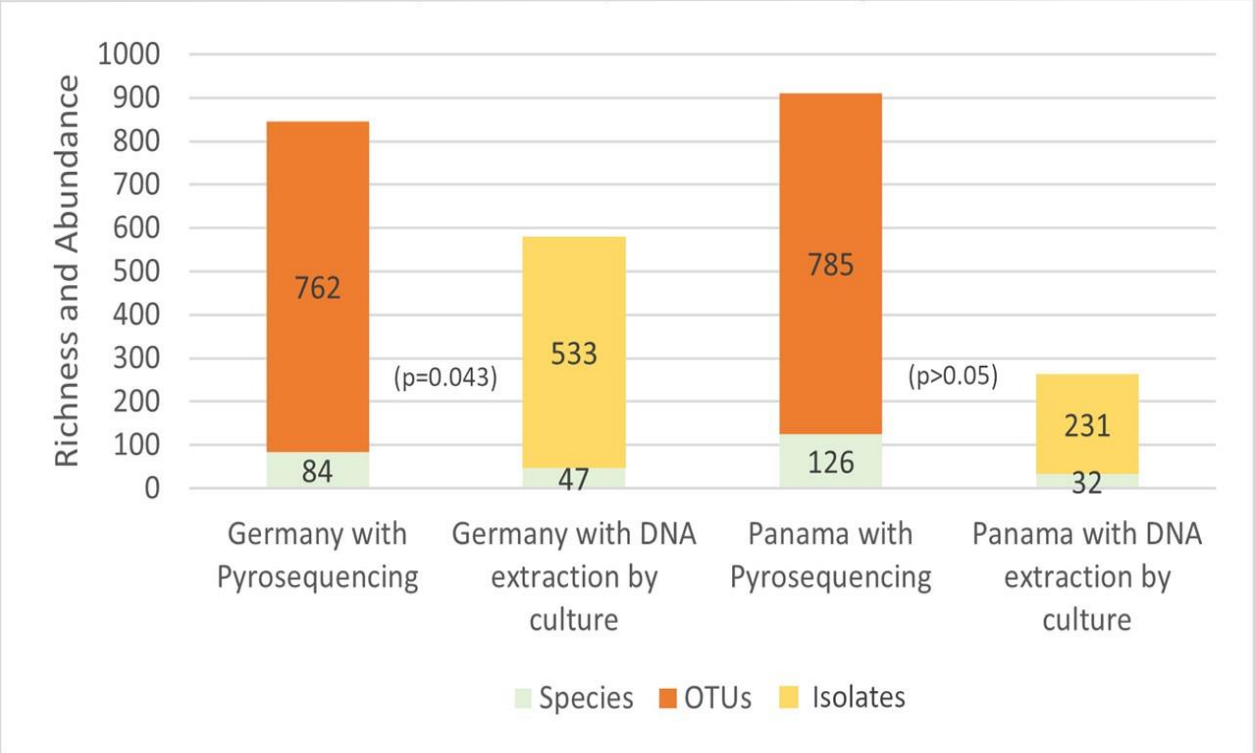


Fig. 24 Comparison of abundances and richness obtained with the two methodologies

Student's t-test showed statistically significant differences between the methodologies used in obtaining abundance in Germany, but no differences were found in species richness. There were no significant differences between the methods in abundance and species richness in Panama. It is worth mentioning that among the methodologies, it shares six species (*Absidia cylindrospora, Cryptococcus podzolicus, Nectria mariannaeae, Paecilomyces carneus, Pochonia bulbillosa, Trichoderma hamatum*).

6.9 Identification of the ten new records found in Panama

Ten new records were found in Western Panama, identified based on morphological and molecular characteristics, five species belong to Eurotiales (*Aspergillus roseoglobulosus*, *A. tamarii*, *Penicillium janczewskii*, *P. miczynskii*, *Talaromyces verruculosus*), four species belong of Hypocreales (*Mariannaea elegans*, *Purpureocillium lilacinum*, *Trichoderma harzianum*, *T. spirale*), and one species belong of Mucorales (*Mucor moelleri*). Four of these species, namely *A. roseoglobulosus*, *M. moelleri*, *P. janczewskii*, and *T. verruculosus*, are also new for Central America.

Phylum: Ascomycota

Order: Eurotiales

Species: *Aspergillus roseoglobulosus* Frisvad & Samson, in Frisvad, Frank, Houbraken, Kuijpers & Samson, Stud. Mycol. 50: 30 (2004).

Macroscopical description. Colonies frequently form agglomerations in circles, pink with white and yellow, cottony. Reverse of culture lightly red to pink. Colony on MEA attaining 1.5 cm diam. after 10 days at 25 °C.

Microscopical description. Hyphae septate, 6−9(−12) µm wide, single hyphal cells (69−)83−102(−120) µm long, hyaline. Vesicles at the tip of the conidiophores globose, (21−)33−102(−120) µm long. Conidial heads biseriate. Metulae (2−)3−4(−5) µm wide and (5−)6−8(−11) µm long. Phialides flask-shaped, 2−3(−4) µm wide and (6−)7−9(−11) µm long. Conidia globose to subglobose, 2–3 µm wide, hyaline, with rough surface.

Material examined: Majagua, bare soil, 18.07.2012, M. Rosas *et al.* P1M37 (FR-0247033), ITS sequence KY320597.

Ecology and distribution. *Aspergillus roseoglobulosus* has been found in subtropical and tropical zones. It has been recorded from Argentina, Brazil, Hawaii, and Pakistan (Frisvad *et al.* 2004). *A. roseoglobulosus* is reported here in Panama for the first time.

Sequencing results

Table 2 shows the results of BLAST search performed with the ITS sequence of the sample number P1M37 including 629 base pairs.

Table 2: BLAST search results for the ITS region of sample P1M37

Number of sequence	Species	Query coverage in %	Maximum identity in %	More sequences with significant alignments
FJ491583	*Aspergillus roseoglobulosus*	57	99	2
FR733827	*Aspergillus sclerotiorum*	57	98	15
EF661408	*Aspergillus sulphureus*	57	98	3
EF200084	*Aspergillus bridgeri*	57	98	2
FR733836	*Aspergillus persii*	57	98	3

Notes. According to the data in GenBank and the BLAST search, the strain P1M37 matches with *Aspergillus roseoglobulosus* (two sequences with significant identities). Molecular identification of strain P1M37 also matches with 98 % with *Aspergillus sclerotiorum*, *A. sulphureus*, *A. bridgeri*, and *A. persii.* However, its morphological characteristics correspond to A*. roseoglobulosus* because of the rose red mycelium and rose red reverse of the colony on MEA (Frisvad *et al*. 2004). *A. sclerotiorum* produces a creamy-yellow layer of conidia and it is frequently observed to occur in soil depths of 30−50 cm (Christensen 1982).

A. roseoglobulosus produces a similar yellow layer of conidia, but its mycelium is pink and it is reported from superficial soil layers (Frisvad *et al.* 2004). On the other hand, *A. sulphureus* is yellow and produces a discoloration zone in agar plates (CDA and MEA medium), while *A. roseoglobulosus* does not present any discoloration in agar plates (Kumar *et al.* 2012). *A. bridgeri* differs from *A. roseoglobulosus* by a colony reverse with ochre color and the diameter of conidial heads (up to 200−260 µm; Christensen 1982). *A. persii* is similar to *A. sclerotiorum* on the basis of morphological characteristics but *A. persii* only grows on certain substrates and at specific temperatures, and *A. persii* differs from *A. roseoglobulosus* by the yellow color of the mycelia (Zotti *et al*. 2010). Morphological and molecular characteristics indicate that the strain number P1M37 corresponds to *A. roseoglobulosus,* but this identification should be complemented by a chemical analysis (Samson *et al.* 2006).

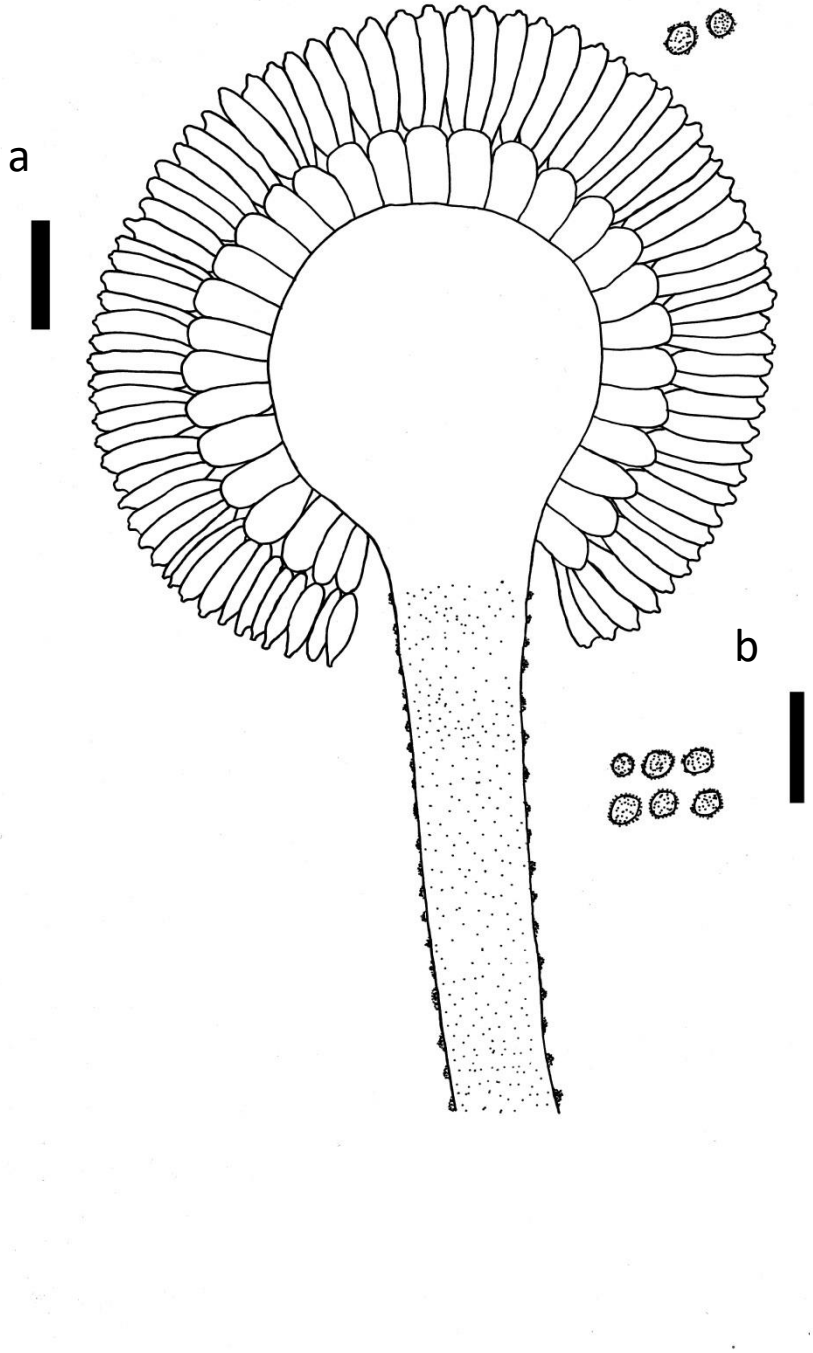


Figure 25: *Aspergillus roseoglobulosus* (P1M37). a. Conidiophore with metulae and phialides. b. Rough conidia. Bars = 10 µm.

Species: *Aspergillus tamarii* Kita, Zentbl. Bakt. Parasitkde, Abt. II 37: 433 (1913).

Macroscopical description. Colonies frequently form agglomerations in circles, olive green, powdery. Reverse of culture greenish to brown. Colony on MYP attaining 1.5 cm diam. after 10 days at 25 °C.

Microscopical description. Hyphae septate, 4−5(−6) µm wide, single hyphal cells (19−)25−62(−86) µm long, hyaline. Conidiophores composed of an unbranched stipe, 5−9 µm wide and up to (120−)154−248(−300) µm long, hyaline, walls rough. Vesicle globose, (11−)34−36(−42) µm wide and up to (15−)36−18(−47) µm long. Conidial heads uni- and biseriate. Metulae present 3−7(−8) µm wide and (3−)4−9(−12) µm long. Phialides flask-shaped, 4−5(−6) µm wide and up to (7−)8−11(−15) µm long. Conidia globose to subglobose, 4–5(−6) µm wide, green, with echinulate walls.

Material examined: Majagua, bare soil, 18.07.2012, M. Rosas *et al.* P1M16 (FR-0247034), ITS sequence KY320598.

Ecology and distribution. *Aspergillus tamarii* has frequently been isolated from cultivated soils (rice, wheat, citrus, and coconut groves), soils under legume crops, and tropical rain forest, Himalayan forest, and estuarine sediments. It has been recorded from Bangladesh, Bahamas, China, Costa Rica, Gambia, India, Iraq, Israel, Japan, Libya, Malaysia, Pakistan, Peru, Somalia, Spain, Syria, and the USA (Domsch *et al.* 2007, Markham & Herren 1990). *A. tamarii* is reported here for Panama for the first time.

Sequencing results

Table 3 shows the results of BLAST search performed with the ITS sequence of the sample number P1M16 including 583 base pairs.

Table 3: BLAST search results for the ITS region of sample P1M16

Number of sequence	Species	Query coverage in %	Maximum identity in %	More sequences with significant alignments
GU362011	*Aspergillus tamarii*	70	99	36
JQ676205	*Aspergillus caelatus*	70	99	8
GQ340557	*Aspergillus parasiticus*	70	99	13
JX852612	*Aspergillus flavus*	70	99	5
JQ812709	*Aspergillus sojae*	70	98	2
JN185451	*Aspergillus chungii*	70	98	1
JF446613	*Aspergillus transmontanensis*	70	98	2
JN217237	*Aspergillus oryzae*	70	98	2
HM560046	*Aspergillus toxicarius*	70	98	1
AF338641	*Aspergillus bombycis*	70	98	3

Notes. According to data in GenBank and BLAST search, the strain P1M16 matches with *Aspergillus tamarii* (36 sequences with significant alignments), *A. caelatus* (eight sequences), *A. flavus* (five sequences), and *A. parasiticus* (13 sequences). According to Raper and Fennell (1965) the section *Flavi* is a group containing nine species and two varieties that include *A. flavus*, *A. tamarii*,

A. caelatus, and *A. parasiticus*, which are closely related but can be distinguished by the color of the conidia, presence or absence of metulae, ornamentation, size of the conidia, and DNA fingerprint (Castillo 2007, Horn 1997, Kurtzman *et al.* 1987, McAlpin *et al*. 2005). Morphologically, *A. caelatus* is closely related to *A. tamarii* because both species have conidia with rough surface (Ito *et al.* 1998). However, they can be distinguished by differences in the ornamentation of the conidia, i.e., conidia of *A. tamarii* have an echinulate surface while conidia of *A. caelatus* have a tuberculate surface, and the color of the colonies after 10 days of growth, i.e., *A. caelatus* shows a change of green to brownish-green and does not produce cyclopiazonic acid (Horn 1997, Ito *et al.* 1999). The other closely related species is *A. parasiticus,* which can be distinguished from *A. tamarii* by the color of the phialides (usually hyaline to pale green), the color of the conidia (yellow-green), and the production of aflatoxin (Kurtzman *et al.* 1987, Samson *et al.* 2004). *A. tamarii* differs from *A. flavus* (with conidia 3.5−4.5 µm wide) by conidia that reach a diameter of up to 6 µm (Domsch *et al.* 2007). Genetically, *A. tamarii* is closely related to *A. caelatus*, but there are some nucleotide differences that allow to distinguish the DNA sequences of these two species in the regions of ITS1, ITS2, 5.8S rDNA, and 28S rDNA (Ito *et al.* 1999). To determine *Aspergillus* species belonging to the section *Flavi* it is necessary to use not only the morphological characteristics but also the characteristics related to the production of mycotoxins, the analysis of the color of colonies using digital images, and DNA sequences (Horn 1997, Horn *et al.* 1996, Samson *et al.* 2006). The morphological characteristics of the strain P1M16 confirm its identification as *A. tamarii*.

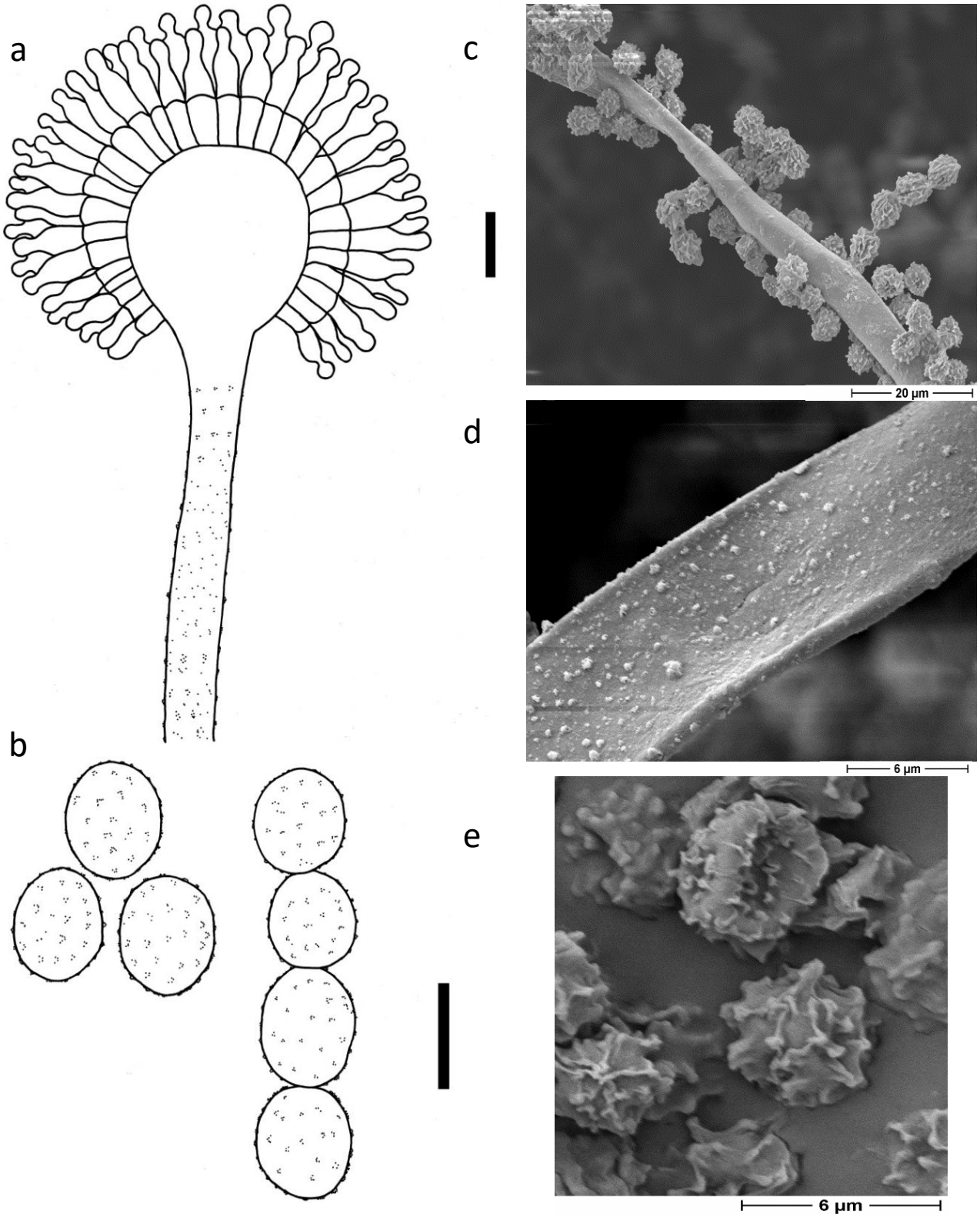


Figure 26: *Aspergillus tamarii* (P1M16). a. Conidiophore with metulae and phialides. b. Echinulate conidia. c. Stipe of a conidiophore with scattered conidia. d. Detail of the conidiophore showing its ornamentation. e. Echinulate conidia. Bars = 5 µm.

Species: *Penicillium janczewskii* K.M.Zaleski, Bull. Acad. Polon. Sci., Math. et Nat., Sér. B: 488 (1927).

Macroscopical description. Mycelium growing fast, covering the Petri dish after 10 days (9 cm diam.) of cultivation at 25 °C. Colonies dark olive green colored, frequently forming agglomerations on their surface.

Microscopical description. Hyphae septate. Stipes of conidiophores 2–3 µm wide and (15−)26−46(−55) µm long. Rami 2–3 µm wide and (7−)13–27(−35) µm long, hyaline. Ramuli 2–3(–4) µm wide and 8–17(−22) µm long. Metulae at the tips of lateral branches, irregularly divergent, with smooth walls, 1–3(−4) µm wide and (3−)4–12(−20) µm long. Phialides flask-shaped, cylindrical, tapering towards the apex, 1–3 µm wide and (4−)6–9(−10) µm long. Conidia in chains, subglobose to globose, 2–4 µm diam., brown, with rough walls.

Material examined: Majagua, bare soil, 18.07.2012, M. Rosas *et al.* P1M5 (FR-0247035), ITS sequence KY320624.

Ecology and distribution. *Penicillium janczewskii* has frequently been isolated from soil of forests in temperate zones, grassland, and arable soils. It has been recorded from Austria, the Bahamas, Brazil, Egypt, India, Italy, Israel, Japan, Peru, and Syria (Daynes *et al.* 2008, Domsch *et al.* 2007). *P. janczewskii* is reported here for Panama for the first time.

Sequencing results

Table 4 shows the results of BLAST search performed with the ITS sequence of the sample number P1M5, including 321 base pairs.

Table 4: BLAST search results for the ITS region of sample P1M5.

Number of sequence	Species	Query coverage in %	Maximum identity in %	More sequences with significant alignments
FJ230987.1	*Penicillium janczewskii*	100	98	3
JN617693.1	*Penicillium jensenii*	100	97	2
KC812360.1	*Penicillium* sp.	99	98	27
JX045775.1	*Penicillium canescens*	98	96	3

Notes. The morphological identification of *Penicillium janczewskii* is based on its conidia, which are rough-walled and brown as seen by light microscopy. These characteristics and the size of the structures agree with those presented for *P. janczewskii* by Domsch *et al.* (2007). Molecular data (table 4) indicate a close relationship of the strain P1M5 with *P. canescens*, *P. janczewskii*, and *P. jensenii*. *P. janczewskii* has strongly divergent metulae and the conidial walls are rough, while conidia are smooth in *P. jensenii* (Domsch *et al.* 2007, Martínez 2003). *P. canescens* is morphologically similar to *P. janczewskii* in size and shape of conidia, but conidia of *P. canescens* are smooth-walled to slightly roughened, while conidia of *P*. *janczewskii* are coarsely warted. Conidiophores of *P. canescens* are conspicuously roughened and those of *P. janczewskii* smooth (Domsch *et al.* 2007, Pessoni *et al.* 2002).

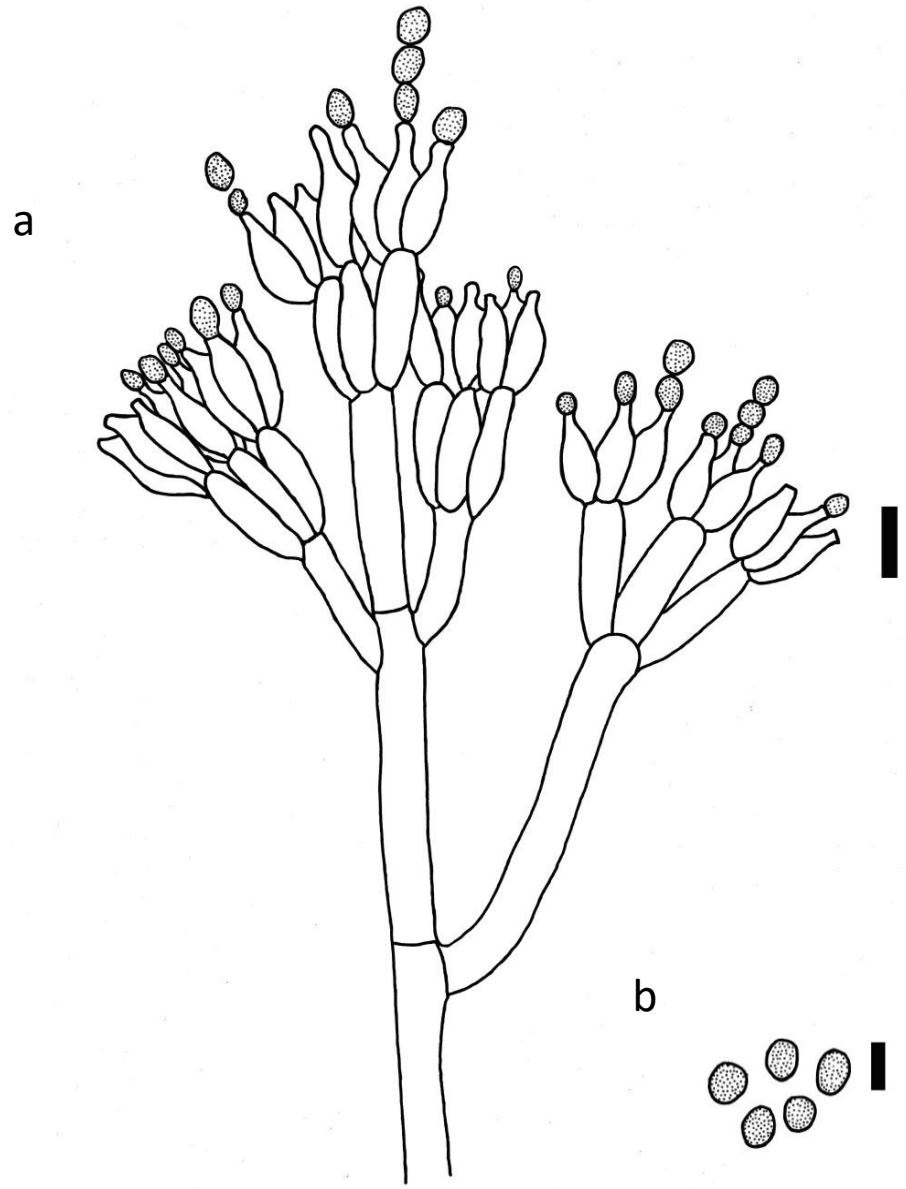


Figure 27: *Penicillium janczewskii* (P1M5). a. Conidiophore with phialides and conidia. b. Conidia. Bars = 5 µm.

Species: *Penicillium miczynskii* K.M.Zaleski, Bull. Acad. Polon. Sci., Math. et Nat., Sér. B: 482 (1927).

Synonym: *Penicillium shearii* Stolk & D.B.Scott, Persoonia 4: 396 (1967).

Macroscopical description. Colonies green with white borders, frequently forming ring-like agglomerations on their surface. Mycelium growing fast, covering the Petri dish after 14 days (9 cm diam.) of cultivation at 25 °C.

Microscopical description. Hyphae septate. Stipe of the conidiophore 3–5 µm wide and (130−)155−181(−210) µm long. Metulae irregularly divergent, 1–3 µm wide and (11−)14–17(−20) µm long. Phialides flask-shaped, cylindrical, tapering towards the apex, 2–3 µm wide and (5−)7–8(−10) µm long. Conidia in tangled chains, subglobose to ovate, 1−2 µm wide and 2–3 µm long, with finely rough ornaments.

Material examined: Majagua, bare soil, 30.07.2012, M. Rosas *et al.* P1M78 (FR-0247036), ITS sequence KY320611.

Ecology and distribution. *Penicillium miczynskii* has been frequently isolated from soil of tropical and subtropical areas (Tuthill & Frisvad 2004). It has been recorded from Austria, Central Africa, Colombia, Honduras, Japan, New Guinea, South Africa, and Sierra Leone (Domsch *et al.* 2007). *P. miczynskii* is reported here for Panama for the first time.

Sequencing results

Table 5 shows the results of BLAST search performed with the ITS sequence of the sample number P1M78 including 636 base pairs.

Table 5: BLAST search results for the ITS region of sample P1M78.

Number of sequence	Species	Query coverage in %	Maximum identity in %	More sequences with significant alignments
KJ439199	*Penicillium shearii*	94	99	7
KJ191428	*Penicillium sanguifluum*	95	97	4
GU566236	*Penicillium roseopurpureum*	95	97	5
JN617685	*Penicillium copticola*	87	97	1
JN617672	*Penicillium manginii*	95	96	1

Notes. Zaleski described *P. miczynskii* in 1927 from the soil of a *Picea* forest in Poland. Both Thom (1930) and Raper and Thom (1949) accepted *P. miczynskii* as valid and unique and provided full descriptions based upon a detailed examination of derivatives of the Zaleski isolates. Pitt (1979) placed *P. chrzaszczii, P. matris-meae*, and *P. miczynskii* (cited as *P. soppii*), also described by Zaleski in 1927, and four additional species (*P. atrosanguineum*, *P. manginii*, *P. pedemontanum*, and *P. syriacum*) into synonymy with *P. miczynskii* and accordingly expanded the species circumscription (Christensen *et al*. 1999). *Penicillium soppii* is the anamorph name for *Eupenicillium shearii* (Stolk & Samson 1983). However, according to Index Fungorum, *Eupenicillium shearii* is a synonym of *P. miczynskii*, and a synonym of *P. manginii* according to Stolk & Samson (1983).

According to data in GenBank, the strain P1M78 corresponds to *P. shearii* with 99 % of identity (Table 5). The sequence of the strain P1M78 showed a relationship with *P. sanguifluum*, *P. roseopurpureum*, and *P. copticola.* Morphologically, *P. miczynskii* resembles *Eu. saturniforme* and *Eu. tropicum*, but it differs from them by strictly velutinous colony texture with abundant conidiogenesis and finely rough conidia (Tuthill & Frisvad 2004). *Eupenicillium saturniforme* can be differentiated from *P. miczynskii* by robust biverticillate penicilli, apically vesiculate metulae and rough-walled stipes (Wang & Zhuang 2009). *Penicillium roseopurpureum* morphologically differs from *P. miczynskii* by its strictly monoverticillate conidiophores (Vega & Posada 2006). *Penicillium copticola* can be differentiated by an analysis of spectroscopic data and by the color of the culture (Daengrot *et al.* 2014). *Eupenicillium bovifimosum* and *Eu. baarnense* are also closely related to *P. miczynskii* especially by similar shape and size of conidia. Nevertheless, they can be differentiated by the production of whorls of up to five metulae (Tuthill 2002). Those species are not represented in GenBank, therefore chemical analysis would be necessary to identify them (Belofsky *et al.* 1998). *Penicillium miczynskii* may be identified by secondary metabolites, this species produces kaitocephalin when cultivated on a solid medium (Shin-ya *et al*. 1997).

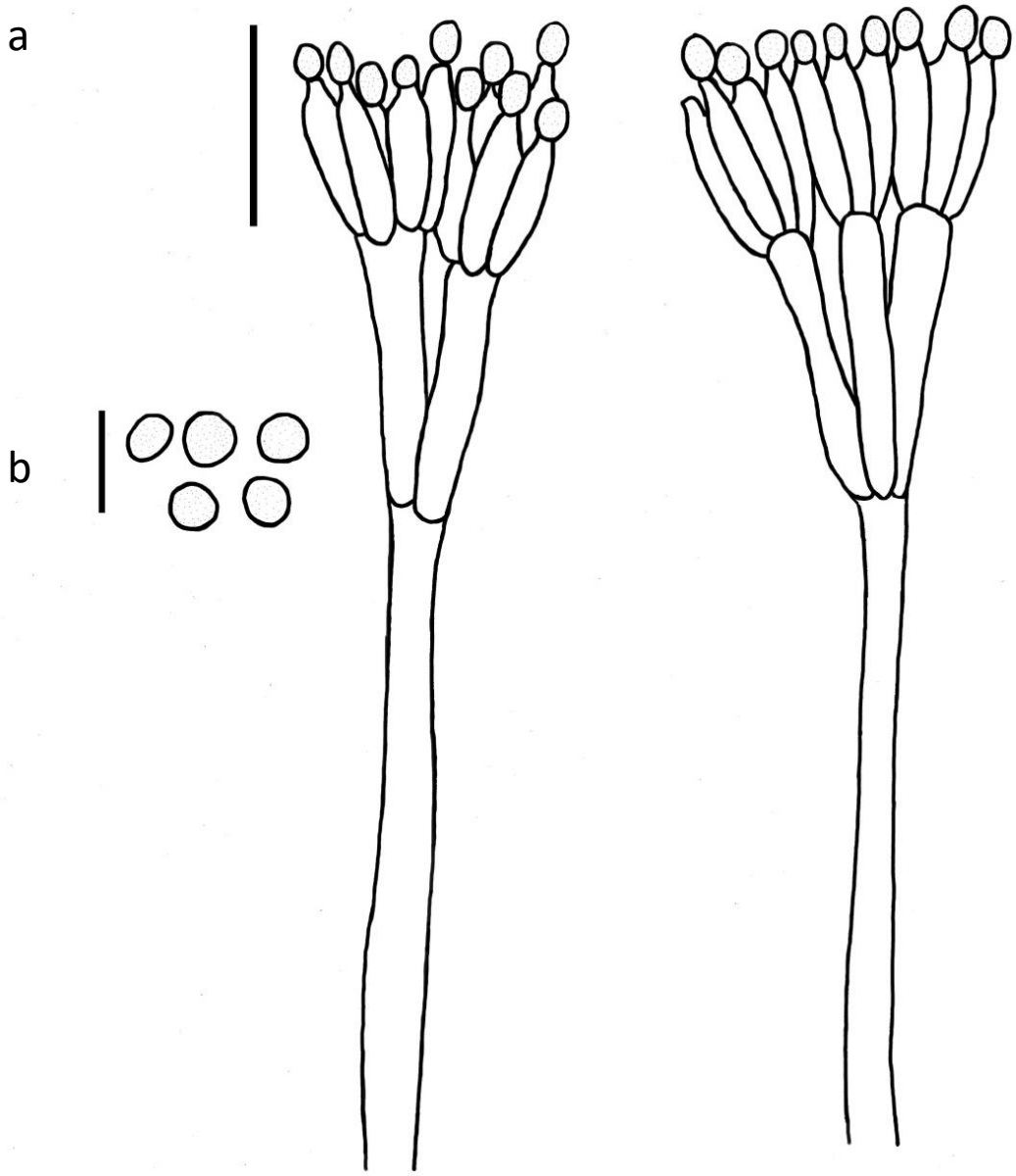


Figure 28: *Penicillium miczynskii* (P1M78). a. Conidiophores with phialides and conidia. b. Conidia. Bars = 5 µm.

Species: *Talaromyces verruculosus* Peyronel, Samson, Yilmaz, Frisvad & Seifert, Studies in Mycology 70: 177 (2011). Fig. 8

Synonym: *Penicillium verruculosum* Peyronel, I germi astmosferici dei fungi con micelio: 22 (1913).

Macroscopical description. Colonies green olive, with white borders, frequently forming ring-like agglomerations on their surface. Reverse of colonies pales reddish yellow. Mycelium growing fast, covering the Petri dish after 7 days (9 cm diam.) of cultivation at 25 °C.

Microscopical description. Hyphae septate. Stipes of conidiophores 2–3 µm wide and (81−)98−136(−144) µm long, smooth. Ramuli 2−3 µm wide and (88−)91–101(−123) µm long. Metulae divergent, 3–4 µm wide and (7−)8–10(−12) µm long. Phialides flask-shaped, cylindrical, tapering towards the apex, 3–4 µm wide and (6−)7–13(−15) µm long. Conidia in disordered chains, spherical to subspherical, 2–3 µm wide, hyaline to subhyaline, with rough walls.

Material examined: Majagua, bare soil, 30.07.2012, M. Rosas *et al.* P1M41 (FR-0247037), ITS sequence KY320632.

Ecology and distribution. *Talaromyces verruculosus* was frequently isolated from soil or roots of plants in grasslands or coniferous forests, in countries like Costa Rica, Denmark, Pakistan, Sweden, the USA, and Taiwan (Bhagobaty *et al.* 2010, Gul Shah *et al.* 2014, Samson *et al.* 2011). In India, it was found among root fungal endophytes in tropical rainforest areas (Bhagobaty *et al.* 2010), whereas in Panama, this species was isolated from grasslands in tropical lowlands.

Sequencing results

Table 6 shows the results of BLAST search performed with the ITS sequence of the sample number P1M41 including 594 base pairs.

Table 6: BLAST search results for the ITS region of sample P1M41.

Number of sequence	Species	Query coverage in %	Maximum identity in %	More sequences with significant alignments
AF510496	*Penicillium verruculosum*	97	99	9
HQ607919	*Penicillium verruculosum* (cited as *Talaromyces verruculosus)*	96	99	7
KF225854	Uncultured *Talaromyces*	97	99	7
JX545189	Uncultured *Penicillium*	97	99	3
KJ461364	Uncultured Ascomycota	96	99	1
HQ392496	*Penicillium aculeatum*	92	99	1

Notes. According to data in GenBank, the strain P1M41 corresponds to *Talaromyces verruculosus* (syn. *Penicillium verruculosum*) with 99 % identity (Table 6). The molecular analysis also shows a 99 % identity of sequences with *Talaromyces aculeatus* (cited as *Penicillium aculeatum*), however the morphological characteristics of the strain P1M41 match with *T. verruculosus*, especially the green olive color on MYP medium, the white color at the margin and the typical pale reddish yellow color of the reverse of the colony (Mayumilto *et al.* 1992). Microscopical characteristics of *T. verruculosus* also match with the description published by Mayumilto *et al.* (1992). *T. verruculosus* is phylogenetically closely related to *T. aculeatus* (Gul Shah *et al.* 2014).

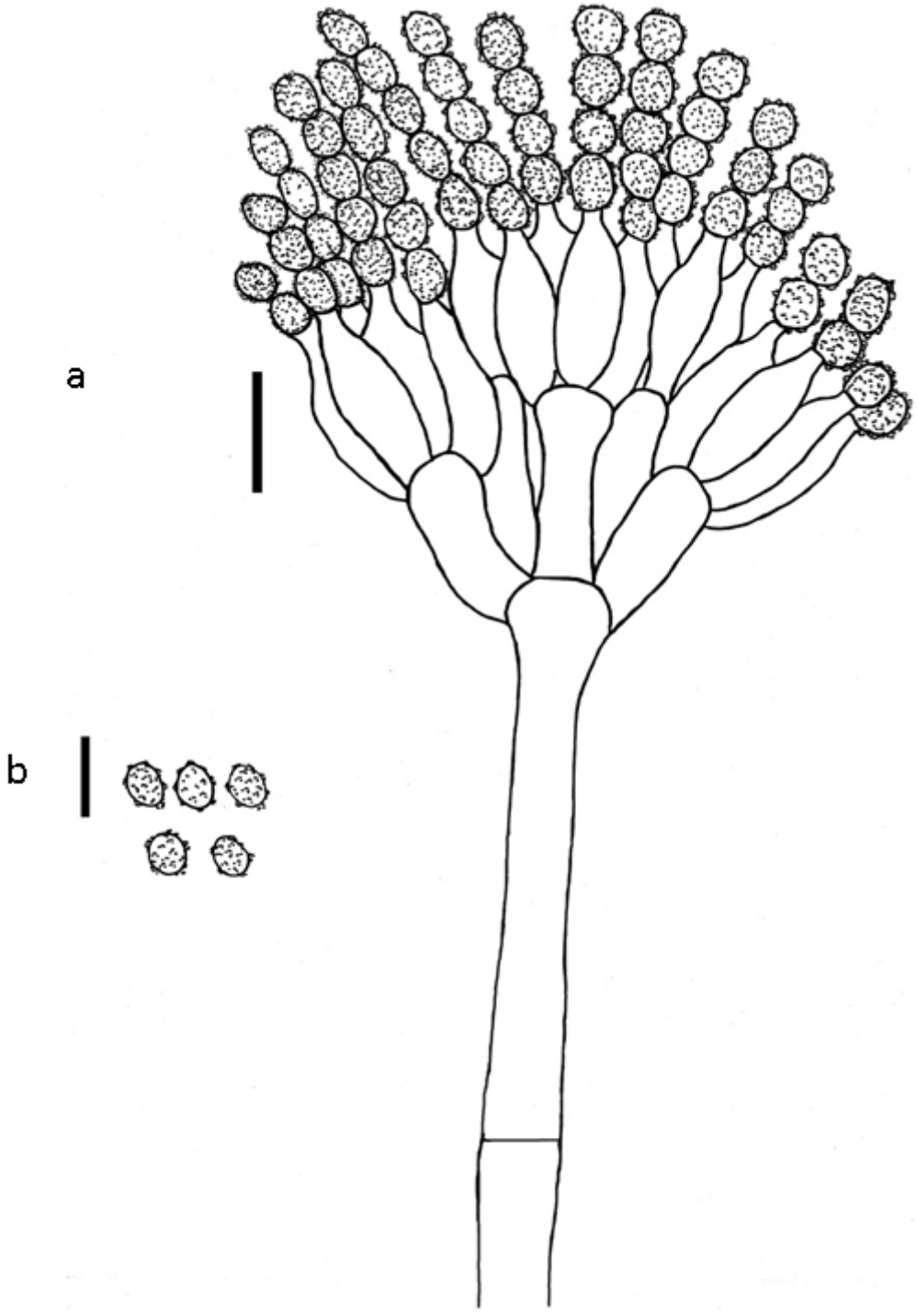


Figure 29: *Talaromyces verruculosus* (P1M41). a. Conidiophore with phialides and conidia. Bars = 10 µm. b. Conidia. Bars = 5 µm.

Oder: Hypocreales

Species: *Mariannaea elegans* (Corda) Samson, Studies in Mycology 6: 75 (1974).

Macroscopical description. Colony with cottony texture, mycelium planar to convex, with aerial mycelium, 2−5 mm high, white, gray, and light brown on the reverse of the culture. Colony on PDA attaining 2.5 cm diam. after 7 days at 25 °C.

Microscopical description. Hyphae septate, individual cells 3−5(−7) µm wide and (13−)16−38(−40) µm long, hyaline. Metulae 3−4(−5) mm wide and 10−18(−20) mm long, smooth, hyaline. Phialides subulate to acerose, (2−)3−4 µm wide and 7−21(−25) µm long. Conidia asymmetrical, fusoid, often apiculate, 2−4(−5) µm wide and (5−)6−10(−15) µm long, hyaline, smooth.

Material examined: Majagua, bare soil, 18.07.2012, M. Rosas *et al.* P1M35 (FR-0247039), ITS sequence KY320619.

Ecology and distribution. *Mariannaea elegans* has been reported from bark or decaying wood and soil in forests (Domsch *et al.* 2007, Samuels & Seifert 1991). *M. elegans* has been isolated from soils of Canada, France, Germany, Italy, Poland, South Africa, the British Isles, the Netherlands, the USA, and Venezuela (Domsch *et al.* 2007, Samuels & Seifert 1991). In Jamaica, a strain of *M. elegans* was isolated from arable soils under similar environmental conditions as those in Panama (Samuels & Seifert 1991). *M. elegans* is reported here for Panama and Central America for the first time.

Sequencing results

Table 7 shows the results of BLAST search performed with the ITS sequence of the sample number P1M35 including 565 base pairs.

Table 7: BLAST search results for the ITS region of sample P1M35

Number of sequence	Species	Query coverage in %	Maximum identity in %	More sequences with significant alignments
AB855778	*Mariannaea elegans*	94	98	11
EU273515	*Mariannaea elegans* (cited as *Nectria mariannaeae*)	94	98	10
AB587666	*Mariannaea camptospora*	94	91	3
JX125048	*Mariannaea samuelsii*	94	91	3
GQ153836	*Mariannaea aquaticola*	94	91	3

Notes. According to data in GenBank, the strain P1M35 matches with *Mariannaea elegans,* which is the anamorph name for *Nectria mariannaeae* (Samuels & Seifert 1991). *M. elegans* isolated from Panamanian soil also matches morphologically with the description published by Domsch *et al*. (2007). *M. elegans* differs from *Mariannaea camptospora* in colony pigmentation, the isolates of *M. camptospora* having a red purple reverse in culture (Domsch *et al.* 2007). Morphologically, *Mariannaea aquaticola* differs from *M. elegans* in the shape of conidia (ellipsoidal to fusiform) and the color of the colony on MEA, which is yellowish to dark brown (Tang *et al.* 2012). Molecular sequences of *M. elegans* and *M. aquaticola* differ, as shown by Cai *et al.* (2010), Samuels (2011), and data presented here. *Mariannaea samuelsii* differs from *M. elegans* by the shape of the conidia, which are cylindrical and 1-septate (Samuels 1989). These two species can be clearly separated from *M. elegans* based on their capacity to produce reddish-purple colonies on MEA (Samuels 2011).

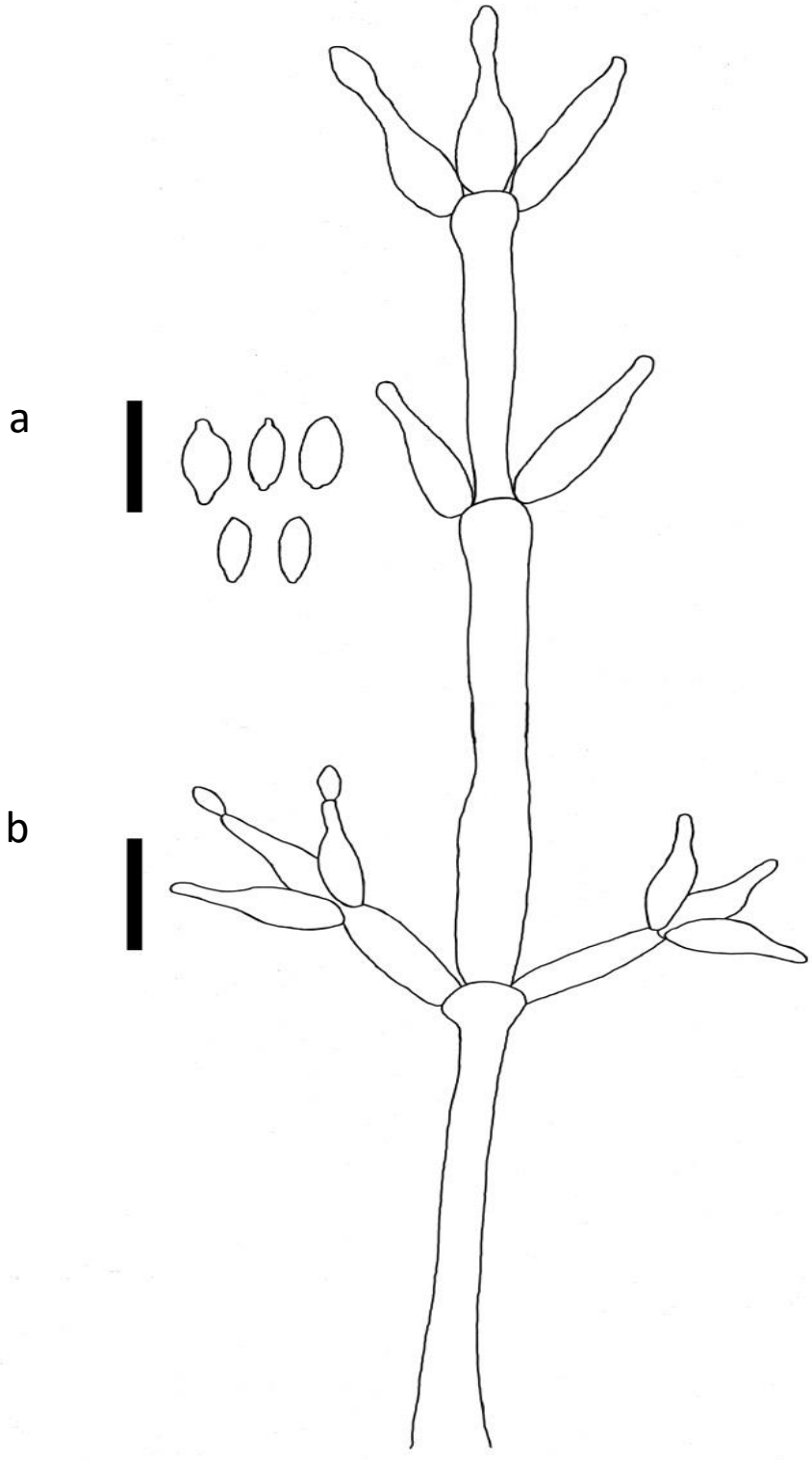


Figure 30: *Mariannaea elegans* (P1M35). a. Conidia. b. Conidiophore with metulae, phialides and conidia. Bars = 10 µm.

Species: *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones and Samson, in Luangsa-ard, Houbraken, Doorn, Hong, Borman, Hywel-Jones and Samson, FEMS Microbiol. Lett. 321: 144 (2011).

Synonyms: *Paecilomyces lilacinus* (Thom) Samson, Stud. Mycol. 6: 58 (1974). *Penicillium lilacinum* Thom, Bull. U.S. Department of Agriculture, Bureau Animal Industry 118: 73 (1910).

Macroscopical description. Colonies frequently forming agglomerations in circles, rose to pink, with cottony texture. Reverse of culture slightly pale pink. Colony on MYP attaining 1.5−3 cm diam. after 10 days at 25 °C.

Microscopical description. Hyphae septate, 2−3(−5) µm wide, single hyphal cells (10−)11−26(−28) µm long, hyaline. Conidiophores composed of an unbranched stipe, 5−7(−8) µm wide and (80−)120−180(−212) µm long, hyaline, with rough walls, with metulae, ramuli, and phialides. Metulae 3−4 µm wide and 8−10(−11) µm long. Phialides flask-shaped, 2−3 µm wide and 4−7(8) µm long, branched. Conidia in long chains, ellipsoidal, 2–3(−6) µm diam., pink, with smooth walls.

Material examined: Majagua, bare soil, 30.07.2012, M. Rosas *et al.* P1M95 (FR-0247040), ITS sequence KY320622.

Ecology and distribution. *Purpureocillium lilacinum* has been frequently isolated from soil of warm regions but it also has been reported from temperate forests (Itin *et al.* 1998). It is recorded from Argentina, Austria, Central Africa, Canada, Chile, Egypt, France, Honduras, Hong Kong, India, Israel, Jamaica, Japan, Libya, Malaysia, Nepal, Pakistan, Poland, Somalia, South Africa, Spain, Turkey, and the USA (Domsch *et al.* 2007). *P. lilacinum* is reported here for the first time in Panama.

Sequencing results

Table 8 shows the results of BLAST search performed with the ITS sequence of the sample number P1M95 including 691 base pairs.

Table 8: BLAST search results for the ITS region of sample P1M95

Number of sequence	Species	Query coverage in %	Maximum identity in %	More sequences with significant alignments
AB103380	*Paecilomyces lilacinus*	92	97	67
AB084157	*Ophiocordyceps heteropoda*	92	96	5
GU980040	*Isaria takamizusanensis*	89	95	2
GU980038	*Akanthomyces* sp.	89	94	1

Notes. According to data in GenBank and the BLAST search, the strain P1M95 matches with *Purpureocillium lilacinum* (partly as *Paecilomyces lilacinus*) (67 sequences with significant alignments), *Ophiocordyceps heteropoda* (five sequences), and *Isaria takamizusanensis* (two sequences). Samson (1974) defined 31 species in the genus *Paecilomyces* including *Paecilomyces lilacinus,* which recently was transferred to *Purpureocillium lilacinum* (Luangsa-ard *et al.* 2011). Species of the new genus *Purpureocillium* differ from species of *Paecilomyces* by the absence of chlamydospores, the lilac conidia (those of *Paecilomyces* are olive-brown), and because the optimum growth temperature is different. *Purpureocillium* spp. show rapid growth on agar media at 25−33 °C, while *Paecilomyces* spp. grow more slowly at 30−45 °C (Luangsa-ard *et al.* 2011). The phylogenetic position using ITS and 1-α (translation elongation factor gene) sequences shows that *P. lilacinum* belongs to the family

Ophiocordycipitaceae (Sung *et al.* 2007, Inglis & Tigano 2006) while species of *Paecilomyces* are located in the family Trichocomaceae (Luangsa-ard *et al.* 2011).

For the morphological identification of the strain P1M95 the keys published by Luangsa-ard *et al.* (2011) and Domsch *et al*. (2007) were used. The genus *Purpureocillium* includes two species: *P. lilacinum* is characterized by ellipsoidal to fusiform conidia, which are hyaline, smooth-walled to slightly roughened and purple *en masse*. *P. lavendulum* differs by subglobose or limoniform conidia with an apiculate base, slightly longer phialides, no growth at 35 °C and the production of a yellow pigment (Luangsa-ard *et al*. 2011, Perdomo *et al.* 2013). *P. lilacinum* is a saprobic species that can be isolated from soil, insects, nematodes, animals, and humans (Itin *et al.* 1998, Luangsa-ard *et al.* 2011). Based on these data, the species is identified as *Purpureocillium lilacinum*.

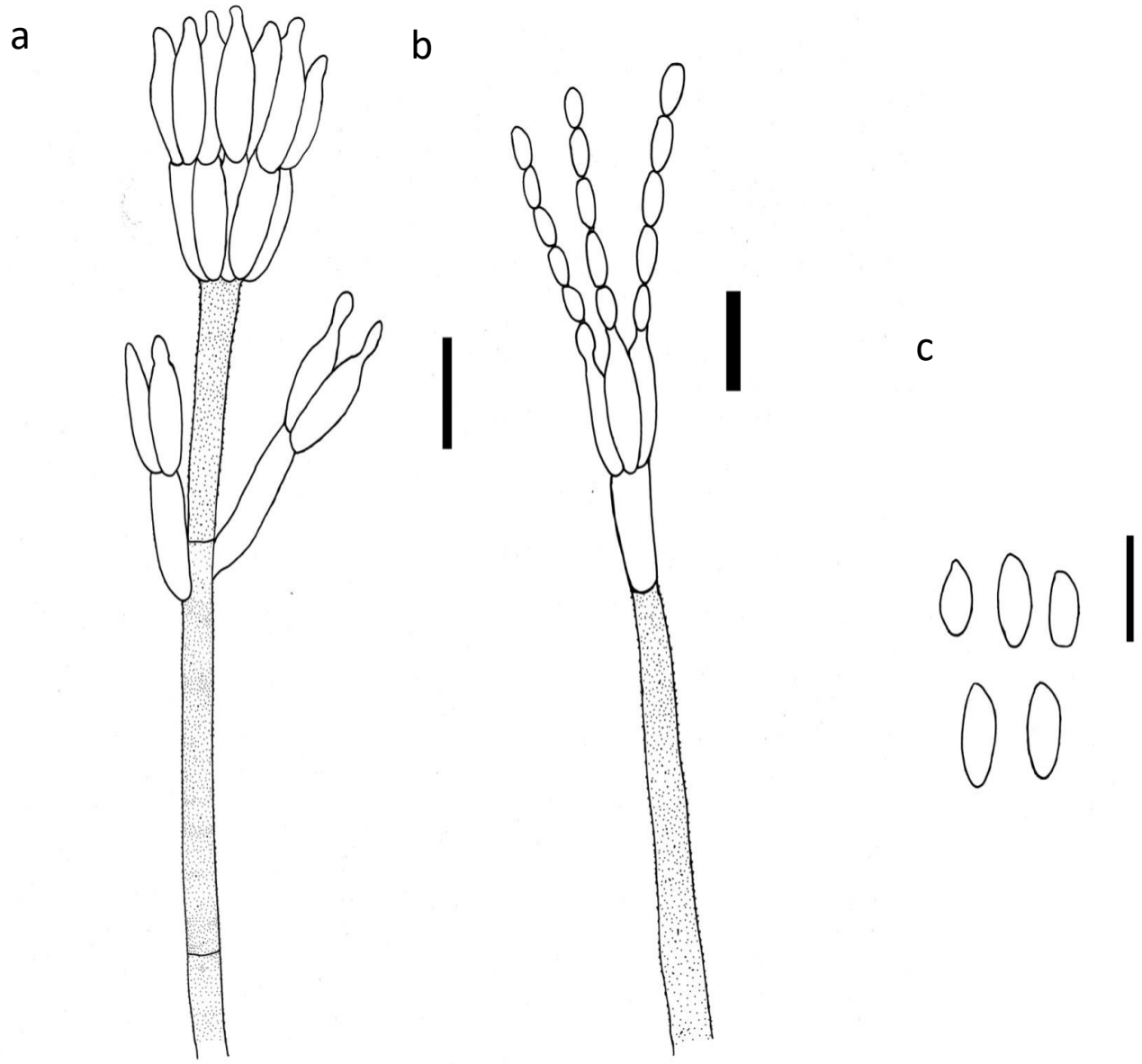


Figure 31: *Purpureocillium lilacinum* (P1M95). a. Conidiophore with phialides, metulae and ramuli. b. Conidiophore with phialides and chains of conidia. c. Conidia. Bars = 5 µm.

Species: *Trichoderma harzianum* Rifai, Mycological Papers 116: 38 (1969).

Macroscopical description. Texture of the colony woolly, colonies with white mycelium and green conidia on their surface. Colony on MYP reaching 5.5 cm diam. after 5 days at 25 °C. Reverse of culture slightly pale yellow.

Microscopical description. Hyphae septate, 3−4 µm wide, single hyphal cells (25−)29−53(−65) µm long, hyaline. Conidiophores 2−3 µm wide and (7−)9−16(−20) µm long, with phialides in dense groups. Phialides flask-shaped, with swollen base, 1−2 µm wide and 4−6(−8) µm long. Conidia globose to subglobose, 2–3 µm wide, green, with smooth walls.

Material examined: Majagua, bare soil, 18.07.2012, M. Rosas *et al.* P1M6 (FR-0247041), ITS sequence KY320639.

Ecology and distribution. *Trichoderma harzianum* (asexual and sexual states) has a worldwide distribution with records from Europe, South America, Africa, and Asia (Chaverri *et al.* 2003, Grondona *et al.* 1997). *T. harzianum* has frequently been isolated from soil of forests, as well as in fields of wheat. It has been recorded from the British Isles, Canada, Costa Rica, India, Italy, Libya, the Netherlands, Nigeria, and the USA (Domsch *et al.* 2007, Holmes *et al.* 2004).

T. harzianum was found in forest soil in Panama resulting as the first report in this country.

Sequencing results

Table 9 shows the results of BLAST search performed with the ITS sequence of the sample number P1M13 including 609 base pairs.

Table 9: BLAST search results for the ITS region of sample P1M13

Number of sequence	Species	Query coverage in %	Maximum identity in %	More sequences with significant alignments
EF191303	*Hypocrea lixii*	94	97	40
JQ775562	*Hypocrea* sp.	95	97	5
KC330218	*Hypocrea lixii* (cited as *Trichoderma harzianum*)	96	96	7

Notes. Chaverri and Samuels (2002) reported that cultures derived from ascospores of *Hypocrea lixii* produced the morphological species *Trichoderma harzianum* in pure culture, so *T. harzianum* is the asexual stage of *H. lixii*. Accordingly, the strain P1M13 matches to sequences labeled as *Hypocrea lixii* as well as *Trichoderma harzianum.* Phylogenetic analysis of ITS region showed that *Trichoderma harzianum*/*Hypocrea lixii* is a cohesive group (Chaverri *et al.* 2003). The identification by morphological characteristics confirms this species identification (Domsch *et al.* 2007). Morphological characteristics of the strain correspond to *T. harzianum,* which is characterized by dense conidiation, colonies rapidly turning yellowish-green to dark green and presenting pustules fringed by sterile white mycelium. Conidiophores tend to be regularly verticillate, forming a pyramidal structure, and are divided by septa. Phialides are ampulliform to lageniform and form verticillate groups of 3–4. Conidia are subglobose and pale green when fully mature (Domsch *et al.* 2007, Kubicek & Harman 1998). *T. harzianum* is somewhat similar to *Trichoderma viride*, but *T. harzianum* has phialides more densely aggregated than *T. viride,* and phylogenetically both species are not closely related (Domsch *et al.* 2007, Samuels 1996). The comparison of the sequences and the morphological description confirm the species identification as *T. harzianum.*

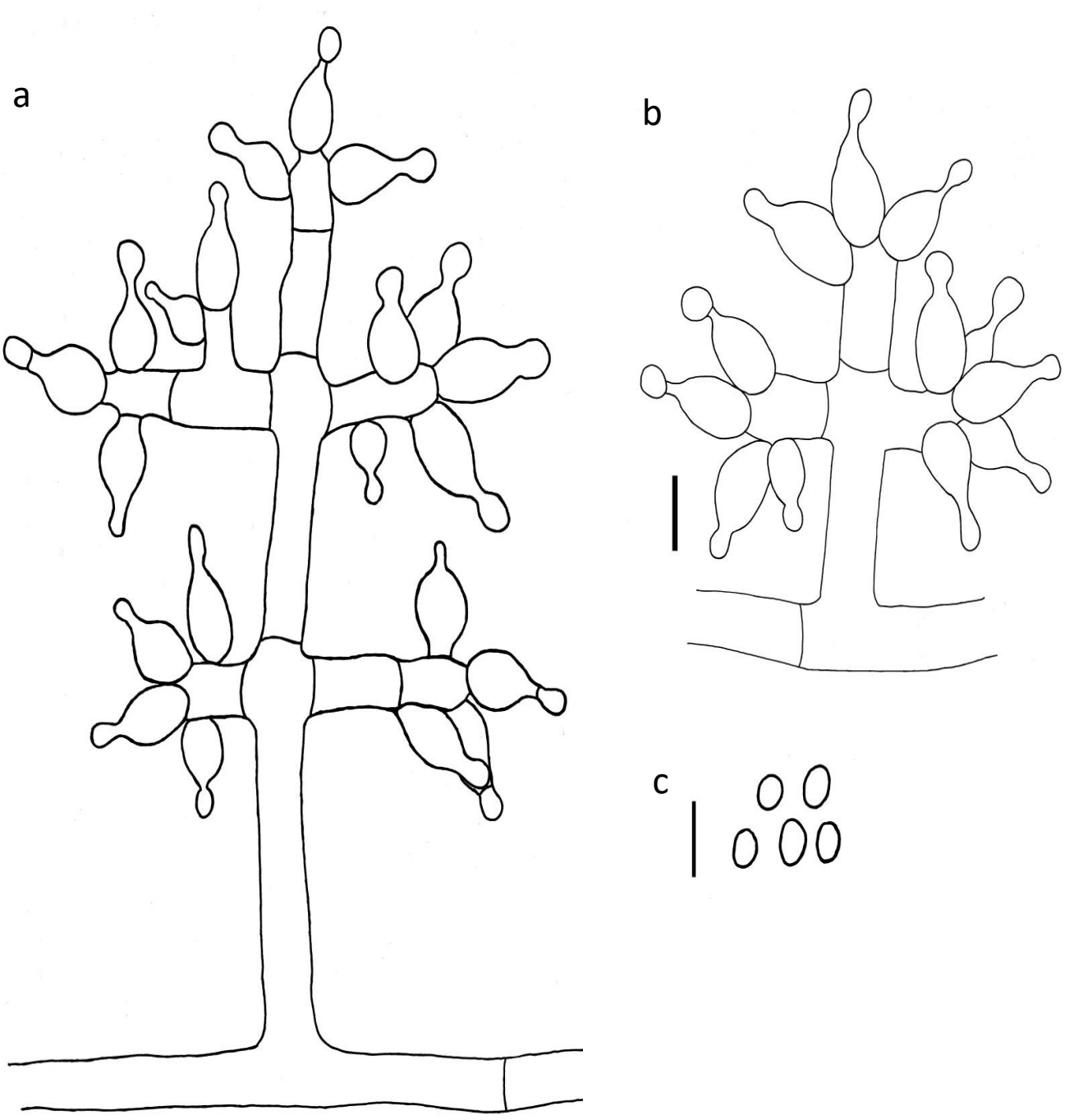


Figure 32: *Trichoderma harzianum* (P1M13). a. Conidiophore with phialides. b. Accumulation of phialides. c. Conidia. Bars = 5 µm.

Species: *Trichoderma spirale* Bissett, Can. J. Bot. 69: 2408 (1992).

Macroscopical description. Texture of the colony woolly, colonies with white mycelium and green conidia on their surface. The color of the reverse side of culture is the same as the upper side (white). Colony on PDA reaching 5.5 cm diam. after 5 days at 25 °C.

Microscopical description. Hyphae septate, 3−4 µm wide, single hyphal cells (15−)19−23(−35) µm long, hyaline, walls smooth. Conidiophores 4−6 µm wide and (30−)38−42(−50) µm long, with 3−5 phialides each. Phialides subglobose, usually swollen at the base, 3−5 µm wide and 4−6(−8) µm long. Conidia ellipsoidal, 2–3 µm diam., green, with smooth walls.

Material examined: Majagua, bare soil, 30.07.2012, M. Rosas *et al.* P1M79 (FR-0247042), ITS sequence KY320642.

Ecology and distribution. *Trichoderma spirale* has frequently been isolated from rain forest soils and bamboo stand soils. It has been recorded from Canada, Costa Rica, New Zealand, Thailand, and the USA (Bissett 1991, NZOR 2013). *T. spirale* is reported here for Panama for the first time.

Sequencing results

Table 10 shows the results of a BLAST search performed with the ITS sequence of the sample number P1M79 including 628 base pairs.

Table 10: BLAST search results for the ITS region of sample P1M79

Number of sequence	Species	Query coverage in %	Maximum identity in %	More sequences with significant alignments
HQ608089	*Trichoderma spirale*	94	97	44
EU280085	*Hypocrea crassa*	94	96	1
JQ040400	*Hypocrea virens*	94	96	2

Notes. In his description of *Trichoderma spirale*, Bissett (1991) mentioned that one of the main morphological characteristics of this species are the spiral sterile conidiophore elongations and the dense production of conidia. Both characteristics are present in the culture P1M79. According to the BLAST search in the NCBI databank, the strain P1M79 matches with a similarity of 97 % to *T. spirale*. *T. spirale* is morphologically similar to *Trichoderma polysporum*, but conidiophores are rough-walled in *T. polysporum* while they are smooth-walled in *T. spirale* (Bissett 1991, Domsch *et al.* 2007).

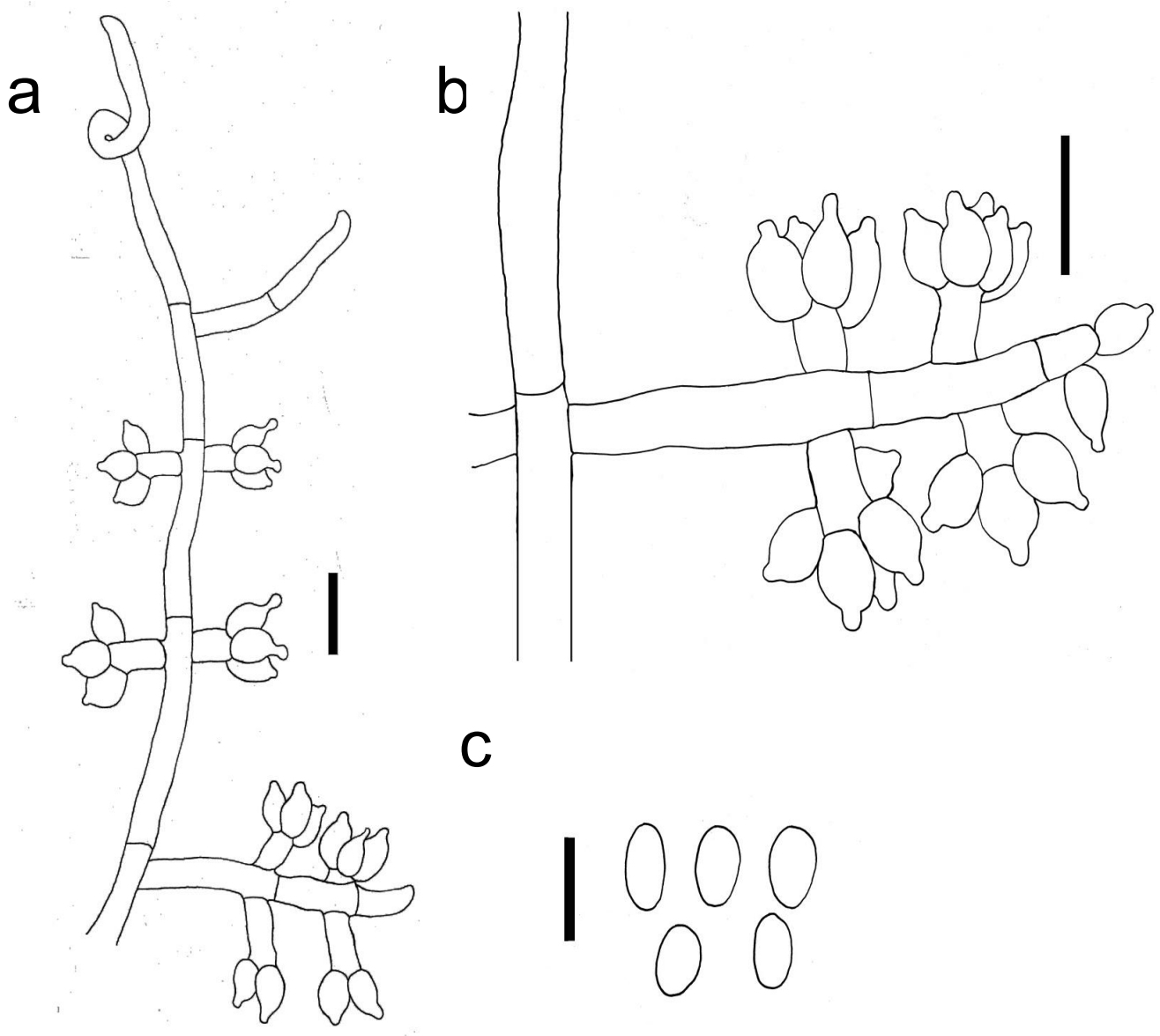


Figure 33: *Trichoderma spirale* (P1M79). a. Conidiophore with phialides and spiral apical extension. b. Part of a conidiophore with dense phialides. Bars = 10 µm. c. Conidia. Bars = 5 µm.

Phylum: Zygomycota

Order: Mucorales

Species: *Mucor moelleri* Vuill., Bull. Soc. mycol. Fr. 19: 117 (1903).

Synonym: *Zygorhynchus moelleri* Vuill., Bull. Soc. mycol. Fr. 19: 117 (1903).

Macroscopic description. The colony is white, forming dense, superficial, woolly colonies. Within the colony numerous zygospores are evident as black dots. The fungal strain covers the entire Petri dish (9 cm diam.) after aprox. 10 days at 25 °C.

Microscopical description. Hyphae without septa, hyaline, 2–5(−7) µm wide. Sporangiophores (5−)8–19(−30) µm long, (2−)3–5(−7) µm wide. Sporangia globose, (8−)11–18(−20) µm diam. Sporangiospores ovoid, (1−)2–3(−4) µm long, hyaline, smooth. Zygospores oval, brown to dark brown, (25−)32–41(−45) µm diam., warty. A zygospore is carried by two unequal suspensors, the small suspensor has a diameter of about 3–6(−8) µm (very difficult to observe), while the larger, ovoid suspensor has a diameter of about (17−)19–28(−32) µm.

Material examined: Majagua, bare soil, 18.07.2012, M. Rosas *et al.* P1M118 (FR-0247043), ITS sequence KY320646.

Ecology and distribution. This species is one of the commonest members of the Mucoraceae and has a worldwide distribution. It has been reported almost exclusively from soils for Austria, the British Isles, Central Africa, Denmark, Germany, India, Italy, New Zealand, Norway, Poland, Rumania, South Africa, and the USA (Domsch *et al.* 2007, Zycha & Siepmann 1969). *M. moelleri* is reported here for Panama and Central America for the first time.

Sequencing results

Table 11 shows the results of a BLAST search performed with the ITS sequence of the sample number P1M118 including 583 base pairs.

Table 11: BLAST search results for the ITS region of sample P1M118

Number of sequence	Species	Query coverage in %	Maximum identity in %	More sequences with significant alignments
KF367556	*Mucor moelleri* (as *Zygorhynchus moelleri*)	100	100	10
KP900327	*Mucor moelleri*	94	100	8
KF367556	*Zygorhynchus* sp.	100	100	2

Notes. According to the BLAST search in GenBank, the ITS sequence of the strain P1M118 matches with a similarity of 100 % with sequences of *Mucor moelleri*. Morphologically, *M. moelleri* is characterized by white to grey, globose to slightly flattened sporangia of 12−40(−60) µm diameter, ellipsoidal to ovoid sporangiospores, and zygospores that are most abundant just above the surface of the substrate, brown to dark brown, mostly 30−35 µm wide, and carried by unequal suspensors (Domsch *et al.* 2007).

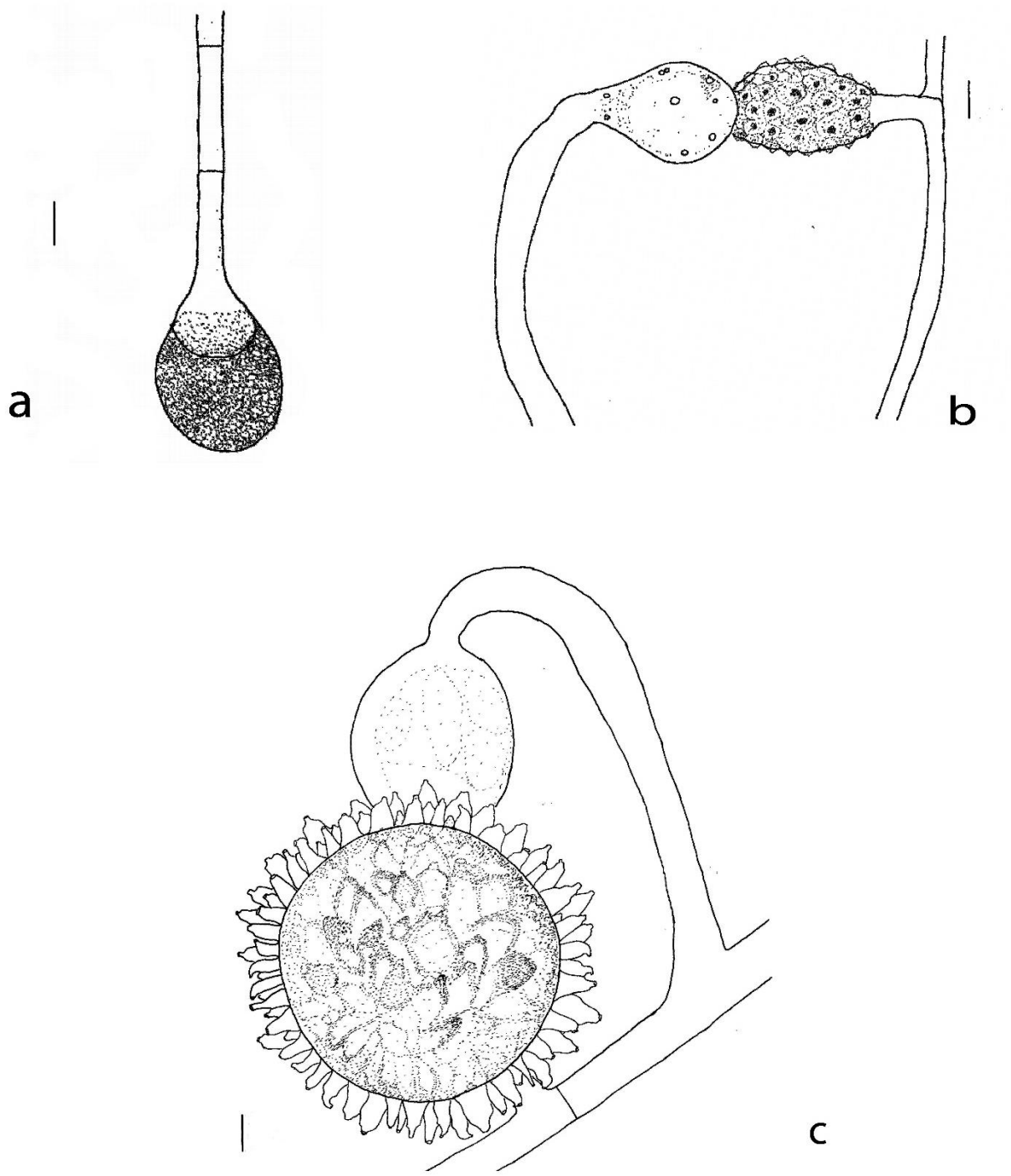


Figure 34: *Zygorhynchus moelleri* a. sporangiophore with sporangium, b. fusing gametangia with gametangiophoes, c. zygospora with two suspensors and adjacent hypha, Bars = 5 μm.

6.10 Levels of soil degradation and soil characteristics

Soil degradation about plant cover is based on three levels: forest without alterations, grassland covered with grass, and bare soil without plant cover. In the case of pH, levels less than six are considered acidic and begin to be considered degraded soils. Based on the above, German soil samples in grassland (4.4-5.5), bare soil (5.1-5.5), and forest (4.9-6.0) were acids. Panama also showed an acid pH in grassland and forest samples (6.0), with slight variations in bare soil (5.0-6.0). Compactation of soil based on bulk density (BD) in Germany was the highest in bare soils (3.0-5.1 BD), followed by that of grasslands (3.2-4.5 BD) and forest soils (2.5-3.8 BD). On the other hand, in the samples of Panama, the lowest and highest compaction was present in forest samples (3.8-5.3), while bare soil had intermediate compaction (4.8-5.1) grasslands was low also (3.9) remained with few changes.

Soil composition was obtained based on the triangle of soil composition, resulting in a classification for soil studied in the two countries. The classification for soils in Germany were for bare soils mostly loam, for grassland and forest were silt loam. In the case of Panama, bare soil was between loam and silt loam, in the case of grassland and forest were silt loam.

6.11 Biotic factors correlation with soil fungi diversity

The correlation between the abiotic factors and the fungal diversity of the soil resulted in a statistically non-significant correlation ($P > 0.05$; Rosas *et al.* 2019)

in both countries. The only factor with a negative effect was compactation in Germany (R^2 = 0.13, *P* = 0.047), as shown in figure 35.

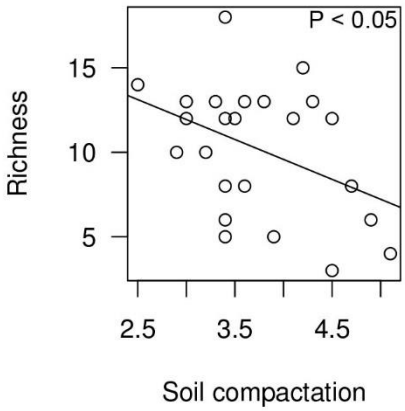


Fig. 35 Correlation between species richness and soil compactation in Germany

6.12 Ecological distance

The non-metric multidimensional scaling (NMDs) results are based on sampling points grouped by ecological distance and then by sampling period, meaning that the ecological distances are closest in sampling points made in the same period (Fig. 36). One clear example is the sampling in group one, found in the first triangle. Every sampling point belongs to the first sampling in Germany, and the same process is repeated with the next triangles. All the sampling points in Panama are grouped by their ecological distance in triangle eight. However, a bridge is formed by rectangle seven between the two principal groups (Germany and Panama). This rectangle represents the eight species that share the two areas.

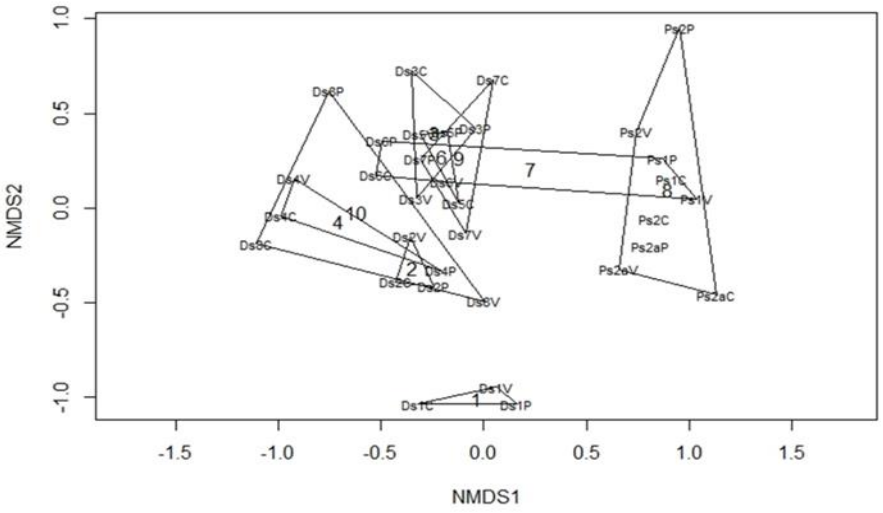


Fig. 36 Ecological distance grouped by sampling points

(Ds = Germany, Ps = Panama, C = bare soil, V = Forest, P = grassland)

The same NMDs also were ordered with the sampling points in two groups based on their ecological distances, the result is evident, the groups are formed according to the country (Fig. 37). This result confirms what was done on site.

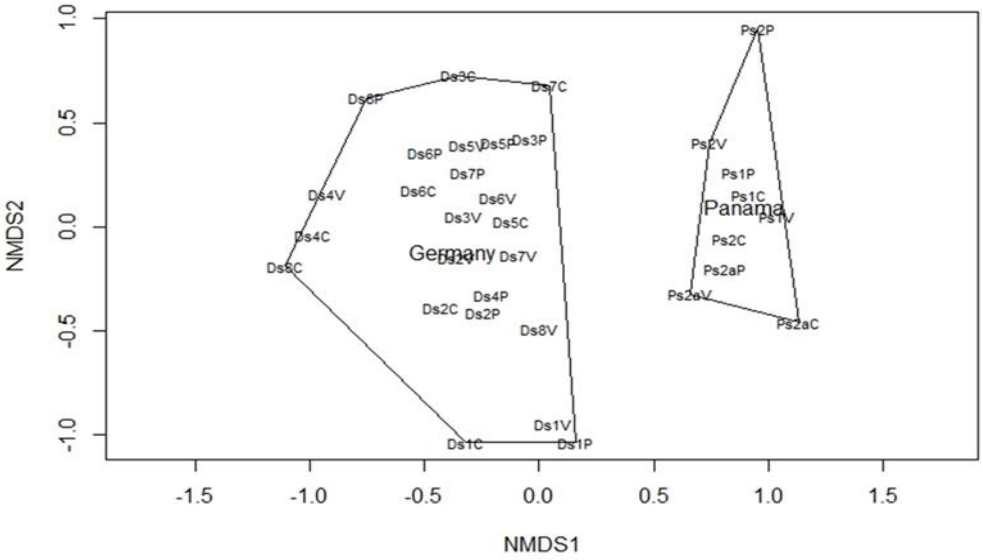


Fig. 37 Ecological distance grouped by sampling points countries

(Ds = Germany, Ps = Panama, C = bare soil, V = Forest, P = grassland)

6.13 Theoretical model

According to the above, a theoretical model was made on how irrigation could be used for the recovery of degraded soils based on fungal diversity. The model is based on abundance, Pareto analysis, alpha-beta, and gamma diversity indices. The periodicity in irrigation is taken based on each country's seasons of the year; likewise, it is considered a theoretical model; this has not been applied and tested. However, it is based on the models of biofertilizers used in soil.

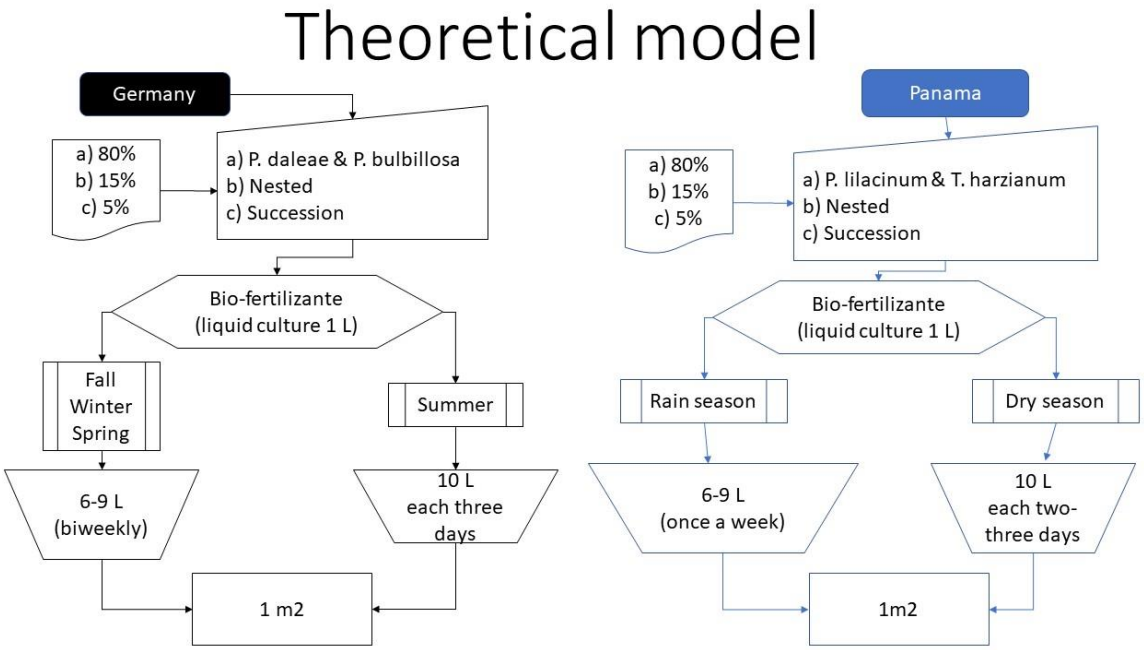


Fig. 38 theoretical model of regeneration of degraded soils based on fungal diversity

The German model starts with the composition of biofertilizers. The composition is 80 % key species plus 15 % nested species plus 5 % successional species. In Germany, the four seasons of the year are presented. In autumn, winter, and spring, it will be watered per square meter from six to nine liters twice a week, while in summer, it will be watered with 10 liters every three days. In the Panama

model, the biofertilizer will be composed of 80 % of the key species, 15 % of the nested species and 5 % of the species in succession. In Panama, there are two marked climatic seasons: the rainy and dry seasons. In the rainy season, six to nine liters per square meter will be watered once a week, while in the dry season, 10 liters per square meter will be watered every two or three days.

6.14 Development of the scientific application cultivation of soil fungi

The main result is the prototype of the application ready to be published. The front-end has an introduction and then the work area opens. In this activity, two buttons lead to the activity of capturing data and taking pictures.

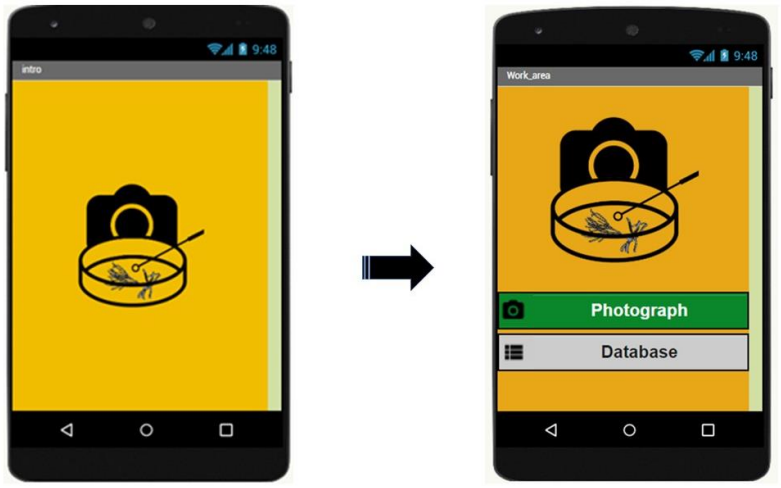


Fig. 39 Introduction and work area

The front-end for the database activity captures the data as follows; the new button sends to another activity where there is a space to record the following data: culture medium, the temperature at which the colony is kept, the day since inoculation and the number of colonies up to the moment of capture.

This activity has the Add-button, which saves the captured data and allows the capture of the following data. It also has the Back-button that allows you to return to the database activity to see the captured data. The following Update-button allows you to write the plate number you want to modify. With the Search-button, your search for the data set for the Petri dish number previously captured, and any data can be changed there. Once modified with the Update-button placed below, the activity is updated, and the Back-button returns to the previous activity. The Delete-button works the same way as Update with the difference of deleting unwanted data. The Display-button displays the list of captured data and can scroll the list of data up and down. The Back-button will return to the previous activity. The last button in this activity is the Export-button, which transforms the database into an Excel format so that it can be sent and worked on other devices.

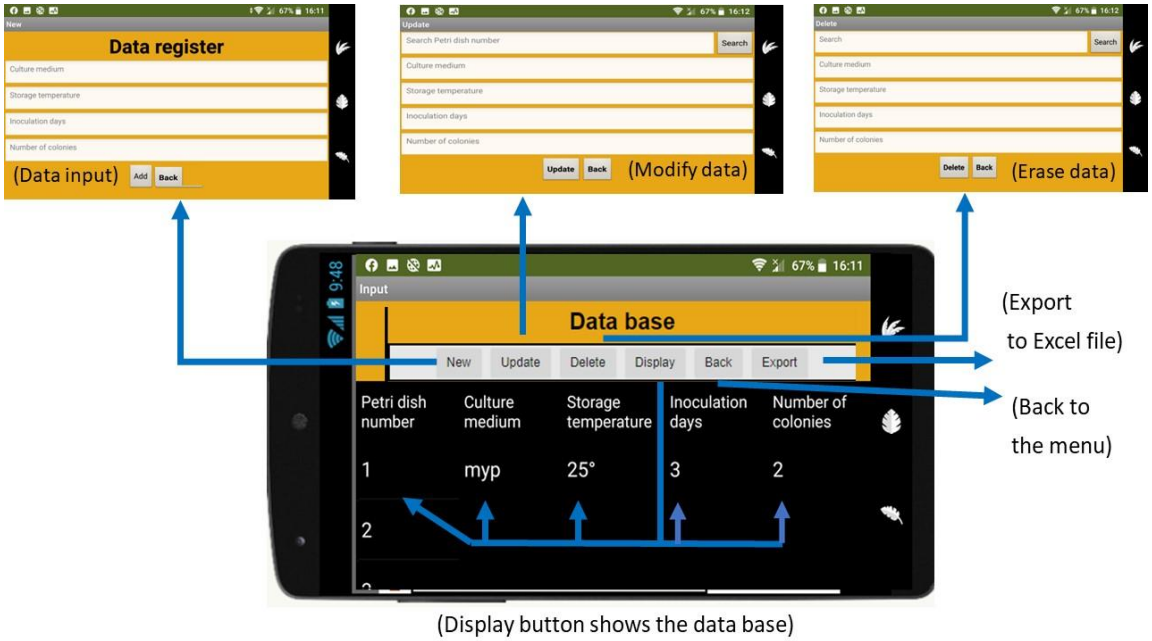


Fig. 40 Front-end of the database activity

The photo activity can be used for capturing photos of Petri dishes to mark and count the colonies that develop day by day. The Image-button is used to see

pictures of previous days and mark the growth of the colonies, thus having a file per day of the changes of the colonies in the Petri dishes. The Photo-button is used to capture the status of the colony, which can then be compared with subsequent photos of colony development. The main function of the Color-button is to differentiate the colonies that grow in the same Petri dish. The Back-button sends the user to the menu in case the user wants to exit the photo activity. The button with the sliding menu is used to choose the tool to mark the photo of the Petri dish. The colony can be located in the Petri dish. The ring is used to highlight the colony of interest. The text is mainly used to write the code of the dish studied in the photo. Finally, the filled point is used to visualize the size and growth of the colony in different images through the days.

The grade bar will be used to grade the size of each tool used in the petri dish photo. The Delete-button has the function of deleting in case of an error in marking colonies. The Save-button saves the image with the changes.

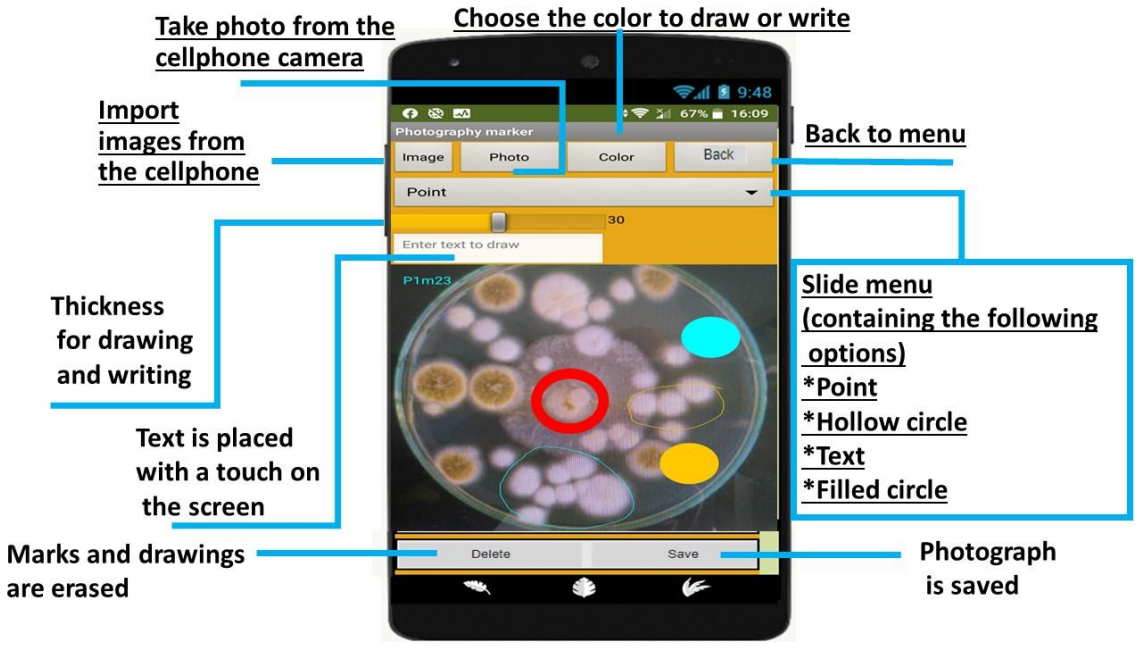


Fig. 41 Front-end of the photography activity

In the search for similar applications, the Colony Counter (CC) app was found, which was evaluated and compared with the App SFC with the help of ten specialized users. Four variables were evaluated; the time of use, which refers to the time required by the user to work on a Petri dish in the application. The navigation flow is evaluated in which activity the user has more problems. For this, a number was assigned to the most relevant and common activities of the applications; 1 for database, 2 for data transfer, 3 for photography activity and 4 for no problem.

The following variable evaluated was the number of Clicks to reach the desired objective and, finally, the general rating from 1 to 10, where 10 is the maximum rating for the App.

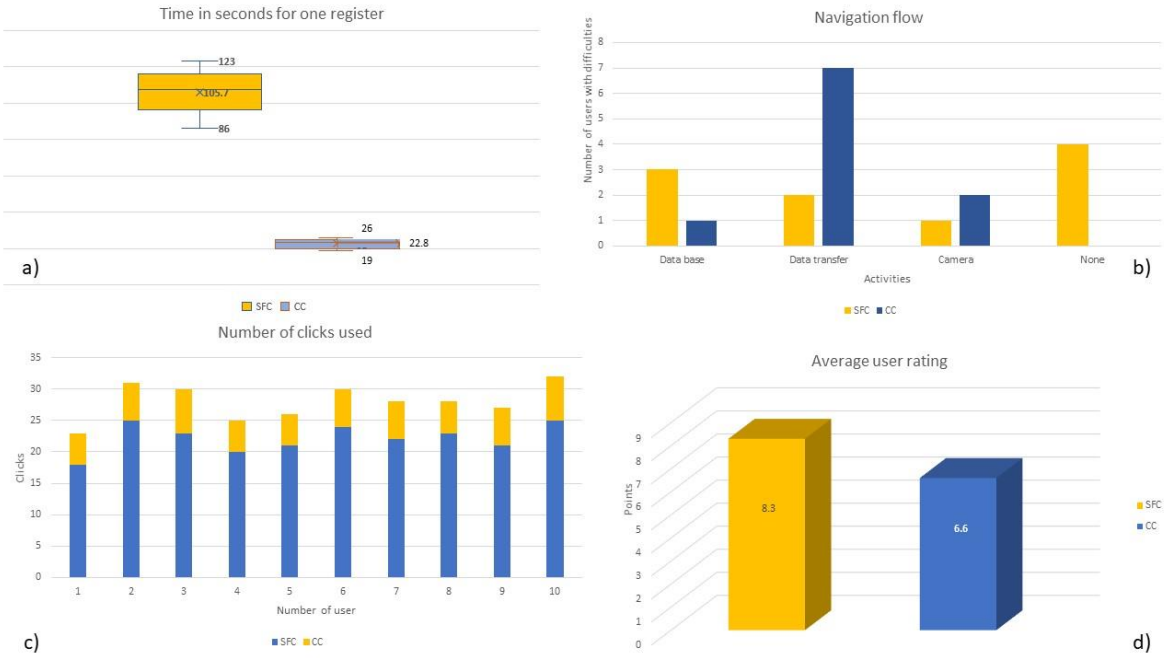


Fig. 42 Graphs, a) time in seconds that the user needs to register in the App, b) navigation flow, activities, where there were problems or the interaction, was not clear, c) the number of clicks used by users in each App, d) user rating for each App

Graph shows that the SFC App was the one that obtained the most time of use. This result shows that the user spends more time making a record because it can record more information. The variability of use in time is greater than the App CC, it goes from 86 to 123 seconds per record, which allows the user to choose what they need in each record. The navigation flow presents three activities and an option that mentions that the user had no problem with any activity. The activity in the database presented a slow flow in the SFC App, while the CC App presented a more constant flow; only one user noticed this activity. The data transfer activity showed that the CC App had the highest number of problems concerning this activity, due to the fact that the CC App cannot export any data.

The camera activity was practically not referenced by users in the two Apps, the activity is user friendly. Finally, the evaluation showed that the flow of navigation was not stopped, only four users mentioned that the SCF App checked in consistently without any hiccups. Graph c compares all the clicks used by users in the two Apps. Clearly, the SFC App had a higher number of clicks because it has more buttons. The SFC App had the best overall rating with an 8.3 rating, while the CC App got 6.6, only a 1.7 difference, which in App usage is not a big difference.

7 Discussion

7.1 Diversity of soil fungi

Over the past century, many studies on soil fungi communities have been published in Panama and Germany. In Panama, most of these studies deal with fungal diversity of the most common and cosmopolitan orders, or with fungi of economic importance (Farrow 1954, Goos 1960, Gualdrón-Arenas *et al.* 1997, Morris & Finley 1956). The species identified and reported in previous check lists are 207 in total (Farrow 1954, Goos 1960, Husband *et al.* 2002, Mangan *et al.* 2004, Morris and Finley 1956, Piepenbring 2006, 2007). However, 24 species were found from 231 isolates in the present study, including ten new records for Panama and four for Central America (Rosas-Medina & Piepenbring 2018). The remaining 14 species are relatively common in tropical forests because these soil fungi share the distribution and diversity of other plant communities (Mangan *et al.* 2004). *For instance, Trichoderma harzianum* is the most abundant species with 27 isolates, representing 3.5 % of total isolates. *Trichoderma* species are dispersed across all latitudes, habitats, and climatic zones (Klein and Eveleigh 1998) and *Trichoderma harzianum* is usually encountered in soils (Domsch *et al.* 2007).

In Germany, research on soil fungi communities is more abundant than in Panama (Anderson & Domsch 1978, Bååth & Anderson 2003, Bonkowski *et al.* 2000, Christ *et al.* 2010, Goldmann *et al.* 2016, Wehner *et al.* 2014, Wubet *et al.* 2012). However, it is still necessary to develop more studies to further our knowledge of these soil fungi communities. Specifically, in the Taunus forest, other studies in mycology have been conducted around different topics such as

diversity, geography, and adaptation of soil fungal communities (Rudolph *et al.* 2018, Schmidt 2016, Tedersoo *et al.* 2014, Weisenborn 2016).

Still, not many studies focus on soil fungi communities that complete the list of diversity in the region. In the present study, 47 species were found originating from soil with 533 isolates, while an identification soil fungi study in the Müggelheim forest, also in Germany, found 32 species with 122 isolates, highlighting *Penicillium* as the most common genera (Kwaśna & Nirenberg 2004). *Pochonia bulbillosa* was the most abundant species in Germany with 81 isolates, representing 10.6 % of total isolates. *Pochonia bulbillosa* is commonly isolated from soil and known to parasitize nematodes (Nonaka *et al.* 2013).

In total, eight species occur both in Germany and Panama: *Absidia cylindrospora*, *Bionectria ochroleuca*, *Penicillium citrinum*, *Purpureocillium lilacinum*, *Penicillium simplicissimum*, *Trichoderma harzianum*, *Trichoderma koningi* and *Zygorhynchus moelleri*. Most of these species are known to be cosmopolitan soil fungi, which are characterized by producing spores in large quantities (Cox *et al.* 2019, Tedersoo *et al.* 2014). Spores production is described, referenced, and cited in each of the specific studies referring to a specific species (Chaverri *et al.* 2002, De Respinis *et al.* 2010, Edelmann and Klomparens 1995, Luangsa-ard *et al.* 2011, Schroers 2001, Tuthill *et al.* 2001, Wallis & Joubert 1974, Wöstemeyer *et al.* 1995).

Diversity of soil fungi in both countries was high regardless of geography. According to the Shannon index with values ranging from 0 to 5, Germany has a diversity value of 3.36 and Panama 3.15, both considerably high values (Kindt & Coe 2005). Although there were differences in the number of samples in

the two countries, the samplings were not a limiting factor to calculate the diversity.

A high diversity value was obtained because the Shannon index is not altered significantly by sample size (Kelly 2016). High diversity in both countries may be due to different factors that were not considered in this study.

7.2 Abundance of species in three different soils

The abundance of species in Germany through time shows the climax in the second sampling in all soils, and then the population declines as time goes by. However, abundance is lower on bare soils than on forested soils. Bare soils can not retain enough water, and the low availability of organic matter affects the microfungal abundance (Purabi & Santa 2012). Forest soils contain a greater abundance of micro-fungi than other soils because forest soils have more significant amounts of water and conditions for the increase in the abundance of species in the diversity of the soil fungal community (Hong *et al.* 2015). What has been said in the previous lines is confirmed in both countries. In Panama, a statistically significant difference was achieved (Fig.23). However, it is assumed that only three samples are few and that with more samples, it would be possible to observe more differences between the different soils studied. In grassland, it was observed that they function as transition areas in the abundance of the fungal community. The Tukey test observed the main statistical differences between Bare and forest soils. In the grasslands, it does not show differences with the Bare soil as well as with the forest soil. The process of increasing abundance is shown to be gradual depending on the type of soil as more degradation

decreases abundance, and as vegetation increases, abundance increases (Meng *et al.* 2021).

7.3 Identification of ten new records in Panama

The morphological identification of the ten new records in Panama was not an easy task. *Aspergillus roseoglobulosus* had only two sequences with a maximum identity of 99 %, while the most similar species was *Aspergillus sclerotiorum*, with 20 similar sequences with a maximum identity of 98 %. However, one of the main morphological differences was the reddish-pink color in the mycelium, which was present in culture, representative of *Aspergillus roseoglobulosus* (Frisvad & Samson 2004). In contrast, *A. sclerotiorum* is characterized by yellow-cream mycelium, which was not present in the culture. The second identification was *Aspergillus tamarii*, which had 36 sequences with a maximum identity of 99 %. The following species was *Aspergillus caelatus* with eight sequences; molecularly, it was clear that the species was A. *tamarii,* and the morphological characteristics confirmed the identification (Castillo 2007, Horn 1997, Kurtzman *et al.* 1987, McAlpin *et al.* 2005). The third identification was *Penicillium janczewskii*, which had only three sequences with a maximum identity of 98 %, while the second option *Penicillium jensenii* had 97 %, which is ruled out because it does not have the minimum, maximum identity of 98 %. In addition to the above, the morphological characteristics in the size of the structures coincided with the species *P. janczewskii* (Domsch *et al.* 2007). The fourth identification was *Penicillium miczynskii*, which had seven sequences with a maximum identity of 99 %, while the second option *Penicillium sanguifluum* had only four sequences

with a maximum identity of 97 %. Culture presented abundant conidiogenesis and finely rough conidia, representative of *Penicillium miczynskii* (Tuthill & Frisvad 2004). The fifth identification was *Talaromyces verruculosus*, which had nine sequences with a maximum identity of 99 %; the following sequences were not identified as species. Morphological analysis reinforced the identification (Mayumilto *et al.* 1992). The sixth identification was *Mariannaea elegans*, which had 21 sequences with an identity of 98 %, while the second referenced species was *Mariannaea camptospora*, with only three sequences with a maximum identity of 91 %; with this proportion, the sequence is identified as *M. elegans* confirmed with the morphological characteristics, *M. elegans* differs from the other species by the color of the crop in the bottom part (Domsch *et al.* 2007). The seventh was *Purpureocillium lilacinum*, which had 67 sequences with a maximum identity of 97 %. Although it does not meet the minimum, maximum identity, the number of sequences reinforces the identification. In addition, the second species *Ophiocordyceps heteropoda* had only five sequences with a maximum identity of 96 %. The morphological characteristics confirm the identification of the species *P. lilacinum* presenting in the cultures ellipsoidal to fusiform conidia, which are hyaline, smooth-walled to slightly roughened, and purple in masse (Luangsa-ard *et al.* 2011, Perdomo *et al.* 2013). The eighth identification was *Trichoderma harzianum*, which had 40 sequences with a maximum identity of 97 %, and the second option was *Hypocrea sp.* With five sequences with a maximum identity of 97 %, the morphological characteristics support the identification of the sequence as *Trichoderma harzianum*. The culture presented dense conidiation, colonies yellowish-green to dark green (Domsch *et al.* 2007, Kubicek & Harman 1998). The ninth identification was *Trichoderma*

spirale, which had 44 sequences with a maximum identity of 97 %, while the second option *Hypocrea crassa*, only had one sequence with a maximum identity of 96 %. The morphological characteristics confirm the molecular analysis by presenting typical spiral sterile conidiophore elongations.

The tenth identification was *Mucor moelleri*, which had 20 sequences with a maximum identity of 100 % divided into ten, eight, and two differently worded sequences with the same name. The morphological characteristics confirmed the identity of the culture; white to gray, globose to slightly flattened sporangia was found, a representative characteristic of *M. moelleri* (Domsch *et al.* 2007).

7.4 Common orders

Four orders were found in Panama with isolation methodology, of which Eurotiales represented 53 % of total isolates. This high percentage is because, in tropical forests, this order is dominant (Mayamor & Poeaim 2014). The second most commonly found order was Hypocreales with 38 %. This order is usually encountered in humid tropical or subtropical forests (Chaverri & Samuels 2002). Next, Mucorales represented 6% of the species found. This order is present in most forests since many species are saprophytes (Almaraz-Sánchez *et al.* 2012). The order with the least amount of species found, Xylariales, amounted to 3% of the total. Usually, this order can be widespread in the tropics, but some species can be found in subtropical and even temperate regions, as the wide distribution extends from southern Mexico to Central America to South America in Argentina (Shearer *et al.* 2007, Davis *et al.* 2003). Eurotiales, Hypocreales, and Xylariales orders have been found in Tropical forest soils with alterations in land use and

non-impacted soils, for which these orders are considered pioneers and resistant to changes in the physicochemical factors of the soil (Ciccolini *et al.* 2015, McGuire *et al.* 2015).

Eight orders were found in Germany with isolation methodology. The three more abundant orders were Eurotiales (38 %), Hypocreales (22 %), and Mucorales (18 %), which are common in temperate forests, such as the Taunus forest. Eurotiales are frequently isolated in areas with a high diversity of trees (Henkel *et al.* 2006). Hypocreales have been found in Europe, for instance, in the Bialowieza forest in Poland, the Utrecht forest in The Netherlands, and the Poltava forest in Ukraine (Jaklitsch 2011, Samuels 2011). It is worth mentioning that these two orders can be found in almost all ecosystems in the world (Battaglia *et al*. 2011, Druzhinina & Kubicek 2016, Visagie *et al.* 2014). Mucorales is commonly found because most of the species are saprophytes and grow in manure, soil, humus, and other organic waste (Almaraz-Sánchez *et al.* 2012). Furthermore, Tremellales had 7 % of total isolates in Germany, which is not surprising, as the presence of this order is related to the presence of grass in the soil, where the Tremellales are predominant (Vishniac 2006). One of the study areas was in grassland, explaining the presence of Tremellales. Next, Capnodiales, with 2 % of the isolates, are represented by species that incorporate plant and human pathogens (Crous *et al.* 2009). Considering that Capnodiales are endophytes, saprobes, and epiphytes, it is congruent to have found them in the sampling. Mortierellales had 2 % of the total number of isolates. Members of the Mortierellales include saprobic fungi and occasionally occur as opportunistic pathogens in animals and humans (Hoffmann *et al.* 2011). This order has been found in Germany (Nuremberg forest) and the Netherlands (Utrecht forest) (Nagy *et al.* 2011); moreover, Onygenales represented 4% of all orders. There are

reports of this order in different soils, as well as in different geographical regions, such as in India, near Stoneleigh (Ontario, Canada), and forests in Italy (Doveri *et al.* 2012, Kirk *et al.* 2008, Scott *et al.* 1993, Sharma *et al.* 2013).

Onygenales utilize keratin, a protein found in hair, nails, and scales from birds, reptiles, and mammals (Sharma *et al.* 2013). This substrate, like cellulose, is abundant in soil in different forests, for this reason, it is possible to find in Germany. Lastly, Pleosporales represents 2 % of all orders from which species occur in various habitats, and can be epiphytes, endophytes or parasites. It is possible to find them in leaves or stems, as hyperparasites on fungi or insects, or as saprobes in dead plant stems, leaves or bark (Zhang *et al.* 2012). Interestingly, this order list was also found in a tropical forest in the Kat Tien National Park in Vietnam, which confirms the dominance and cosmopolitan of these orders in different forests around the world (Тиунова 2011).

Three orders were dominant in both study areas in Germany and Panama: Eurotiales, Hypocreales, and Mucorales. There are different reasons why these orders were found, Eurotiales for example have an aggressive colonization strategy, adaptable to extreme environmental conditions, cosmopolitan distribution, ubiquitous in soil, and common associates of decaying plant and food material (Tedersoo *et al.* 2010), factors that can be found in both countries. Members of Hypocreales are common in all types of moist forests, with different temperature degrees (Chaverri & Samuels 2002), making it possible to find them in both Germany and Panama. Lastly, species from the third order, Mucorales, are included in the decomposition process of leaf roots and other organic matter (Morace & Borghi 2012) in the soil of both areas. These three orders can be considered cosmopolitan and extremophiles because they can be found in

extreme conditions in different countries, including Germany and Panama. They appear on the most well-known soil microfungi diversity lists (Devi *et al.* 2012, Khusnullina *et al.* 2018, Kyaschenko *et al.* 2017).

7.5 Pyrosequencing identification and factors prediction of soil fungi diversity

The global analysis of soil fungi diversity based on pyrosequencing identification (Tedersoo *et al.* 2014) which was a wide-ranging study incomparable to any prior studies, resulted in 80,486 fungal species in an Operational Taxonomic Units (OTUs) level classified as Fungi. Although many reviews of fungi diversity had been done prior to this study (Bridge & Spooner 2001, Mangan *et al.* 2004, Porras-Alfaro *et al.* 2011, Wahegaonkar *et al.* 2011, Zhang *et al.* 2015), the ground-breaking study from Tedersoo *et al.* (2014) had a great reach and coverage with a global influence, through sampling around the world, which provides useful insights into the relationship between soil fungi diversity and the environmental factors present at each sampling area.

The representativeness of the dominant phyla was evident with Ascomycota (48.7 %) and Basidiomycota (41.8 %). These OTUs had more presence in environmental areas like tropical forests and grasslands. However, in the temperate forest, the OTUs registered were lower in comparison with the other environments.

Egidi *et al.* (2019) surveyed 235 soils around the world. They found that 83 phylotypes belonging to Ascomycota (which represent <0.1 % of the collected fungi) dominate the soil at the global level. In contrast, the highest average

abundance was found in tropical and temperate forests. In the case of Basidiomycota, some studies confirm the second place in dominance in the forest of different regions (Liu *et al.* 2015, Ludley & Robinson 2008, Moll *et al.* 2016, Porras-Alfaro *et al.* 2011, Wubet *et al.* 2012).

Of special importance is finding relationships between environmental factors and soil fungi diversity to predict fungal richness. On a global scale, the climate factors can be used as predictors of fungal richness because it is possible to find climate-driven patterns in soil fungal distribution (Větrovský *et al.* 2019, Xiang *et al.* 2016). Moreover, edaphic and spatial variables can be used on the same scale as predictors of fungal community composition. However, in microscale, the small changes in the factors (deep, moisture, and pH) can have significant changes in the soil fungi community composition (Morris 1999, O'Brien *et al.* 2005, Taylor *et al.* 2014). Considering the climatic factors and fungal diversity on a global scale, it is possible to observe Rapoport's rule with marked exceptions. Rapoport's rule is a biogeographic principle that predicts a decrease in the geographic extent of fungi species if the latitude drops (Raspor & Zupan 2006, Veter *et al.* 2013). In the Raspor and Zupan (2006) study, it was found that the soil fungi richness increased towards the equator. Seeing all fungi species on a global scale, the distance from Equator (P-value 0.033) plays an essential role in fungal diversity and its prediction.

However, an increase towards the poles of the mean latitudinal range of fungi was also observed (Blackwell 2011), which can be explained by the endemic fungi proportion from tropical areas (Arnold & Lutzoni 2007, Hillebrand 2004). In the case of Ascomycota, the diversity decreased with distance from the equator,

while the diversity of saprotrophic species increased with low latitudes. In the case of ectomycorrhizal fungi, greater diversity was observed at mid-latitudes.

Notably, the principal, decisive, and most active predictor factor of total fungal diversity on a global scale is the mean annual precipitation ($P < 0.001$). In some groups, the influence of this factor can be more evident, such as the case of saprotrophs, where moisture and precipitation have a strong positive effect. Taylor *et al.* (2014) mention that the fungal community composition is directly correlated with plant community and indirectly correlated with environmental factors such as soil moisture. However, results for some studies suggest that precipitation can be the principal factor in the composition of soil fungi communities, far above other factors such as soil nutrient status or plant diversity (Braga-Neto *et al.* 2008, Hawkes *et al.* 2011, McGuire *et al.* 2012).

Among edaphic variables, one of the factors that showed a positive correlation in the fungal OTU richness on a global scale was pH ($P < 0.001$). However, on a local scale, in specific points and for some groups, it is not possible to observe the pH correlation (Rosas *et al.* 2019). Soil pH factor was the strongest predictor in the ectomycorrhizal fungus and saprotroph community (Erland *et al.* 1990, Pec *et al.* 2017, Tedersoo *et al.* 2016). Generally, changes in the pH in the soil have direct and indirect effects on fungal diversity (Lauber *et al.* 2009, Rousk *et al.* 2010). Competition, growth, and reproduction can be influenced by pH in different groups of fungi. One example is the saprotrophs that exhibit optimal activities at pH < 5, meaning a more significant advantage against competitors (Tedersoo *et al.* 2016, Yamanak 2003, Yang *et al.* 2017).

Biogeographic regions showed that the north of Europe, North America, East, and West Asia had similar fugal communities. The same situation happened in boreal, temperate coniferous, and temperate deciduous forests, whereas in grasslands and shrublands, many fungi and most functional groups were found. Patterns in the soil fungi community were found in different works around the world (Matsuoka *et al.* 2019, Meiser *et al.* 2014, Wang *et al.* 2019, Zeng *et al.* 2019).

Based on the above, the biogeographic analyses suggest ecological patterns in fungi communities on a global scale.

7.6 Methodologies

Pyrosequencing is a sequencing method based on the motorization in real time of DNA synthesis (Lentendu *et al.* 2011). For this reason, it is possible to sequence enormous amounts of DNA in a short period of time, which is an advantage in comparison to other techniques (Buée *et al.* 2009). However, this technology is expensive, and some of the fungal species are not picked up by pyrosequencing, which suggests that the pyrosequencing technique might have technical glitches such as primer mismatches, differential sequence lengths, and precision loss in the homopolymer regions (Tedersoo *et al.* 2014). Additionally, the variation in the ability to break open cells or fungal structures can lead to biases in the number of species in molecular-based diversity studies (Kirk *et al.* 2004). Contrarily, morphological identification is a process that requires a large amount of time, as it is necessary to use a microscope for the identification of each species in each culture, but this technique is also more specific because it

gives more information about specific morphological characteristics. Although sometimes, the main problem in isolation is the development of the fungi material in agar cultures (Kim *et al.* 2014). Many fungal species elude culturing in the laboratory (Van Elsas *et al.* 2000). In contrast, molecular identification by sequencing the ITS is a cheaper technique with optimal results (Mueller *et al.* 2004). However, the ITS region of several taxa was not enough for the identification of species. In some cases, it is necessary to use other regions to complete the identification. Also, the level within and between species variations in the ITS1 region varies among different groups of fungi (Ovaskainen *et al.* 2010), one example being the variability between Trichoderma species. Another problem in the comparison of sequences is the BLASTN searches against the INSD database, which provided an incomplete solution to compare and identify fungi by ITS regions (Tedersoo *et al.* 2010). Also, the level of similarity represents another difficult situation for the interpretation in the results because the reference to be considered as a reliable identification is when the sequences match with 98 % or more of maximum identity, according to the data in GenBank and the BLAST algorithm (Kõljalg *et al.* 2013). The latter is debatable if we consider the comparison of these results with the morphological identification, for example, in the case of the strain P1M74, which matches in 98 % maximum identity with *Aspergillus flavipes*. However, according to Domsch *et al.* (2007), *A. flavipes* grows slowly (10-14 days) while the strain P1M74 grows faster (4-7 days for 3.5 cm) on the same media. Furthermore, the colour of the strain P1M74 is brown, while the colony of *A. flavipes* is yellow, according to the description. However, the main difference is the conidiophores: the diameter of the strain P1M74 presents a conidiophore of 5-8 µm diam. In comparison, Domsch *et al.* (2007)

reported that the diameter of the conidiophore of *A. flavipes* is around 2-3 µm. Furthermore, the conidiophore of the strain P1M74 has rough while A. flavipes has smooth walls (Horn *et al.* 1996, Kurtzman *et al.* 1987, McAlpin *et al.* 2005). On the other hand, the strand P1M79 had a match with *Trichoderma spirale* with less than 98 % in the maximum identity, but the morphological analysis confirms its identity as being *T. spirale* because in this case, the spiral in the conidiophore is a specific characteristic of this species (Domsch *et al.* 2007, Bissett 1991). Sometimes both identifications can only reach identification up to genera, or in the best cases, both techniques match with the same species identification.

A complimentary T-test was used to find statistical differences between the methodologies. Species richness did not present statistically significant differences in using the two methodologies in any country. However, only six species share the two methodologies in both countries, which shows that pyrosequencing is more effective in identifying the orders Agaricales and Russulales in Germany (Fig. 8), while in Panama Hipocreales and Agaricales (Fig. 9), while morphological identification is more effective in the orders Eurotiales, Hypocreales and Mucorales in both countries (Fig. 14 and 15). In the case of abundance, there were only statistically significant differences in the samples from Germany. The ability to identify individuals is different in each methodology, and each methodology has different scopes for identifying different species in soil fungal diversity.

Taking both advantages and disadvantages of the techniques into consideration, one can conclude that while one might not be particularly better than the other, they are certainly very different techniques. Given the fact that environmental soils are highly diverse, it is highly unlikely that a single technique can be

universally applicable in assessing fungal diversity. In this context, we agree when Anderson and Cairney (2004) mentions in his publication that: "*the profiteering of new technologies will help to increase our understanding and knowledge of soil fungal communities in the future*". Based on the above, it is very useful to combine morphological and molecular identification to obtain optimal results. In this study, it is possible to observe the result of pyrosequencing in a large amount of species identified; however, with the molecular technique, we found different species. This is the reason why it is essential to combine both techniques, as they can be complementary to each other.

7.7 Environmental factors

Environmental factors in relation to the soil fungi diversity, richness and species composition had inconclusive results. This study did not find a statistically significant correlation between all the factors studied and the fungal diversity, just compactation in Germany was statistically significant, this can be explained because Germany had more samplings than Panama. Melo (2019) found that environmental factors are related to spore production and colonization. While other studies show that compaction has a negative influence on diversity (Kheyrodin 2014, Li *et al.* 2004), as mentioned in the bibliography, when the compaction increases, the diversity comes down.

The statistical result in this work is not conclusive. Therefore, it is necessary to acquire more samplings across time to obtain possibly significant data, considering that changes due to environmental factors take a long time to develop, and each species has an individual relationship with environmental

factors. In the case of microbial communities, the relation between the bacterial community and environmental soil factors remains unclear (Wang *et al.* 2019). In other studies, it is possible to see the relationship between soil fungi communities with each factor, Collins *et al.* (2008) mentioned that in forest ecosystems, fungi diversity had a relation with water availability present as moisture, these abiotic characteristics create patchy distributions of roots and biological soil crust which influence and select fungi communities. In Panama, results indicate a positive tendency (without statistically significant) between fungi soil diversity and moisture increment. With more samples and more time, it might be possible to find a statistical significance because, in other research, the relationship is strong and statistically significant, the difference is the number of the samples across time (Canini *et al.* 2019, Frąc *et al.* 2018). Temperature is one of the strongest influences on soil fungal community composition (Shi *et al.* 2013). An upward tendency was observed between increases in the temperature with more diverse soil fungi in Germany (with a gradient between 7 to 24 degrees). Studies of the effect of the increment in the temperature caused by global warming in the soil fungi communities mentioned the relationship between temperature and soil fungi, diversity is closely correlated regardless of forest type (Newsham *et al.* 2016, Tedersoo *et al.* 2014). However, suppose the temperature increased above 25 degrees. In that case, it can be appreciated as a detriment in the soil fungi diversity because the water availability, nutriments, fungi metabolisms are affected, factors that influence the fungi diversity (Kivlina *et al*. 2011, Tedersoo *et al.* 2012). The temperature graphic in Panama was observed with a detriment in the soil fungi diversity with the increment in the temperature with a gradient between 25 to 30 degrees. Seasonality is another critical factor that influences

the soil fungi diversity because, over time, the soil fungi diversity changes with the biogeochemical cycles (Schadt *et al.* 2003). It is possible to see the grouping by season in the multidimensional analysis.

7.8 Theoretical model

The theoretical model is an abstract mental representation expressed through flow diagrams (Pons *et al.* 2010). In this case, a model is represented to combat soil degradation based on fungal diversity. Different fungal cultures have been used for soil fertilization and to stop soil degradation in the Mediterranean, obtaining optimal results (Cornejo 2006). The model presented is based on a combination of methodologies: seasonal irrigation system, use of keystone species, and identification of needs (Schroder 2005, Leiro 2006). The theoretical model applied to biofertilizers is relatively new; its primary function is to solve problems based on the combination of methodologies and using theoretical knowledge based on the literature (Rojas López 2015). The advantages of this model are the analysis, planning, and decision-making, the models, applied to fertilizers must have these characteristics (Aguilera *et al.* 2013). The proposed model is based on Pareto analysis for decision making and identification of the type of species; likewise, the statistical analysis showed that abundance increases as degradation decreases and the proportion of keystone species, nested species, and species in succession. The β-diversity analysis is essential in ecology and biogeography to understand the values of biological diversity. How the ecosystem works this concept is the key to conserving biodiversity and managing ecosystems (Legendre *et al.* 2008). The analysis of β diversity in the

project made it possible to find species constantly changing over time and in the different study areas (Calderón-Patrón *et al.* 2012). However, it was not studied, nor did the project focus on the reasons for the change in diversity, either due to succession or loss of species, because it was not the objective of the study. Gamma (γ) diversity analysis showed which species nested in all soils over time. The work of nested species in diversity is to maximize the number of shared interactions, which provides cohesion and stability in the ecological environment in which they are found (Toju *et al.* 2015). By finding the nested species, it was possible to theoretically shape the proportion and components of the biofertilizer. The keystone species that shape part of the nested species were identified based on abundance. It has been found that the abundance of keystone species contributes more than the richness of species in the formation of biofilms of multiple species in the soil, which suggests that the "mechanism of selection" plays a more important role than the "complementarity mechanism" (Xiong *et al.* 2021). Keystone species help develop the abundance and richness of colonizing species and the stability of diversity (Han *et al.* 2021). Thus, by forming specific biofertilizers for the studied areas, the fight against soil degradation can be started with the help of fungal diversity.

7.9 Soil-Fungi-Cultures (SFC) Application

The Soil-Fungi-Cultures (SFC) Application is new and innovative because it combines computer science and mycology focused on data management. In general, informatics is combined with biology in bioinformatics, defined as the application of computational tools and the analysis of the capture and

interpretation of biological data (Bayat 2002). However, the main focus of bioinformatics is on handling biological and medical data focused on DNA sequences (Cannataro 2019), while the development of software to handle other biological data outside of genetic data in mycology is innovative. Within the SFC App, the database area is easy to use, practical, cheap, and portable. Before the App, the most common thing was to write the data in notebooks and then make a database on a computer (Canedo & Ames 2004, Rosas-Medina and Piepenbring 2018, Ali *et al.* 2013), which required more time. The SCF App makes it more practical as it can be managed within a smartphone. Photography is essential in mycology in many areas; the photos are taken with a professional camera to download later and work with editing software (Senanayake *et al.* 2020). This work represents more costs and time, while the App uses the phone's camera and presents an activity to edit it, thus making the process more efficient and at no cost to the user.

The use of this App for data management for scientific purposes in mycology represents a significant advance in this area because the use and development of Apps for scientific and educational purposes in other areas have been developing in this decade (Brown & Hocut 2015, Liu *et al.* 2021, Zydney & Warner 2016). The advantage of this tool being a telephone App is the possibility that it can be used anywhere in the world where there is a telephone and the SFC App downloaded. Especially in Central America and Africa, where fungal diversity is high, researchers and students have limited resources for their study (Valero-Fernández 2018).

The SCF App is the first to focus on mycology in managing data for cultivation. However, in the area of bacteria cultivation, there is the Colony Counter App (CC)

with a similar objective to help manage and capture data. These two Apps share two main characteristics, database, and photography. The photography area is the one that shows the most remarkable differences between one another; in the case of the CC App, it takes a photograph, but it cannot be worked on or modified at the user's convenience, while the SCF App gives the option to make annotations and remarks on the photo and save it for later observations for the user.

The most similar App on the market is the CC App. However, it must be considered that it is focused on bacteria. Therefore, the problem of contaminating crops and then purifying them is not as common as the case of growing micro-fungi from the soil, especially when it is done directly with grams of soil on the culture medium (Rosas-Medina and Piepenbring 2018).

The differences between the Apps and their applications became clearer when observing the results of the surveys in the graphs. The data recording time and the use of activities are related to the more significant number of buttons and options that the SFC App presents. Each user can decide which activity they dedicate more time according to their particular interests, hence the time; while the CC App, the variability of options to register a crop is not much, so the registration time varies little and is shorter, this means that for practical and simple use it can also be used in the field of mycology, without being so specific in the area of photography. The navigation flow showed that by giving more options in the registry to form the database, the SFC App stops or slows down the navigation flow, even though the database is more detailed, which was done with the users in mind. They are scientists who need the most information from the crops. In practice, they behave like regular users, and the fewer clicks are

better (Bhamidipati *et al.* 2017). The CC App, in this aspect, was more friendly to the user by allowing a more constant navigation flow than the SFC App.

The data transfer variable was the most mentioned activity that affected the navigation flow in the two Apps. It is considered necessary by users to transfer data outside the App. The above is not possible in the CC App, while in the SFC App it is, where the user has to search the memory of his phone for the certain file is. It is an area of potential improvement for the two Apps.

The camera activity in the user interaction is successful because only one user stopped the navigation flow there, considering that it is the activity of the user.

8 Conclusions

In conclusion, it is considered that the characterization of fungi soil community by orders is essential to understand the diversity at a region level and have a global vision of the soil fungi community. Documentation of biodiversity and further research on the effect of disturbance on the functionality of their ecosystem is necessary. Also, it is necessary to take into account in the future the abundance as well as the keystone species to be able to propose accessible and efficient solutions in the fight against soil degradation.

The proposed theoretical model intends to offer a specific solution based on the study carried out in the studied locality, focused on the fungal abundance found. Based on this study, it is possible to develop more research projects toward repairing soils and testing the effect of varying environmental factors on other fungi species. On the other hand, scientific mobile application development is an underexploited and futuristic area as it allows the development of specific tools to solve specific scientific methodological problems. The SFC App is a handy and efficient tool for the user who cultivates microfungi from the soil for various studies.

Finally, it is essential to mention this study is a pioneer in Panama; one of the principal problems in this region is the lack of studies. They do not give any background about the situation in the region. For this reason, it is necessary to develop more studies in the region of Central America to understand the important function of soil fungi communities in these regions.

In Germany, this study is a contribution to soil fungi research made in the Taunus area. Also, the research contributes to the list of species identified in the Taunus

region. In the context of global diversity, it is assisted in studies of representative regions; such is the case of this study that considers the Taunus as representative of Germany. Finally, the study of soil fungi diversity in both forests must continue to find the relationship between the environment and the fungal community.

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11 ANNEXES

The results of this study were compiled in the following three publications:

First publication

1.- Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou N, Wijesundera R, Ruiz L, Vasco-Palacios A, Thu P, Suija A, Smith M, Sharp C, Saluveer E, Saitta A, **Rosas M**, Riit T, Ratkowsky D, Pritsch K, Põldmaa K, **Piepenbring M**, Phosri C, Peterson M, Parts K, Pärtel K, Otsing E, Nouhra E, Njouonkou A, Nilsson R, Morgado L, Mayor J, May T, Majuakim L, Lodge D, Lee S, Larsson K, Kohout P, Hosaka K, Hiiesalu I, Henkel T, Harend H, Guo L, Greslebin A, Grelet G, Geml J, Dunstan G, Dunk C, Drenkhan R, Dearnaley J, Kesel A, Dang T, Chen X, Buegger F, Brearley F, Bonito G, Anslan S, Abell S, Abarenkov K. (2014) Global diversity and geography of soil fungi. Science. 346: 1256688.

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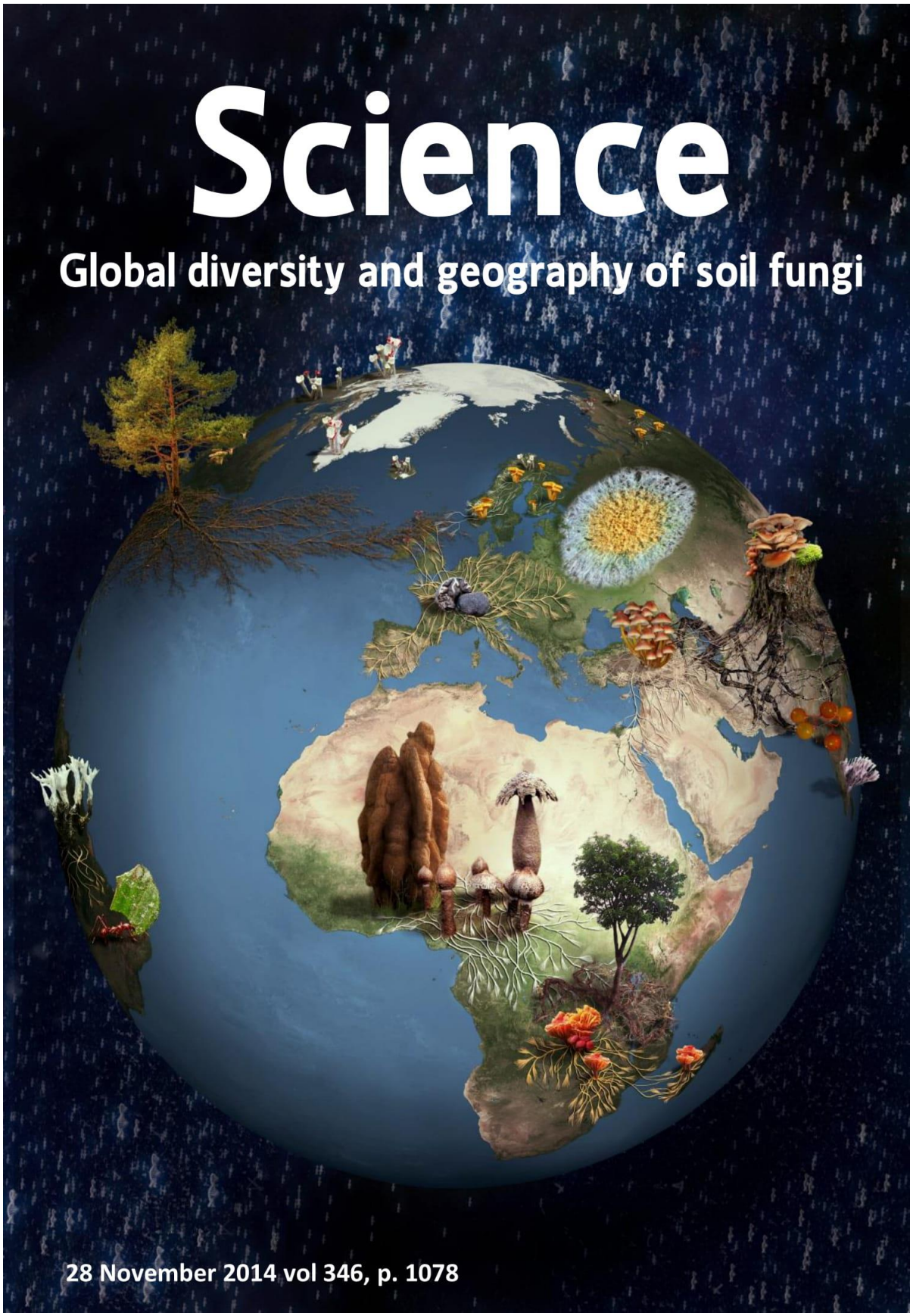
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RESEARCH ARTICLE

FUNGAL BIOGEOGRAPHY

Global diversity and geography of soil fungi

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Fungi play major roles in ecosystem processes, but the determinants of fungal diversity and biogeographic patterns remain poorly understood. Using DNA metabarcoding data from hundreds of globally distributed soil samples, we demonstrate that fungal richness is decoupled from plant diversity. The plant-to-fungus richness ratio declines exponentially toward the poles. Climatic factors, followed by edaphic and spatial variables, constitute the best predictors of fungal richness and community composition at the global scale. Fungi show similar latitudinal diversity gradients to other organisms, with several notable exceptions. These findings advance our understanding of global fungal diversity patterns and permit integration of fungi into a general macroecological framework.

Fungi are eukaryotic microorganisms that play fundamental ecological roles as decomposers, mutualists, or pathogens of plants and animals; they drive carbon cycling in forest soils, mediate mineral nutrition of plants, and alleviate carbon limitations of other soil organisms. Fungi comprise some 100,000 described species (accounting for synonyms), but the actual extent of global fungal diversity is estimated at 0.8 million to 5.1 million species (*1*).

Globally, the biomass and relative proportions of microbial groups, including fungi, co-vary with the concentration of growth-limiting nutrients in soils and plant tissues. Such patterns suggest that the distribution of microbes reflects latitudinal variation in ecosystem nutrient dynamics (*2*–*4*). Richness of nearly all terrestrial and marine macroorganisms is negatively related to increasing latitude (*5*)—a pattern attributed to the combined effects of climate, niche conservatism, and rates of evolutionary radiation and extinction (*6*). Although morphological species of unicellular microbes are usually cosmopolitan (*7*), there is growing evidence that the distribution of microorganisms is shaped by macroecological and community assembly processes (*8*). Only a few of these biogeographic processes have been demonstrated for fungi at the local scale (*9*). Despite their enormous diversity and importance in ecosystem function, little is known about general patterns of fungal diversity or functional roles over large geographic scales. We used a global data set to disentangle the roles of climatic, edaphic, floristic, and spatial variables governing global-scale patterns of soil fungal diversity. We also address macroecological phenomena and show that fungi largely exhibit strong biogeographic patterns that appear to be driven by dispersal limitation and climate.

Materials and methods

We collected 40 soil cores from natural communities in each of 365 sites across the world using a uniform sampling protocol (Fig. 1A and data file S1). Most plots (2500 m^2) were circular, but in steep mountain regions and densely forested areas, some plots were oblong. We randomly selected 20 trees located at least 8 m apart. In two opposite directions, 1 to 1.5 m from each tree trunk, loose debris was removed from the forest floor. Polyvinyl chloride (PVC) tubes (5 cm in diameter) were hammered into the soil down to 5 cm depth. These soil cores almost always included fine roots and comprised both the organic layer and top mineral soil. Although deep soil may contain some distinctive organisms adapted to anoxic conditions or low nutrient levels, our sampling was limited to topsoil for the following reasons. First, in the vast majority of soil types, >50% of microbial biomass and biological activity occur in the topmost organic soil layer. Second, deeper sampling was impossible in shallow, rocky soils or those with high clay concentrations and hardpans. Third, differences among soil horizons may be masked by other variables across large geographic scales (*10*). The 40 soil cores taken in each site were pooled, coarse roots and stones were removed, and a subset of the soil was air-dried at <35°C. Dried soil was stored in zip-lock plastic bags with silica gel in order to minimize humidity during transit. In the laboratory, we ground dried soil into fine powder using bead beating.

We extracted DNA from 2.0 g of soil using the PowerMax Soil DNA Isolation kit (MoBio, Carlsbad, CA) following manufacturer's instructions. We performed polymerase chain reaction (PCR) using a mixture of six forward primers (in equimolar concentration) analogous to ITS3 and a degenerate reverse primer analogous to ITS4 (hereafter referred to as ITS4ngs). We shortened and modified forward and reverse primers to

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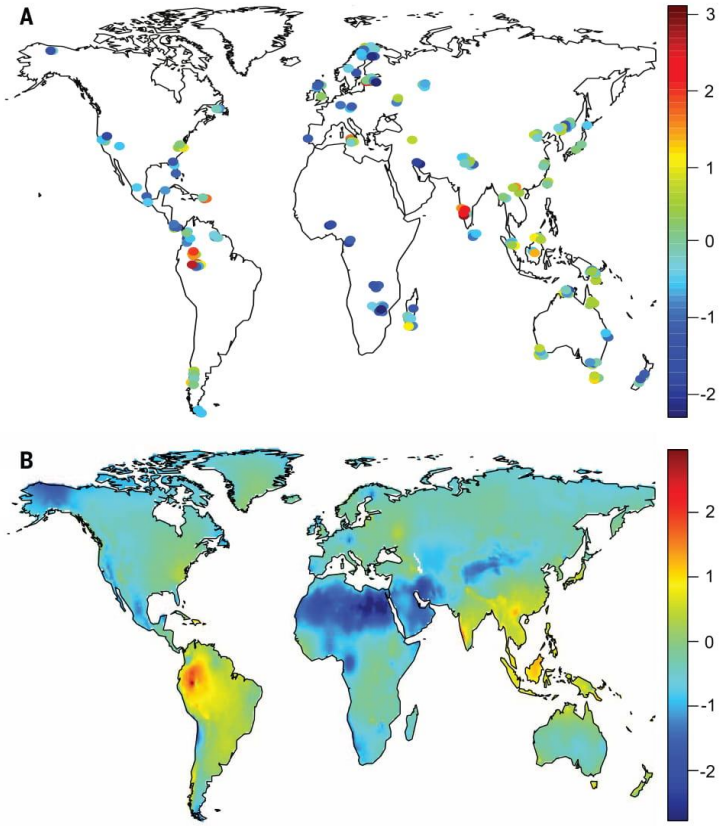

Fig. 1. Maps of global sampling and interpolated taxonomic richness of all fungi. (**A**) Map of global sampling. Circles indicate study sites. (**B**) Interpolated taxonomic richness of all fungi using IDW algorithm and accounting for the relationship with mean annual precipitation (based on the best multiple regression model). Different colors depict residual OTU richness of all fungi accounting for sequencing depth. Warm colors indicate OTU-rich sites, whereas cold colors indicate sites with fewer OTUs.

completely match >99.5% of all fungi [except ~60% of Tulasnellaceae that exhibit highly divergent 5.8*S* ribosomal DNA (rDNA) and Microsporidia that exhibit rearrangements in rDNA] (table S1). The ITS4ngs primer was tagged with one of 110 identifiers (multiplex identifiers, 10 to 12 bases) that were modified from those recommended by Roche (Basel, Switzerland) to differ by >3 bases, start only with adenosine, and consist of between 30 and 70% adenosine and thymidine in order to optimize the adaptor ligation step. The PCR cocktail consisted of 0.6 μl DNA extract, 0.5 μl each of the primers (20 pmol), 5 μl 5xHOT FIREPol Blend Master Mix (Solis Biodyne, Tartu, Estonia), and 13.4 μl double-distilled water. We carried out PCR in four replicates using the following thermocycling conditions: an initial 15 min at 95°C, followed by 30 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 1 min, and a final cycle of 10 min at 72°C. PCR products were pooled, and their relative quantity was estimated by running 5 μl amplicon DNA on 1% agarose gel for 15 min. DNA samples yielding no visible band were reamplified by using 35 cycles in an effort to obtain sufficient PCR product, whereas samples with a very strong band were reamplified with only 25 cycles. It is important to use as few cycles as possible to minimize chimera formation and to be able to interpret sequence abundance in a semiquantitative manner (*11*). We used negative (for DNA extraction and PCR) and positive controls throughout the experiment. Amplicons were purified with Exonuclease I and FastAP thermosensitive alkaline phosphatase enzymes (Thermo Scientific, Pittsburgh, PA). Purified amplicons were subjected to quantity normalization with a SequalPrep Normalization Plate Kit (Invitrogen, Carlsbad, CA) following manufacturer's instructions. We divided normalized amplicons into five pools that were subjected to 454 adaptor ligation, emulsion PCR, and 454 pyrosequencing by using the GS-FLX+ technology and Titanium chemistry as implemented by Beckman Coulter Genomics (Danvers, MA).

Bioinformatics

Pyrosequencing on five half-plates resulted in 2,512,068 reads with a median length of 409 bases. The sequences were reassigned to samples in mothur 1.32.2 (www.mothur.org) based on the barcodes and then trimmed (parameters: minlength = 300; maxambigs = 1; maxhomop = 12; qwindowaverage = 35; qwindowsize = 50; and bdiffs = 1) to exclude short and low-quality sequences, resulting in 2,231,188 high-quality sequences. We used ITSx 1.0.7 (http://microbiology.se/software/itsx) to remove the flanking 5.8*S* and 28*S* rRNA genes for optimal resolution of ITS2 clustering and removal of compromised and nontarget sequences. As a filter to remove most of the partial sequences, we retained only sequences >99 base pairs (bp) in length. Chimera control was exercised through UCHIME 4.2 (www.drive5.com/uchime). After these filtering steps, 1,397,679 sequences were retained and further clustered at 90.0% and 95.0 to 99.0% sequence similarity thresholds (*12*) as implemented in CD-Hit 4.6.1 (www.cd-hit.org). Clustering revealed 37,387, 59,556, 66,785, 77,448, 94,255, and 157,956 taxa based on 90.0, 95.0, 96.0, 97.0, 98.0, and 99.0% sequence similarity thresholds, respectively. The longest sequence of each Operational Taxonomic Unit (OTU), based on clustering at 98.0% sequence similarity, was selected as the representative for BLASTn searches (word size = 7; penalties: gap = −1; gap extension = −2; and match = 1) against the International Nucleotide Sequence Databases Collaboration (INSDC; www.insdc.org) and UNITE (unite.ut.ee) databases. In addition, we ran BLASTn searches against established reference sequences of all fungi in 99.0% similarity clusters that include third-party taxonomic and metadata updates (*12*) as implemented in the PlutoF workbench (*13*). For each query, we considered the 10 best-matching references to annotate our global sequences as accurately as possible. If no reliable taxon name was available, we ran manual BLASTn searches against INSDC with 500 best-matching sequences as output. We typically relied on 90, 85, 80, and 75% sequence identity as a criterion for assigning OTUs with names of a genus, family, order, or class, respectively. Sequence identity levels were raised in subsets of Sordariomycetes, Leotiomycetes, and Eurotiomycetes, because these taxa contain multiple genera and families that have unusually conserved internal transcribed spacer (ITS) sequences. As a rule, we considered *e*-values of BLASTn search results e^{-50} reliable to assign sequences to the fungal kingdom, whereas those $>e^{-20}$ were considered "unknown." E-values between e^{-20} and e^{-50} were manually checked against the 10 best matches for accurate assignment. We followed INSDC for higher-level taxonomy of eukaryotes (*14*) and the Index Fungorum (www.indexfungorum.org) for species through class-level taxonomy of fungi. Our group of taxonomic experts assigned each fungal genus, family, or order to functional categories (data file S2). If different functional categories

were present within a specific genus, we chose the dominant group (>75% of species assigned to a specific category) or considered its ecology unknown (<75% of species assignable to a single category). All Glomeromycota were considered to be arbuscular mycorrhizal (AM). Taxa were considered to be ectomycorrhizal (EcM) if they best matched any sequences of known EcM lineages (*15*) and exhibited sequence length/BLASTn scores above lineage-specific thresholds. For several taxonomic groups, we constructed phylogenetic trees to assess the performance of clustering, sequence quality of singletons, accuracy of OTU separation, and taxonomic assignments (fig. S1). In the course of this project, we provided 10,232 third-party taxonomic reannotations to INSDC sequences to improve subsequent identification of fungal sequences and made these available through the UNITE database.

Statistical analyses

Estimates of the mean annual temperature (MAT), mean annual precipitation (MAP), soil moisture, and soil carbon at 30 arc second resolution were obtained from the WorldClim database (www.worldclim.org). Estimates of potential evapotranspiration (PET) and net primary productivity (NPP) at 30 arc minute resolution were obtained from the Atlas of the Biosphere (www.sage.wisc.edu/atlas/maps.php). Variation coefficients for MAT and MAP were computed based on the average monthly values to represent seasonality of temperature and precipitation. We also calculated the difference of MAP to PET in order to evaluate the effect of rainfall surplus or deficit. On the basis of vegetation type and geographical distribution, sites were categorized into biogeographic regions and biomes following the classification of the World Wildlife Foundation (http://worldwildlife.org) with a few exceptions: (i) temperate deciduous forests in the Northern and Southern hemispheres were treated separately; (ii) tropical montane forests (>1500 m elevation) were separated from the tropical lowland moist forests; and (iii) grasslands and shrublands of all geographic origins were pooled. At each site, we also determined the age of vegetation, time since the last fire, and EcM plant species along with their relative contribution to stand basal area. EcM plants are usually conspicuous trees or prominent shrubs that are relatively easy to identify, and their mycorrhizal status is verifiable in the field by using root excavation and microscopy. Complete lists of tree species were available for <10% of the sites, so we did not directly include plant community composition parameters in our analyses.

Concentrations of N, C, $^{13}C/^{12}C$, and $^{15}N/^{14}N$ were determined from 1 to 20 mg of soil by using GC-combustion coupled to isotope-ratio mass spectrometry (*16*). Concentrations of soil calcium, potassium, magnesium, and phosphorus were determined as in (*16*). Soil pH was measured in 1 N KCl solution.

For analyses of fungal richness, we calculated residuals of OTU richness in relation to the square root of the number of obtained sequences to account for differences in sequencing depth. This method outperformed the commonly used rarefaction to the lowest number of sequences method, which removes most of the data (*17*). We also calculated the richness of major class-level taxonomic and functional groups (comprising >100 OTUs). We excluded outlying samples dominated by a few OTUs of molds, which are indicative of poor sample preservation (relative abundance of sequences belonging to Trichocomaceae >5%, Mortierellaceae >20%, or Mucoraceae >20% that exceeded three times the mean + SD). Although these samples were fairly homogeneously distributed across the world, they had conspicuously lower fungal richness. We also excluded samples that yielded less than 1200 sequences per sample.

To determine the relationship between plant and fungal richness, we relied on co-kriging values from the global vascular plant species richness data set (*18*), which covered 96.7% of our sites. These scale-free values of plant richness were then regressed with residuals from the best-fit models for fungal richness and fungal functional groups. We further calculated the ratio of relative plant richness to fungal richness and fitted this ratio with latitude using polynomial functions to test the assumed uniformity of plant-to-fungal richness ratios at the global scale (*1*, *19*, *20*). To account for potential latitudinal biases in plant-to-fungal diversity estimates, we took into account the nonuniform distribution of land surfaces by calculating an inverse distance weighting (IDW) spatial interpolation of standardized ratios of plant-to-residual fungal diversity using the "gstat" package in R (*21*). We then used IDW to interpolate total fungal diversity beyond sampling sites by accounting for MAP as based on the best-fitting multiple regression model.

Distance from the equator, altitude, age of vegetation, time since last fire, climatic variables, and concentrations of nutrients were log-transformed before analyses in order to improve the distribution of residuals and reduce nonlinearity. To account for potential autocorrelation effects, we calculated spatial eigenvectors using SAM version 4 (*22*). To determine the best predictors of global fungal diversity, we included edaphic, climatic, floristic, and spatial variables in multiple regression models. Because of the large number of predictors, we preselected 16 candidate predictors that were revealed through exploratory multiple linear and polynomial regression analyses based on coefficients of determination and forward selection criteria. The most parsimonious models were determined according to the corrected Akaike information criterion (AICc), which penalizes over-fitting. Last, components of the best models were forward-selected to determine their relative importance as implemented in the "packfor" package in R.

To test the direct effects of climatic variables on richness of fungi and their functional groups, and indirect climatic effects (via soil nutrients and vegetation), we used Structural Equation Modeling (SEM) in Amos version 22 (SPSS Software, Chicago, IL). Model fits were explored based on both χ^2 test and root-mean-square error of approximation (RMSEA). First, we included all potentially important variables (inferred from both the multiple regression models and correlations for individual response variables) to construct separate SEM models. We tested all direct and indirect relations between exogenous and endogenous variables, including their error terms. Then, we used backward elimination to remove nonsignificant links to maximize whole-model fit. Last, we combined the obtained SEM models in a unified path model, following the same elimination procedure.

In addition to full models, we specifically tested the relationships between OTU richness and distance from the equator and soil pH because these or closely related variables were usually among the most important predictors. For these analyses, we calculated residuals of richness that accounted for other significant variables of the best models. To address nonlinear relationships, we fitted up to fifth-order polynomial functions and selected best-fit models on the basis of AICc values.

The relative effects of climatic, edaphic, spatial, and floristic variables on the total fungal community composition and on particular functional groups were determined by using Hellinger dissimilarity (calculated if >90% sites were represented by >1 shared OTUs), exclusion of all OTUs that occurred once, and a multistage model selection procedure as implemented in the DISTLM function of Permanova+ (www.primer-e.com/permanova.htm). Considering computational requirements, 15 candidate variables were preselected based on unifactorial (marginal test based on largest F_{pseudo} values) and multifactorial (forward selection) models. Spatial eigenvectors were not included in these analyses because they typically were of minor importance in variation partitioning analyses and to avoid making the models computationally prohibitive. Optimal models were selected based on the AICc. To obtain coefficients of determination (cumulative $R^2_{adjusted}$) and statistics (F_{pseudo} and P values) for each variable, components of the best models were forward selected. In parallel, we prepared Global Nonmetric Multidimensional Scaling (GNMDS) graphs using the same options. Significant variables were fitted into the GNMDS ordination space by using the "envfit" function in the "vegan" package of R. We also grouped all climatic, edaphic, spatial, and floristic variables into a variation partitioning analysis by integrating procedures in the "vegan" and "packfor" packages of R. Besides group effects, variation partitioning estimates the proportion of shared variation among these groups of predictors.

For global biogeographic analyses, we excluded OTUs from the order Hypocreales and family Trichocomaceae (both Ascomycota) because the ITS region provides insufficient taxonomic resolution, and known biological species are grouped together within the same OTU (*23*). We tested the differences among fungal taxonomic and functional groups for the occurrence frequency (number of sites detected) and latitudinal range of OTUs using a nonparametric Kruskal-Wallis

test and Bonferroni-adjusted multiple comparisons among mean ranks. To test the validity of Rapoport's rule in soil fungi, we calculated the average latitudinal range of OTUs for each site (*24*). The average latitudinal range was regressed with the latitude of study sites by means of polynomial model selection based on the AICc criterion. This analysis was run with and without OTUs only detected at a single site (range = 0). Because the results were qualitatively similar, we report results including all OTUs. To construct biogeographic relationships among major regions and biomes, we generated cross-region and cross-biome networks based on the number of shared OTUs. We excluded occurrences represented by a single sequence per site. Ward clustering of biogeographic regions and biomes were constructed by using the Morisita-Horn index of similarity, which is insensitive to differences in samples size, by use of the "pvclust" package of R. In this procedure, *P* values are inferred for nonterminal branches based on multiscale bootstrap resampling with 1000 replicates.

Taxonomic and functional diversity

Pyrosequencing analysis of global soil samples revealed 1,019,514 quality-filtered sequences that were separated into 94,255 species-level OTUs (supplementary materials). Altogether, 963,458 (94.5%) sequences and 80,486 (85.4%) OTUs were classified as Fungi. Most other taxa belonged to animals (Metazoa, 3.3%), plants (Viridiplantae, 3.1%), alveolates (Alveolata, 2.8%), and amoebae (mostly Rhizaria, 1.3%). Kingdom-level assignment of 3.8% OTUs remained elusive. The fungal subset included 35,923 (44.6%) OTUs that were represented by a single sequence; these were removed from further analyses in order to avoid overestimating richness based on these potentially erroneous sequences (*25*). The remaining 44,563 nonsingleton fungal OTUs in our data set numerically correspond to approximately half of the described fungal species on Earth (*1*). For comparison, there are currently 52,481 OTUs based on 98.0% similarity clustering of all fungal ITS sequences in publicly available databases (*12*). Global soil sampling revealed representatives of all major phyla and classes of Fungi. Of fungal taxa, Basidiomycota (55.7%), Ascomycota (31.3%), Mortierellomycotina (6.3%), and Mucoromycotina (4.4%) encompassed the

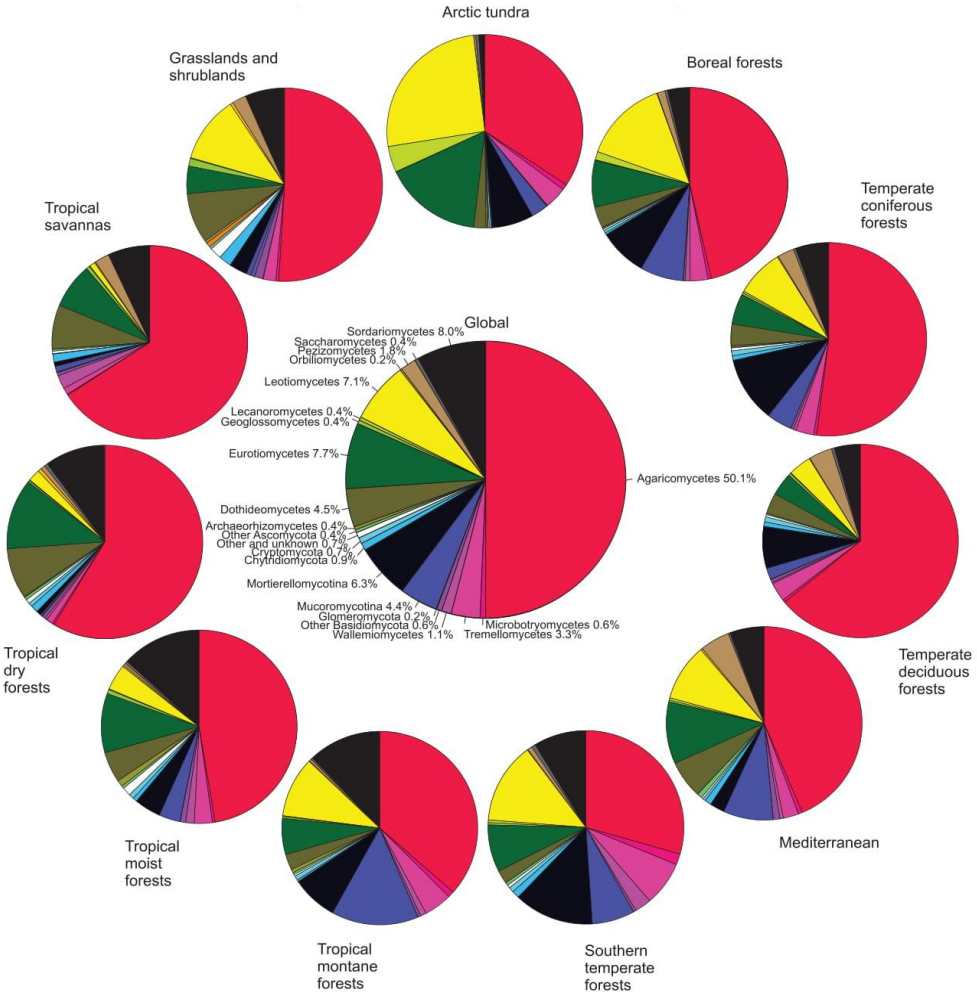

Fig. 2. Relative proportion of fungal sequences assigned to major taxonomic groups in different biomes.

largest proportion of sequences (Fig. 2), whereas the most OTU-rich phyla were the Ascomycota (48.7%), Basidiomycota (41.8%), Chytridiomycota (2.3%), and Cryptomycota (syn. Rozellida; 2.1%) (fig. S2 and data file S1). Except for the recently described phylum Cryptomycota (*26*), the relative proportions of major phyla correspond to the proportional distribution of taxa described and sequenced to date (www.indexfungorum.org) (*12*). Below the phylum level, ~6% of all fungal OTUs could not be assigned to any known class of fungi. Further clustering of unidentified fungal sequences at 70% sequence similarity revealed 14 distinct taxonomic groups comprising >7 OTUs, suggesting that there are several deeply divergent class-level fungal lineages that have not yet been described or previously sequenced.

Our classification revealed that 10,801 (24.2%) fungal OTUs exhibited >98% sequence similarity, and 33.8% exhibited >97% similarity, to preexisting ITS sequences in public databases. This is consistent with (*19*), reporting 48% of OTUs amplified from Alaskan soils with >97% similarity to any database sequences. In our study, only 4353 fungal OTUs (9.8%) were matched to sequences from herbarium specimens or fully described culture collections at >98.0% sequence similarity. Although many type collections are yet to be sequenced, the paucity of matches to database entries indicates that a majority of soil-inhabiting fungal taxa remain undescribed (*19*, *20*). These results highlight the current lack of data from understudied tropical and subtropical ecosystems. The phenomenon of high cryptic diversity and low success in naming OTUs at the genus or species level have been found in other groups of soil microbes and invertebrates, emphasizing our poor overall knowledge of global soil biodiversity (*27*, *28*).

The main fungal phylogenetic and functional groups were present in all ecosystems, but their relative proportions varied severalfold across biomes (Fig. 2 and figs. S2 to S4). The ratio of Ascomycota to Basidiomycota OTUs was highest in grasslands and shrublands (1.86) and tropical dry forests (1.64) but lowest in the temperate deciduous forests (0.88). Chytridiomycota, Cryptomycota, and Glomeromycota were relatively more diverse in the grasslands and shrublands, accounting for 4.6, 3.6, and 1.4% of OTU richness, respectively. The relative OTU richness of Mortierellomycotina and Mucoromycotina (including most fast-growing molds but also some plant symbionts) peaked in the tundra biome (4.8 and 2.7%, respectively), but their abundance was lowest in tropical dry forests (1.0 and 0.6%, respectively). Archaeorhizomycetes, a recently described class of Ascomycetes from a boreal forest (*29*), was most diverse in tropical moist and montane forests, particularly in northern South America and New Guinea.

Among all fungal taxa, OTUs assigned to saprotrophs, EcM mutualists, and plant pathogens comprised 19,540 (43.8%), 10,334 (23.2%), and 1770 (4.0%), respectively (fig. S4). Other trophic categories contained <1% of remaining OTUs. EcM fungi contributed 34.1% of all taxa in the northern temperate deciduous forests but accounted for a relatively low proportion (11.9%) in grasslands and shrublands, reflecting the paucity of host plants in these ecosystems. Similarly, the proportion of EcM fungal taxa was lowest in northern South America (8.0%), where AM trees often dominate. Plant pathogens were relatively more abundant and diverse in lowland tropical moist (6.2%) and dry (6.3%) forests.

Predictors of global richness

Structural equation models revealed that climate has both a strong direct effect on plant and fungal richness and functional groups, but it also indirectly affects these metrics by altering edaphic conditions (fig. S5). Both SEM and regression models suggest that the best predictors of diversity differed among phylogenetic and functional groups of fungi. Positive effects of mean annual precipitation (MAP) and soil calcium (Ca) concentration were the strongest predictors of total fungal diversity, explaining 7.2 and 8.9% of residual richness, respectively (table S2). Richness of EcM fungi responded positively to the relative proportion and species richness of EcM plants (explaining 18.3 and 8.5% of variance, respectively), as well as soil pH (13.0%). EcM host species richness (5.9%) and soil pH (20.4%) remained the strongest predictors in the best model for sites with EcM vegetation, accounting for >60% of basal area, a critical point above which the proportion of EcM plants had no further effect on EcM fungal richness. MAP had a strong positive effect (14.8%) on richness of saprotrophs. Diversity of plant pathogens declined with increasing distance from the equator (17.8%) and soil carbon/nitrogen (C/N) ratio (11.6%). Animal parasites responded positively to MAP (20.3%), whereas monthly variation of precipitation (MAP CV) had a negative impact on richness of mycoparasites (fungus-parasitic fungi; 8.2%). Richness of the AM Glomeromycota was negatively related to the age of vegetation (7.3%) but positively related to PET (3.5%) and soil pH (4.3%). Of the major taxonomic groups, the richness of Ascomycota in general (18.5%) and that of Archaeorhizomycetes (21.7%) were negatively related to distance from the equator in best-fit models. Climatic variables were the best predictors for richness of Mortierellomycotina (MAT, negative effect, 26.1%) and the ascomycete classes Dothideomycetes (MAT, positive effect, 20.9%), Lecanoromycetes (MAT, negative effect, 26.7%), Leotiomycetes (MAT, negative effect, 30.1%), Orbiliomycetes (MAT, positive effect, 12.8%), and Sordariomycetes (MAP, positive effect, 33.4%). The richness of Chytridiomycota and the ascomycete class Pezizomycetes was best explained by a positive response to soil pH (8.6 and 40.5%, respectively). Concentration of soil nutrients or their ratio to other nutrients were the strongest predictors for OTU richness of Cryptomycota (N concentration, positive effect, 10.1%), Geoglossomycetes [N/phosphorus (P) ratio, positive effect, 3.7%], Mucoromycotina (C/N ratio, positive effect, 19.0%), and Wallemiomycetes (P concentration, negative effect, 14.9%). The richness of Basidiomycota and its class Agaricomycetes were best explained by a positive response to soil Ca concentration (13.5 and 12.8%, respectively).

Although geographical distance per se had negligible effects on richness (Moran's $I = 0.267$), spatial predictors were included in the best richness models of nearly all functional and phylogenetic groups (except Glomeromycota), indicating regional- or continental-scale differences in OTU richness (Fig. 1B). Compared with other tropical regions, richness of fungi was conspicuously lower in Africa, independent of biome type. These results might reflect the relatively lower MAP in much of Africa as compared with other tropical continents. Alternatively, lower fungal richness could be related to the disproportionately strong shifts in biomes during the Pleistocene, which impoverished the African flora (*18*).

Among edaphic variables, soil pH and Ca concentration were typically the most important predictors of fungal OTU richness. These variables positively correlated with fungal richness at the global scale ($F_{1,335} = 290.7$; $R_{Pearson} = 0.682$; $P < 0.001$). The strong positive influence of soil Ca concentration on richness of fungi, in particular Basidiomycota, is congruent with a similar positive relationship found for Ca and EcM fungal richness associated with Northern Hemisphere *Alnus* spp. (*30*). Exchangeable Ca is important for many physiological processes in plants and microorganisms and influences the turnover rate of soil organic matter (*31*). In soil geochemical processes, pH and Ca concentration affect each other and thus may have both direct and indirect effects on soil biota. Fungal functional groups were differentially affected by pH. Richness of EcM fungi was greatest in slightly acidic to neutral soils (fig. S6), whereas saprotrophs, especially white rot decomposers, were more diverse in moderately to strongly acidic soils. Richness of Pezizomycetes peaked distinctly in neutral soils.

Macroecological patterns

In general agreement with biogeographic patterns of plants, animals, and foliar endophytic fungi (*5*, *32*), the overall richness of soil fungi increased toward the equator (Fig. 3A). However, major functional and taxonomic groups showed dramatic departures from the general latitudinal richness patterns (Fig. 3 and fig. S7). Namely, diversity of saprotrophic fungi, parasites, and pathogens increased at low latitudes, whereas richness of EcM fungi peaked at midlatitudes, especially in temperate forests and Mediterranean biomes of the Northern Hemisphere (40° to 60°N) (fig. S8). In contrast, saprotrophic fungi had a broad richness peak spanning from ~45°S to 25°N. Richness of Ascomycota—in particular, that of Archaeorhizomycetes, Dothideomycetes, Eurotiomycetes, Orbiliomycetes, and Sordariomycetes—peaked in tropical ecosystems (fig. S7). Conversely, the ascomycete classes Lecanoromycetes and Leotiomycetes, as well as Microbotryomycetes (basidiomycete yeasts), Mortierellomycotina, and Mucoromycotina increased in diversity toward

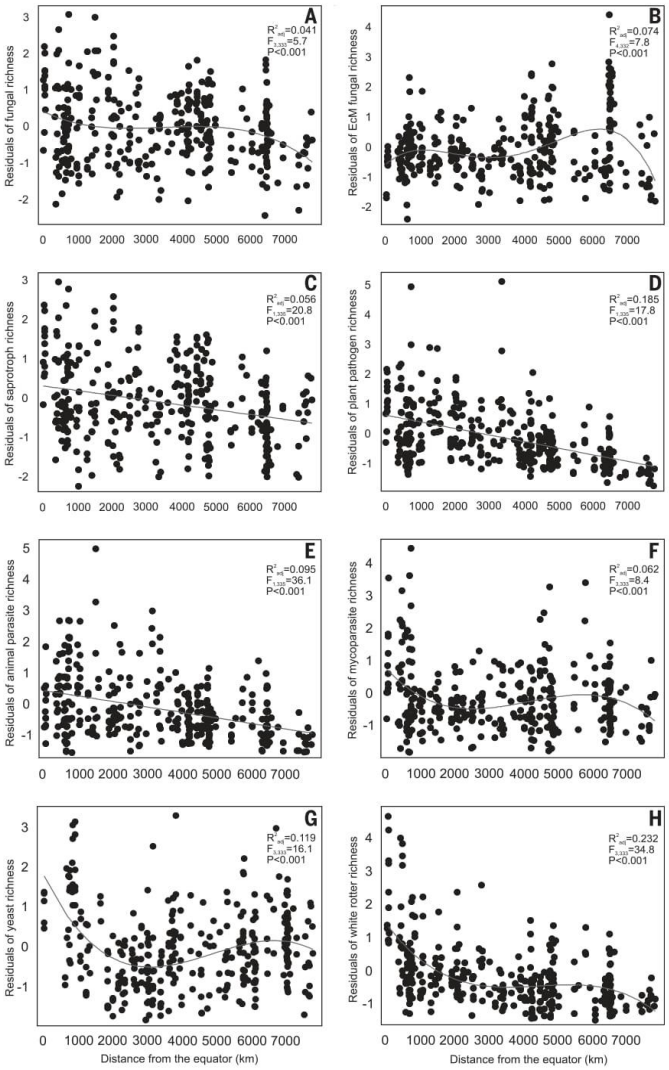

Fig. 3. Relationships between residual richness of fungal taxonomic or functional groups and distance from the equator. (**A**) All fungi. (**B**) EcM fungi. (**C**) Saprotrophic fungi. (**D**) Plant pathogens. (**E**) Animal parasites. (**F**) Mycoparasites. (**G**) White rot decomposers. (**H**) Yeasts. Lines indicate best-fitting linear or polynomial functions.

the poles, with no noticeable decline in boreal forests and tundra biomes. Agaricomycetes, Pezizomycetes, and Tremellomycetes exhibited distinct richness peaks at mid-latitudes. Richness of Agaricomycetes was greater in the Northern Hemisphere, whereas that of Microbotryomycetes, Tremellomycetes, and Wallemiomycetes peaked in the Southern Hemisphere temperate ecosystems (fig. S8).

All of these phylogenetic groups originated >150 million years ago on the supercontinent Pangaea (*33*) and have had sufficient time for long-distance dispersal. However, our data suggest that particular regional biotic or abiotic conditions (such as soil pH and favorable climatic conditions) have likely stimulated evolutionary radiations in certain geographic areas and not in others. Adaptation to cold climate in younger fungal phyla has been suggested to explain differential latitudinal preferences among fungal groups (*34*). However, our global analysis provided no support for this hypothesis (fig. S9). Instead, it revealed that ancient lineages are relatively more common in nonwooded ecosystems.

Relation of plant and fungal richness

Plant and fungal richness were positively correlated (fig. S10), but plant richness explained no residual richness of fungi according to the best regression model ($R^2_{adj} < 0.01$; $P > 0.05$). These results and SEM path diagrams suggest that correlations between plant and fungal richness are best explained by their similar response to climatic and edaphic variables (covariance) rather than by direct effects of plants on fungi. However, when separating functional categories, trophic groups of fungi exhibited differential response to plant diversity and relative proportion of potential hosts.

Plant pathogens usually attack a phylogenetically limited set of host plants (*35*), suggesting that plant pathogens have at least partly coevolved with their hosts and may have radiated more intensively in the tropics, where high plant diversification and richness permit greater diversification. Strong phylogenetic signals in soil feedbacks, adaptive radiation, and negative density dependence (the Janzen-Connell hypothesis) have probably contributed to the pronounced richness of both plants and their pathogens at low latitudes (*36*, *37*). However, our analyses revealed no significant effects of plant richness per se on residual richness of pathogens in soil. Similarly to pathogens, richness of AM fungi was unrelated to the proportion of AM host trees or interpolated host richness, which may result from nonspecific associations with tree and understory species. Hence, both AM and soil pathogen richness were unaffected by plant richness. In contrast, host richness explained 6% of variation in EcM fungal richness, indicating either niche differentiation of fungi in forests of mixed hosts or sampling effects (forests with higher host diversity are more likely to include plant species that harbor high fungal diversity). With a few notable exceptions, most studies have found low levels of host preference or host specificity among EcM fungi (*38*). We found that relative EcM host density had a strong influence on EcM fungal richness, suggesting that greater availability of colonizable roots in soil provides more carbon for EcM fungi and thereby yields greater species density and local-scale richness regardless of latitude. The peak of EcM fungal taxonomic and phylogenetic richness in northern temperate biomes coincides with the geographical distribution and dominance of Pinaceae, which is the oldest extant EcM plant family (*15*, *39*).

The ratio of plant-to-fungal richness decreased exponentially with increasing latitude because plant diversity dropped precipitously toward the poles relative to fungal diversity (Fig. 4). This finding calls into question present global fungal richness estimates. These estimates assume similar spatial turnover of plant and fungal species and a constant plant-to-fungus ratio and have been formulated mostly based on data from temperate and boreal ecosystems (*1*, *19*, *20*). Yet, local-scale beta diversity of both plants and fungi

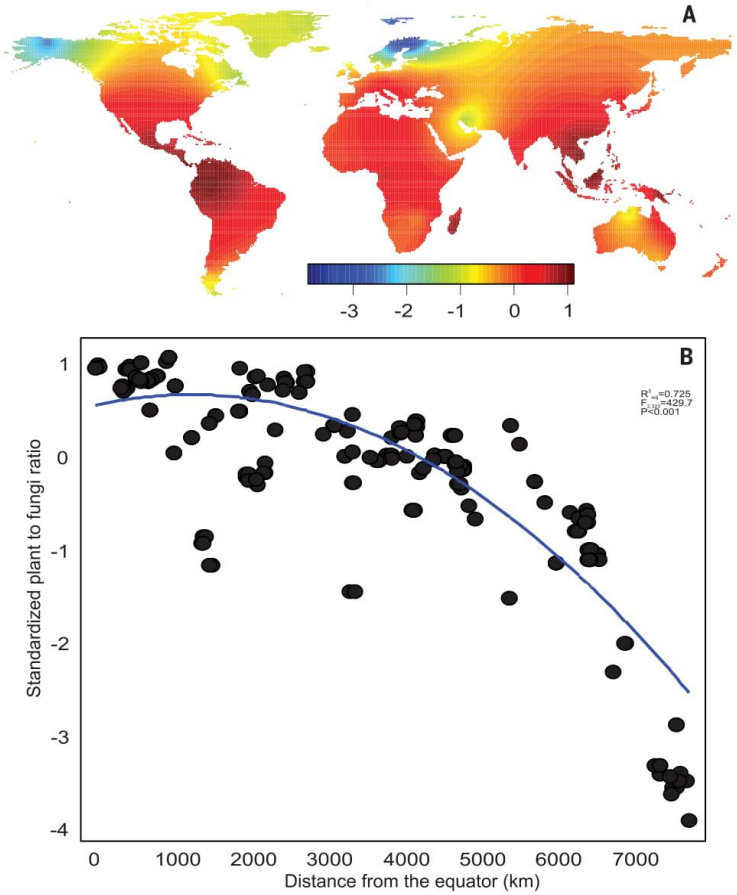

Fig. 4. Relationship between standardized plant richness to fungal richness ratio and distance from the equator. (**A**) Interpolated values. (**B**) Polynomial regression. Residuals of fungal richness are taken from the best linear regression model accounting for other significant predictors. Warm colors indicate high plant-to-fungal richness ratio, whereas cold colors indicate low plant-to-fungal richness.

differ among temperate and tropical sites (*40*, *41*), and there are profound differences in plant species turnover depending on propagule size (*42*). Natural distribution of very few vascular plant species encompass several continents, but there are multiple fungal species with circumpolar or cosmopolitan distribution (*43*, *44*). Although we cannot directly compare plant and fungal beta diversity, spatial turnover of plant species is inarguably greater (*42*). Based on the function of fungi-to-plant richness ratio to latitude and latitudinal distribution of land, we calculated that fungal richness is overestimated by 1.5- and 2.5-fold on the basis of constant temperate (45° latitude) and boreal (65° latitude) richness ratios, respectively.

Because richness estimates are calculated based on the frequency of the rarest species, the reliability of singleton data call into question biologically meaningful extrapolations (*11*). In metabarcoding studies such as ours, sequencing errors tend to give rise to singleton sequences, and the number of rare artificial taxa grows rapidly with increasing sequencing depth (*25*). Therefore, despite the size of our data set, it cannot readily be used to produce reliable taxonomic richness extrapolations.

Community ecology

Variation partitioning analysis revealed that climatic, edaphic, and floristic variables (and their shared effects) are the strongest predictors for community composition of all fungi and most of their functional groups (fig. S11). However, the saprotroph community composition was most strongly explained by purely spatial variables. More specifically, PET and soil pH explained 2.4 and 1.5%, respectively, of the variation in total fungal community composition (table S3 and fig. S12). PET contributed 3.8, 2.8, and 11.7% to community structure of saprotrophs, plant pathogens, and yeasts, respectively. Distance from the equator (1.3%) and soil pH (0.7%) were the strongest predictors of EcM fungal community composition, whereas mean annual temperature (4.0%) was the strongest predictor for animal parasites, and distance from the equator (3.5%) was the best predictor for mycoparasites (table S3 and fig. S12).

These results indicate that both environmental and spatial predictors generally have a minor influence on species-level composition of fungi at the global scale. Nonetheless, the significant global-scale pH effect in several groups of fungi is consistent with the substantial influence of pH on the phylogenetic structure of soil fungal and bacterial communities in both local and continental scales (*27*, *45*). The relatively stronger climatic and edaphic drivers of richness at the class and phylum level suggest that phylogenetic niche conservatism in fungal lineages is similar to cross-biome distribution patterns in vascular plants (*46*) and protists (*47*).

Global biogeography

Consistent with Rapoport's rule formulated for macro-organisms (*24*) and later applied to marine bacteria (*48*), the mean latitudinal range of fungi strongly increased toward the poles (fig. S13). These results also suggest that a greater proportion of fungi are endemic within tropical rather than extratropical ecosystems.

Major taxonomic and functional groups of fungi differed markedly in their distribution range (figs. S14 and S15). Animal parasites were more widely distributed as compared with all other groups, suggesting that there are many generalist OTUs with global distribution. Saprotrophs and plant pathogens had broader distribution ranges than EcM and AM root symbionts. Taxa belonging to Mortierellomycotina, Mucoromycotina, Tremellomycetes, and Wallemiomycetes—groups that include a large proportion of saprotrophs and parasites that produce exceptionally large quantities of aerially dispersed mitospores—were generally most widely distributed. Besides the AM Glomeromycota, OTUs belonging to the ascomycete classes Archaeorhizomycetes, Geoglossomycetes, and Orbiliomycetes were detected from the fewest sites.

The northernmost biogeographic regions (Europe, West Asia, East Asia, and North America) had the most similar fungal communities as revealed by shared fungal OTUs (Fig. 5). According to the Morisita-Horn similarity index, the northern and southern temperate regions clustered together with marginally nonsignificant support (P = 0.064) (Fig. 6A). In spite of the large geographical distance separating them, paleo- and neotropical biogeographic regions clustered together (P = 0.059). However, biogeographic clustering of regions deviated markedly in certain functional groups of fungi (Fig. 6). For instance, EcM fungi in the southern temperate and tropical regions had greater similarity as compared with northern temperate ecosystems

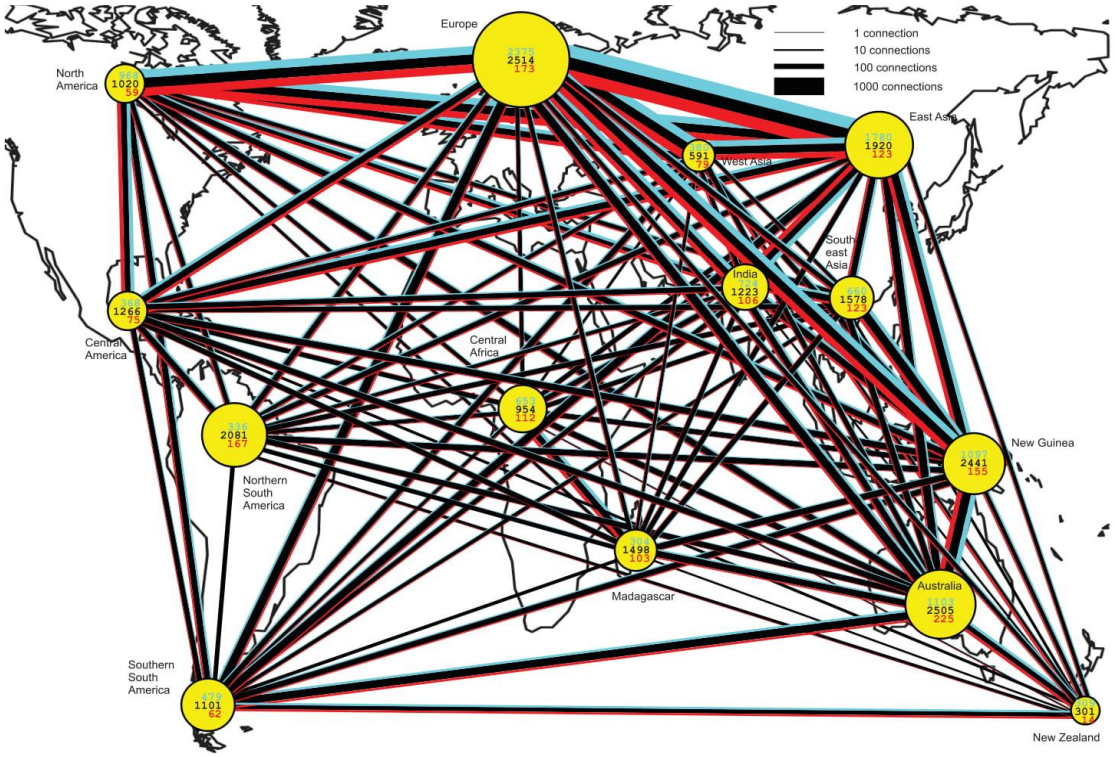

Fig. 5. Connectedness of biogeographic regions by shared OTUs of EcM fungi, saprotrophs, and plant pathogens. Blue, EcM fungi; Black, saprotrophs; and red, plant pathogens. The width of lines and diameter of circles are proportional to the square root of the number of connections and sample size (number of sites), respectively. Numbers in circles indicate the number of OTUs found in each region. OTUs with a single sequence per site and OTUs belonging to Hypocreales and Trichocomaceae (in which the ITS region is too conservative for species-level discrimination) were excluded.

(P = 0.001). Among biomes, boreal forests, temperate coniferous forests, and temperate deciduous forests shared the largest numbers of fungal OTUs (fig. S16). Fungal OTUs in temperate deciduous forests were highly similar to Mediterranean and tropical montane forests, whereas fungal OTUs in tropical montane forests were linked to tropical moist forests, which in turn exhibited substantial connections with tropical dry forests and savannas. As a result, cluster analysis supported separation of tropical and nontropical biomes (Fig. 6B). Consistent with biogeographic region-level analysis, lowland tropical biomes, arctic tundra and boreal forests biomes, and temperate biomes formed three well-supported clusters. Tropical montane forests and grasslands and shrublands were clustered with temperate biomes according to distribution of all fungi and most functional groups. However, in EcM fungi, taxa from southern temperate forests, tropical montane forests, and grass/shrublands clustered with tropical lowland and Mediterranean biomes. A relatively large proportion of EcM fungal taxa were shared across various biomes in Australia and New Guinea, which explains these deviating patterns. In contrast, plant pathogens from tropical montane forests clustered with tropical lowland biomes rather than with temperate biomes.

Our biogeographic analyses complement the community-level results, suggesting that both climate and biogeographic history shape macroecological patterns of fungi. Comigration with hosts over Pleistocene land bridges (such as Beringia, Wallacea, and Panamanian) and long-distance dispersal by spores appear to have played important roles in shaping current fungal distribution patterns (*30*, *43*). The relative influence of climate and biotrophic associations with host plants of varying extant distributions probably contribute to differences in the range and biogeographic relationships among fungal functional groups (*49*). In addition, taxon-specific constraints for dispersal, such as shape and size of propagules and sensitivity to ultraviolet light, may differentially affect long-distance dispersal among taxa (*7*). For instance, Glomeromycota OTUs, which form relatively large non–wind-dispersed asexual spores, had the lowest average geographical range. In general, region-based distribution patterns of fungi are somewhat conflicting with clustering of plants and animals, where Holarctic lineages are deeply nested within larger tropical groups (*50*). Consistent with macroorganisms, fungi from the Southern Hemisphere temperate landmasses cluster together. Differences observed in macroecological patterns among fungi, plants, and animals may originate from the relative strength of dispersal limitation and phylogeographic history, but exaggeration from methodological differences among studies cannot be discounted. The use of homogenous sampling and analytical methods, as done in this study, are necessary to confidently compare macroecological patterns among distinct life forms and to reliably test degrees of consistency among all kingdoms of life.

Conclusions and perspectives

Climatic variables explained the greatest proportion of richness and community composition in fungal groups by exhibiting both direct and indirect effects through altered soil and floristic variables. The strong driving climatic forces identified here open up concerns regarding the

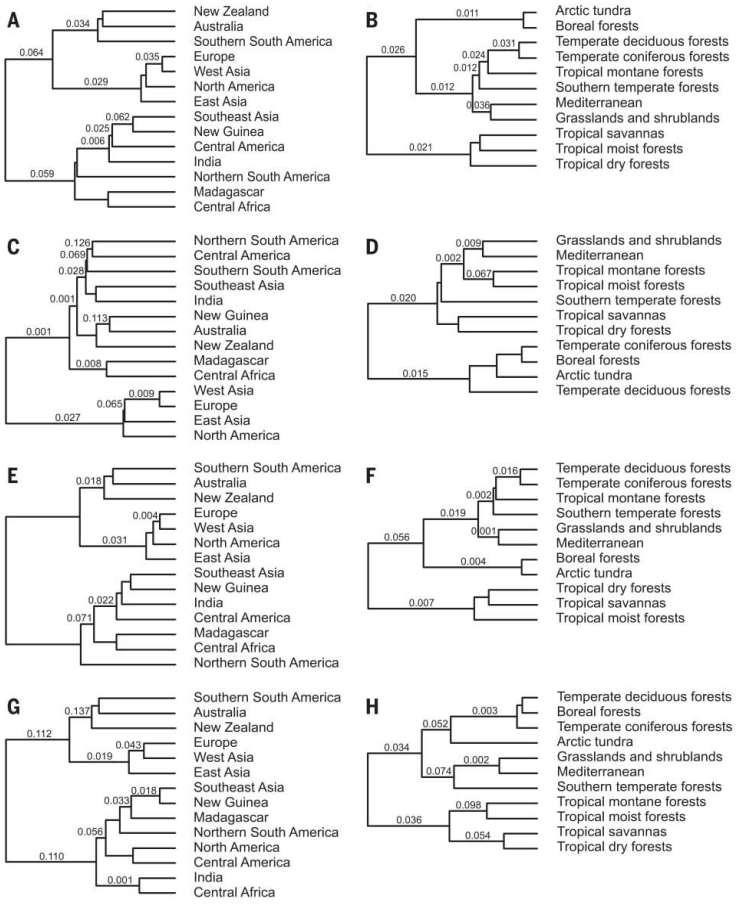

Fig. 6. Ward clustering of biogeographic regions and biomes based on the Morisita-Horn pairwise similarity index. Left, biogeographic regions; right, biomes. (**A** and **B**) All fungi. (**C** and **D**) EcM fungi. (**E** and **F**) Saprotrophs. (**G** and **H**) Plant pathogens. Numbers above branches indicate *P* values.

impact of climate change on the spread of disease (*51*) and the functional consequences of altered soil microorganism communities (*52*). The observed abrupt functional differences between fungal communities in forested and treeless ecosystems, despite spatial juxtaposition, suggests that plant life form and mycorrhizal associations determine soil biochemical processes more than plant species per se. Loss of tree cover and shrub encroachment resulting from drying and warming may thus have a marked impact on ecosystem functioning both above- and belowground.

In addition to natural mechanisms, such as long-distance dispersal and migration over past land bridges, global trade has enhanced the spread of some non-native soil organisms into other ecosystems, where they sometimes become hazardous to native biota, economy, and human health (*53*). Our results highlight how little insight we still have into natural microbial distribution patterns, and this undermines our ability to appraise the actual role of humans in shaping these biogeographic processes. Even larger-scale sampling campaigns are needed to provide data for establishing natural distributions and building species distribution models (*52*), which will enable us to predict the spread and habitat suitability of non-native microorganisms.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/346/6213/1256688/suppl/DC1
Figs. S1 to S17
Tables S1 to S3
Data Files S1 and S2

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New records of microfungi from degraded soil close to dry seasonal forest in Western Panama

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With 11 figures and 2 tables

Abstract: Microfungi of degraded soil at the border of a tropical seasonal dry forest in Western Panama were isolated and cultivated. These fungi were identified based on morphological characteristics and molecular sequences of the internal transcribed spacer (ITS) region. 24 species of soil fungi were identified among which 10 species are new reports for Panama, i.e., five species of Eurotiales (*Aspergillus roseoglobulosus*, *A. tamarii*, *Penicillium janczewskii*, *P. miczynskii*, *Talaromyces verruculosus*), four species of Hypocreales (*Mariannaea elegans*, *Purpureocillium lilacinum*, *Trichoderma harzianum*, *T. spirale*), and one species of Mucorales (*Mucor moelleri*). Four of these species, namely *A. roseoglobulosus*, *M. moelleri*, *P. janczewskii*, and *T. verruculosus*, are also new for Central America. The strain P1M27 probably belongs to *Ascopolyporus* and may represent a species new to science.

Key words: biodiversity, biogeography, environmental isolates, fungal ecology, fungal morphology.

Introduction

The most widely accepted number of fungal species existing worldwide is estimated to correspond to about 1.5 million (Hawksworth 2001), but only about 100,000 species of fungi are known (González et al. 2003, Hawksworth 1991, Kirk et al. 2001). It can be assumed that a large number of still unknown fungal species are soil microfungi, especially in tropical areas like Panama, where few studies have been carried out up to now (Guzmán & Piepenbring 2011). One of the first and most extensive studies on soil fungi in Panama is that of Farrow (1954), who isolated 135 species of fungi from soils of Panama and Costa Rica. More recently, Mangan et al. (2004) found a total of 27 arbuscular mycorrhizal fungal species in a study on the diversity of arbuscular

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mycorrhizal fungi across a fragmented forest in Panama. Piepenbring (2006, 2007) presented a checklist of fungi reported for Panama which includes 1802 species of micro- and macrofungi based on more than 300 publications that represent around 100 years of mycological history in Panama. It is estimated that about 50,000 species of fungi exist in Panama but only about 4% are known. The great need of basic field research, especially in remote areas such as many neotropical regions, is evidenced by many known and the newly described species being only represented by one or very few preserved collections (Piepenbring 2000). Tropical regions of the world are considered to have the highest diversity for most groups of organisms (Hillebrand 2004), and this is generally true for fungi as well (Arnold & Lutzoni 2007, Tedersoo et al. 2014). Fungi constitute an important part of the soil ecosystem, playing central roles in biotic and abiotic interactions, cycling processes, and decomposition (Pompéia et al. 2013). The immense phenotypic and genetic diversity of soil fungal communities makes it one of the most difficult communities to study (Kirk et al. 2004). It is difficult to isolate all the fungi from a particular soil representative of one area, and due to a lack of taxonomic knowledge it is difficult to identify all the species (Kirk et al. 2004). However, our knowledge and understanding of soil fungi diversity has changed in the last decades due to advances in molecular technology (Christ et al. 2010). Molecular methods are used to assess fungal communities giving a more realistic view of their diversity (Kirk et al. 2004). For the present study, new reports of species of soil fungi in Panama are presented, with species identified based on a combination of morphological and molecular analyses.

Material and methods

The samples were obtained from soil at the border of a tropical, semicaducifolious forest in the Majagua valley, close to the village Los Algarrobos, in the province Chiriquí in Western Panama (08°29'33"N, 82°25'59"E, approx. 120 m a.s.l.). Soil samples were collected in the forest, in grassland, and from bare soil at 10 m distance from each other from the upper surface of the A1 horizon with a metal tube of 7 cm diameter. Three core samples of the first five centimeters of the soil were collected at each site, where the exact points were selected randomly. The three cores were kept in plastic bags and mixed for a total sample for each site, following the protocol published by Carrasco et al. (2002). Microfungi were cultivated from soil samples in Petri dishes with different media (malt extract agar – MEA, potato dextrose agar – PDA, Czapek agar – CzA, and malt extract-yeast-peptone agar – MYP). Minute quantities of about 0.005–0.02 mg of the different soil samples diluted in distilled water were dispersed on the surface of sterile media. Fungal colonies growing out on these media kept at 25°C for 2 to 25 days were used to isolate pure cultures.

Morphological identification of fungi was performed by using different keys (Booth 1971, Chaverri et al. 2003, Domsch et al. 2007, Hesseltine & Ellis 1964, Martínez 2003, Samuels 2011, Samson et al. 2006, Stolk & Samson 1983). For every plate, the colony was described macroscopically with the aid of a stereomicroscope (Muller et al. 2004). Microscopical structures were observed by light microscopy in preparations in potassium hydroxide (KOH) stained with lactophenol, cotton blue, or phloxin (Baxter & Van der Linde 1999).

Molecular identification was done with 0.25–0.5 g of pure fungal cultures for DNA extraction, which was done according to the protocol published by Talbot (2001) modified by Weisenborn (2016). The PCR reaction was realized with ITS1 and ITS4 primers (White et al. 2013) to obtain sequence data from the ribosomal DNA (rDNA), as this region is one of the regions most commonly used in phylogenetic studies and barcoding approaches to distinguish fungal species. The DNA purification was done with the Cycle pure kit (VWR) and the samples were sent to the laboratory

of the Biodiversity and Climate Center (BiK-F) for sequencing. The sequences were aligned with the software CodonCode Aligner version 8.1. (CodonCode Corporation, www.codoncode.com) and MEGA (Tamura et al. 2013). After that, these sequences were compared with other reference sequences from GenBank (NCBI, http://www.ncbi.nlm.nih.gov). According to the data in GenBank and the BLAST algorithm, strains that matched 98% or more of maximum identity were considered as reliable identifications, while sequences with less than 98% identity were subjected to critical morphological analysis (Kõljalg et al. 2005). The principal reference for the selection of the currently valid name was MycoBank (http://es.mycobank.org).

Dry colonies of the strains were deposited at the Herbarium Senckenbergianum in Frankfurt/Main, Germany (FR).

Results

A total of 24 species of soil microfungi were identified (Table 1), including 10 species that are new reports for Panama and one species that is probably new to science. Nine species reported for the first time for Panama belong to Ascomycota (five species to Eurotiales and four species to Hypocreales) and one to Zygomycota (Mucorales). In the following, new records for Panama and a species probably new to science are presented by morphological descriptions and molecular sequence data (Table 2).

Descriptions of species new to Panama

Aspergillus roseoglobulosus Frisvad & Samson, in Frisvad, Frank, Houbraken, Kuijpers & Samson, Stud. Mycol. 50: 30 (2004). Fig. 1

Macroscopical description: Colonies frequently forming agglomerations in circles, pink with white and yellow, cottony. Reverse of culture lightly red to pink. Colony on MEA attaining 1.5 cm diam. after 10 days at 25°C.

Microscopical description: Hyphae septate, 6–9(–12) µm wide, single hyphal cells (69–)83–102(–120) µm long, hyaline. Vesicles at the tip of the conidiophores globose, (21–)28–33(–38) µm long. Conidial heads biseriate. Metulae (2–)3–4(–5) µm wide and (5–)6–8(–11) µm long. Phialides flask-shaped, 2–3(–4) µm wide and (6–)7–9 (–11) µm long. Conidia globose to subglobose, 2–3 µm wide, hyaline, with rough surface.

Material examined: Majagua, bare soil, 18.07.2012, M.Rosas et al. P1M37 (FR-0247033), ITS sequence KY320597.

Ecology and distribution: *Aspergillus roseoglobulosus* has been found in subtropical and tropical zones. It has been recorded from Argentina, Brazil, Hawaii, and Pakistan (Frisvad et al. 2004). *A. roseoglobulosus* is reported here for Panama for the first time.

Notes: According to the data in GenBank and the BLAST search, the strain P1M37 matches with *Aspergillus roseoglobulosus* (two sequences with significant identities). Molecular identification of strain P1M37 also matches with 98% with *Aspergillus sclerotiorum*, *A. sulphureus*, *A. bridgeri*, and *A. persii.* However, its morphological characteristics correspond to A. *roseoglobulosus* because of the rose red mycelium and rose red reverse of the colony on MEA (Frisvad et al. 2004). *A. sclerotiorum* produces a creamy-yellow layer of conidia and it is frequently observed to occur in soil depths of 30–50 cm (Christensen 1982). *A. roseoglobulosus* produces a similar yellow layer of

Table 1. Species of fungi isolated from soil from Western Panama and identified by morphology and molecular sequence data. Names of species reported for the first time for Panama are written in bold.

Ascomycota
Eurotiales
Aspergillus japonicus Saito
Aspergillus roseoglobulosus Frisvad & Samson
Aspergillus tamarii Kita
Penicillium citrinum Thom
Penicillium janczewskii K.M.Zaleski
Penicillium javanicum J.F.H.Beyma
Penicillium miczynskii K.M.Zaleski
Penicillium simplicissimum (Oudem.) Thom
Talaromyces verruculosus (Peyronel) Samson, N.Yilmaz, Frisvad & Seifert
Hypocreales
Acremonium zonatum (Sawada) W.Gams
Ascopolyporus sp. P1M27
Bionectria ochroleuca (Schwein.) Schroers & Samuels
Fusarium oxysporum Schltdl. species complex
Fusarium solani (Mart.) Sacc. species complex
Lecanicillium saksenae (Kushwaha) Kurihara & Sukarno
Mariannaea elegans (Corda) Samson
Metarhizium marquandii (Massee) Kepler, S.A.Rehner & Humber
Purpureocillium lilacinum (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson
Trichoderma harzianum Rifai
Trichoderma koningii Oudem.
Trichoderma koningiopsis Samuels, C.Suárez & H.C.Evans
Trichoderma spirale Bissett
Zygomycota
Mucorales
Absidia cylindrospora Hagem
Gongronella butleri (Lendn.) Peyronel & Dal Vesco
Mucor moelleri (Vuill.) Lendn.

conidia, but its mycelium is pink and it is reported from superficial soil layers (Frisvad et al. 2004). On the other hand, *A. sulphureus* is yellow and produces a discoloration zone in agar plates (CDA and MEA medium), while *A. roseoglobulosus* does not present any discoloration in agar plates (Kumar et al. 2012). *A. bridgeri* differs from *A. roseoglobulosus* by a colony reverse with ochre colour and the diameter of conidial heads (up to 200–260 µm; Christensen 1982). *A. persii* is similar to *A. sclerotiorum* on the basis of morphological characteristics but *A. persii* only grows on certain substrates and at specific temperatures, and *A. persii* differs from *A. roseoglobulosus* by the yellow color of the mycelia (Zotti et al. 2010). Morphological and molecular characteristics indicate that the strain number P1M37 corresponds to *A. roseoglobulosus,* but this identification should be complemented by a chemical analysis (Samson et al. 2006).

Aspergillus tamarii Kita, Zentbl. Bakt. Parasitkde, Abt. II 37: 433 (1913). Fig. 2

MACROSCOPICAL DESCRIPTION: Colonies frequently forming agglomerations in circles, olive green, powdery. Reverse of culture greenish to brown. Colony on MYP attaining 1.5 cm diam. after 10 days at 25 °C.

MICROSCOPICAL DESCRIPTION: Hyphae septate, 4–5(–6) µm wide, single hyphal cells (19–)25–62(–86) µm long, hyaline. Conidiophores composed of an unbranched stipe, 5–9 µm wide and up to (120–)154–248(–300) µm long, hyaline, walls rough. Vesicle globose, (11–)34–36(–42) µm wide and up to (15–)36–18(–47) µm long. Conidial heads uni- or biseriate. Metulae when present, 3–7(–8) µm wide and (3–)4–9 (–12) µm long. Phialides flask-shaped, 4–5(–6) µm wide and up to (7–)8–11(–15) µm long. Conidia globose to subglobose, 4–5(–6) µm wide, green, with echinulate walls.

MATERIAL EXAMINED: Majagua, bare soil, 18.07.2012, M.Rosas et al. P1M16 (FR-0247034), ITS sequence KY320598.

ECOLOGY AND DISTRIBUTION: *Aspergillus tamarii* has frequently been isolated from cultivated soils (rice, wheat, citrus, and coconut groves), soils under legume crops, and tropical rain forest, Himalayan forest, and estuarine sediments. It has been recorded from Bangladesh, Bahamas, China, Costa Rica, Gambia, India, Iraq, Israel, Japan, Libya, Malaysia, Pakistan, Peru, Somalia, Spain, Syria, and the USA (Domsch et al. 2007, Markham & Herren 1990). *A. tamarii* is reported here for Panama for the first time.

NOTES: According to data in GenBank and BLAST search, the strain P1M16 matches with *Aspergillus tamarii* (36 sequences with significant alignments), *A. caelatus* (eight sequences), *A. flavus* (five sequences), and *A. parasiticus* (13 sequences). According to Raper and Fennell (1965) the section *Flavi* is a group containing nine species and two varieties that include *A. flavus*, *A. tamarii*, *A. caelatus*, and *A. parasiticus*, which are closely related, but can be distinguished by the color of the conidia, presence or absence of metulae, ornamentation, size of the conidia, and DNA fingerprint (Castillo 2007, Horn 1997, Kurtzman et al. 1987, McAlpin et al. 2005). Morphologically, *A. caelatus* is closely related to *A. tamarii* because both species have conidia with rough surface (Ito et al. 1998). However, they can be distinguished by differences in the ornamentation of the conidia, i.e., conidia of *A. tamarii* have an echinulate surface while conidia of *A. caelatus* have a tuberculate surface, and the color of the colonies after 10 days of growth, i.e., *A. caelatus* shows a change of green to brownish-green and does not produce cyclopiazonic acid (Horn 1997, Ito et al. 1999). The other closely related species is *A. parasiticus,* which can be distinguished from *A. tamarii* by the color of the phialides (usually hyaline to pale green), the color of the conidia (yellow-green), and the production of aflatoxin (Kurtzman et al. 1987, Samson et al. 2004). *A. tamarii* differs from *A. flavus* (with conidia 3.5–4.5 µm wide) by conidia that reach a diameter of up to 6 µm (Domsch et al. 2007). Genetically, *A. tamarii* is closely related to *A. caelatus*, but there are some nucleotide differences that allow to distinguish the DNA sequences of these two species in the regions of ITS1, ITS2, 5.8S rDNA, and 28S rDNA (Ito et al. 1999). To determine *Aspergillus* species belonging to the section *Flavi* it is necessary to not only use the morphological characteristics, but also the characteristics related to the production of mycotoxins, the analysis of the color of colonies using digital images, and DNA sequences (Horn 1997, Horn et

Table 2. BLAST search results for ITS sequence data of strains isolated from soil in Panama.

Species (strain, base pairs)	GenBank accession number	Species name in GenBank	Query coverage in %	Maximum identity in %	Number of sequences of the same species with significant identity
Aspergillus roseoglobulosus (P1M37, KY320597, 629 bp)	FJ491583	*Aspergillus roseoglobulosus*	57	99	2
	FR733827	*Aspergillus sclerotiorum*	57	98	15
	EF661408	*Aspergillus sulphureus*	57	98	3
	EF200084	*Aspergillus bridgeri*	57	98	2
	FR733836	*Aspergillus persii*	57	98	3
Aspergillus tamarii (P1M16, KY320598, 583 bp)	GU362011	*Aspergillus tamarii*	70	99	36
	JQ676205	*Aspergillus caelatus*	70	99	8
	GQ340557	*Aspergillus parasiticus* (also cited as *A. sojae* or *A. toxicarius*)	70	99	18
	JX852612	*Aspergillus flavus* (also cited as *A. oryzae*)	70	99	8
	JN185451	*Aspergillus chungii*	70	98	1
	JF446613	*Aspergillus transmontanensis*	70	98	2
	AF338641	*Aspergillus bombycis*	70	98	3
Mariannaea elegans (P1M35, KY320619, 565 bp)	AB855778	*Mariannaea elegans* (also cited as *Nectria mariannaeae*)	94	98	22
	AB587666	*Mariannaea camptospora*	94	91	3
	JX125048	*Mariannaea samuelsii*	94	91	3
	GQ153836	*Mariannaea aquaticola*	94	91	3
Mucor moelleri (P1M118, KY320646, 583 bp)	KF367556	*Mucor moelleri* (also cited as *Zygorhynchus moelleri*)	100	100	19
Penicillium janczewskii (P1M5, KY320624, 321 bp)	JN617693	*Penicillium jensenii*	100	97	2
	JX045775	*Penicillium canescens*	98	96	3
Penicillium miczynskii (P1M78, KY320611, 636 bp)	KJ439199	*Penicillium miczynskii* (cited as *P. shearii*)	94	99	7
	KJ191428	*Penicillium sanguifluum*	95	97	4
	GU566236	*Penicillium roseopurpureum*	95	97	5
	JN617685	*Penicillium copticola*	87	97	1
	JN617672	*Penicillium atrosanguineum* (cited as *P. manginii*)	95	96	1

Purpureocillium lilacinum (P1M95, KY320622, 691 bp)	AB103380	*Purpureocillium lilacinum* (also cited as *Paecilomyces lilacinus*)	92	97	67
	AB084157	*Ophiocordyceps heteropoda*	92	96	5
	GU980040	*Purpureocillium takamizusanense* (cited as *Isaria takamizusanensis*)	89	95	2
	GU980038	*Akanthomyces* sp.	89	94	1
Talaromyces verruculosus (P1M41, KY320632, 594 bp)	AF510496	*Talaromyces verruculosus* (also cited as Penicillium verruculosum)	97	99	17
	HQ392496	*Talaromyces aculeatus* (cited as *Penicillium aculeatum*)	92	99	1
	FJ230987	*Penicillium janczewskii*	100	98	3
Trichoderma harzianum (P1M6, KY320639, 609 bp)	EF191303	*Trichoderma harzianum* (also cited as *Hypocrea lixii*)	94	97	48
Trichoderma spirale (P1M79, KY320642, 628 bp)	HQ608089	*Trichoderma spirale*	94	97	44
	EU280085	*Trichoderma crassum* (cited as *Hypocrea crassa*)	94	96	1
	JQ040400	*Trichoderma virens* (cited as *Hypocrea virens*)	94	96	2
cf. ***Ascopolyporus* sp.** (P1M27, KY320593, 556 bp)	AY886545	*Ascopolyporus moellerianus* (cited as *Ascopolyporus philodendri*)	62	89	2
	JN943305	*Cordyceps pseudomilitaris*	59	86	2
	AB360356	*Lecanicillium kalimantanense*	68	86	1
	EU284721	*Lecanicillium fusisporum*	60	86	2

al. 1996, Samson et al. 2006). The morphological characteristics of the strain P1M16 confirm its identification as *A. tamarii*.

Mariannaea elegans (Corda) Samson, Studies in Mycology 6: 75 (1974). Fig. 3

Macroscopical description: Colony with cottony texture, mycelium planar to convex, with aerial mycelium, 2–5 mm high, white, gray, and light brown on the reverse of the culture. Colony on PDA attaining 2.5 cm diam. after 7 days at 25°C.

Microscopical description: Hyphae septate, individual cells 3–5(–7) µm wide and (13–)16–38(–40) µm long, hyaline. Metulae 3–4(–5) mm wide and 10–18(–20) mm long, smooth, hyaline. Phialides subulate to acerose, (2–)3–4 µm wide and 7–21 (–25) µm long. Conidia asymmetrical, fusoid, often apiculate, 2–4(–5) µm wide and (5–)6–10(–15) µm long, hyaline, smooth.

Material examined: Majagua, bare soil, 18.07.2012, M.Rosas et al. P1M35 (FR-0247039), ITS sequence KY320619.

Ecology and distribution: *Mariannaea elegans* has been reported from bark or decaying wood and soil in forests (Domsch et al. 2007, Samuels & Seifert 1991). *M. elegans* has been isolated from soils of Canada, France, Germany, Italy, Poland, South Africa, the British Isles, the Netherlands, the USA, and Venezuela (Domsch et al. 2007, Samuels & Seifert 1991). In Jamaica, a strain of *M. elegans* was isolated from arable soils under similar environmental conditions as those in Panama (Samuels & Seifert 1991). *M. elegans* is reported here for Panama and Central America for the first time.

Notes: According to data in GenBank, the strain P1M35 matches with *Mariannaea elegans,* which is the anamorph name for *Nectria mariannaeae* (Samuels & Seifert 1991). *M. elegans* isolated from Panamanian soil also matches morphologically with the description published by Domsch et al. (2007). *M. elegans* differs from *Mariannaea camptospora* in colony pigmentation, the isolates of *M. camptospora* having a red purple reverse in culture (Domsch et al. 2007). Morphologically, *Mariannaea aquaticola* differs from *M. elegans* in the shape of conidia (ellipsoidal to fusiform) and in the color of the colony on MEA, which is yellowish to dark brown (Tang et al. 2012). Molecular sequences of *M. elegans* and *M. aquaticola* differ as shown by Cai et al. (2010), Samuels (2011), and data presented here. *Mariannaea samuelsii* differs from *M. elegans* by the shape of the conidia, which are cylindrical and 1-septate (Samuels 1989). These two species can be clearly separated from *M. elegans* based on their capacity to produce reddish-purple colonies on MEA (Samuels 2011).

Mucor moelleri Vuill., Bull. Soc. mycol. Fr. 19: 117 (1903). Fig. 4

Synonym: *Zygorhynchus moelleri* Vuill., Bull. Soc. mycol. Fr. 19: 117 (1903).

Macroscopic description: The colony is white, forming dense, superficial, woolly colonies. Within the colony numerous zygospores are evident as black dots. The fungal strain covers the entire Petri dish (9 cm diam.) after approx. 10 days at 25°C.

Microscopical description: Hyphae without septa, hyaline, 2–5(–7) µm wide. Sporangiophores (5–)8–19(–30) µm long, (2–)3–5(–7) µm wide. Sporangia globose,

(8–)11–18(–20) µm diam. Sporangiospores ovoid, (1–)2–3(–4) µm long, hyaline, smooth. Zygospores oval, brown to dark brown, (25–)32–41(–45) µm diam., warty. A zygospore is carried by two unequal suspensors, the small suspensor has a diameter of about 3–6(–8) µm (very difficult to observe), while the larger, ovoid suspensor has a diameter of about (17–)19–28(–32) µm.

Material examined: Majagua, bare soil, 18.07.2012, M.Rosas et al. P1M118 (FR-0247043), ITS sequence KY320646.

Ecology and distribution: This species is one of the commonest members of the Mucoraceae and has a worldwide distribution. It has been reported almost exclusively from soils for Austria, the British Isles, Central Africa, Denmark, Germany, India, Italy, New Zealand, Norway, Poland, Rumania, South Africa, and the USA (Domsch et al. 2007, Zycha & Siepmann 1969). *M. moelleri* is reported here for Panama and Central America for the first time.

Notes: According to the BLAST search in GenBank, the ITS sequence of the strain P1M118 matches with a similarity of 100% with sequences of *Mucor moelleri*. Morphologically, *M. moelleri* is characterized by white to grey, globose to slightly flattened sporangia of 12–40(–60) µm diameter, ellipsoidal to ovoid sporangiospores, and zygospores that are most abundant just above the surface of the substrate, brown to dark brown, mostly 30–35 µm wide, and carried by unequal suspensors (Domsch et al. 2007).

Penicillium janczewskii K.M.Zaleski, Bull. Acad. Polon. Sci., Math. et Nat., Sér. B: 488 (1927). Fig. 5

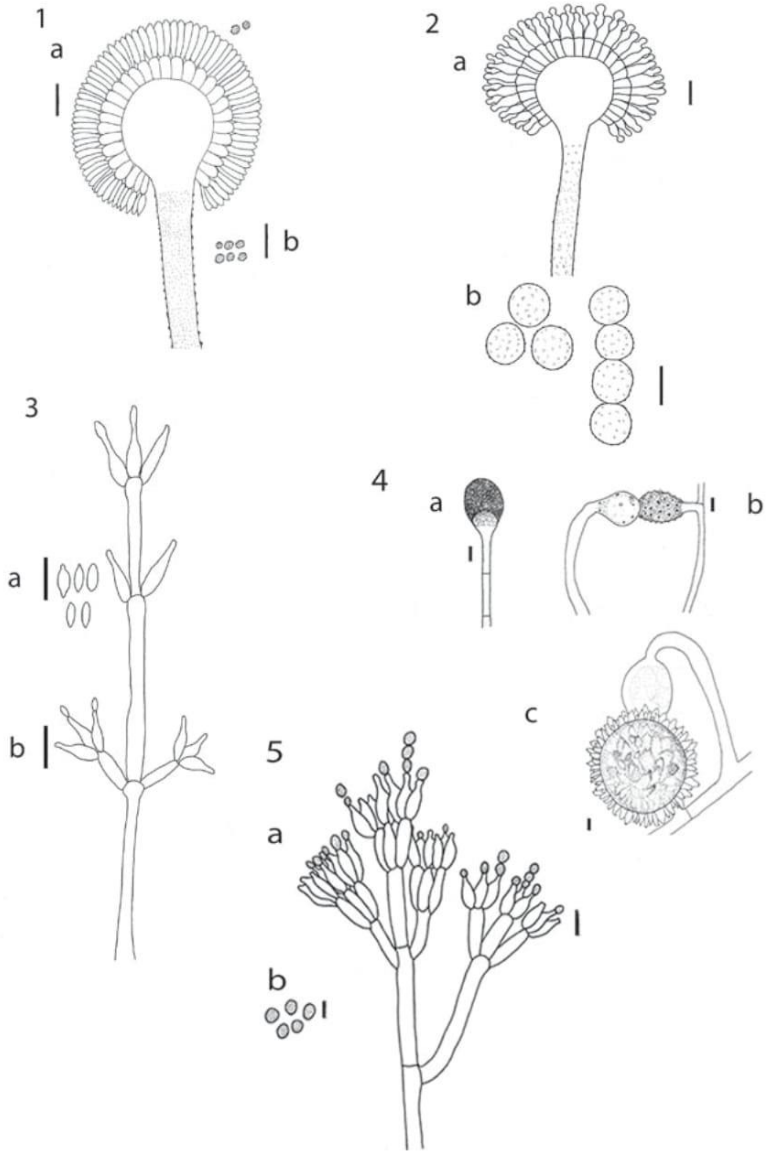
Macroscopical description: Mycelium growing fast, covering the Petri dish after 10 days (9 cm diam.) of cultivation at 25°C. Colonies dark olive green colored, frequently forming agglomerations on their surface.

Microscopical description: Hyphae septate. Stipes of conidiophores 2–3 µm wide and (15–)26–46(–55) µm long. Rami 2–3 µm wide and (7–)13–27(–35) µm long, hyaline. Ramuli 2–3(–4) µm wide and 8–17(–22) µm long. Metulae at the tips of lateral branches, irregularly divergent, with smooth walls, 1–3(–4) µm wide and (3–)4–12(–20) µm long. Phialides flask-shaped, cylindrical, tapering towards the apex, 1–3 µm wide and (4–)6–9(–10) µm long. Conidia in chains, subglobose to globose, 2–4 µm diam., brown, with rough walls.

Material examined: Majagua, bare soil, 18.07.2012, M.Rosas et al. P1M5 (FR-0247035), ITS sequence KY320624.

Ecology and distribution: *Penicillium janczewskii* has frequently been isolated from soil of forests in temperate zones, grassland, and arable soils. It has been recorded from Austria, the Bahamas, Brazil, Egypt, India, Italy, Israel, Japan, Peru, and Syria (Daynes et al. 2008, Domsch et al. 2007). *P. janczewskii* is reported here for Panama for the first time.

Notes: The morphological identification of *Penicillium janczewskii* is based on its conidia, which are rough-walled and brown as seen by light microscopy. These characteristics and the size of the structures agree with those presented for *P. janczewskii*



Figs 1–5. *Aspergillus roseoglobulosus* (P1M37). Fig. 1. a. Conidiophore with metulae and phialides. b. Rough conidia. Bars = 10 µm. Fig. 2. *Aspergillus tamarii* (P1M16). a. Conidiophore with metulae and phialides. b. Echinulate conidia. Bars = 5 µm. Fig. 3. *Mariannaea elegans* (P1M35). a. Conidia. b. Conidiophore with metulae, phialides and conidia. Bars = 10 µm. Fig. 4. *Mucor moelleri* (P1M118). a. Sporangiophore with sporangium. b. Fusing gametangia with gametangiophores, c. Zygospore with two suspensors and adjacent hypha. Bars = 5 µm. Fig. 5. *Penicillium janczewskii* (P1M5). a. Conidiophore with phialides and conidia. b. Conidia. Bars = 5 µm.

by Domsch et al. (2007). Molecular data (table 2) indicate a close relationship of the strain P1M5 with *P. canescens*, *P. janczewskii*, and *P. jensenii*. *P. janczewskii* has strongly divergent metulae and the conidial walls are rough, while conidia are smooth in *P. jensenii* (Domsch et al. 2007, Martínez 2003). *P. canescens* is morphologically similar to *P. janczewskii* in size and shape of conidia, but conidia of *P. canescens* are smooth-walled to slightly roughened, while conidia of *P. janczewskii* are coarsely warted. Conidiophores of *P. canescens* are conspicuously roughened and those of *P. janczewskii* smooth (Domsch et al. 2007, Pessoni et al. 2002).

Penicillium miczynskii K.M.Zaleski, Bull. Acad. Polon. Sci., Math. et Nat., Sér. B: 482 (1927). Fig. 6

Synonym: *Penicillium shearii* Stolk & D.B.Scott, Persoonia 4: 396 (1967).

Macroscopical description: Colonies green with white borders, frequently forming ring-like agglomerations on their surface. Mycelium growing fast, covering the Petri dish after 14 days (9 cm diam.) of cultivation at 25°C.

Microscopical description: Hyphae septate. Stipe of the conidiophore 3–5 µm wide and (130–)155–181(–210) µm long. Metulae irregularly divergent, 1–3 µm wide and (11–)14–17(–20) µm long. Phialides flask-shaped, cylindrical, tapering towards the apex, 2–3 µm wide and (5–)7–8(–10) µm long. Conidia in tangled chains, subglobose to ovate, 1–2 µm wide and 2–3 µm long, with finely rough ornaments.

Material examined: Majagua, bare soil, 30.07.2012, M.Rosas et al. P1M78 (FR-0247036), ITS sequence KY320611.

Ecology and distribution: *Penicillium miczynskii* has been frequently isolated from soil of tropical and subtropical areas (Tuthill & Frisvad 2004). It has been recorded from Austria, Central Africa, Colombia, Honduras, Japan, New Guinea, South Africa, and Sierra Leone (Domsch et al. 2007). *P. miczynskii* is reported here for Panama for the first time.

Notes: Zaleski described *P. miczynskii* in 1927 from the soil of a *Picea* forest in Poland. Both Thom (1930) and Raper and Thom (1949) accepted *P. miczynskii* as valid and unique and provided full descriptions based upon detailed examination of derivatives of the Zaleski isolates. Pitt (1979) placed *P. chrzaszczii, P. matris-meae*, and *P. miczynskii* (cited as *P. soppii*), also described by Zaleski in 1927, and four additional species (*P. atrosanguineum*, *P. manginii*, *P. pedemontanum*, and *P. syriacum*) into synonymy with *P. miczynskii* and accordingly expanded the species circumscription (Christensen et al. 1999). *Penicillium soppii* is the anamorph name for *Eupenicillium shearii* (Stolk & Samson 1983). However, according to Index Fungorum, *Eupenicillium shearii* is a synonym of *P. miczynskii*, and a synonym of *P. manginii* according to Stolk & Samson (1983). According to data in GenBank, the strain P1M78 corresponds to *P. shearii* with 99% of identity (Table 2). The sequence of the strain P1M78 showed relationship with *P. sanguifluum*, *P. roseopurpureum*, and *P. copticola.* Morphologically, *P. miczynskii* resembles *Eu. saturniforme* and *Eu. tropicum*, but it differs from them by strictly velutinous colony texture with abundant conidiogenesis and finely rough conidia (Tuthill & Frisvad 2004). *Eupenicillium saturniforme* can be differentiated from *P. miczynskii* by robust biverticillate penicilli, apically vesiculate metulae and rough-

walled stipes (Wang & Zhuang 2009). *Penicillium roseopurpureum* morphologically differs from *P. miczynskii* by its strictly monoverticillate conidiophores (Vega & Posada 2006). *Penicillium copticola* can be differentiated by an analysis of spectroscopic data and by the color of the culture (Daengrot et al. 2014). *Eupenicillium bovifimosum* and *Eu. baarnense* are also closely related to *P. miczynskii* especially by similar shape and size of conidia. Nevertheless, they can be differentiated by the production of whorls of up to five metulae (Tuthill 2002). Those species are not represented in GenBank, therefore chemical analysis would be necessary to identify them (Belofsky et al. 1998). *Penicillium miczynskii* may be identified by secondary metabolites, this species produces kaitocephalin when cultivated on a solid medium (Shin-ya et al. 1997).

Purpureocillium lilacinum (Thom) Luangsa-ard, Houbraken, Hywel-Jones and Samson, in Luangsa-ard, Houbraken, Doorn, Hong, Borman, Hywel-Jones and Samson, FEMS Microbiol. Lett. 321: 144 (2011). Fig. 7

Synonyms: *Paecilomyces lilacinus* (Thom) Samson, Stud. Mycol. 6: 58 (1974).

Penicillium lilacinum Thom, Bull. U.S. Department of Agriculture, Bureau Animal Industry 118: 73 (1910).

Macroscopical description: Colonies frequently forming agglomerations in circles, rose to pink, with cottony texture. Reverse of culture slightly pale pink. Colony on MYP attaining 1.5–3 cm diam. after 10 days at 25°C.

Microscopical description: Hyphae septate, 2–3(–5) µm wide, single hyphal cells (10–)11–26(–28) µm long, hyaline. Conidiophores composed of an unbranched stipe, 5–7(–8) µm wide and (80–)120–180(–212) µm long, hyaline, with rough walls, with metulae, ramuli and phialides. Metulae 3–4 µm wide and 8–10(–11) µm long. Phialides flask-shaped, 2–3 µm wide and 4–7(8) µm long, branched. Conidia in long chains, ellipsoidal, 2–3(–6) µm diam., pink, with smooth walls.

Material examined: Majagua, bare soil, 30.07.2012, M.Rosas et al. P1M95 (FR-0247040), ITS sequence KY320622.

Ecology and distribution: *Purpureocillium lilacinum* has been frequently isolated from soil of warm regions but it also has been reported from temperate forest (Itin et al. 1998). It is recorded from Argentina, Austria, Central Africa, Canada, Chile, Egypt, France, Honduras, Hong Kong, India, Israel, Jamaica, Japan, Libya, Malaysia, Nepal, Pakistan, Poland, Somalia, South Africa, Spain, Turkey, and the USA (Domsch et al. 2007). *P. lilacinum* is reported here for the first time for Panama.

Notes: According data in GenBank and the BLAST search, the strain P1M95 matches with *Purpureocillium lilacinum* (partly as *Paecilomyces lilacinus*) (67 sequences with significant alignments), *Ophiocordyceps heteropoda* (five sequences), and *Isaria takamizusanensis* (two sequences). Samson (1974) defined 31 species in the genus *Paecilomyces* including *Paecilomyces lilacinus,* which recently was transferred to *Purpureocillium lilacinum* (Luangsa-ard et al. 2011). Species of the new genus *Purpureocillium* differ from species of *Paecilomyces* by the absence of chlamydospores, the lilac conidia (those of *Paecilomyces* are olive-brown), and because the optimum growth temperature is different. *Purpureocillium* spp. show rapid growth on agar media

at 25–33°C, while *Paecilomyces* spp. grow more slowly at 30–45°C (Luangsa-ard et al. 2011). The phylogenetic position using ITS and 1-α (translation elongation factor gene) sequences shows that *P. lilacinum* belongs to the family Ophiocordycipitaceae (Sung et al. 2007, Inglis & Tigano 2006) while species of *Paecilomyces* are located in the family Trichocomaceae (Luangsa-ard et al. 2011).

For the morphological identification of the strain P1M95 the keys published by Luangsa-ard et al. (2011) and Domsch et al. (2007) were used. The genus *Purpureocillium* includes two species: *P. lilacinum* is characterized by ellipsoidal to fusiform conidia which are hyaline, smooth-walled to slightly roughened and purple *en masse*. *P. lavendulum* differs by subglobose or limoniform conidia with an apiculate base, slightly longer phialides, no growth at 35°C and the production of a yellow pigment (Luangsa-ard et al. 2011, Perdomo et al. 2013). *P. lilacinum* is a saprobic species that can be isolated from soil, insects, nematodes, animals, and humans (Itin et al. 1998, Luangsa-ard et al. 2011). Based on these data the species is identified as *Purpureocillium lilacinum*.

Talaromyces verruculosus Peyronel, Samson, Yilmaz, Frisvad & Seifert, Studies in Mycology 70: 177 (2011). Fig. 8

Synonym: *Penicillium verruculosum* Peyronel, I germi astmosferici dei fungi con micelio: 22 (1913).

Macroscopical description: Colonies green olive, with white borders, frequently forming ring-like agglomerations on their surface. Reverse of colonies pale reddish yellow. Mycelium growing fast, covering the Petri dish after 7 days (9 cm diam.) of cultivation at 25°C.

Microscopical description: Hyphae septate. Stipes of conidiophores 2–3 µm wide and (81–)98–136(–144) µm long, smooth. Ramuli 2–3 µm wide and (88–)91–101 (–123) µm long. Metulae divergent, 3–4 µm wide and (7–)8–10(–12) µm long. Phialides flask-shaped, cylindrical, tapering towards the apex, 3–4 µm wide and (6–)7–13 (–15) µm long. Conidia in disordered chains, spherical to subspherical, 2–3 µm wide, hyaline to subhyaline, with rough walls.

Material examined: Majagua, bare soil, 30.07.2012, M.Rosas et al. P1M41 (FR-0247037), ITS sequence KY320632.

Ecology and distribution: *Talaromyces verruculosus* was frequently isolated from soil or roots of plants in grasslands or coniferous forest, in countries like Costa Rica, Denmark, Pakistan, Sweden, the USA, and Taiwan (Bhagobaty et al. 2010, Gul Shah et al. 2014, Samson et al. 2011). In India, it was found among root fungal endophytes in tropical rainforest areas (Bhagobaty et al. 2010), whereas in Panama, this species was isolated from grasslands in tropical lowlands.

Notes: According to data in GenBank, the strain P1M41 corresponds to *Talaromyces verruculosus* (syn. *Penicillium verruculosum*) with 99% identity (Table 2). The molecular analysis also shows a 99% identity of sequences with *Talaromyces aculeatus* (cited as *Penicillium aculeatum*), however the morphological characteristics of the strain P1M41 match with *T. verruculosus*, especially the green olive color on MYP medium, the white color at the margin and the typical pale reddish yellow color of the reverse

of the colony (Mayumi et al. 1992). Microscopical characteristics of *T. verruculosus* also match with the description published by Mayumi (1992). *T. verruculosus* is phylogenetically closely related to *T. aculeatus* (Gul Shah et al. 2014).

Trichoderma harzianum Rifai, Mycological Papers 116: 38 (1969). Fig. 9

Macroscopical description: Texture of the colony woolly, colonies with white mycelium and green conidia on their surface. Colony on MYP reaching 5.5 cm diam. after 5 days at 25°C. Reverse of culture slightly pale yellow.

Microscopical description: Hyphae septate, 3–4 µm wide, single hyphal cells (25–) 29–53(–65) µm long, hyaline. Conidiophores 2–3 µm wide and (7–)9–16(–20) µm long, with phialides in dense groups. Phialides flask-shaped, with swollen base, 1–2 µm wide and 4–6(–8) µm long. Conidia globose to subglobose, 2–3 µm wide, green, with smooth walls.

Material examined: Majagua, bare soil, 18.07.2012, M.Rosas et al. P1M6 (FR-0247041), ITS sequence KY320639.

Ecology and distribution: *Trichoderma harzianum* (asexual and sexual states) has a worldwide distribution with records from Europe, South America, Africa, and Asia (Chaverri et al. 2003, Grondona et al. 1997). *T. harzianum* has been frequently been isolated from soil of forests, as well as in fields of wheat. It has been recorded from the British Isles, Canada, Costa Rica, India, Italy, Libya, the Netherlands, Nigeria, and the USA (Domsch et al. 2007, García et al. 1992, Holmes et al. 2004). *T. harzianum* was found in forest soil in Panama resulting as the first report in this country.

Notes: Chaverri and Samuels (2002) reported that cultures derived from ascospores of *Hypocrea lixii* produced the morphological species *Trichoderma harzianum* in pure culture, so *T. harzianum* is the asexual stage of *H. lixii*. Accordingly, the strain P1M13 matches to sequences labeled as *Hypocrea lixii* as well as *Trichoderma harzianum.* Phylogenetic analysis of ITS region showed that *Trichoderma harzianum*/*Hypocrea lixii* is a cohesive group (Chaverri et al. 2003).

The identification by morphological characteristics confirms this species identification (Domsch et al. 2007). Morphological characteristics of the strain correspond to *T. harzianum,* which is characterized by dense conidiation, colonies rapidly turning yellowish-green to dark green and presenting pustules fringed by sterile white mycelium. Conidiophores tend to be regularly verticillate forming a pyramidal structure and are divided by septa. Phialides are ampulliform to lageniform and form verticillate groups of 3–4. Conidia are subglobose and pale green when fully mature (Domsch et al. 2007, Kubicek & Harman 1998). *T. harzianum* is somewhat similar to *Trichoderma viride*, but *T. harzianum* has phialides more densely aggregated than *T. viride* and phylogenetically both species are not closely related (Domsch et al. 2007, Samuels 1996). The comparison of the sequences as well as the morphological description allow to confirm the species identification as *T. harzianum.*

Trichoderma spirale Bissett, Can. J. Bot. 69: 2408 (1992). Fig. 10

Macroscopical description: Texture of the colony woolly, colonies with white mycelium and green conidia on their surface. The color of the reverse side of culture

is the same as of the upper side (white). Colony on PDA reaching 5.5 cm diam. after 5 days at 25°C.

Microscopical description: Hyphae septate, 3–4 µm wide, single hyphal cells (15–)19–23(–35) µm long, hyaline, walls smooth. Conidiophores 4–6 µm wide and (30–)38–42(–50) µm long, with 3–5 phialides each. Phialides subglobose, usually swollen at the base, 3–5 µm wide and 4–6(–8) µm long. Conidia ellipsoidal, 2–3 µm diam., green, with smooth walls.

Material examined: Majagua, bare soil, 30.07.2012, M.Rosas et al. P1M79 (FR-0247042), ITS sequence KY320642.

Ecology and distribution: *Trichoderma spirale* has frequently been isolated from rain forest soils and bamboo stand soils. It has been recorded from Canada, Costa Rica, New Zealand, Thailand, and the USA (Bissett 1991, NZOR 2013). *T. spirale* is reported here for Panama for the first time.

Notes: In his description of *Trichoderma spirale*, Bissett (1991) mentioned that one of the main morphological characteristics of this species are the spiral sterile conidiophore elongations and the dense production of conidia. Both characteristics are present in the culture P1M79. According to the BLAST search in NCBI databank, the strain P1M79 matches with a similarity of 97% to *T. spirale*. *T. spirale* is morphologically similar to *Trichoderma polysporum*, but conidiophores are rough-walled in *T. polysporum* while they are smooth-walled in *T. spirale* (Bissett 1991, Domsch et al. 2007).

cf. *Ascopolyporus* sp. (P1M27) Fig. 11

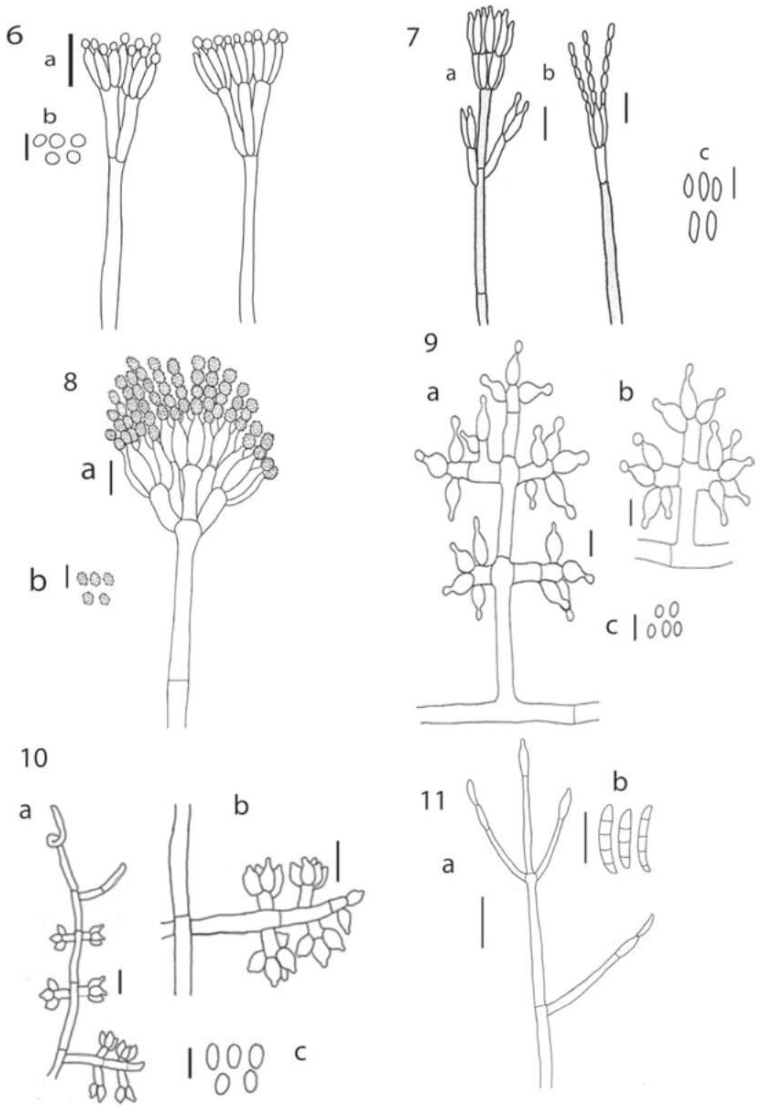
Macroscopical description: Colony with cottony texture, mycelium 2–5 mm high, white. Reverse of culture not pigmented. Colony on MEA reaching 5.5 cm diam. after 25 days at 25°C.

Microscopical description: Hyphae septate, 1–3(–5) µm wide, individual cells (25–)28–55(–61) µm long, hyaline. Conidiophores composed of a stipe with ramifications at its tip, each tip with one phialide, stipe 2–3(–6) µm wide and (40–)45–50(–58) µm long, hyaline, with smooth walls. Phialides flask-shaped, 3–4(–5) µm wide and 7–10(–15) µm long. Conidia subcylindrical, (1–)2–5(–6) µm wide and (2–)4–24(–26) µm long, with 1–4 septa, hyaline, smooth.

Material examined: Majagua, bare soil, 18.07.2012, M.Rosas et al. P1M27 (FR-0247038), ITS sequence KY320593.

Ecology and distribution: Species of *Ascopolyporus* in their asexual states have been isolated from soils of Brazil, Costa Rica, and the USA (Sullivan et al. 2000). For Panama, only *Ascopolyporus moellerianus* has been reported in its sexual state, cited as *Ascopolyporus philodendri* ("*philodendrus*", Bischoff et al. 2005, Guzmán & Piepenbring 2011).

Notes: According to data in GenBank, sequence data of the ITS region of the strain P1M27 match with data from *Ascopolyporus moellerianus* over 89%. This percentage is rather low and does not help to identify the species, considering the 98% threshold (Ovaskainen et al. 2010, Tedersoo et al. 2014). According to Bischoff et al. (2005),



Figs 6–11. Fig. 6. *Penicillium miczynskii* (P1M78). a. Conidiophores with phialides and conidia. b. Conidia. Bars = 5 µm. Fig. 7. *Purpureocillium lilacinum* (P1M95). a. Conidiophore with phialides, metulae, and ramuli. b. Conidiophore with phialides and chains of conidia. c. Conidia. Bars = 5 µm. Fig. 8. *Talaromyces verruculosus* (P1M41). a. Conidiophore with phialides and conidia. Bars = 10 µm. b. Conidia. Bars = 5 µm. Fig. 9. *Trichoderma harzianum* (P1M6). a. Conidiophore with phialides. b. Part of a conidiophore with phialides. c. Conidia. Bars = 5 µm. Fig. 10. *Trichoderma spirale* (P1M79). a. Conidiophore with phialides and spiral apical extension. b. Part of a conidiophore with dense phialides. Bars = 10 µm. c. Conidia. Bar = 5 µm. Fig. 11. cf. *Ascopolyporus* sp. (P1M27). a. Conidiophore with phialides and two young conidia. b. Mature conidia. Bars = 25 µm.

A. moellerianus (cited as *A. philodendri*, syn. according to Guzmán & Piepenbring 2011) and *A. polychrous* are closely related, but can be distinguished by the color and the size of the stromata in the teleomorphic state. A further characteristic used for the identification of the species is the host (Baum et al. 2013). None of these characteristics can be used to determine the present species in culture because only the anamorphic state was isolated from soil. The anamorphic state of *Cordyceps pseudomilitaris* is similar to the strain P1M27 concerning the form and size of the phialides (Zare & Gams 2001), however, the shape of the conidia does not match (subcylindrical and multiseptate vs subglobose and without septa). The anamorphic states of *Ascopolyporus* and *Hyperdermium* are the only taxa in Clavicipitaceae, to our knowledge, to produce multiseptate conidia (Bischoff et al. 2005, Möller 1901). Further molecular data, information on the teleomorph of the present species, and a broader knowledge on morphology of asexual forms of species in this group are needed to identify this fungus isolated from soil in Panama.

Discussion

Records of soil microfungi are scarce for Panama and they come from a few decades ago (Farrow 1954, Goos 1960, Morris & Finley 1956). Research including species identification mostly refers to fungal diversity, selected orders, or fungi of economic importance (Gualdrón-Arenas et al. 1997). The checklist of fungi in Panama (Piepenbring 2006, 2007) includes 207 species of soil microfungi reported for Panama (Farrow 1954, Goos 1960, Husband et al. 2002, Mangan et al. 2004, Morris & Finley 1956). In the context of the present study, 24 species of soil fungi were identified including 10 species that are new reports for the country. Four species among the 10 new reports for Panama are even new records for Central America, namely *Aspergillus roseoglobulosus*, *Mucor moelleri, Penicillium janczewskii*, and *Talaromyces verruculosus*.

Information on soil microfungi in the neighbouring country Costa Rica is available from Bills & Polishook (1994), Chaverri et al. (2010), Goos (1960), and Polishook et al. (2000). Other studies provide information on mycorrhizal fungi or fungi of phytopathological relevance (Gualdrón-Arenas et al. 1997, Chaverri & Vílchez 2006). For Colombia, mycologists focused on deforestation effects and species diversity (Vasco-Palacios & Franco-Molano 2013, Gualdrón-Arenas et al. 1997). Information on soil microfungi for Honduras (Goos 1963) and Nicaragua (Delgado 2011) is scarce, for this reason it is necessary to further investigate this topic.

There are different methods to isolate soil microfungi, with the most common techniques being direct soil inoculation and soil dilution (Bills & Polishook 1994, Farrow 1954, Mueller et al. 2004, Polishook et al. 2000). Fungal species belonging to selected orders can be isolated by using specific, selective agar, while other techniques use the centrifugation of the solution across a density gradient to obtain the spores (Bills & Polishook 1994, Mangan et al. 2004). Other isolation and identification techniques focus on fungi of the rhizosphere (Goos & Timonin 1962).

Results obtained by direct inoculation of soil onto complete media are limited because the fast growth of some species implies that other species cannot develop. However, this technique allows identifying species with great potential of development under laboratory conditions on agar media with all nutrients available.

Pure cultures of microfungi permit a study of morphological characteristics and the identification of the isolated species. Morphological characteristics are combined with molecular data of ITS sequences which are often used as markers for species identification of fungi (Haug et al. 2010, Kõljalg 2005, Piepenbring et al. 2012, Polishook et al. 2000, Seifert 2009). However, sequences of other genes are better markers for species differentiation in certain orders, and multilocus sequence data provide more concise results. The molecular sequences are not always helpful for the identification of species in the tropics, because GenBank includes sequences only for about 16% of the 100,000 known fungal species (Hawksworth 2004). In addition to this problem, there are sequences without names resulting from taxonomic identification (Buée et al. 2009). Therefore, the combination of morphological and molecular data is essential to obtain reliable identifications that can be used in future works.

In order to know all species of microfungi that exist in the soil in a given region, it is necessary to take into consideration the depth of the sample, which has an impact on the species community (Gualdrón-Arenas et al. 1997, Mangan et al. 2004). Also, we keep in mind that weather changes in the different seasons most likely have an impact on species that can be found (Bills & Polishook 1994).

Since several years, environmental sequencing of fungal DNA present in soil is performed for the analysis of fungal diversity (e.g., Lumini et al. 2009, Tedersoo et al. 2010, 2014). By performing pyrosequencing 454 on soil samples of the same area as in the present study, Tedersoo et al. (2014) obtained 418 OTUs of fungi that correspond to 126 species identified to genus level. In this context, species can be identified only tentatively, because sequences obtained by pyrosequencing are relatively short and only from one locus (ITS). Nevertheless, tentative species identifications based on pyrosequencing data were compared to species identifications provided by the present study. As a result, apparently only one species, i.e., *Mariannaea elegans*, was tentatively detected by pyrosequencing and identified as pure culture. A direct comparison of sequence data obtained by pyrosequencing and from isolated cultures did not yield reliable results, because the overlapping areas of sequences consisted of less than 200 base pairs and the sequence identity was approximately 94% for sequences of *M. elegans*.

This lack of congruence of results obtained by pyrosequencing and direct isolation can be attributed to several factors. All samples were taken from the border of a forest along 500 m during the same season. However, samples were taken from locally different places with different micro-spatial characteristics concerning the presence of plants, activities of animals, and the amount of organic matter, among other factors. Apparently, microfungal communities are highly diverse and heterogeneous at local scale (e.g., de Bellis et al. 2007). The fact that most species identified as cultures were not detected by pyrosequencing may also be explained by deficiencies of the pyrosequencing technique, which can show technical bias by the primers, relatively

high divergence of the ITS region, and problems resulting from different lengths of the sequences (Lumini et al. 2009). Different species were identified using different techniques, suggesting that the two techniques are complementary and thereby can provide relatively complete and representative results.

The documentation of the diversity of soil fungi in Panama is still work in progress. For this reason, it is necessary to realize more investigation that includes the application of further techniques of isolation and identification.

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RESEARCH ARTICLE

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Diversity of Fungi in Soils with Different Degrees of Degradation in Germany and Panama

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ABSTRACT

Soil degradation can have an impact on the soil microbiota, but its specific effects on soil fungal communities are poorly understood. In this work, we studied the impact of soil degradation on the richness and diversity of communities of soil fungi, including three different degrees of degradation in Germany and Panama. Soil fungi were isolated monthly using the soil-sprinkling method for 8 months in Germany and 3 months in Panama, and characterized by morphological and molecular data. Soil physico-chemical properties were measured and correlated with the observed values of fungal diversity. We isolated a total of 71 fungal species, 47 from Germany, and 32 from Panama. Soil properties were not associated with fungal richness, diversity, or composition in soils, with the exception of soil compaction in Germany. The geographic location was a strong determinant of the soil fungal species composition although in both countries there was dominance by members of the orders Eurotiales and Hypocreales. In conclusion, the results of this work do not show any evident influence of soil degradation on communities of soil fungi in Germany or Panama.

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1. Introduction

Fungi constitute an important part of the soil ecosystem, playing a central role in the biotic and abiotic interactions in this environment, participating in the decomposition of organic matter and the recycling of soil nutrients to make them available to plants [1]. Therefore, communities of soil fungi are involved in soil fertility [2] and contribute to the alleviation of soil degradation [3].

Soil fungi are an immensely diverse group of organisms. A recent study on the diversity of soil fungi revealed around 80,500 operational taxonomic units (OTUs) occurring in soils worldwide [4]. Soil fungal diversity is affected by the local environmental conditions [5], including the chemical and physical soil characteristics, which determine to a great extent the composition of extant fungal communities [6].

Soil degradation is the decline in soil quality (physical, chemical, and biological deterioration) caused by its improper use, usually due to agricultural, grazing, or industrial pressures [7]. Soil degradation can result in changes in its physical properties, such as soil texture [8]; its chemistry, often caused by the application of fertilizers and pesticides [9] that lead to soil acidification [10]; and its biological components, such as losses in vegetation cover that prevent soil erosion [11,12]. Soil degradation is triggered by human activities, which influence the biodiversity of soil [13]. Lands with different levels of soil degradation are estimated to cover between one billion to over six billion hectares worldwide [14].

Soil degradation impacts fungal diversity because soil characteristics influence the presence, distribution, and abundance of fungal species, and the soil characteristics depend on the soil degradation level. Every soil particle has a different micro-spatial composition of fungal species, which is influenced by different micro-habitats in the soil [15]. Every species of fungi requires specific conditions for development, reproduction, and propagation, including different ranges of temperature, moisture, carbon reservoirs, seasons, soil depth, or chemical factors [16]. Without bacteria and fungi, the soil degrades [9]. Soil compaction decreases soil fertility through decreasing storage and supply of water and nutrients, which entails a reduction in the activities and diversity of fungal communities [17]. Soil moisture is assumed to be very important for microorganisms, because water availability is fundamental for different processes. The soil pH has a strong

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Supplemental data for this article can be accessed here.

influence on species richness of soil fungi, diversity, and community structure [10]. The composition and proportion of the soil components have appreciable effects on nutrient concentrations and soil texture, thereby influencing the community of soil fungi [18].

Analyses that include fungal morphological and molecular data in correlation with environmental conditions of soils are not extensive [19]. In Germany, the knowledge of soil fungi is more developed, but there are still many gaps. The principal biodiversity research project in the area is the German Biodiversity Exploratory (www.biodiversity-exploratories.de), which is focused on research related to forest management, on the biodiversity, and on the functions of forest ecosystems [20]. These studies focused on invertebrates in the soil, soil bacterial communities, wood-inhabiting fungi, and yeasts [21] as well as soil fungi [22]. Specifically, for Panama, research is limited. The province of Chiriquí in Panama has been recently declared as one of the four critical areas subject to soil degradation processes in the country, which affects directly the soil organisms and the biophysical system [23], making necessary the study of fungi in this region. Also, it is estimated that Panama has about 50,000 species of fungi of which only about 3.6% are known [24]. Recently, 24 species of soil fungi were identified in Western Panama including 10 new reports for the country, and 4 of these species are also new to Central America [25]. However, analyses that include soil fungal diversity data in correlation with soil environmental conditions are not extensive [19].

The objectives of this study are to (1) assess the impact of soil degradation on communities of soil fungi, (2) establish the diversity of species and their relationship with environmental factors, (3) test whether the effects of degradation are consistent across geographic and environmental conditions taking into consideration one area in Germany and one in Panama, and (4) determine which soil factors are most important for changes in fungal communities caused by degradation.

2. Materials and methods

2.1. Sampling sites

Two geographically separated study areas were selected. The first location was selected within a temperate forest in the Taunus mountain range located in the north-west of Frankfurt am Main, Hesse, Germany (50° 08′ 28.0″N, 8° 16′ 21.1″ E, ca. 360–380 m a.s.l.). The local climate comprises ranges of the temperature of 5–17 °C and of precipitation of 600–1300 mm per year [26]. The second location was within a tropical, semi-deciduous forest in the Majagua valley in the province of Chiriquí, in Panama (08° 29′ 33.5″ N, 82° 25′ 59.4″ W, ca. 120 m a.s.l.). The local climate in this province varies between ranges of temperature of 25–32 °C, and average precipitation of 3700 mm per year [27].

2.2. Collection of soil samples

Three sites were selected in each location in Germany and Panama, representing areas of dense forest (without disturbance); grasslands, indicative of biological degradation because the areas are covered by grass only [6]; and bare soils on the paths [28]. The sampling sites in each country were inside an area of less than 1000 square meters. Samplings in Germany were done across 8 months (January, March, April, May, June, September, October, and November) in 2012. In Panama, samplings were done across 3 months (February, July, and August) in 2012. At each sampling event and site, three cores were collected and pooled, resulting in a total of 24 soil samples for Germany and 9 for Panama. The collection of samples followed the protocol described by Carrasco et al. [29]. In brief, a tube was introduced in the first 5 cm of soil and this core was introduced into a plastic bag after removing roots and stones from the samples. Samples were brought to the lab for processing within 3 d. One portion of the samples was used to determine the soil characteristics.

2.3. Physico-chemical soil analysis

Physico-chemical soil characteristics were measured from one soil sample at each site. The pH of all samplings zones was determined with a litmus paper in a soluble extraction of the soil samples. Moisture content in the soil was measured by the water balance value, which was calculated from the sample weight before and after drying at 105 °C for 48 h [30]. Compaction was measured by the determination of the ratio of the mass to the bulk or macroscopic volume of soil particles plus pore spaces in a sample [Bulk density (g cm^{-3}) = Mass of dry soil (g)/Volume of core (cm^3)]. Finally, the composition of soil was measured by the sedimentation method [31]. All environmental factors measurement for each sampling event is showing in Supplementary Table S2.

2.4. Isolation of soil fungi

For the isolation of soil fungi, plates were prepared by dispersing minute quantities (of around 0.05 mg) of the different soil samples on the surface of a

sterile Petri dish with a cultivation medium following the procedure described by Rosas-Medina and Piepenbring [25]. The method is a variant of Warcup soil plates [32], and consists of spreading a minute quantity of soil in a water suspension on the surface of the agar medium. Each soil sample was cultivated in duplicate on three media: potato dextrose agar (PDA; Panreac, Darmstadt, Germany), malt extract agar (MEA; Roth, Karlsruhe, Germany), and malt yeast peptone agar (MYP, Roth), all these amended with 0.5 g l^{-1} tetracycline. The plates were incubated at 25 °C for up to 25 d in an incubation chamber until colonies developed. This process involved the observation of cultures every day, making dilutions in water to separate spores, and re-cultivating until obtaining pure cultures. Re-cultivations were done on different media (PDA, MEA, MYP, and glucose yeast peptone liquid medium) [33,34].

2.5. Identification of fungal cultures

The identification of fungal isolates by morphological characteristics was done using morphological identification keys [35–42]. Macroscopical characteristics (form, size, color, and growth rates of cultures) and microscopical characteristics (forms and sizes of hyphal cells, conidiophores, metulae, conidia, ornamentation, etc.) of the isolated fungi were compared to corresponding information in the descriptions (see examples of morphological identifications in the Supplementary material). Morphological identifications were complemented by a molecular assessment of representative isolates of each morphospecies. DNA extraction by the cetyltrimethylammonium bromide (CTAB) method, polymerase chain reaction (PCR) amplification and sequencing of the internal transcribed spacer region of ribosomal DNA (rDNA ITS1-5.8S-ITS2) with primers ITS1f and ITS4 [43,44] followed the procedure outlined by Rosas-Medina and Piepenbring [25]. The ITS sequences were compared by BLAST with other reference sequences from NCBI GenBank (http://www.ncbi.nlm.nih.gov) and the User-friendly Nordic ITS Ectomycorrhizal database group (UNITE; http://unite.ut.ee/) database for fungal ITS sequences [44]. Upon BLAST searches, sequences matching with 98% or higher of maximum identity with database records were considered as reliable identifications, while sequences with less than 98% identity were subjected to critical morphological analysis [45]. Results from BLAST were compared with morphological identification to confirm the identifications. The principal reference for the selection of currently valid names was Mycobank (http://www.mycobank.org/). Several isolates could not be identified to species level based on their ITS sequences or by their morphology. In these cases, fungi were identified morphologically to the smallest possible taxonomic category.

Fungal cultures are maintained in the Integrative Fungal Research (IPF) culture collection at Goethe University Frankfurt am Main. All the sequences obtained were deposited in the NCBI GenBank nucleotide database under accession numbers KY320587–KY320646 (Supplementary Table S2).

2.6. Data analysis

The dataset for fungal diversity analyses included the occurrence of all fungal species across sampling events and sites, expressed as the percentages of isolation per sample. Diversity analyses from the isolation dataset were performed with the community ecology library vegan version 2.4-4 [46] in the statistical program R version 3.1.1 [47]. Species richness and species accumulation curves were calculated for each treatment, including forest, grassland, and bare soil for both countries Germany and Panama. Analyses of the diversity of fungal communities were carried out with the Shannon diversity index [48]. Comparisons between richness and environmental factors were done with linear regression models. A matrix of dissimilarities in fungal community composition among samples was obtained using the Jaccard's index based on presence/absence data. Differences in species composition were compared across samples using non-metric multidimensional scaling (NMDS), and they were correlated with environmental factors using the function *envfit* of vegan.

3. Results

3.1. Fungal species diversity and systematics

A total of 764 strains of soil fungi were isolated, 533 from Germany and 231 from Panama. The isolates from Germany were distributed in three divisions: Ascomycota, Zygomycota, and Basidiomycota, the latter represented only by the order Tremellales (12 isolates). In Panama, only members of the divisions Ascomycota and Zygomycota were found. The distribution of isolates in fungal orders is shown in Figure 1.

The isolates were classified in 71 species, 47 from Germany and 32 from Panama, of which eight were shared by both countries (Supplementary Table S2). These data resulted in Shannon's index diversity values of 3.36 for Germany and 3.15 for Panama.

The number of isolates and fungal species found in each sampling event changed in both countries (Figure 2). In Germany, high variability in the number of isolates was observed between the first and

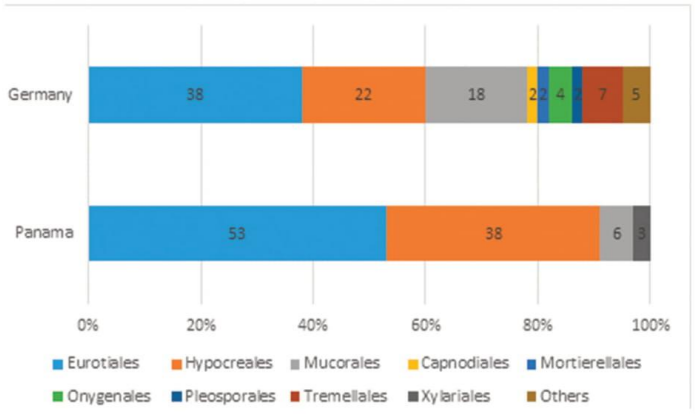

Figure 1. Affiliation of fungal strains isolated from soil in Germany or Panama to orders of fungi.

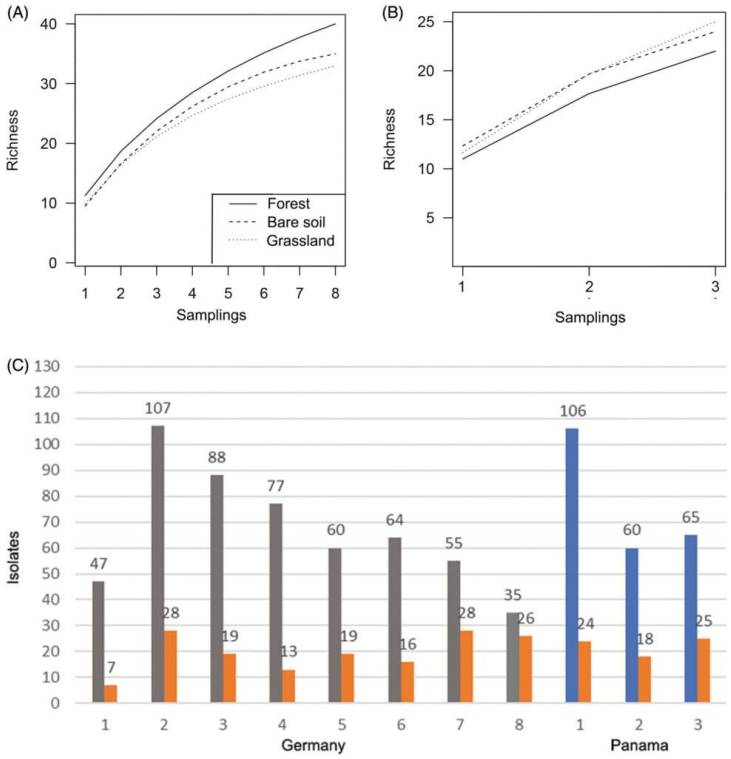

Figure 2. Species diversity and richness of soil fungi in Germany and Panama recorded during the samplings and number of isolates and species in both countries. (A) Species accumulation curves for each of the three soil types sampled in Germany, respect to sampling events; (B) Species accumulation curves for each of the three soil types sampled in Panama, respect to sampling events; (C) Numbers of isolates and numbers of species (orange bars) obtained by samplings in Germany (grey bars) and Panama (blue bars).

the second samplings, with an increment from 47 to 107 isolates. In the subsequent six sampling events, lower numbers of isolates were obtained (Figure 2(c)). The number of species ranged from 7 to 28 species (SD 3.97 bare soil, 3.22 grassland, and 3.96 forests). Two species were present in nearly all samplings, *Trichoderma hamatum* and *Mucor moelleri* (Supplementary Table S2).

In Panama, the differences between the first and second sampling are 46 isolates, and the differences between second and third sampling are 5 isolates (Figure 2(c)). The number of species ranged from 18 to 25 species (SD 3.09 bare soil, 3.56 grassland, and 2.16 forest). Three species of the order Eurotiales (*Aspergillus aculeatus, Penicillium citrinum,* and *Penicillium simplicissimum*) and three species of the order Hypocreales (*Ophiocordyceps heteropoda, Purpureocillium lilacinum,* and *Trichoderma harzianum*) were present in all sampling events. Species accumulation curves show differences in richness between Germany and Panama (Supplementary Figure S1).

3.2. Effect of soil compaction on fungal diversity

None of the environmental factors measured were significantly correlated with fungal richness or diversity ($p > .05$; Supplementary Figure S2), with the exception of soil compaction (Figure 3). This association was negative and found only in Germany ($R^2 = 0.13$, $p = .047$), whereas in Panama no effect was detected ($p > 0.5$; Figure 3).

The NMDs ordination showed clustering of samples in two groups with similar species compositions, one group for Germany and another for Panama (Supplementary Figure S2). Ordinations by countries in relationship with different vegetation covers showed no clustering pattern (Supplementary Figure S3).

4. Discussion

In this study we analyzed the fungal diversity in soils from areas in Germany and Panama with different vegetation covers, to determine which factors have an influence on the communities of soil fungi in the context of soil degradation. We did not find important effects of ecological factors related to soil degradation on fungal communities, although we found an important effect of the geographic location on fungal species composition. For both countries, however, similar orders were dominant, i.e., Eurotiales and Hypocreales.

The impact of soil degradation on fungal community composition was negligible. Previous studies have shown a similar lack of effects. For instance, Saxena and Stotzky [49] found no significant differences in the culturable fungi between soil with plants and bare soil, similar to the forest and bare soil assessed in our study. However, they mentioned that these results should be considered as preliminary, because only culturable fungi were evaluated. Our study has a similar result, considering that many fungal species dwelling in soil cannot be isolated or cultivated and hence need to be assessed with complementary methodologies [50,51]. Evaluating the effects of soil degradation on fungal communities, Samaniego-Gaxiola and Chew-Madinaveitia [52] found that in two of three cultivation areas, soil degradation had no impact on the community of soil fungi, but in the more saline area, fungal diversity was lower, showing a soil degradation effect on fungi diversity. Consequently, we cannot rule out an effect of degradation on soil fungal communities, based on results from other studies [53–55].

Species diversity of communities of soil fungi in relation to ecological factors varies according to the specific conditions of each soil. Some studies with a similar methodology to the one in this study have shown varying results. Wahegaonkar et al. [56] found 45 genera distributed in 85 species in agricultural soils. Gaddeyya et al. [57] found a total of 15 species belonging to six genera from cultivable fields, where the dominant species were *Aspergillus flavus*, *Trichoderma viride*, *T. harzianum*, *Fusarium oxysporum*, and *Fusarium solani*, that were also found in this study. In our samplings in Germany, the dominant order was the Eurotiales, with 38% of the total of isolated species. Eurotiales have a

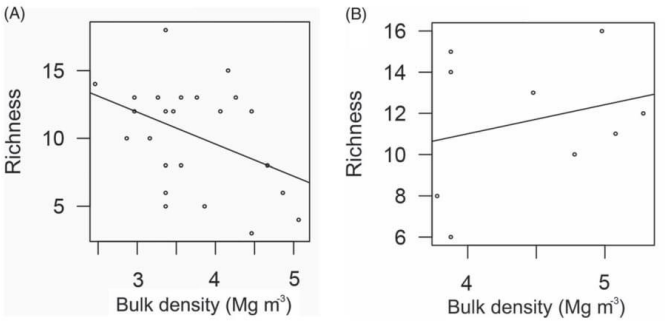

Figure 3. Richness of species of soil fungi in relation to soil compaction measured by bulk density. (A) in Germany, with 24 samples from bare soil, grassland or forest; (B) in Panama, with nine samples from bare soil, grassland, or forest.

cosmopolitan distribution with records in many habitats all over the world [37,58,59]. The second most abundant order was the Hypocreales with 22% of the total of isolated species. Species of Hypocreales can be found in different types of soil worldwide [36,41,60], and are common in all types of moist forests. In the same regions of this study in Germany and Panama, Tedersoo et al. [4] found that Eurotiales and Hypocreales were within the ten most common orders according to data obtained by environmental sequencing. Most species of Eurotiales have a pioneer colonization strategy in the soil, and are adapted to extreme environmental conditions, have cosmopolitan distribution, and are common associates of decaying plant and food material [61]. Species of Hypocreales are commonly encountered in humid tropical or subtropical forests although they also occur in arid, temperate, or boreal forests, even in the most extreme north and south latitudes, with some genera in the order being considered cosmopolitan soil fungi [36,62].

We did not find an important effect of environmental factors on species richness, except for soil compaction in Germany. Soil compaction has negative influences on soil microfungi because it reduces pore space which, in turn, affects the growth, distribution, and development of fungi [63]. Harris et al. [64] showed that soil bulk density has a negative influence on the spread and spatial distribution of *Rhizoctonia solani*.

The lack of correlation between soil factors and the diversity of soil fungi may be due to different causes. One can be that our samplings spanned a temporal but not a spatial variation, because the spatial variation implies extension in the sampling area entailing differences in environmental factors. Although the fungal communities changed across time, at the same time the values of soil factors did not vary significantly. Soil depth can influence the correlation between environmental soil factors and the biomass of soil fungi based on microclimates [65,66]. Another reason can be that fungal communities are influenced by other factors like the amount of rain per year, soil nutrient content, or total and labile organic carbon [67].

We did not find degradation effects on communities of soil fungi across geographical locations. Usually, the effects of soil degradation vary with geographic location. Goldmann et al. [68] mention that communities of soil fungi similar in one place change with increasing geographical distance. This could be driven by three main mechanisms; the first one, environmental conditions become increasingly different with increasing geographical distance, the second one is the modulation of dispersal rates of taxa influenced by the limitations of landscape heterogeneity, and the last one is the dispersal limitations of organisms in homogenous landscapes.

This study has some methodological limitations. The isolation of fungi in culture is not comprehensive because many species are not easily detected in agar media. When soil particles are scattered onto the surface of the medium, some species develop more quickly than others given their fast growth rates and, in some cases, their parasitism on other fungi [69]. The statistical analysis is also limited because it is not quantitative, as it is focused on the presence/absence of fungi. For this reason, some fungal groups are more likely to be detected than others without relationship to their abundance. However, despite the above limitations, our methodology is sufficient to find differences across soils and environmental factors. Other methodologies can be used to complement the ones in our study. For example, Tedersoo et al. [4] used 454 pyrosequencing to identify the soil fungal communities in the same areas of our study. However, with this method, 281 of the species [22] were detected by 454 pyrosequencing and cultivation, suggesting that high-throughput sequencing has its own technical biases, such as primer mismatches, differential sequence length and precision loss in the homopolymer regions [70,71]. Therefore, it is important to combine both techniques, since they provide complementary information.

In summary, our results do not show the evident effects of soil degradation on communities of soil fungi from Germany and Panama. However, our study sets the basis to develop further studies in the same direction or to test other fungal relations with more environmental factors. This study is pioneer for Panama since it is one of few studies focusing on soil mycobiota in this country. Finally, in both countries further work in this direction is needed to assess human impacts on understudied areas in terms of fungal diversity.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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12. Appendices

Table of Species

			BLAST results			Germany									Panama		
Species	Order	GenBank accession	Description	Identity (%)	Ds1	Ds2	Ds3	Ds4	Ds5	Ds6	Ds7	Ds8	Overall	Ps1	Ps2	Overall	Overall
Absidia cylindrospora	Mucorales	KY320587	*Absidia cylindrospora* SH199695.07FU	98	3	9	0	1	2	0	2	1	18	0	4	4	22
Absidia glauca	Mucorales	KY320588	*Absidia glauca* SH190044.07FU	88	9	18	0	1	1	0	1	1	31	0	0	0	31
Acremonium cellulolyticus	Hypocreales	KY320589	*Talaromyces pinophilus* SH119577.07FU	97.5	0	0	0	0	0	0	0	0	0	0	8	8	8
Alternaria alternata	Pleosporales	KY320590	*Alternaria alternata* SH429908.07FU	100	1	0	0	0	0	0	1	1	3	0	0	0	3
Arthrinium cf. *arundinis*	Hypocreales	KY320591	A species of Apiosporaceae SH183134.07FU	99.2	0	0	0	1	2	1	1	1	6	0	0	0	6
Arthroderma vanbreuseghemii	Onygenales	KY320592	*Trichophyton mentagrophytes* SH450601.07FU	99.5	0	0	0	0	0	1	0	1	2	0	0	0	2
Ascopolyporus sp.	Hypocreales	KY320593	*Lecanicillium kalimantanense* SH194043.07FU	87.1	0	0	0	0	0	0	0	0	0	2	1	3	3
Aspergillus aculeatus	Eurotiales	KY320594	*Aspergillus japonicus* SH205971.07FU	99.4	0	0	0	0	0	0	0	0	0	2	3	5	5
Aspergillus cf. *flavipes*	Eurotiales	KY320595	*Aspergillus polyporicola* SH194611.07FU	100	0	5	9	0	0	0	0	1	15	0	0	0	15
Aspergillus japonicus	Eurotiales	KY320596	*Gongronella butleri* SH186911.07FU	99.8	0	0	0	0	0	0	0	0	0	2	3	5	5
Aspergillus roseoglobulosus	Eurotiales	KY320597	*Aspergillus sclerotiorum* SH207596.07FU	97.8	0	0	0	0	0	0	0	0	0	1	1	2	2
Aspergillus tamarii	Eurotiales	KY320598	*Aspergillus bombycis* SH432428.07FU	99.3	0	0	0	0	0	0	0	0	0	1	1	2	2
Bionectria ochroleuca	Hypocreales	KY320599	*Clonostachys rosea* SH182678.07FU	99.8	0	1	0	3	1	2	1	0	8	3	2	5	13
Chrysosporium merdarium	Onygenales	KY320600	*Geomyces auratus* SH183331.07FU	99	0	1	0	0	0	0	0	0	1	0	0	0	1
Cladosporium cf. *ossifragi*	Capnodiales	KY320601	*Mycosphaerella tassiana* SH216250.07FU	99.3	0	4	1	2	1	0	1	0	9	0	0	0	9
Clonostachys candelabrum	Hypocreales	KY320602	A species of Bionectriaceae SH211202.07FU	100	0	1	2	0	0	0	1	0	4	0	0	0	4
Clonostachys cf. *rossmaniae*	Hypocreales	KY320604	A species of Bionectriaceae SH211202.07FU	99.7	0	0	0	1	1	0	1	1	4	0	0	0	4
Clonostachys rosea	Hypocreales	KY320603	*Clonostachys rosea* SH182678.07FU	98.8	0	0	0	0	0	0	0	1	1	0	0	0	1
Cryptococcus podzolicus	Tremellales	KY320605	*Cryptococcus podzolicus* SH181879.07FU	99.8	0	1	0	0	0	0	0	1	2	0	0	0	2
Cryptococcus terreus	Tremellales	KY320607	*Cryptococcus terreus* SH190018.07FU	99.6	0	1	0	1	0	0	0	1	3	0	0	0	3
Cunninghamella elegans	Mucorales	KY320606	*Cunninghamella elegans* SH216493.07FU	100	0	2	0	0	5	0	3	0	10	0	0	0	10
Eupenicillium javanicum	Eurotiales	KY320610	*Penicillium javanicum* SH237551.07FU	100	0	0	0	0	0	0	0	0	0	0	3	3	3
Eupenicillium shearii	Eurotiales	KY320611	*Penicillium miczynskii* SH205215.07FU	100	0	0	0	0	0	0	0	0	0	2	1	3	3
Fusarium oxysporum	Hypocreales	KY320613	A species of Sordariomycetes SH219102.07FU	99.6	0	0	0	0	0	0	0	0	0	4	1	5	5
Fusarium solani	Hypocreales	KY320612	*Fusarium pseudensiforme* SH181399.07FU	98.5	0	0	0	0	0	0	0	0	0	4	1	5	5
Geomyces pannorum	Leotiomycetes I	KY320608	*Geomyces auratus* SH183331.07FU	99	0	3	2	16	2	4	4	1	32	0	0	0	32
Gliocladium viride	Hypocreales	KY320614	A species of Sordariomycetes SH177686.07FU	98.8	0	5	1	2	0	2	0	1	11	0	0	0	11
Gongronella butleri	Mucorales	KY320615	*Gongronella butleri* SH186911.07FU	99.8	0	0	0	0	0	0	0	0	0	6	0	6	6
Lecanicillium saksenae	Hypocreales	KY320616	*Lecanicillium saksenae* SH212427.07FU	99.4	0	0	0	0	0	0	0	0	0	2	1	3	3
Mortierella verticillata	Mortierellales	KY320617	*Mortierella humilis* SH196779.07FU	99.8	0	0	0	0	0	2	1	0	3	0	0	0	3
Mucor hiemalis	Mucorales	KY320618	*Mucor hiemalis* SH187073.07FU	99.6	0	1	0	0	0	0	0	1	2	0	0	0	2
Nectria mariannaeae	Hypocreales	KY320619	A species of Hypocreales SH217623.07FU	99	0	0	0	0	0	0	0	0	0	1	1	2	2
Ophiocordyceps heteropoda aff.	Hypocreales	KY320620	*Purpureocillium lilacinum* SH184932.07FU	92.3	0	0	0	0	0	0	0	0	0	3	5	8	8

Paecilomyces carneus	Eurotiales	KY320621	A species of Ascomycota SH214904.07FU	99.4	0	2	0	0	3	0	0	1	6	0	0	0	6
Paecilomyces lilacinus	Eurotiales	KY320622	*Purpureocillium lilacinum* SH184932.07FU	99.8	0	0	0	0	0	0	0	0	0	13	0	13	13
Paecilomyces marquandii	Eurotiales	KY320623	A species of Ascomycota SH217931.07FU	97.3	0	0	0	0	0	0	0	0	0	0	8	8	8
Penicillium canescens	Eurotiales	KY320624	*Penicillium herquei* SH207149.07FU	96.8	0	0	0	0	1	0	3	0	4	0	0	0	4
Penicillium cf. *pulvillorum*	Eurotiales	KY320630	*Penicillium mariae-crucis* SH237726.07FU	82.5	0	0	0	0	0	0	0	0	0	1	1	2	2
Penicillium citrinum	Eurotiales	KY320625	*Penicillium citrinum* SH190215.07FU	100	0	0	4	0	1	1	2	1	9	6	11	17	26
Penicillium daleae	Eurotiales	KY320626	*Penicillium daleae* SH182497.07FU	95.8	0	3	16	22	15	9	5	4	74	0	0	0	74
Penicillium decaturense	Eurotiales	KY320627	*Penicillium westlingii* SH259266.07FU	97.8	0	0	0	0	1	1	2	0	4	0	0	0	4
Penicillium kapuscinskii	Eurotiales	XXXX	n.d.	n.d.	0	0	1	8	0	0	0	1	10	0	0	0	10
Penicillium miczynskii	Eurotiales	XXXX	*Penicillium westlingii* SH259266.07FU	97.8	0	1	0	0	0	2	1	0	4	0	0	0	4
Penicillium pinophilum	Eurotiales	KY320629	*Talaromyces pinophilus* SH119577.07FU	98	0	0	0	0	0	0	0	0	0	0	5	5	5
Penicillium simplicissimum	Eurotiales	KY320631	*Penicillium mariae-crucis* SH237726.07FU	99.2	0	0	0	0	4	8	5	0	17	16	5	21	38
Penicillium madriti	Eurotiales	KY320628	*Penicillium madriti* SH279507.07FU	95.2	0	0	2	0	2	0	1	0	5	0	0	0	5
Penicillium manginii	Eurotiales	XXXX	*Penicillium westlingii* SH259266.07FU	94.8	0	0	0	0	0	0	0	1	1	0	0	0	1
Penicillium verruculosum	Eurotiales	KY320632	*Talaromyces oumae-annae* SH522559.07FU	97.7	0	0	0	0	0	0	0	0	0	1	1	2	2
Penicillium westlingii	Eurotiales	KY320633	*Penicillium westlingii* SH259266.07FU	99.6	0	2	0	0	0	0	1	0	3	0	0	0	3
Penicillum janczerskii	Eurotiales	XXXX	n.d.	n.d.	0	0	0	0	0	0	0	0	0	1	0	1	1
Pestalotiopsis sp.	Xylariales	XXXX	A species of *Pestalotiopsis* SH205618.07FU	87.9	0	0	0	0	0	0	0	0	0	0	2	2	2
Pochonia bulbillosa	Hypocreales	XXXX	A species of *Metapochonia* SH528250.07FU	77.3	0	13	18	15	10	14	4	7	81	0	0	0	81
Pochonia chlamydosporia	Hypocreales	XXXX	n.d.	n.d.	3	1	0	0	0	0	0	0	4	0	0	0	4
Pochonia suchlasporia	Hypocreales	XXXX	n.d.	n.d.	0	1	0	0	0	0	0	1	2	0	0	0	2
Purpureocillium lilacinum	Hypocreales	KY320634	*Purpureocillium lilacinum* SH184932.07FU	100	0	4	1	0	1	0	1	0	7	10	25	35	42
Rhodotorula mucilaginosa	Sporidiobolales	KY320609	*Rhodotorula mucilaginosa* SH216361.07FU	99.8	0	0	8	0	1	0	2	0	11	0	0	0	11
Talaromyces verruculosus	Eurotiales	KY320635	*Talaromyces pinophilus* SH119577.07FU	97.3	0	0	0	0	0	0	0	0	0	0	2	2	2
Trichoderma asperellum	Hypocreales	KY320636	*Trichoderma yunnanense* SH327524.07FU	99.7	0	3	1	0	1	4	1	1	11	0	0	0	11
Trichoderma deliquescens	Hypocreales	KY320637	A species of Sordariomycetes SH177686.07FU	100	0	2	0	0	3	5	4	1	15	0	0	0	15
Trichoderma hamatum	Hypocreales	KY320638	*Trichoderma pubescens* SH085192.07FU	98.9	2	13	2	0	2	2	4	0	25	0	0	0	25
Trichoderma harzianum	Hypocreales	KY320639	*Trichoderma harzianum* SH177701.07FU	99.3	0	2	4	2	0	0	1	0	9	20	7	27	36
Trichoderma koningi	Hypocreales	KY320640	*Trichoderma koningiopsis* SH327523.07FU	100	21	1	11	1	0	0	0	0	34	4	1	5	39
Trichoderma koningiopsis	Hypocreales	KY320641	*Trichoderma cerinum* SH252855.07FU	98.9	0	0	0	0	0	0	0	0	0	0	2	2	2
Trichoderma spirale	Hypocreales	KY320642	*Trichoderma spirale* SH190868.07FU	97.3	0	0	0	0	0	0	0	0	0	1	1	2	2
Trichoderma viride	Hypocreales	KY320643	*Trichoderma koningiopsis* SH327523.07FU	99.4	0	0	1	0	0	0	0	1	2	0	0	0	2
Trichosporon asahii	Tremellales	KY320644	*Trichosporon asahii* SH103015.07FU	100	0	2	0	0	0	3	1	1	7	0	0	0	7
Umbelopsis ramanniana	Mucorales	KY320645	*Umbelopsis ramanniana* SH524399.07FU	93.9	0	0	0	0	0	0	1	1	2	0	0	0	2
Zygorhynchus moelleri	Mucorales	KY320646	*Mucor moelleri* SH188374.07FU	100	8	5	3	1	1	2	0	1	21	0	18	18	39

Table of Soil Factors

			Soil conditions							
Country	Site	Date	Vegetation cover	Temperature (°C)	pH	Moisture (%)	Compactation	Sand content (%)	Lime content (%)	Clay content (%)
Germany	Ds1	January	C	8	5.2	0.23	5.1	0.44	0.49	0.07
	Ds1	January	P	9	4.5	0.27	4.5	0.28	0.62	0.11
	Ds1	January	V	10	5.1	0.46	3.4	0.33	0.56	0.11
	Ds2	February	C	13	5.5	0.39	4.2	0.37	0.51	0.12
	Ds2	February	P	13	5.1	0.29	3.6	0.31	0.55	0.14
	Ds2	February	V	12	6.0	0.39	3.4	0.39	0.54	0.08
	Ds3	March	C	13	5.1	0.21	3.9	0.44	0.43	0.13
	Ds3	March	P	13	5.5	0.31	3.5	0.28	0.59	0.13
	Ds3	March	V	12	5.5	0.47	3.0	0.37	0.53	0.10
	Ds4	April	C	17	5.1	0.35	4.9	0.45	0.47	0.08
	Ds4	April	P	19	4.8	0.31	3.2	0.26	0.64	0.40
	Ds4	April	V	13	5.7	0.36	3.4	0.34	0.54	0.12
	Ds5	June	C	23	5.1	0.4	4.3	0.45	0.49	0.06
	Ds5	June	P	20	4.4	0.33	4.5	0.29	0.62	0.09
	Ds5	June	V	18	5.3	0.46	2.9	0.35	0.55	0.11
	Ds6	July	C	19	5.1	0.47	3.0	0.44	0.46	0.08
	Ds6	July	P	19	4.5	0.18	3.4	0.27	0.62	0.10
	Ds6	July	V	17	5.3	0.66	3.4	0.34	0.55	0.11
	Ds7	September	C	14	5.1	0.53	4.1	0.45	0.49	0.07
	Ds7	September	P	15	4.6	0.26	3.3	0.29	0.61	0.10
	Ds7	September	V	8	5.4	0.64	2.5	0.40	0.52	0.08
	Ds8	October	C	10	5.1	0.24	4.7	0.46	0.47	0.07
	Ds8	October	P	9	4.5	0.23	3.6	0.32	0.60	0.07
	Ds8	October	V	10	4.9	0.42	3.8	0.33	0.52	0.15
Panama	Ps1	July	C	25.6	5.5	0.38	5.0	0.34	0.40	0.26
	Ps1	July	P	28.95	6.0	0.23	3.9	0.37	0.58	0.05
	Ps1	July	V	26.95	6.0	0.3	4.5	0.42	0.52	0.06
	Ps2a	August	C	25.1	6.0	0.39	4.8	0.33	0.58	0.09
	Ps2a	August	P	29.6	6.0	0.27	3.9	0.35	0.63	0.02
	Ps2a	August	V	27.3	6.0	0.33	5.3	0.35	0.61	0.04
	Ps2	August	C	26.1	5.0	0.38	5.1	0.35	0.22	0.43
	Ps2	August	P	28.3	6.0	0.19	3.9	0.39	0.53	0.09
	Ps2	August	V	26.6	6.0	0.28	3.8	0.48	0.44	0.08

13. Eidesstattliche Versicherung

E R K L Ä R U N G

Ich, Miguel Angel de Jesus Rosas-Medina versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: Diversity of fungi in soils of different degrees of degradation in Panama and Germany selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren/innen beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.

Ich versichere, die Grundsätze der guten wissenschaftlichen Praxis beachtet.

Frankfurt am Main, den .....

(Unterschrift)