140. Jahrgang (2023), Heft 2, S. 99–120



**Centralblatt** für das gesamte Forstwesen

# **Ectomycorrhizal Symbionts of Scots Pine and Silver Birch Forest Ecosystems in the Natural Reserve Ertis Ormany in Kazakhstan**

# **Ektomykorrhiza-Symbionten in Waldökosystemen der Waldkiefer und Hängebirke im Naturschutzgebiet Ertis Ormany in Kasachstan**

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- **Keywords:** *Betula pendula*, *Pinus sylvestris*, fungal diversity, symbiosis, symbiotic relationships, species saturation coefficient, *Agaricales*, *Russulales*, *Boletales*
- **Schlüsselbegriffe:** *Betula pendula*, *Pinus sylvestris*, Pilzdiversität, Symbiose, symbiotische Beziehungen, Artensättigungskoeffizient, *Agaricales*, *Russulales*, *Boletales*

# **Abstract**

Symbiotic relationships maintain the balance of forest ecosystems, with ectomycorrhizal (EcM) symbiosis being one of the most significant. EcM symbiosis in forests dominated by Scots pine (*Pinus sylvestris* L.) and silver birch (*Betula pendula* Roth.) has been often observed, but remain understudied in parts of world. This study was conducted in the State Forest Natural Reserve "Ertis Ormany" in Kazakhstan. The aim of this study was to identify EcM symbionts on Scots pine and silver birch by analyzing ribosomal DNA sequences isolated from hyphal endings. Our results show that among all EcM symbionts the order *Agaricales* (13 species/28.8% of total species numer) had highest species diversity in Scots pine, followed by *Boletales* (9/20%) and *Russulales* (8/17.7 %). For silver birch, the order *Russulales* (7/15.5%) had highest species diversity, followed by *Agaricales* (5/11.1%), and *Boletales* (3/6.7%). A total of 15

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symbiont families were identified: *Agaricaceae* (2 species/4,4% of the total number of species identified in the study area), *Amanitaceae* (4/8.9%), *Boletacea* (5/11.1%), *Cortinariaceae* (3/6.7%), *Gloeophyllaceae* (1/2.2%), *Inocybaceae* (1/2.2%), *Lycoperdaceae* (1/2.2%), *Lyophyllaceae* (2/4.4%), *Paxillaceae* (1/2.2%), *Polyporaceae* (3/6.7%), *Psathyrellaceae* (2/4.4%), *Pseudoclitocybaceae* (2/4.4%), *Russulaceae* (8/17.8%), *Suillaceae* (6/13.3%), and *Tricholomataceae* (4/8.9). A higher symbiotic association with EcM was found in Scots pine (66.5%) compared to silver birch (33.3%).

### **Zusammenfassung**

Symbiotische Beziehungen sind wichtig für die Stabilität von Waldökosystemen, wobei Ektomykorrhiza (EcM)-Symbiose einen besonderen Stellenwert hat. Die EcM-Symbiose an Waldkiefer (*Pinus sylvestris* L.) und Hängebirke (*Betula pendula* Roth.) wurde recht häufig beobachtet, ist aber in vielen Teilen der Welt wenig erforscht. Das Forschungsgebiet war das Naturschutzgebiet "Ertis Ormany" in Kasachstan. Unsere Ergebnisse zeigen, dass unter allen Symbionten der Waldkiefer die Ordnung *Agaricales* (13 Arten/28,8 % der gesamten Artenzahl) und *Boletales* (9 Arten/20 %) die höchste Artenvielfalt aufwiesen, gefolgt von Russulales (8/17,7 %). Bei der Hängebirke waren die Ordnungen *Agaricales* (5/11,1 %), *Boletales* (3/6,7 %) und *Russulales* (7/15,5 %) führend. Insgesamt wurden hier bei den Symbionten der Waldkiefer und Hängebirke 16 Familien identifiziert: *Agaricaceae* (2/4,4 %), *Amanitaceae* (4/8,9 %), *Boletacea* (5/11,1 %), *Cortinariaceae* (3/6,7 %), *Gloeophyllaceae* (1/2,2 %), *Inocybaceae* (1/2,2 %), *Lycoperdaceae* (1 /2,2 %), *Lyophyllaceae* (2/4,4 %), *Paxillaceae* (1/2,2 %), *Polyporaceae* (3/6,7 %), *Psathyrellaceae* (2/4,4 %), *Pseudoclitocybaceae* (2/4,4 %), *Russulaceae* (8/17,8 %), *Suillaceae* (6/13,3 %) und *Tricholomataceae* (4/8,9 %). Es gibt einen Trend zu einem höheren Koeffizienten der Artensättigung mit EcM-Symbionten bei der Waldkiefer (66,5 %) im Vergleich zu der Hängebirke (33,3 %).

### **1 Introduction**

Symbiotic relationships are one of the most important components for maintaining the balance and stability of forest ecosystems (Courty *et al.*, 2010). A common form of symbiotic relationship in forest ecosystems is the ectomycorrhizal (EcM) symbiosis. It is well known that the diversity and structure of EcM symbiosis are influenced by a number of biotic and abiotic factors such as host tree genotype, mycosymbiont interaction strategy, soil characteristics, availability of nutrients, the activity of pathogens, and quality and quantity of soil organic matter (Vaishlya *et al.*, 2019). The impacts of climate change may alter belowground fungal communities (Pickles *et al.*, 2012), so that they may become a limiting factor in tree migration. Furthermore, knowledge of the linkages among soil fungal diversity, community structure, and environmental factors is critical to understand the organization and stability of forest ecosystems (Simard and Austin, 2010; Karst *et al.*, 2014).

The EcM symbiosis of Scots pine (*Pinus sylvestris* L.) and silver birch (*Betula pendula* Roth) is quite often observed in forest ecosystems. Ectomycorrhiza is formed by about 5-6 thousand plant species (Voronina, 2006; 2008), almost exclusively woody or shrub species from Gymnosperms (family *Pinaceae*, *Cupressaceae*) and Angiosperms (18 families, most important are *Fagaceae*, *Betulaceae*, *Salixaceae*, *Myrtaceae*, *Aceraceae*, *Tiliaceae*, *Ulmaceae*) (Molina *et al.*, 1992).

EcM fungi are obligate mutualistic symbionts and hence rely entirely on carbon supply from host plants (Smith and Read, 2008). While they are abundant in root systems of herbaceous plants (Hiiesalu *et al.*, 2014), they are also associated with various tree species (Liu *et al.*, 2015). EcM fungi play a major role in forest community dynamics (Smith and Read, 2008) as they promote the dominance of specific plant families e.g., Pinaceae, Fagaceae, Betulaceae, and Dipterocarpaceae (Tedersoo and Smith, 2013). Ectomycorrhizae are found in the upper layer of the soil, which mostly contains humus, rather than the lower layers which contain mineral-rich substances (Dogmus Lehtijarvi, 2007). These fungi improve plant nutrition in exchange for carbohydrates (Smith and Read, 1997; Sebastiana *et al.*, 2018). They have an important role in the intake of Zn, Cu, Mn, Fe, Ca, K, N, and particularly P, due to slow uptake from the soil. Moreover, mycorrhiza increase water use efficiency, as well as improve soil structure and protect against soil erosion (Dogmus Lehtijarvi, 2007). Previous studies stated that the use of mycorrhizal seedlings in afforestation, rehabilitation and restoration works in marginal sites can significantly increase the success of plantations (Arocena and Glowa, 2006; Qiang-Sheng and Ren-Xue, 2006; Bennett *et al.*, 2017; Kharuk *et al.*, 2019). This subject is important in marginal sites with extreme ecological conditions and higher climate change influence (Barbati *et al.*, 2018). The environmental conditions on poor sites favour numerous harmful biotic factors including pathogens (Haavik *et al.*, 2015).

Studies of the ecology and physiology of EcM have been concentrated mainly in Europe, North America, and Australia (Smith and Read, 1997; 2008; Read, 1999; Finlay, 2005; Polenov, 2013). In the Republic of Kazakhstan, first studies of mycobiota and EcM macromycetes were carried out by Nam (1998), Abiev *et al.* (2000), and Abiev (2015). Applied aspects of the mycorrhization were investigated by Meshkov (2010). He obtained four types of macromycetes from the investigated culture and developed a technology for their scaling and application in inocculated compost for reforestation in Zailiysky Alatau (Meshkov *et al.*, 2009; Meshkov 2010). However, some researchers emphasize that for optimal development of trees, specialized strains of macromycetes fungi forming EcM are needed (Alvarez and Trappe, 1983; Valdes, 1986). Especially, coniferous seedlings are not able to achieve adequate growth, if they are excluded from mycorrhizal occurrence (Kais *et al.*, 1981; Alvarez and Trappe, 1983; Valdes, 1986).

Preservation of environmentally favorable living conditions in various regions of the world directly depends on the rational and careful use of forest resources. Forest ecosystems of Central and Northeast Kazakhstan are one of the most important components of the Earth's biosphere, contributing to the ecological balance on the entire planet (Sarsekova *et al.*, 2021). In this context, Godbold *et al.* (2006) draw attention that an increase in mycorrhizal hyphal biomass and turnover would increase carbon sequestration in terrestrial ecosystems. The assessment of EcM symbionts of forest trees in Kazakhstan's forest ecosystems could help to improve regeneration, afforestation, rehabilitation, and restoration efforts in this region which is affected by climate change and extreme ecological conditions. The main objectives of this study were:

- (1) to establish basic information about EcM associated with Silver birch and Scots pine forests in the protected area "Ertis Ormany" in Kazakhstan, using molecular methods isolating DNA from hyphal endings of the EcM and subsequent sequencing of ribosomal DNA,
- (2) to determine whether there are differences in the quantity of mycorrhizal symbiosis and in the preference of forest tree species, and
- (3) to investigate if there are new undescribed ectomycoriza species in these silver birch and Scots pine forests.

# **2 Materials and Methods**

### **2.1 Study area**

The Republican State Institution State Forest Natural Reserve "Ertis Ormany" is located on the eastern part of the Pavlodar region on the right bank of the Irtysh river in Kazakhstan (Figure 1) (Northern latitude 51º23˝ – 52º 15˝, Eastern longitude 78º 01˝ – 79º 21˝). It is within the borders of Shcherbaktinsky district and Akkulinsky district (Anonymous, 2009) and covers an area of 277,961 hectares.



*Figure 1: The natural distribution area of* Pinus sylvestris *L. and* Betula pendula *Roth. (Caudullo* et al., *2018) and the location of the study area in Kazakhstan.*

Abbildung 1: Das natürliche Verbreitungsgebiet von *Pinus sylvestris* L. und *Betula pendula* Roth. (Caudullo *et al.*, 2018) und die Lage des Untersuchungsgebiets in Kasachstan.



*Figure 2: Satellite image map showing sampling areas of the study area.* 

Abbildung 2: Satellitenbild mit Position der Probenahmen im Untersuchungsgebiet.

Territories of the state nature reserves are divided into zones according to different types of protection and use:

- i) a protected area is a core zone intended for the long term conservation of genetic resources, biological diversity, ecological systems, and landscapes, having sufficient features to achieve such goals;
- ii) buffer zone a section of territory that is used for conducting environmentally oriented economic activities and sustainable reproduction of biological resources (Figure 2, 3) (Forest Code of the Kazakhstan Republic, 2003).



*Figure 3: Sample areas of Scots pine and silver birch; a) № 1 sample area of Scots pine in Shaldai forest; b) № 6 Scots pine in Taybagarskoe forest; c) example of selected seedlings of Scots pine; d) № 3 sample area of Silver birch in Taybagarskoe forest; e) № 8 silver birch in Zavodskoe forest; f) example of soil blocks under crown of silver birch.* 

Abbildung 3: Untersuchungsflächen der Waldkiefer und der Hängebirke; a) Nr. 1 Untersuchungsgebiet der Waldkiefer im Shaldai-Wald; b) Nr. 6 Waldkiefer im Taybagarskoe-Wald; c) Beispiel eines ausgewählten Sämlings der Waldkiefer; d) Nr. 3 Untersuchungsgebiet mit Hängebirke im Pervomayskoe-Wald; e) Nr. 8 Versuchsfläche der Hängebirke im Zavodskoe-Wald; f) Beispiel eines Bodenblocks unter der Krone von Hängebirke.

### **2.2 Natural and climatic conditions of the study area**

The climate in the study area is continental characterized by dry conditions due to dry and hot winds blowing from the Central Asian deserts during summer and winter. The area is open to cold air flows coming from the north. Winters are cold and last long (5.5 months) and summers are short and hot with low precipitation, with extreme temperature fluctuations between winter and summer (range 88 ºC) and between day and night (range 22 ºC). The average annual air temperature is between +2.5 and +3 ºC, average temperature of the coldest month (January) ranges between -17 and -19 ºC and about +21 ºC in the warmest month (July). The absolute minimum and maximum temperatures ever recorded at the Shaldai weather station were -49 ºC and +41ºC, respectively. The growing season lasts 137 days on average. In summer, relative air humidity (at 13:00) is about 40%, sometimes decreasing to 10% causing intensive transpiration of plants and a large loss of moisture from the soil. Mean annual precipitation sum ranges between 240 to 310 mm (Anonymous, 2015).

#### **2.3 Methods**

The "Ertis Ormany" reserve is covered by various forest ecosystems – pure and mixed forests with silver birch and Scots pine (Figure 1). Within this forests, seven permanent transects were established. In each transect, macromycetes were collected and inventoried every 10-15 days during the spring season from May to September in 2020-2022. A total of 110 samples were collected, 60 of these were soil samples and the other 50 samples were collected from the root systems of tree seedlings. Transects were established according to the methodology of Villarreal-Ruiz and Neri-Luna (2018). In total, 60 soil cores were taken about every 2 meters along each 125 m transect.

Collection and identification of the EcM fruit bodies was performed following standard methods, using available determinants and different web sources (http://www. indexfungorum.org and http://www.mycobank.org). Micromorphological studies were carried out using a light microscope ("Altami SM745-T", Russia) at a magnification of 400-1000. To identify the natural color of microstructures, samples were viewed in distilled water and in 3-5% KOH solution. Hyaline structures were stained with a 5% aqueous solution of safranin, and the presence or absence of amyloid and dextrinosis structures were determined using Meltzer reagent (Vaishlya *et al.*, 2012).

To determine the type of fungal symbiont of the EcM, the Agerer morphotyping method was used (Agerer, 1987; 1991; Agarer *et al.*, 2002). For this purpose, soil samples were selected within the projection of the crown of mature trees according to the method of concentric sampling (Vaishlya *et al.*, 2019). Before sampling, the upper layer of litter was removed. The seedlings were extracted from the soil without disturbing their root systems. In total, 210 root ends were examined, 113 for Scots pine and 97 for silver birch.

The samples were wrapped in aluminum foil and stored at a temperature of  $+4$  °C. The roots were washed with running water and cut into segments of 3-5 cm. EcM endings were separated under a magnifying glass with tweezers and scissors. Morphotyping of ectomycorrhizae was performed using a binocular microscope "*Alta*

*mi SM745-T*" with a camera Altami UCMOSO3100KPA and according to the DEEMY (http://deemy.de) system, based on the nature of branching, the color of the EcM termination, features of the mantle surface, the presence or absence of external mycelium and rhizomorphs. The selected EcM samples (113 for *Pinus sylvestris* and 97 for *Betula pendula*) were photographed and fixed in 70% ethanol for DNA isolation. DNA isolation from the sampled EcM hyphal endings was performed using a standard Quiagen kit based on the attached protocol (Vaishlya, 2017). ITS-1F and ITS-4B primers were used for amplification and sequencing of the ITS nuclear DNA site. Sequencing was performed on the ABI 3130 Genetic Analyzer (Applied Biosystems) with subsequent processing of the data obtained in the BioEdit Sequence Alignment Editor application. Identification of EcM fungi to generic and species taxa was performed using the м algorithm by comparison with reference sequences from the open GenBank database (http://www.ncbi.nlm.nih.gov/genbank/), taking into account the lower threshold of 97-98% established for ITS regions of ribosomal genes of fungal DNA (Kõljalg *et al*., 2013).

The nucleotide sequences were checked and manually edited using SeqScape V3.0 or Vector NTI Advance 11.5.1 software. Subsequently, they were compared with sequences deposited in GenBank using the BlastN search (Altschul *et al.*, 1990). The newly obtained ITS sequences were deposited in GenBank under unique accession numbers.

Of all the known DNA marker sequences, the International Fungal Barcoding Consortium has recognized the ITS region of ribosomal genes of nuclear DNA as the main marker in the molecular genetic method for fungi species identification (Schoch *et al.,* 2014). When determining the boundaries of the studied operational taxonomic units, we proceeded from the previously established lower cutoff value (treshold)  $=$ 97–98% for fungi' ITS (Kõljal *et al.*, 2013).

# **3 Results and Discussion**

The distribution of ectomycorrhizas (EcM species) according to tree species is given in table 1. Our results reveal, that among all the symbionts of Scots pine forests (Figure 4), the orders *Agaricales* (13 species/28.8%) and *Boletales* (9/20%) had the highest species diversity, followed by the order *Russulales* (8/17.7%) with a smaller number of species (Table 1).



*Figure 4: Photos of various ectomycorrhizae morphotypes of the root system of Scots pine and silver birch. a- Lyophyllum decastes (Fr.) Singer, b- Lactarius sp., c- Lactarius rimosellus Peck, d- Russula sp.1, e-Amanita sp., f- Suillus sp., g- Candolleomyces candolleanus (Fr.) D. Wächt. & A. Melzer, h- Psathyrella sp., i-Pseudosperma sp., j- Lycoperdon norvegicum Demoulin, k- Russula medullata Romagn, l- Tricholoma sp.* 

Abbildung 4: Fotos verschiedener Ektomykorrhizae-Morphotypen des Wurzelsystems von Waldkiefer und Hängebirke. a- *Lyophyllum decastes* (Fr.) Singer, b- *Lactarius* sp., c- *Lactarius rimosellus* Peck, d-*Russula* sp.1, e- *Amanita* sp., f- *Suillus* sp., g- *Candolleomyces candolleanus* (Fr.) D. Wächt. & A. Melzer, h- Psathyrella sp., i- *Pseudosperma* sp., j- *Lycoperdon norvegicum* Demoulin, k- *Russula medullata*  Romagn, l- *Tricholoma* sp.

*Table 1: Ectomycorrhizal symbionts identified from root endings and macromycetes of* Pinus sylvestris

Tabelle 1: Ektomykorrhiza-Symbionten identifiziert aus Wurzelenden und Makromyceten von *Pinus sylvestris* und *Betula pendula*.

*and* Betula pendula*.* 







A similar ratio of the number of species in the orders was found in Russia, for example, for the Stolby Nature Reserve of the Krasnoyarsk Territory (Kosheleva, 2007), the Urals (Fefelov, 2005) and the Lower Volga Region (Zemlyanskaya, 2003). Regional differences are manifested only at the level of species saturation of genera and families. According to recent studies, EcM has a close functional connection with the formation of the structure, diversity, and stability of plant communities (Püschel *et al.*, 2007; Lambers *et al.*, 2008; Veselkin, 2012a; 2012b). Different forest types and favorable environmental conditions in forests promote high EcM species richness in top soil presumably with both high functional diversity and phylogenetic redundancy while stressful environmental conditions lead to lower species richness and functional redundancy (Pena *et al.*, 2017). The ratio of symbionts in EcM may depend on factors such as the functional age state and the phenological state of the tree (Veselkin, 2013). Bahram et al. (2012) emphasized that EcM fungal diversity became lower at the elevated sites and was correlated with the soil temperature. In addition, Sizonenko and Dubrovskiy (2018) stated that a decline in the ectomycorrhiza linear dimensions accompanied by a compensatory growth of the ectomycorrhiza density with the raised elevation and the deterioration of environmental conditions. The composition of EcM communities is affected by large-scale patterns such as stand age, climatic conditions, soil-fauna and spore dispersal. The relationship between mycorrhizal fungi and soil acidity has long been discussed in the literature (Kalliokoski *et al.*, 2010; Pena *et al.*, 2017). EcM fungi are an important source of organic acids in soil (Griffiths *et al.*, 2009) and affect the pH of the rhizosphere (Smith and Read, 2008). On the other hand, soil conditions affect species diversity of EcM fungi as demonstrated by the dramatic decrease in species richness along a gradient of increasing nitrogen deposition (Lilleskov *et al.*, 2002). Altering nitrogen conditions in soil affects the outcome of species interactions possibly disadvantaging species adapted to the pre-change conditions while favoring generalists (Koide *et al.*, 2005). Mycorrhiza has been noted to decrease the pH in the rhizosphere, because of the selective absorption of ammonium NH+ ions and the release of H+ ions (Eremin and Popova, 2016). Smith *et al.* (2003) stated that the low effectiveness of root and mycorrhizal functions in the cold soils is due to insufficient mineral elements supply.

Otgonsuren *et al.* (2020) detected 24 different ectomycorrhizal morphotypes associated with *Pinus sylvestris* and *Pinus sibirica*, and 18 morphotypes were identified to taxa in forests in Mongolia. The two *Pinus* species had dissimilar ectomycorrhizal communities, and only 2 ectomycorrhizal fungal taxa were common to both species. In another survey conducted by Sarsekova *et al.* (2020), 30 ectomycorrhizas belonging to *Agaricomycetes* were identified on the right bank of the Irtysh River in a mixed forest of *Pinus sylvestris*, *Picea obovata* and *Betula pendula* trees of northern and central Kazakhstan. In their study, the distribution of 30 species into families was as follows; *Suillaceae* (8 species), *Russulaceae* (7), *Cortinariaceae* (4), *Boletaceae* (3), *Tricholomataceae* (2), *Amanitaceae* (1), *Cantharellaceae* (1), *Gomphaceae* (1), *Gomphidiaceae* (1), *Paxillaceae* (1), and *Bankeraceae* (1). The genus with the highest number of recorded species was *Suillus* (8). At the same time Sarsekova *et al.* (2020)'s study, 17 ectomycorrhizas were recorded under *Pinus sylvestris*, 8 under *Betula pendula*, 6 under *Populus tremula*, 1 under *Picea obovata*, 1 under *Quercus robur*, 1 under *Salix* sp., and 1 under *Pinus densiflora*. It should be noted that some species such as *Paxillus involutus*, *Russula undulata*, *Cortinarius* sp. and *Cantharellus cibarius* formed a symbiotic relationship with both coniferous and broadleaved tree species (Sarsekova *et al.*, 2020). According to Garcia *et al.* (2016) the basidiomycete *Rhizopogon* spp. is typical in the root systems of the genus *Pinus* which is represented by the most species in the dominant regions of the *Pinaceae* family in the Pacific northwest of the USA. However, the symbiotic relationship of Scots pine and *Rhizopogon* sp. has not been found within the scope of our study. Massicotte *et al.* (1992) stated that the ascomycete *Cenococcum graniforme* (Sow.) Fred and Winge is the most common ectomycorrhizal fungus in the World, surprisingly this species has not been found in our study.

In the present study, five ectomycorrhiza families were identified in the silver birch stands: (5 species/25% of the total number of species identified in the studied area), *Amanitaceae* (3/6.7%), *Boletaceae* (3/6.7%), *Lycoperdaceae* (1/2.2%), *Paxillaceae* (1/2.2%), and *Russulaceae* (3/6.7%) (Tab. 1).

The list of families (with number and proportion) for all recorded species in the investigated area is as follows: *Agaricaceae* (2 species 2/4.4%), *Amanitaceae* (4/8.9%), *Boletacea* (5/11.1%), *Cortinariaceae* (3/6.7%), *Gloeophyllaceae* (1/2.2%), *Inocybaceae* (1/2.2%), *Lycoperdaceae* (1/2.2%), *Lyophyllaceae* (2/4.4%), *Paxillaceae* (1/2.2%), *Polyporaceae* (3/6.7%), *Psathyrellaceae* (2/4.4%), *Pseudoclitocybaceae* (2/4.4%), *Russulaceae* (8/17.8%), *Suillaceae* (6/13.3%), and *Tricholomataceae* (4/8.9). The coefficient of species saturation orders is: *Agaricales* 28.8, *Boletales* 20, *Russulales* 17.7. They included 45 of all species defined and identified with DNA 28 species in the study area (Figure 4). The remaining 16 genera included 6.2 % of all identified species.

The average species saturation of pine forests is 10.0, which is lower than the average species saturation of 18.6 in the Republic of Kazakhstan (Novozhilov, 2005). This trend is explained by the locality of the territory and the absence of many types of substrates that have been studied in other regions of the Republic of Kazakhstan. For example, those that are often found in the steppe and desert (excrement of herbivorous animals, remnants of succulents, wormwood *Artemísia absínthium* L.). The leading families are those families where the number of species exceeds the average species saturation of families in the study area. As a result of this study, fundamental differences were found between the species saturation of EcM symbionts of Scots pine and Silver birch forests. In addition, Shi *et al.* (2016) stated that there are still critical gaps remaining in our understanding of biogeographic patterns of mycorrhizal associations. Further, our limited knowledge of the anthropogenic factors responsible for shifting plant-mycorrhizal distributions has hindered the efforts to predict ecosystem feedbacks to climate change. Mäkelä *et al.* (2022) suggest that EcM can use a significant proportion of the carbon assimilated by vegetation and hence be an important driver of the decline in carbon use efficiency at higher latitudes. Within this context, Godbold *et al.* (2006) emphasized that the turnover of the mycorrhizal external mycelium may be a fundamental mechanism for the transfer of root-derived C to soil organic material.

In total, 110 samples were collected during field work. Seventeen species were identified using classical methods via macromycete determinants, including EcM list of De Roman *et al.* (2005), and 28 were identified using DNA sequencing analysis (Figure 5). In the 60 soil samples 22 morphotypes of ectomycorrhizal fungal taxa associated with Scots pine and silver birch were determined. For the identify, a list from the study by De Roman *et al.* (2005), Tedersoo (2007), electronic resources (Ilyina, 2019; Polenov, 2013), as well as the Index Fungorum database and MycoBank (Robert *et al.*, 2020) were used.



*Figure 5: The number of occurrences of taxa of EcM fungi in soil samples of Scots pine and silver birch.* 

Abbildung 5: Die Anzahl der Vorkommen von Taxa von EcM-Pilzen in Bodenproben von Waldkiefer und Hängebirke.

# **4 Conclusion**

Five EcM families (*Psathyrellaceae*, *Russulaceae*, *Amanitaceae*, *Tricholomataceae*, and *Boletaceae*) were predominant in the Scots pine and silver birch forests studied here, with in total 11 species. These species are *Candolleomyces pseudocandolleanus* (A.H. Sm.) D. Wächt. & A. Melzer, *Amanita muscaria* (L.) Lam., *A. pantherina* (DC.) Krom., *Russula medullata* Romagn, *Suillus bovinus* (L.) Roussel, *Tricholoma robustum* (Alb. & Schwein.) Ricken, *Russula* sp.1, *Leccinum scabrum* (Bull.) Gray, L. *versipelle* (Fr. & Hök) Snell, *Psathyrella* sp., *Cortinarius croceus* (Schaeff.) Gray. In total, we managed to isolate the DNA of only 28 fungi species and the remaining species were determined by classical method using macromycetes.

In this study, a higher symbiotic association with EcM was found in Scots pine forests compared to Silver birch forests. A similar trend is observed not only in this study area but also in other parts of Kazakhstan. Our study allow us to conclude that the species and quantitative composition of Scots pine and silver birch forests vary considerably. The findings of this study are important for restoring unforested areas. In this context, EcM may be used as a major performance-enhancing tool in afforestation practices in the Irtysh River basin, where extreme ecological conditions and climate change effects prevail.

### **Acknowledgements**

The authors would like to thank to İlkim ÇAVUŞ KARA from Kastamonu-Ilgaz Mountain National Park Directorate, and Assoc. Prof. Dr. Bulent TOPRAK and Dr. Aylin GÜ-NEY, İzmir Katip Çelebi University, Faculty of Forestry, Turkey, for their valuable contributions to the paper.

#### **Funding Acknowledgement Statement**

The project (The state registration No: 13005/844) titled "*Mycorrhiza macromycetes of the main forest-forming species of Central and North-East Kazakhstan and their use for artificial mycorrhization of seedlings of forest tree species*" has been financed by the Ministry of Agriculture of the Republic of Kazakhstan.

# **Conflict of Interest**

The authors declare that they have no conflict of interest.

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