Table S1. Overview of geographical origin of used fungal material, including the information about vegetative cover.

	Čepkelių Nature Reserve	Puszcza Drawska Forest	Puszcza Zielonka Forest	Czerniejewo Forest District	Białowieża Forest
location	South Lithuania 54°01'50.0" N 24°37'00.0" E	West Poland 52°53'10.0" N 15°44'50.0" E	Central-West Poland 52°33'10.0" N 17°08'35.0" E	Central-West Poland 52°25'20.0" N 17°24'20.0" E	North-East Poland 52°51'30.0" N 23°53'00.0" E
climate	humid continental climate (Dfa, Köppen climate classification)				
vegetative cover					
biome	temperate broadleaf forest				
dominant form of vegetation	peatbog/pine forest	decidious forest	decidious forest	decidious forest	corniferous forest
dominant plant	Scots pine	beech with hornbeam	oak	hornbeam	Norway spruce
forest management	absent	present	present	present	present

Table S2. Used PCR protocol.

	Stage	Temperature (°C)	Time (min)
1	Initial denaturation	94	3
2	Denaturation	94	1
3	Alignment	56	1
4	Synthesis	72	3
	Repeat steps 2-4, 35 X		
5	Final synthesis	72	10
6	Hold	4	∞
	Lid temperature: 105 °C		

Table S3. Results of sequencing the PCR amplification products of the prepared ectomycorrhizal DNA isolates. The "Used sample" column refers to the sample used, with the first letter referring to the used morphotype, and roman numeral and second letter to the isolation and purification method used respectively. The red color marks samples from which sequencing was unsuccessful. The yellow color marks samples from which DNA of contaminating yeast was isolated. Reference sequences and species hypotheses were assigned in reference to the UNITE database.

Used sample	Name	Reference Sequence	Species Hypothesis	BLAST Score	%
A IV B	*Debaryomyces hansenii*	KY103269	SH190089.07FU	752	100
A V B	*Elaphomyces muricatus*	EU784198	SH190236.07FU	953	99,62
A VI B	*Elaphomyces muricatus*	EU784198	SH190236.07FU	968	99,4
A V C	-	-	-	-	-
B V B	-	-	-	-	-
B V C	*Pachyphloeus*	UDB032978	SH631019.07FU	361	98,54
C V B	*Genea hispidula*	KJ938839	SH194536.07FU	835	99,78
C V C	*Genea hispidula*	KX168651	SH194536.07FU	344	100
D V B	*Humaria*	EU024888	SH179624.07FU	1011	99,46
E V B	*Tuber puberulum*	HM190013	SH216305.07FU	749	100
E V C	*Tuber puberulum*	HM190013	SH216305.07FU	723	99,75
F IV B	*Amanita rubescens*	FJ890031	SH221016.07FU	1061	99,83

F	**V**	**B**	*Amanita rubescens*	KX449422	SH221016.07FU	819	99,12
F	**VI**	**B**	*Amanita rubescens*	FJ890031	SH221016.07FU	1158	99,84
F	**V**	**C**	*Amanita rubescens*	UDB000038	SH221016.07FU	1129	100
G	**V**	**A**	*Cortinarius torvus*	AJ889977	SH188541.07FU	640	99,71
G	**IV**	**B**	*Cortinarius torvus*	AJ889977	SH188541.07FU	917	99,6
G	**V**	**B**	*Cortinarius torvus*	AJ889977	SH188541.07FU	911	99,4
G	**VI**	**B**	*Cortinarius torvus*	AJ889977	SH188541.07FU	953	99,62
G	**V**	**C**	*Cortinarius torvus*	AJ889977	SH188541.07FU	667	98,16
H	**V**	**B**	*Laccaria amethystina*	KX449421	SH220959.07FU	1170	100
H	**V**	**C**	*Laccaria amethystina*	KX449421	SH220959.07FU	963	99,81
I	**III**	**B**	*Piloderma sphaerosporum*	JQ711966	SH196824.07FU	941	99,81
I	**IV**	**B**	*Piloderma sphaerosporum*	JQ711966	SH196824.07FU	942	99,61
I	**V**	**B**	*Piloderma sphaerosporum*	JQ711966	SH196824.07FU	941	100
I	**V**	**4**	*Piloderma sphaerosporum*	JQ711966	SH196824.07FU	854	100
J	**V**	**A**	*Imleria badia*	LN877746	SH216653.07FU	516	91,51
J	**III**	**B**	*Imleria badia*	HM190036	SH216653.07FU	1101	99,5
J	**V**	**B**	*Imleria badia*	HM190050	SH216653.07FU	1031	99,47
J	**III**	**C**	*Imleria badia*	HM190036	SH216653.07FU	928	97,78
J	**V**	**C**	*Imleria badia*	HM190036	SH216653.07FU	1092	99,83
K	**III**	**B**	*Xerocomellus cisalpinus*	HM190056	SH221249.07FU	1249	99,71
K	**IV**	**B**	*Xerocomellus cisalpinus*	HM190056	SH221249.07FU	1293	98,9
K	**V**	**B**	*Xerocomellus cisalpinus*	HM190056	SH221249.07FU	1131	98,43
K	**V**	**C**	*Xerocomellus cisalpinus*	HM190056	SH221249.07FU	1175	99,23
L	**V**	**B**	*Paxillus involutus*	UDB015588	SH210482.07FU	1210	98,83
M	**III**	**B**	*Suillus variegatus*	L54081	SH176741.07FU	688	100
M	**IV**	**B**	*Suillus variegatus*	L54081	SH176741.07FU	715	99
M	**V**	**B**	*Suillus variegatus*	L54081	SH176741.07FU	566	99,68
M	**III**	**C**	*Suillus variegatus*	L54081	SH176741.07FU	571	98,76
M	**V**	**C**	-	-	-	-	-
N	**V**	**B**	*Craterellus cornucopioides*	KX449405	SH181204.07FU	931	99,22
N	**VI**	**B**	*Craterellus cornucopioides*	KX449405	SH181204.07FU	1194	99,83
N	**V**	**C**	*Craterellus cornucopioides*	KX449405	SH181204.07FU	959	100
O	**III**	**B**	*Clavulina coralloides*	UDB031967	SH220213.07FU	702	98,26
O	**V**	**B**	*Clavulina coralloides*	AY292292	SH220213.07FU	808	100
O	**VI**	**B**	*Clavulina coralloides*	UDB031967	SH220213.07FU	974	98,9
O	**III**	**C**	*Clavulina coralloides*	EU862223	SH220213.07FU	706	99,23
O	**V**	**C**	*Clavulina coralloides*	AY292292	SH220213.07FU	926	96,92
P	**V**	**B**	*Lactarius aurantiacus*	KP783446	SH182376.07FU	1092	98,24

Q	**III**	**B**	*Russula nigricans*	KM085390	SH219259.07FU	808	98,68
Q	**V**	**B**	*Russula nigricans*	KM085390	SH219259.07FU	849	99,78
Q	**III**	**C**	*Russula nigricans*	KM085390	SH219259.07FU	833	99,35
R	**III**	**B**	*Envir: Tomentella*	UDB027188	SH217498.07FU	972	99,44
R	**V**	**B**	*Envir: Tomentella*	UDB027188	SH217498.07FU	928	98,85
R	**III**	**C**	*Envir: Tomentella*	UDB027188	SH217498.07FU	952	100
R	**V**	**C**	*Envir: Thelephoraceae*	UDB017056	SH184514.07FU	828	97,72
S	**V**	**A**	*Tomentella*	UDB014251	SH177961.07FU	1024	99,82
S	**III**	**B**	*Tomentella*	UDB014251	SH177961.07FU	974	99,44
S	**IV**	**B**	*Tomentella*	UDB014251	SH177961.07FU	987	99,81
S	**V**	**B**	*Tomentella*	UDB014251	SH177961.07FU	654	95,42
S	**V**	**C**	*Tomentella*	UDB014251	SH177961.07FU	915	99,4
T	**V**	**A**	*Tomentella terrestris*	UDB003315	SH189365.07FU	505	97,95
T	**III**	**B**	*Tomentella*	UDB016650	SH189365.07FU	874	98,98
T	**IV**	**B**	*Tomentella terrestris*	UDB003315	SH189365.07FU	961	98,71
T	**V**	**B**	*Tomentella terrestris*	UDB003315	SH189365.07FU	869	98,01
T	**VI**	**B**	*Tomentella*	UDB016650	SH189365.07FU	1201	100
T	**V**	**C**	*Tomentella terrestris*	UDB003315	SH189365.07FU	875	98,59
T	**VI**	**C**	*Tomentella terrestris*	UDB003315	SH189365.07FU	979	99,08

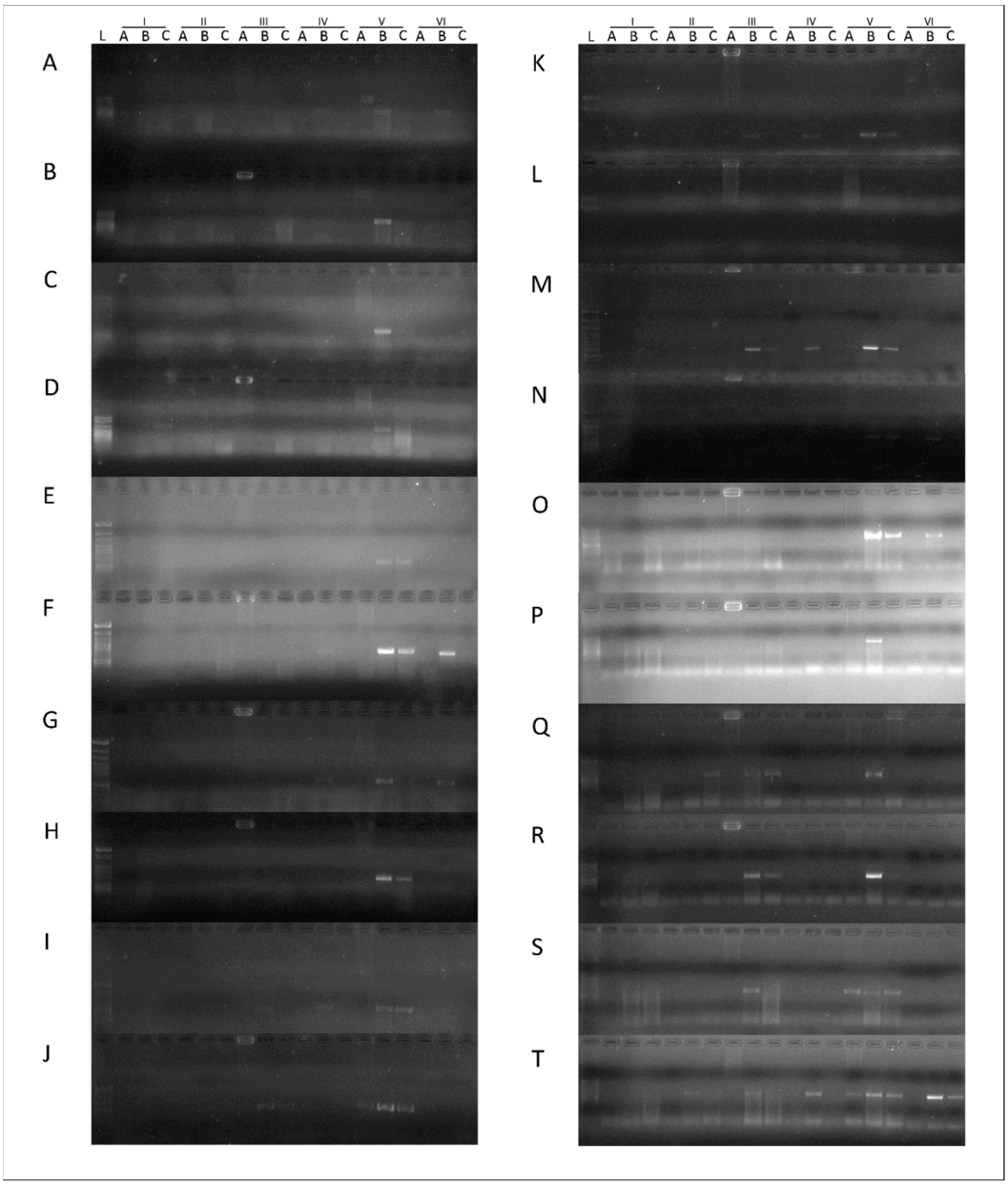

Figure S1. Electrophoresis results: the columns were loaded with: (L) DNA ladder (peqGOLD 50 bp DNA-Ladder, PEQLAB); and (IA–VIC) PCR products prepared using respective isolation methodproduct as a reaction matrix; the gels (A–T) were prepared with samples from respective fungal taxa, as assigned in the table 1.

Cell lysis protocols:

I. No lysis

1. ECM root tips were placed in empty Eppendorf tubes; 100 µL of mili-Q water was added to each

II. Mechanical cell disruption

1. ECM root tips were placed in empty Eppendorf tubes; 100 µL of mili-Q water was added to each
2. Samples were homogenized with a sterile disposable micro pestle

III. Chemical lysis

1. ECM root tips were placed in empty Eppendorf tubes; 50 µL of Extraction buffer (Extract-N-Amp™ Plant PCR Kit, Sigma) was added to each

2. Tubes were incubated at 95 °C for 10 min
3. 50 µL of Dilution buffer (Extract-N-Amp™ Plant PCR Kit, Sigma) was added to each tube

IV. Enzymatic lysis

1. ECM root tips were placed in empty Eppendorf tubes; 100 µL of proteinase buffer (10mM Tris-HCl; 1mM EDTA; 0.5% Tween-20; 1 mg/mL proteinase K; pH 7.5) was added to each tube
2. Tubes were incubated at 60 °C overnight

V. Mechanical + chemical lysis

1. ECM root tips were placed in empty Eppendorf tubes; 50 µL of Extraction buffer was added to each tube
2. Samples were homogenized with a disposable micro pestle
3. Tubes were incubated at 95 °C for 10 min
4. 50 µL of Dilution buffer was added to each tube

VI. Enzymatic + chemical lysis

1. ECM root tips were placed in empty Eppendorf tubes; 100 µL of proteinase buffer (10mM Tris-HCl; 1mM EDTA; 0.5% Tween-20; 1 mg/mL proteinase K; pH 7.5) was added to each
2. Samples were homogenized with a disposable micro pestle
3. Tubes were incubated at 60 °C overnight

Lysate purification protocols:

A. No purification

1. Samples were used for further downstream applications

B. Silica columns purification

1. 40 µL of the Buffer P was added to the provided isolation mini columns; minicolumns were incubated for 15 min in room temperature (RT)
2. 350 µL of the Sol P buffer and 250 µL of 96% ethanol were added to the samples; samples were mixed
3. Samples were centrifuged for 1 min at 14000 × g, RT
4. From each sample, 600 µL of the supernatant was transferred to the activated mini column placed in a collection tube
5. Samples were centrifuged for 1 min at 14000 × g, RT
6. The filtrate was discarded from the collection tubes; the remaining supernatant was added to the minicolumns
7. Samples were centrifuged for 1 min at 14000 × g, RT
8. The filtrate was discarded from the collection tubes; 500 µL of the Wash PX buffer was added to the minicolumns
9. Samples were centrifuged for 1 min at 14000 × g, RT
10. The filtrate was discarded from the collection tubes; 500 µL of the Wash PX buffer was added to the minicolumns
11. Samples were centrifuged for 2 min at 14000 × g, RT
12. The minicolumns were moved to clean Eppendorf tubes; 100 µL of the Elution buffer preheated to 70 °C was added to each of the minicolumns
13. Samples were incubated for 3 min in RT
14. Samples were centrifuged for 1 min at 14000 × g, RT

15. The solution collected in the Eppendorf tubes was used for further downstream applications

C. Chloroform extraction purification

1. 25 μL of preparation buffer (1M Tris-HCl; 6M NaCl; 0.5% (w/v) PVP; pH 8) was added to the samples; samples were incubated for 30 min in 60 °C
2. 125 μL of chloroform was added to each sample; samples were vortexed for 2 min
3. Samples were centrifuged for 15 min at 10000 × g, RT
4. From each sample, 90 μL of aqueous supernatant was transferred to new tube
5. 45 μL of 4 M NaCl was added to each sample; samples were incubated on ice for 5 min
6. 270 μL of cold 96% ethanol was added to each sample; samples were incubated for 2 min in RT
7. Samples were centrifuged for 8 min at 8000 × g, RT
8. The supernatant was discarded; the pellets were washed with 100 μL 75% ethanol
9. The pellets were dried and dissolved in 100 μL mili-Q water
10. The final solution was used for further downstream applications