

Mycobiome diversity: high-throughput sequencing and identification of fungi

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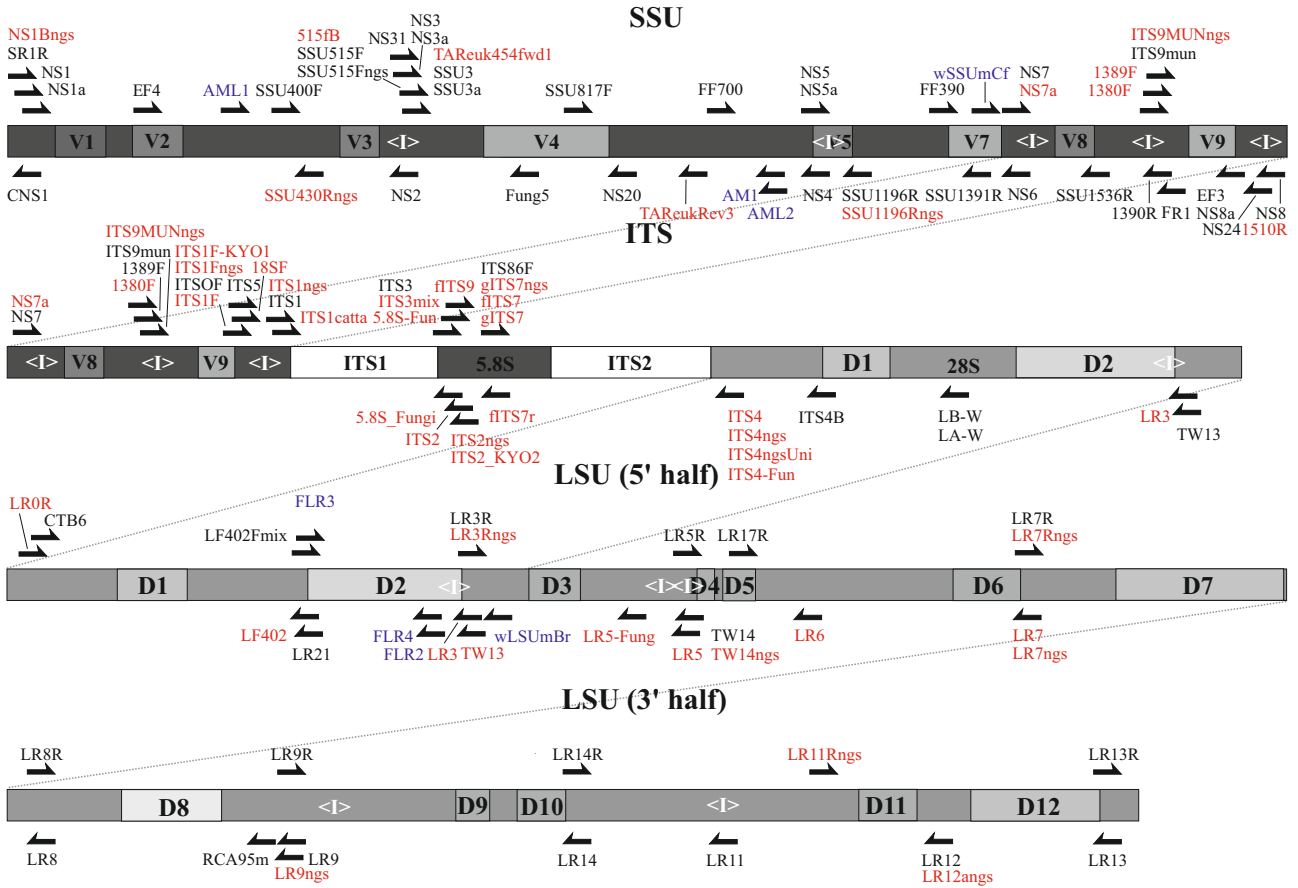
Microbiome series

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Supplementary Information

Supplementary Figure 1 – Primer map indicating the relative placement of primers used for fungal taxonomy and biodiversity analyses along the nuclear rRNA SSU gene (*Saccharomyces cerevisiae* positions 1-1800), the ITS region, and the LSU gene (5' part, 1-1800; 3' part 1801-3396). Primers used for metabarcoding are shown in red, except those used specifically for Glomeromycota, which are depicted in blue. ITS1, ITS2, V1-V9, and D1-D12 variable domains of the rRNA operon are indicated in light grey, with lighter tones indicating relatively lower conservation across Fungi. <I> indicates common intron sites that may disrupt some primer sites (NS31, NS2, NS3, 1380F, NS8, LR5, TW14, and LR11) and cause discrimination against, or no amplification of, intron-containing taxa. Primer references and additional technical information are provided in Supplementary Table 1.



Supplementary Table 1 – Additional technical information and references for the primers shown in Supplementary Figure 1. The primer positions correspond to the mid-points in sequences of *Saccharomyces cerevisiae* SSU, 5.8S, and LSU rRNA genes. Percentages of coverage were calculated following Tedersoo & Lindahl (2016).

Primer	Position	Sequence	Marker	Orientation	Target taxa	Reference
1380F	1625	CCCTGCCHTTTGTACACAC	SSU	fwd	Fungi (~100%), other eukaryotes	Amaral-Zettler et al. 2009
1389F	1630	TTGTACACACCGCCC	SSU	fwd	Fungi (~100%), other eukaryotes	Amaral-Zettler et al. 2009
1390R	1630	GACGGGCGGTGTGTAC	SSU	rev	Fungi (~100%), other eukaryotes	Amaral-Zettler et al. 2009
1510R	1780	CCTTCYGCAGGTTACCTAC	SSU	rev	Eukaryotes	Amaral-Zettler et al. 2009
18SF	55	GTAAAAGTCGTAACAAGGTTTC	SSU	fwd	Eukaryotes	Findley et al. 2013
5.8S_Fungi	10	CAAGAGATCCGTTGTTGAAAGTT	ITS	rev	Fungi (80%)	Epp et al. 2012
5.8S-Fun	10	AACTTYRRC AAYGGATCWCT	ITS	fwd	Fungi (95%)	Taylor et al. 2016
AM1	1075	GTTTCCCGTAAGGCGCCGAA	SSU	rev	Glomeromycota	Helgason et al. 1998
AML1	320	ATCAACTTTTCGATGGTAGGATAGA	SSU	fwd	Glomeromycota	Lee et al. 2008
AML2	1080	GAACCCAAACACTTTGGTTTCC	SSU	rev	Glomeromycota	Lee et al. 2008
CNS1	25	GAGACAAGCATATGACTACTG	IGS	rev	Eukaryotes	White et al. 1990
CTB6	40	GCATATCAATAAGCGGAGG	LSU	fwd	Eukaryotes	Garbelotto et al. 1997
EF3	1735	TCCTCTAAATGACCAAGTTTG	SSU	rev	Eukaryotes	Smit et al. 1999
EF4	200	GGAAGGGRTGTATTTATTAG	SSU	fwd	Eukaryotes	Smit et al. 1999
FF390	1325	CGATAACGAACGAGACCT	SSU	fwd	Eukaryotes	Vainio & Hantula 2000
FF700	1010	GATACCGTIGTAGTCT	SSU	fwd	Eukaryotes	Vainio & Hantula 2000
fITS7	70	GTGARTCATCGAATCTTTG	ITS	fwd	Fungi (98%)	Ihrmark et al. 2012
fITS7r	70	CAAAGATTCGATGAYTCAC	ITS	rev	Fungi (98%)	L. Tedersoo, unpublished
fITS9	55	GAACACAGCGAAATGTGA	ITS	fwd	Fungi (96%)	Ihrmark et al. 2012
FLR2	610	GTCGTTTAAAGCCATTACGTC	LSU	rev	Glomeromycota	Trouvelot et al. 1999
FLR3	420	TTGAAAGGGAAACGATTGAAGT	LSU	fwd	Glomeromycota	Gollotte et al. 2004
FLR4	595	TACGTCAACATCCTTAACGAA	LSU	rev	Glomeromycota	Gollotte et al. 2004
FR1	1655	AICCATTC AATCGGTAIT	SSU	rev	Eukaryotes	Vainio & Hantola 2000
Fung5	735	GTAAAAGTCCTGGTTCCCC	SSU	rev	Fungi (80%)	Smit et al. 1999
gITS7	70	GTGARTCATCGARTCTTTG	ITS	fwd	Fungi (99%), plants, protists	Ihrmark et al. 2012
gITS7ngs	70	GTGARTCATCRARTYTTTG	ITS	fwd	Fungi (~100%), plants, protists	Tedersoo & Lindahl 2016
ITS1	1770	TCCGTAGGTGAACCTGCGG	ITS	fwd	Fungi (90%), other eukaryotes	White et al. 1990
ITS1catta	1795	ACCWGC GGARGGATCATT A	ITS	fwd	Fungi (95%), few plants	L. Tedersoo, unpublished
ITS1F	1710	CTTGGTCATTTAGAGGAAGTAA	ITS	fwd	Fungi (90%)	Gardes & Bruns 1993
ITS1F_KYO1	90	CTHGGTCATTTAGAGGAASTAA	ITS	fwd	Fungi (92%)	Toju et al. 2012
ITS1Fnsgs	1710	GGTCATTTAGAGGAAGTAA	ITS	fwd	Fungi (95%)	Tedersoo et al. 2015a,b
ITS1ngs	1770	TCCGTAGGTGAACCTGC	ITS	fwd	Fungi (90%)	Tedersoo et al. 2015a,b
ITS2	40	GCTGCGTTCTTCATCGATGC	ITS	fwd	Fungi (95%), other eukaryotes	White et al. 1990
ITS2_KYO2	45	TTYRCTR CGTTCTTCATC	ITS	rev	Fungi (99%), other eukaryotes	Toju et al. 2012

ITS2ngs	35	TTYRCKRCGTTCTTCATCG	ITS	rev	Fungi (~100%), other eukaryotes	L. Tedersoo, unpublished
ITS3	40	GCATCGATGAAGAACGCAGC	ITS	fwd	Fungi (95%), other eukaryotes	White et al. 1990
ITS3mix1-5	40	CANCGATGAAGAACGYRG	ITS	fwd	Fungi (~100%), other eukaryotes	Tedersoo et al. 2014
ITS4	40	TCCTCCGCTTATTGATATGC	ITS	rev	Fungi (98%), other eukaryotes	White et al. 1990
ITS4B	200	CAGGAGACTTGACACGGTCCAG	ITS	rev	Basidiomycota (70%)	Gardes & Bruns 1993
ITS4-Fun	35	AGCCTCCGCTTATTGATATGCTTAART	ITS	rev	Fungi (90%)	Taylor et al. 2016
ITS4ngs	40	TCCTSCGCTTATTGATATGC	ITS	rev	Fungi (99%), other eukaryotes	White et al. 1990
ITS4ngsUni	40	CCTSCSCTTANTDATATGC	ITS	rev	Fungi (~100%), other eukaryotes	Tedersoo & Lindahl 2016
ITS5	50	GGAAGTAAAAGTCGTAACAAGG	ITS	fwd	Eukaryotes	White et al. 1990
ITS86F	70	GTGAATCATCGAATCTTTGAA	ITS	fwd	Eukaryotes	Turenne et al. 1999
ITS9mun	1635	TGTACACACCGCCCGTCG	ITS	fwd	Fungi (~100%), other eukaryotes	Egger 1995
ITS9MUNngs	1635	TACACACCGCCCGTCG	ITS	fwd	Fungi (~100%), other eukaryotes	Tedersoo & Lindahl 2016
ITSOF-T	90	ACTTGGTCATTTAGAGGAAGT	ITS	fwd	Fungi (95%)	Taylor & McCormick, 2008 Tedersoo et al. 2008
LA-W	360	CTTTTCATCTTTTCGATCACTC	ITS	rev	Ascomycota (95%)	Tedersoo et al. 2008
LB-W	360	CTTTTCATCTTTCCCTCACGG	ITS	rev	Fungi excl. Ascomycota	Tedersoo et al. 2008
LF402	410	TTCCCTTTYARCAATTTAC	LSU	rev	Fungi (95%)	Tedersoo et al. 2015a,b
LF402F	410	GTGAAATTGYTRAAAGGGAA	LSU	fwd	Fungi (95%)	Tedersoo et al. 2015a,b
LR0R	30	ACCCGCTGAACTTAAGC	LSU	fwd	Fungi (98%), other eukaryotes	Hopple & Vilgalys 1994
LR11	2810	GCCAGTTATCCCTGTGGTAA	LSU	rev	Eukaryotes	Vilgalys & Hester 1990
LR12	3115	GACTTAGAGGCGTTCAG	LSU	rev	Eukaryotes	Vilgalys & Hester 1990
LR13	3350	CGTAACAACAAGGCTACT	LSU	rev	Eukaryotes	Vilgalys lab
LR13R	3350	GCATTGTTGTTCCGATG	LSU	fwd	Eukaryotes	Vilgalys lab
LR14	2600	AGCCAAACTCCCCACCTG	LSU	rev	Eukaryotes	Vilgalys & Hester 1990
LR14R	2600	CAGGTGGGGAGTTTGGCT	LSU	fwd	Eukaryotes	Vilgalys lab
LR17R	1040	TAACCTATTCTCAAACCTT	LSU	fwd	Eukaryotes	Vilgalys & Hester 1990
LR21	420	ACTTCAAGCGTTTCCCTTT	LSU	rev	Fungi (90%)	Hopple & Vilgalys 1994
LR3	640	CCGTGTTTCAAGACGGG	LSU	rev	Eukaryotes	Hopple & Vilgalys 1994
LR3R	645	GTCTTGAAACACGGACC	LSU	fwd	Eukaryotes	Hopple & Vilgalys 1994
LR3Rngs	670	CGTCTTGAAACAYGGACC	LSU	fwd	Eukaryotes	L. Tedersoo, unpublished
LR5	955	TCCTGAGGGAAACTTCG	LSU	rev	Eukaryotes	Hopple & Vilgalys 1994
LR5-Fung	880	CGATCGATTGTCACGTCAGA	LSU	rev	Fungi (99%), no plants	Tedersoo et al. 2008
LR5R	955	CGAAGTTTCCCTCAGGA	LSU	fwd	Eukaryotes	Hopple & Vilgalys 1994
LR6	1130	CGCCAGTTCTGCTTACC	LSU	rev	Eukaryotes	Hopple & Vilgalys 1994
LR7	1435	TACTACCACCAAGATCT	LSU	rev	Eukaryotes	Hopple & Vilgalys 1994

LR7ngs	1440	TGYTACTACCWCCAAGATC	LSU	rev	Eukaryotes	L. Tedersoo, unpublished
LR7R	1435	AGATCTTGGTGGTAGTA	LSU	fwd	Eukaryotes	Vilgalys lab
LR7Rngs	1440	GATCTTGGWGGTAGTARC	LSU	fwd	Eukaryotes	L. Tedersoo, unpublished
LR8	1850	CACCTTGGAGACCTGCT	LSU	rev	Eukaryotes	Vilgalys & Hester 1990
LR8R	1850	AGCAGGTCTCCAAGGTG	LSU	fwd	Eukaryotes	Vilgalys & Hester 1990
LR9	2200	AGAGCACTGGGCAGAAA	LSU	rev	Eukaryotes	Vilgalys & Hester 1990
LR9ngs	2195	ASCACTGGGCAGAAATC	LSU	rev	Eukaryotes	L. Tedersoo, unpublished
LR9R	2200	TTTCTGCCCAGTGCTCT	LSU	fwd	Eukaryotes	Vilgalys lab
NS1	25	GTAGTCATATGCTTGTCTC	SSU	fwd	Eukaryotes	White et al. 1990
NS1a	40	TCTCAAAGAYTAAGCCATGC	sSU	fwd	Eukaryotes	L. Tedersoo, unpublished
NS1B1ngs	10	CCTNGTTGATYCTGCCAGT	SSU	fwd	Eukaryotes	L. Tedersoo, unpublished
NS2	560	GGCTGCTGGCACCAGACTTGC	SSU	rev	Eukaryotes	White et al. 1990
NS20	860	CGTCCCTATTAATCATTACG	SSU	rev	Eukaryotes	White et al. 1990
NS24	1760	AAACCTTGTTACGACTTTTA	SSU	rev	Fungi (95%)	White et al. 1990
NS2a	560	TGCTGGCACCAGACTTGC	SSU	rev	Eukaryotes	L. Tedersoo, unpublished
NS3	560	GCAAGTCTGGTGCCAGCAGCC	SSU	fwd	Eukaryotes	White et al. 1990
NS31	555	TTGGAGGGCAAGTCTGGTGCC	SSU	fwd	Eukaryotes	Simon et al. 1992
NS3a	560	AGTCTGGTGCCAGCAGCC	SSU	fwd	Eukaryotes	Tedersoo et al. 2017
NS3b	580	CCGCGGTAATTCCAGCTC	sSU	fwd	Eukaryotes	L. Tedersoo, unpublished
NS4	1140	CTTCCGTCAATTCTTTAAG	SSU	rev	Eukaryotes	White et al. 1990
NS5	1140	AACTTAAAGGAATTGACGGAAG	SSU	fwd	Eukaryotes	White et al. 1990
NS5a	1140	CTTAAAGGAATTGACGGAAG	SSU	fwd	Eukaryotes	Tedersoo et al. 2017
NS6	1425	GCATCACAGACCTGTTATTGCCTC	SSU	rev	Eukaryotes	White et al. 1990
NS7	1425	GAGGCAATAACAGGTCTGTGATGC	SSU	fwd	Eukaryotes	White et al. 1990
NS7a	1425	CAATAACAGGTCTGTGATGC	SSU	fwd	Eukaryotes	Tedersoo et al. 2017
NS8	1780	TCCGCAGGTTACCTACGGA	SSU	rev	Eukaryotes	White et al. 1990
NS8a	1735	CCTCTAAATGACCRAGTTTG	SSU	rev	Eukaryotes	L. Tedersoo, unpublished
RCA95m	2145	CTATGTTTTAATTAGACAGTCAG	LSU	rev	Fungi (98%)	Wurzbacher et al. 2018
SR1R	10	TACCTGGTTGATYCTGCCAGT	SSU	fwd	Eukaryotes	Vilgalys & Hester 1990
SSU0817f	805	TTAGCATGGAATAATRRATAGGA	SSU	fwd	Eukaryotes	Borneman & Hartin 2000
SSU1196ngs	1205	TCTGGACCTGGTGAGTTT	SSU	rev	Eukaryotes	Tedersoo et al. 2015a,b
SSU1196R	1205	TCTGGACCTGGTGAGTTTCC	SSU	rev	Eukaryotes	Borneman & Hartin 2000
SSU1391R	1360	GACGGGCGGTGWGTRCA	SSU	rev	Eukaryotes	Borneman & Hartin 2000
SSU1536R	1545	ATTGCAATGCYCTATCCCCA	SSU	rev	Eukaryotes	Borneman & Hartin 2000
SSU3	575	GCCAGCAGCCGCGGTAATTC	SSU	fwd	Eukaryotes	Atlan et al. 2012

SSU3a	575	CAGCAGCCGCGGTAATTC	SSU	fwd	Eukaryotes	Tedersoo et al. 2017
SSU400F	390	GGAGARRGAGCCTGAGA	SSU	fwd	Eukaryotes	L. Tedersoo, unpublished
SSU430Rngs	430	AATTTDCGYSCCTGCTGC	SSU	rev	Eukaryotes	L. Tedersoo, unpublished
SSU515f	570	GTGCCAGCMGCCGCGGTAA	SSU	fwd	Cross-domain	Turner et al. 1999
SSU515Fngs	570	GCCAGCMRCCGCGGTAA	SSU	fwd	Cross-domain	Tedersoo et al. 2015a,b
TAREuk454fwd1	565	CCAGCASCYGCGGTAATTCC	SSU	fwd	Eukaryotes	Stoeck et al. 2010
TAREukRev3	970	ACTTTCGTTCTTGATYRA	SSU	rev	Eukaryotes	Stoeck et al. 2010
TW13	645	GGTCCGTGTTTCAAGACG	LSU	rev	Eukaryotes	T.J. White, unpublished
TW14	960	GCTATCCTGAGGGAAACTTC	LSU	rev	Eukaryotes	T.J. White, unpublished
TW14ngs	960	CTATCCTGRGRGAAAYTTC	LSU	rev	Eukaryotes	Tedersoo & Lindahl 2016
wLSUmBr	670	AACACTCGCAYAYATGYTAGA	ITS	rev	Glomeromycota	Schlaeppi et al. 2017
wSSUmCf	1380	TATYGYTCTTNAACGAGGAATC	ITS	fwd	Glomeromycota	Schlaeppi et al. 2016

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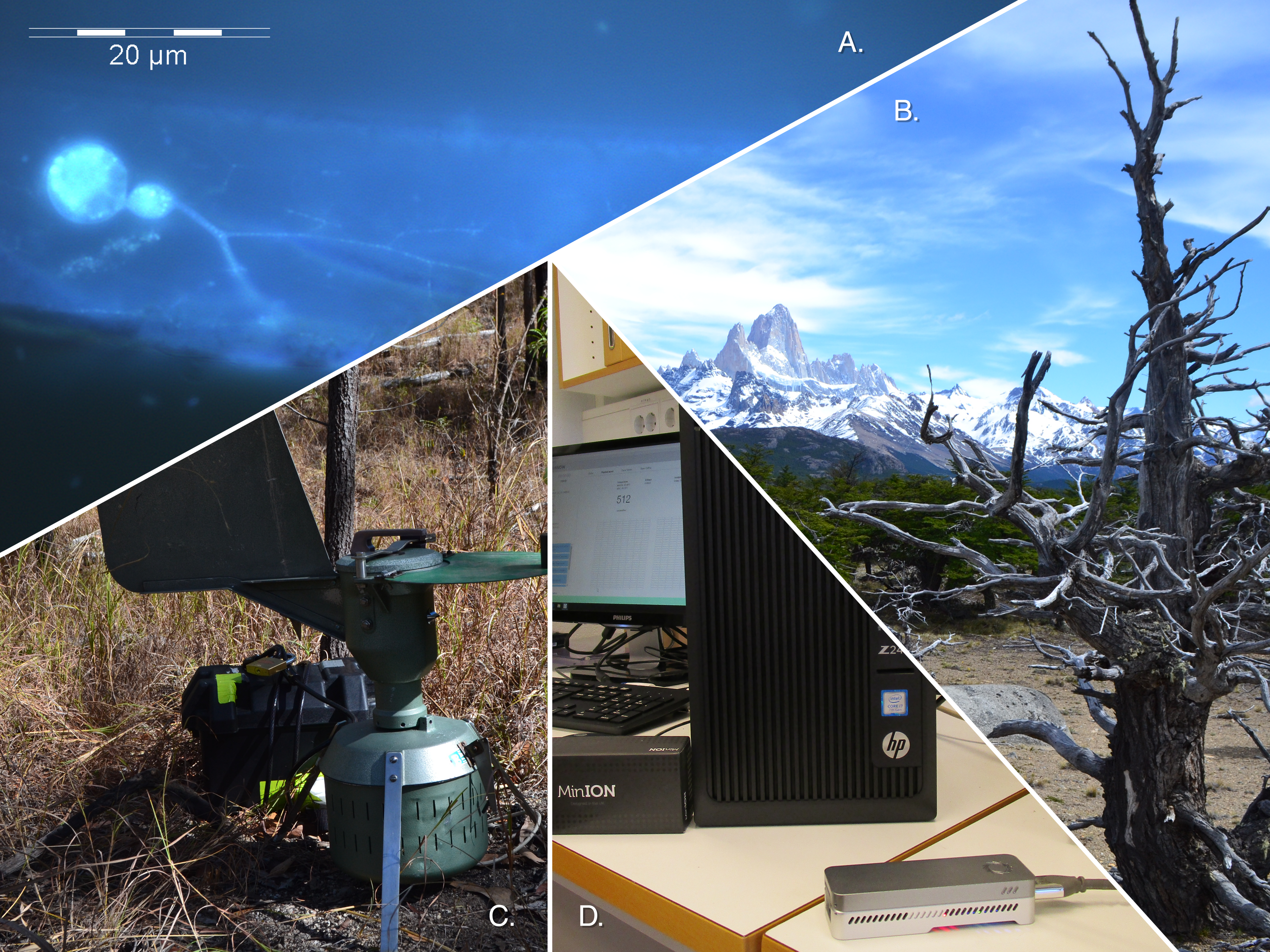
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Supplementary Figure 2 – Image plate showing HTS applications and environments that have been explored for fungal community structure. A) Aquatic fungus from the Chytridiomycota growing on a chitinous exoskeleton of a water flea from a freshwater lake in Germany. B) Soil sampling in Patagonia, near the Andes in Argentina, which is known for a unique mycobiota connected to southern beeches (*Nothofagus* spp.) C) A battery-powered spore sampler in open eucalypt woodland in Queensland, Australia. Sampling of airborne spores can be used to monitor fruitification patterns over extended periods of time. D) The Oxford Nanopore MinION sequencer running in a laboratory setting on a mid-range stationary computer. This remarkably portable third-generation HTS device excels at long read lengths and is beginning to find its way into applications such as A-C.



A.

B.

C.

D.

20 μm