SUPPLEMENTARY MATERIAL

Article title: Greenland and Svalbard glaciers host unknown basidiomycetes: the yeast *Camptobasidium arcticum* sp. nov. and the dimorphic *Psychromyces glacialis* gen. and sp. nov.

Authors: Laura Perini¹, Kristina Andrejašič¹, Cene Gostinčar^{1,2}, Nina Gunde-Cimerman¹, Polona Zalar^{1,*}

¹ Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia; ² Lars Bolund Institute of Regenerative Medicine, BGI-Qingdao, Qingdao 266555, China

*Corresponding author: Polona Zalar, polona.zalar@bf.uni-lj.si

TABLES:

<u>Table S1</u>. List of strains considered in the present study, their isolation source, GenBank accession numbers of: LSU rDNA, ITS rDNA, SSU rDNA, cytochrome B (CYTB), translation elongation factor 1 alpha (TEF), and two subunits of the RNA polymerase II (RPB1 and RPB2). http://www.ncbi.nlm.nih.gov.

<u>Table S2</u>. Results on assimilation tests of *Camptobasidium gelus*, *C*, *arcticum*, and *Psychromyces glacialis*.

FIGURES:

<u>Figure S1</u>. Maximum likelihood phylogeny of the concatenated rDNA genes (SSU, LSU, 5.8 S rDNA), estimated by PhyML. aLRT as Chi2 based support was used for calculation of branch supports.

Figure S2. Phylogenetic trees based on alignment of ITS rDNA, estimated by Bayesian inference. The tree includes representatives of (A) *Camptobasidium.*, unrooted, and (B) *Psychromyces glacialis* gen. et sp. nov. rooted with *Phenoliferia psychrophenolica*. A total 10 million generations were calculated in each case and the first 25% were discarded before the shown consensus trees were calculated from trees sampled every 100 generations. The numbers given on branches are frequencies at which the individual clades appeared in the sampled trees.

<u>Figure S3</u>. Maximum likelihood tree based on ITS2 sequences obtained in NGS study of glacial environments in Svalbard and the Greenland Ice Sheet, and on ITS sequences of *Camptobasidium gelus, Camptobasidium arcticum, Camptobasidium* sp. and *Psychromyces glacialis* pure cultures.

<u>Figure S4</u>. Minimum spanning network calculated from the alignment of *Camptobasidium* ITS sequences. The similarity between the strains is shown with the thickness of the lines connecting the circles representing the strains.

<u>Figure S5</u>. Minimum spanning network calculated from the alignment of *Psychromyces* ITS sequences. The similarity between the strains is shown with the thickness of the lines connecting the circles representing the strains.

Figure S6. Morphology of Psychromyces glacialis EXF-12419

(a–b): Cultures of *P. glacialis* EXF-12419 on PDA at 15 °C: (a) after 14 days, (b) after 8 months; (c–e) synnemata and hyphae after 14 days in 60% lactic acid; (k) teliospores of EXF-12454 after 2 months; (f, g–j, l) hyphae with clamps stained with calcofluor white after 14 days (f, g, i) and after 2 months (h, j, l).

Table S1. List of strains considered in the present study, their isolation source, GenBank accession numbers of: LSU rDNA, ITS rDNA, SSU rDNA, cytochrome B (CYTB), translation elongation factor 1 alpha (TEF), and two subunits of the RNA polymerase II (RPB1 and RPB2). http://www.ncbi.nlm.nih.gov.

EXF-					Ger	nBank Accession numbers			
accession Isol. Geographical origin number medium (*)	Isolation source	LSU rDNA	ITS rDNA	SSU rDNA	CYTB/TEF	RPB1/ RPB2			
Camptobasi	Camptobasidium gelus								
12745⊺	MM	GrIS	Cryoconite	MK454802	MN983240	MT248410	-/-	-/MT260387	
12575	SNA	SV, Midtre Lovénbreen	Subglacial ice	MK670456	-	-	-	-	
12576	ММ	SV, Midtre Lovénbreen	Subglacial ice	MK670454	MN983222	MW036178	-/MW122989	-/MW036260	
12582	DRBC	SV, Pedersenbreen	Subglacial ice	MK670478	MN983223	-	-	-	
12584	DRBC	SV, Pedersenbreen	Subglacial ice	MK670480	MN983224	-	-	-	
12594	DRBC	SV, Vestre Brøggerbreen	Subglacial ice	MK670498	MN983225	MW036179	-/-	MW122991/ MW036261	
12597	DRBC	SV, Vestre Brøggerbreen	Subglacial ice	MK670502	MN983226	-	-	-	
12948	SNA	SV, Midtre Lovénbreen	Subglacial ice	MK670457	MN983227	-	-	-	
12950	SNA	SV, Midtre Lovénbreen	Subglacial ice	MK670455	-	-	-	-	
12952	ММ	SV, Midtre Lovénbreen	Subglacial ice	MK670453	MN983228	-	-	-	
12959	SNA	SV, Pedersenbreen	Subglacial ice	MK670474	-	-	-	-	
12967	DRBC	SV, Vestre Brøggerbreen	Subglacial ice	MK670494	MN983229	-	-	-	
12968	DRBC	SV, Vestre Brøggerbreen	Subglacial ice	MK670495	MN983230	-	-	-	
12969	DRBC	SV, Vestre Brøggerbreen	Subglacial ice	MK670492	-	-	-	-	
(12970)	ММ	SV, Vestre Brøggerbreen	Subglacial ice	MK670500	MN983231	-	-	-	
13102	ММ	SV	Glacial meltwater	MK670451	MN983232	MW036180	-/-	MW122993/ MW036262	
13103	ММ	SV, Pedersenbreen	Subglacial ice	MK670469	MN983233	MW036177	-/-	MW122992/ MW036267	
13104	SNA	SV, Pedersenbreen	Subglacial ice	MK670473	-	-	-	-	

13113	SNA	SV, Vestre Brøggerbreen	Subglacial ice	MK670503	MN983234	-	-	-	
(12394)	R2A	GrlS	Supraglacial water	MK454824	-	-	-	-	
12401	R2A	GrIS	Dark ice	MK454807	MN983235	-	-	-	
12406	R2A	GrIS	Dark ice	MK454810	MN983236	-	-	-	
12527	R2A	GrIS	Clear ice	MK454796	-	-	-	-	
12693	R2A	GrIS	Supraglacial water	MK454821	-	-	-	-	
(12694)	R2A	GrIS	Supraglacial water	MK454817	MN983237	-	-	-	
(12698)	R2A	GrIS	Supraglacial water	MK454823	-	-	-	-	
12712	SNA	GrIS	Clear ice	MK454797	MN983238	-	-	-	
12714	SNA	GrIS	Clear ice	MK454799	-	-	-	-	
(12716)	SNA	GrIS	Clear ice	MK454801	-	-	-	-	
12717	R2A	GrlS	Clear ice	MK454794	MN983239	-	-	-	
15272	SNA	GrIS	Dark ice	MK454811	-	-	-	-	
15273	R2A	GrIS	Clear ice	MK454795	-	-	-	-	
Camptobasi	Camptobasidium arcticum								
12713⊺	SNA	GrIS	Clear ice	MK454798	MN983248	MT304813	MT260394/ MT260390	-/ MT260386	
(12392)	R2A	GrIS	Supraglacial water	MK454819	MN983241	-	-	-	
(12405)	R2A	GrlS	Dark ice	MK454808	-	-	-	-	
12516	R2A	GrIS	Dark ice	MK454809	MW019434	-	-	-	
12519	MM	GrlS	Supraglacial water	MK454812	MW019433	-	-	-	
12522	R2A	GrlS	Supraglacial water	MK454816	MW019432	MW036182	MW036274/-	-/ MW036263	
12524	R2A	GrIS	Supraglacial water	MK454818	MN983242	MW036181	MW036272/ MW122987	MW122995/ MW036264	
12686	SNA	GrlS	Supraglacial water	MK454827	MN983243	-	-	-	
12687	MM	GrlS	Supraglacial water	MK454813	MN983244	-	-	-	
(12688)	MM	GrlS	Supraglacial water	MK454814	-	-	-	-	
12689	ММ	GrlS	Supraglacial water	MK454815	MN983245	-	-	-	
(12692)	R2A	GrIS	Supraglacial water	MK454820	MN983246	-	-	-	
12696	R2A	GrIS	Supraglacial water	MK454825	-	-	-	-	
(12697)	R2A	GrIS	Supraglacial water	MK454822	MN983247	-	-	-	
12711	DRBC	GrlS	Dark ice	MK454805	MW019431	MW036183	MW036273/ MW122988	MW122994/ MW036265	

12715	SNA	GrIS	Clear ice	MK454800	MW019430	-	-	-
12728	DRBC	GrIS	Dark ice	MK454804	MN983249	-	-/ MW122986	-/ MW036266
(12868)	SNA	GrIS	Cryoconite	MK454803	MN983250	-	-	-
12872	R2A	GrIS	Supraglacial water	MK454826	MN983251	-	-	-
13086	R2A	GrIS	Clear ice	MK454828	MN983252	-	-	-
(15271)	R2A	GrIS	Dark ice	MK454806	MN983253	-	-	-
Camptobasi	Camptobasidium sp.							
(12685)	SNA	GrIS	Supraglacial water	MK454829	MN983221	MT248411	-	-
Psychromyc	es glacialis	•		1		1	L	
13111 [⊤] =CBS 16467	DRBC	SV, Vestre Brøggerbreen	Suglacial ice	MT301949	MK671633	MT248408	MT260392/ MT260389	-/ MW036268
12419	MM	GrIS	Dark ice	MK454861	MK460401	MT248407	MT260391/-	-/ MW036270
12886	DG18	GrIS	Cryoconite	MT301950	MK460394	MT248409	MT260393/ MT260388	-/ MW036269
12398	DRBC	GrIS	Clear ice	MK454857	MK460391	-	-	-
12420	MM	GrIS	Dark ice	-	MK460402	-	-	-
12452	MM	GrIS	Cryoconite	MK454858	MK460396	-	-	-
12454	MM	GrIS	Cryoconite	MK454859	MK460397	-	-	-
12545	MM	GrIS	Cryoconite	-	MK460395	-	-	-
12604	DRBC	GrIS	Dark ice	MK454862	MK460403	-	-	-
12623	ММ	SV, Midtre Lovénbreen	Suglacial ice	-	MK671639	-	-	-
12626	DRBC	SV, Midtre Lovénbreen	Suglacial ice	-	MK671638	-	-	-
12627	SNA	SV, Midtre Lovénbreen	Suglacial ice	-	MK671636	-	-	-
12647	DRBC	SV, Vestre Brøggerbreen	Suglacial ice	-	MK671634	-	-	-
12708	DRBC	GrIS	Dark ice	MK454860	MK460399	-	-	-
12718	DRBC	GrlS	Clear ice	MW026145	MK460392	-	-	-
12890	DG18	GrIS	Cryoconite	-	MK460398	-	-	-
12984	DRBC	SV, Midtre Lovénbreen	Suglacial ice	MW026144	MK671637	-	-	-
12991	DRBC	SV, Vestre Brøggerbreen	Suglacial ice	MW026141	MK671635	MW036176	MW036271/ MW122990	-
13154	DRBC	GrIS	Dark ice	MW026143	MK460400	-	-	-
13156	SNA	GrIS	Clear ice	MW026142	MK460393	-	-	-

Legend: GrIS: Greenland Ice Sheet, SV: Svalbard; (*) the accession numbers of strains that didn't survive freezing period of 3 years at -80 °C are written in the brackets.

Table S2. Results on assimilation tests of *Camptobasidium gelus, C. arcticum*, and *Psychromyces* glacialis.

	C-SOURCES	Camptobasidium gelus	Camptobasidium arcticum	Psychromyces glacialis (EXF- 13111-yeast)	Psychromyces glacialis (filamentous)
	a-D-glucose	+	+	+	+,D; YT:-
	inulin	V(-,+)	-	-	-
	sucrose	+	+	+	+
	D-raffinose	+	D	+	+, YT:-, D
	D-melibiose	-	V(+,-), YT:-	-	-
	D-galactose	V (-,W, D)	-	D	-
	Lactose	V(-,W)	V(-,+,W)	-	V(-, WD)
	D-trehalose	V(-,+,W)	+,W; YT:-	+	D
SS	maltose	W,+; YT:-	+	D	+, YT: W, D
nrce	D-melezitose	-	+	+	+
sol	a-methyl D-glucoside	-	-	-	-
oon	Starch	-	-	-	-
carl	cellobiose	-	+	W; YT:-	W,+
ed	salicin	W,+	V(-,W)	W,D; YT:-	V(-, WD)
-bas	L-sorbose	V(-,W,D)	-	+	-
ose-	L-rhamnose	-	-	-	-
Нехо	gentiobiose	-	+	-	V(+,-)
	maltotriose	-	+	D	V(+,-)
	D-psicose	-	-	-	-
	palatinose	-	V(+,-,D)	-	-
	stachyose	+	D	+	V(-,D)
	b-methyl D-glucoside	-	+	-	V(-,D)
	turanose	-	-	-	-
	dextrin	-	+,D	D	D
	arbutin	-	-	-	-
	amygdalin	-	-	-	-
	Г		1	1 1	
e	D-xylose	W,D	V(-, W, D)	W,D	-
itos	L-arabinose	-	-	-	V(-,W)
per	D-arabinose	-	-	-	-
	D-ribose	D, W, +	-	-	-
	glycerol	V(-, W,+), YT: -	-	-	-
	i-erythritol	-	-	-	-
<u>v</u>	xylitol	-	-	-	-
polyols	D-mannitol	-	-	-	-
	D-sorbitol	-	-	W,D	-
	maltitol	-	V(+,-,W,D)	-	-
	adonitol (ribitol)	-	-	-	-
	D-arabitol	-	-	-	-
	Г				1
	methyl succinate	-	-	-	-
ids	fumaric acid	-	-	-	V(+,-)
ac	L-malic acid	-	-	-	-

	bromo succinic acid	-	-	-	-
	L-glutamic acid	-	-	-	V(-,W)
	g-amino butyric acid	V(D,-)	W	D	-
	D-gluconic acid	-	-	-	-
	a-keto-glutaric acid	-	-	-	-
	2-keto-D-gluconic				
	acid	-	-	-	-
sed nes	D-glucosamine	-	-	W,D	-
gluc bas ami	N-acetyl-D-	_			
	glucosamme	_	_	_	_
	methyl succinate +				
	D-xylose	-	-	-	-
	N-acetyl-L-glutamic				
S	acid + D-xylose	-	V(+,-,W,D)	D	-
urce	quínic acid + D- xylose	-	V(+,-,W,D)	D	-
l so	D-glucuronic acid +				
ona	D-xylose	D	V(+,-,D)	+	-
ditio	dextrin + D-xylose	-	+, D	+	V(-, D)
h ad	a-D-lactose + D- xylose	-	V(+,-,D)	-	V(-, D)
witl	D-melibiose + D-				
se	xylose	-	V(+,-,W,D)	D	-
(ylo	D-galactose + D-				
- -	xylose	V(-, W,D)	V(+,-,W,D)	+	-
	m-inositol + D-xylose	-	V(+,-,W,D)	-	-
	xylose	-	V(+,-,D)	W,D	-
	acetoin + D-xylose	-	-	W,D	-

N-SOURCES

nitrate	+	V(+,-,W)	+	+
nitrite	V(-,W)	-	+	+/WD
L-lysine	+	V(W,-)	W	V(W,-)
cadaverine	+	+,WD	+	+

OTHER TESTS

tween 80	V(-, W,+)	-	+	+
10% NaCl	-	-	-	-
15% NaCl	-	-	-	-
50% glc	-	-	-	-

LEGEND:

black, bold: classic assimilation, Biolog tests in agreement

red, bold: classic assimilation, BIOLOG YT tests NOT in agreement

normal, black: Biolog YT tests only

blue, bold: classic assimilation tests only

+: assimilation, -: no assimilation, w: weak assimilation, v: variable results, d: delayed assimilation, YT - BIOLOG YT plate results

Figure S1. Maximum likelihood phylogeny of the concatenated rDNA genes (SSU, LSU, 5.8 S rDNA), estimated by PhyML. aLRT as Chi2 based support was used for calculation of branch supports.



Figure S2. Phylogenetic trees based on alignment of ITS rDNA, estimated by Bayesian inference. The tree includes representatives of (A) *Camptobasidium*. unrooted, and (B) *Psychromyces glacialis* gen. et sp. nov. rooted with *Phenoliferia psychrophenolica*. A total 10 million generations were calculated in each case and the first 25% were discarded before the shown consensus trees were calculated from trees sampled every 100 generations. The numbers given on branches are frequencies at which the individual clades appeared in the sampled trees.



Figure S3. Maximum likelihood tree based on ITS2 sequences obtained in NGS study of glacial environments in Svalbard and the Greenland Ice Sheet, and on ITS sequences of *Camptobasidium gelus, Camptobasidium arcticum, Camptobasidium* sp. and *Psychromyces glacialis* pure cultures.



0.020

Figure S4. Minimum spanning network calculated from the alignment of *Camptobasidium* ITS sequences. The similarity between the strains is shown with the thickness of the lines connecting the circles representing the strains.



Figure S5. Minimum spanning network calculated from the alignment of *Psychromyces* ITS sequences. The similarity between the strains is shown with the thickness of the lines connecting the circles representing the strains.



Figure S6. Morphology of Psychromyces glacialis EXF-12419

X5 (a–b): Cultures of *Psychromyces glacialis* EXF-12419 on PDA at 15 °C: (a) after 14 days, (b) after 2 months; (c–e) synnemata and hyphae after 14 days in 60% lactic acid; (k) teliospores of EXF-12454 after 2 months; (f, g–j, l) hyphae with clamps stained with calcofluor white after 14 days (f, g, i) and after 2 months (h, j, l).

