

Psychrophilic yeasts from Antarctica and European glaciers: description of *Glaciozyma* gen. nov., *Glaciozyma martinii* sp. nov. and *Glaciozyma watsonii* sp. nov.

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Abstract Field campaigns in Antarctica, Greenland and the Italian glaciers aiming to explore the biodiversity of these disappearing environments identified several undescribed yeast strains unable to grow at temperature above 20°C and belonging to unknown species. Fourteen of these strains were selected and grouped based on their morphological and physiological characteristics. Sequences of the D1/D2 and ITS regions of the ribosomal RNA

demonstrated that the strains belong to unknown species related to *Leucosporidium antarcticum*. The new genus *Glaciozyma* is proposed and two new species are described, namely *Glaciozyma martinii* sp. nov. and *Glaciozyma watsonii* sp. nov. Additionally, re-classification of *Leucosporidium antarcticum* as *Glaciozyma antarctica* is proposed. Strains of *Glaciozyma* form a monophyletic clade and a well separated lineage within class Microbotryomycetes (Pucciniomycotina, Basidiomycota). The description of *Glaciozyma* genus and the re-classification of *L. antarcticum* reduce the polyphyletic nature of the genus *Leucosporidium*.

Keywords *Glaciozyma* gen. nov. · *Glaciozyma martinii* sp. nov. · *Glaciozyma watsonii* sp. nov. · Psychrophilic yeasts · Antarctica · European glaciers · Cold-adapted biodiversity

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Introduction

Extremophilic yeasts are eukaryotic microorganisms that are able to thrive in physically and/or chemically extreme environments; low temperature is one of the parameters that identifies a habitat as extreme. The presence of yeasts colonising cold environments, as either psychrophiles or psychrotolerating organisms, depending on their maximum growth temperature, is well documented and reviewed (Raspor and Zupan 2006; Vishniac 2006; Fell et al. 2006; Shivaji and Prasad 2009).

Many of the habitats from which these species were isolated are now considered to be in danger of disappearance due to global warming. The increase in temperature of these ecosystems causes the melting of ice in glacial environments that may result in the eventual disappearance of entire glacier ecosystems (Oerlemans et al.

1998). Psychrophilic and psychrotolerant microorganisms thrive in stressful (cold) conditions thereby limiting the active fungal communities to those most adapted to fluctuations in extreme environments (Vishniac 2006). The adaptation to such habitats requires the development of protective mechanisms altering the physiology or enhancing repair capabilities (Deming 2002; Shivaji and Prasad 2009). Many of these properties have already been exploited by biotechnological industries and others are under development (Margesin et al. 2007; Shivaji and Prasad 2009).

Although a few ascomycetous yeast species (*Candida famata*, *Candida intermedia*, *Candida psychrophila*, *Candida santamariae*, *Clavispora lusitaniae*, *Debaryomyces hansenii*, *Dipodascus australiensis*, *Pichia guilliermondii*, *Wickerhamomyces patagonicus*) were found, most of the yeast species isolated from glacial habitats were basidiomycetes. They may be better able to survive under extreme conditions in order to explain this taxonomically skewed distribution.

The distribution of both ascomycetous and basidiomycetous yeast species in glacial habitats has been recently reviewed and discussed by some authors (Connell et al. 2008; Branda et al. 2010; de Garcia et al. 2010). These studies showed that the dominant species differ with geographical area and that the most common yeast genus isolated from glaciers and related habitats is *Cryptococcus*. Other isolated basidiomycetous genera were: *Bulleromyces*, *Cystofilobasidium*, *Dioszegia*, *Erythrobasidium*, *Filobasidium*, *Guehomyces*, *Leucosporidiella*, *Leucosporidium*, *Malassezia*, *Mastigobasidium*, *Mrakia*, *Mrakiella*, *Rhodosporeidium*, *Rhodotorula*, *Sporobolomyces*, *Sporidiobolus*, *Trichosporon* and *Udeniomyces* (Connell et al. 2008; Branda et al. 2010; de Garcia et al. 2010).

Additionally, several new species isolated from cold environment have been recently described: viz., *Mrakia robertii*, *Mrakia blollopis*, *Mrakiella niccombsii* (Thomas Hall et al. 2010), *Wickerhamomyces patagonicus* (de Garcia et al. 2010), *Rhodotorula arctica* (Vishniac and Takashima 2010), as well as *Dioszegia antarctica* and *Dioszegia cryoxerica* (Connell et al. 2010). This further illustrates the rich, and not yet fully explored yeast biodiversity that occurs in such extreme and disappearing habitats.

During recent years field campaigns in Antarctica, Greenland (Thomas-Hall and Watson 2002; Connell et al. 2008) and in the glaciers of Italian Alps (Turchetti et al. 2008) and Apennines (Branda et al. 2010) several yeast strains that belong to unknown species have been isolated. Here, we describe two, viz., *Glaciozyma martinii* and *Glaciozyma watsonii*, which are phylogenetically related to *Leucosporidium antarcticum*. Further we propose a novel genus *Glaciozyma* to accommodate those species.

Materials and methods

Yeasts isolation

The yeast strains described in this study are listed in Table 1.

In a recent study on the fungal diversity of the Vestfold Hills area at Davis Base, Antarctica, 327 yeast isolates were identified from 185 soil samples (Thomas-Hall and Watson 2002). Soil samples were stored at -10°C in quarantine at the University of Tasmania, Hobart. In particular, five strains were isolated and grouped based on morphological characters: the first group consisting of CBS 8932, CBS 8940, CBS 8944, and the second group consisting of CBS 8928 and CBS 8929.

Strains CBS 10633, CBS 10641, CBS 10620 and DBVPG 8014 were isolated from Antarctica, South Victoria Land during the 2003–2004 austral summer season (Connell et al. 2008). Isolate CBS 10633 was obtained from near the foot of Mount Discovery where soil consisted primarily of a thin layer of ground shells and rock (~ 5 cm) overlaying blue glacial ice; CBS 10641 was obtained from soil collected on the marine side of Commonwealth Glacier; isolate CBS 10620 was collected from a dead sponge that was found on top of the Ross Ice Shelf near Black Island; DBVPG 8014 was collected from a moss sample on the marine side of Commonwealth Glacier in Taylor Valley.

The strain DBVPG 4841 was isolated from sediments collected in Calderone Glacier (altitude 2,630–2,830 m a.s.l.) that is located in the Gran Sasso d'Italia group (Apennines, Italy) and represents the southernmost glacier of Europe (Branda et al. 2010).

The remaining strains, DBVPG 4726^T, DBVPG 4760, DBVPG 4799 and DBVPG 4802, were isolated from sediments of the Forni, and Sforzellina glaciers in the Italian Alps, and identified as described in Turchetti et al. (2008). All cultures were maintained on YEP (yeast extract 10%, peptone 10%, glucose 20%, agar 20%) plates and as aqueous stocks or lyophilised at 4°C , with long-term storage in glycerol stocks at -80°C .

In order to clarify the phylogenetic position of the new two species, nine more strains were also included in the selected strains panel. In particular, four additional strains (CBS 6581, CBS 7054, CBS 9639, CBS 7009) conserved in Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands and identified as *L. antarcticum*, and five strains (CBS 10636, CBS 10638, CBS 10639, CBS 8939 and CBS 8943) collected in Antarctica and related to *L. antarcticum*.

Physiological tests and morphology

Physiological tests were performed according to the protocols described by Yarrow (1998). All tests were

Table 1 List of the yeast strains of *Glaciozyma martinii* and *Glaciozyma watsonii* considered in the present study: strain accession numbers, substrate and locality of isolation, GenBank accession number of 26S rRNA gene (LSU) and that of the ITS regions of the rRNA sequences, <http://www.ncbi.nlm.nih.gov>

Species	Strain number	Isolation source	Coordinates	Sample	GenBank accession number	
					LSU	ITS1&2
<i>Glaciozyma martinii</i>	CBS 8928	Lake Fletcher (Antarctica)	68°27'06"S, 78°15'24"E	Soil	AY033640	AY033641
	CBS 8929	Lichen Valley (Antarctica)	68°29'48"S, 78°25'07"E	Soil	AY040646	AY040664
	CBS 10620 ^T	South Victoria Land (Antarctica)	78°8'57"S, 166°6'39"E	Dead sponge	HQ676604	EU149807
	DBVPG 4841	Calderone Glacier (Apennines, Italy)	42°28'15"N, 12°27'08"E	Sediment	EU287888	HQ432816
	CBS 6581	Antarctica		Sea water	JF900363	JF900359
	CBS 7054	Antarctica	64°16'S, 150°06'E	Sea water	JF900365	JF900361
	CBS 9639	Taylor Valley (Antarctica)		Soil	JF900366	JF900362
	CBS 8932	Lichen Lake (Antarctica)	68°28'32"S, 78°25'8"E	Soil	AY040645	AY040662
	CBS 8940	Chelnok Lake (Antarctica)	68°38'44"S, 78°20'03"E	Benthic mat on ice top	AY040643	AY040660
<i>Glaciozyma watsonii</i>	CBS 8944	Marine Plain (Antarctica)	68°37'30"S, 78°7'4"E	Soil	AY033639	AY033638
	CBS 10633	South Victoria Land (Antarctica)	78°20'48"S, 165°43'24"E	Soil	HQ852042	EU149802
	DBVPG 8019	South Victoria Land (Antarctica)	77°34'18"S, 163°22'12"E	Soil	HQ700372	DQ402535
	DBVPG 8014	South Victoria Land (Antarctica)	77°33'36"S, 163°22'3"E	Soil	HQ852043	EU149804
	CBS 10641	South Victoria Land (Antarctica)	46°23'N, 10°35'E	Sediment	HQ432817	HQ432818
	CBS 10986 ^T	Forni glacier (Italian Alps)	46°20'N, 10°30'E	Sediment	HQ432819	HQ432820
	CBS 11006	Sforzellina Glacier (Italian Alps)	46°20'N, 10°30'E	Sediment	EF643742	HQ432821
	CBS 10999	Sforzellina Glacier (Italian Alps)	46°20'N, 10°30'E	Sediment	HQ432822	HQ432823
	CBS 10997	Sforzellina Glacier (Italian Alps)	64°10'S, 150°03'E	Sea water	JF900364	JF900360
	CBS 7009	Antarctica				
	CBS 5942 ^T	Weddell Sea near Joinville Island (Antarctica)		Sea water	AF189906	AF444529
	Undescribed yeast	CBS 10638	South Victoria Land (Antarctica)	78°20'49"S, 165°43'25"E	Soil	HQ676603
CBS 10639		South Victoria Land (Antarctica)	77°35'19"S, 163°19'58"E	Soil	HQ676605	EU149806
CBS 10636		South Victoria Land (Antarctica)	78°20'49"S, 165°43'25"E	Soil	HQ676602	EU149808
CBS 8943		Lake Fletcher (Antarctica)		Soil	AY033636	AY033637
CBS 8939		Moss Cirque (Antarctica)		Soil	AY040642	AY040663

CBS Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, *DBVPG* Industrial Yeast Collection, University of Perugia, Italy, *T* type strain

performed in duplicate at 10–15°C and results were recorded at 2 and 4 weeks post inoculation. Images of the colony morphology were taken using a stereo microscope coupled with the Dage 3-chip CCD camera while colonies were photographed directly with a Nikon zoom digital camera, COOLPIX950. Standard light microscopy was employed using a Zeiss Axioskop microscope and Nomarski optics coupled with a Dage 3-chip CCD camera, Model DC-330E video camera for imaging. Cell size and morphology were determined by scanning electron microscopy (JEOL model JSM 5800LV, accelerating voltage 15 kV) using an improved fixation procedure for yeasts (Hanschke and Schauer 1996). Transmission electron microscopy (TEM) was done with strain DBVPG 4726 according to the methods described in Müller et al. (1998).

For determination of sexual reproduction, pairs of 4-day-old cultures were crossed on SGA (soytone 0.2%; glucose 0.2%; agar 2%) and malt extract agar (MEA) and incubated at 10°C for 12 months. The production of hyphae and teliospores was checked regularly. All possible combinations between strains belonging to the same

species and also between those belonging to different species described in this paper were investigated. The germination of the teliospores was induced by incubating blocks of agar containing the teliospores in dH₂O at 10°C for 2 weeks and then transferring them to 2% water agar. These were incubated at 10°C for 6 months with regular microscopic checks for teliospores.

Phylogenetic analysis

The D1/D2 domains of the large subunit (LSU) rRNA gene region and the Internal Transcribed Spacers (ITS 1 + 2 regions) including the 5.8S rRNA gene were amplified by cycle sequencing using the following forward and reverse primers, respectively: NL1 5'-GCATATCAATAAGCGG AGGAAAAG and RLR3 5'-GGTCCGTGTTTCAAGAC for LSU; ITS1 5'-TCCGTAGGTGAACCTGCGG and ITS4 5'-TCCTCCGCTTATTGATATGC for the ITS regions. The sequences were obtained with an ABI PRISM 3730XL Capillary Sequencer using the standard protocol. The sequences were aligned, analysed and corrected using

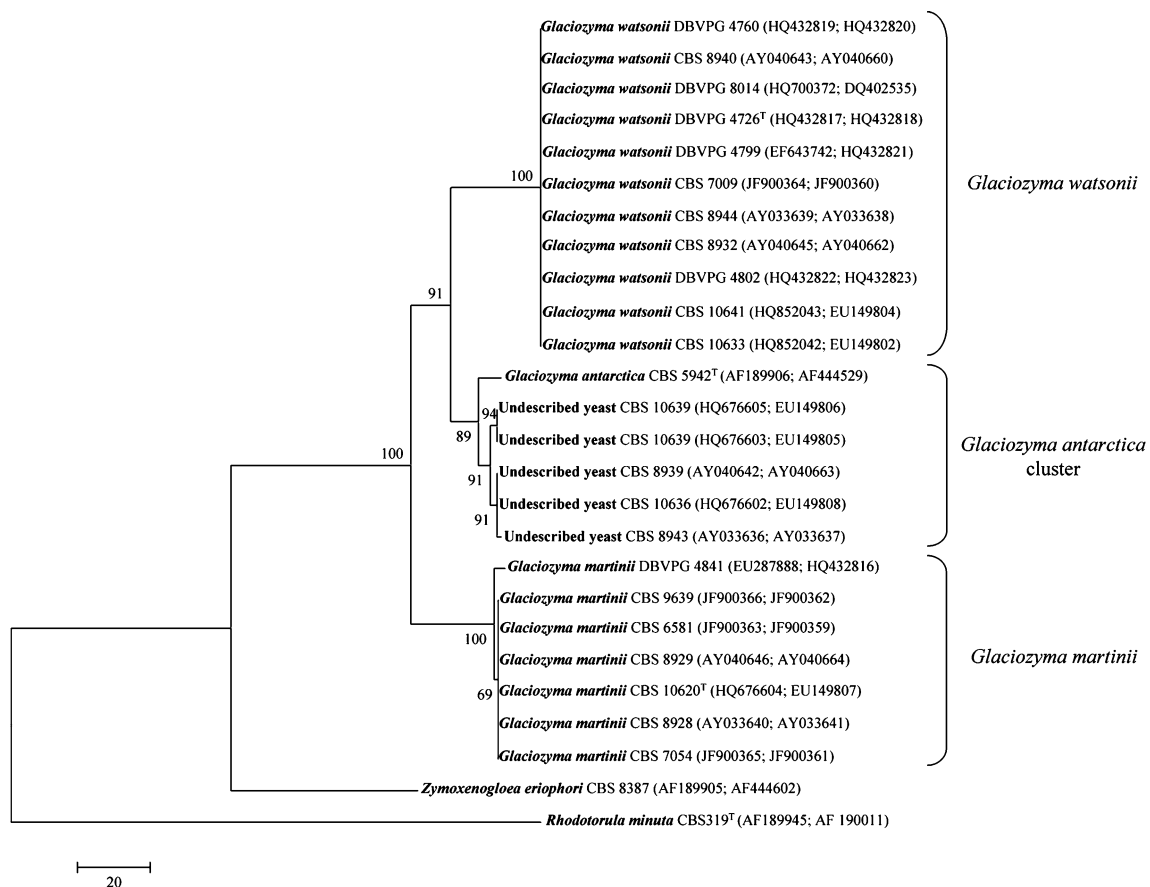


Fig. 1 Phylogeny of the *Glaciozyma* clade including the representative strains of the related species *K. eriophori* and *C. hydrophilum*. Maximum parsimony tree of D1/D2 region of LSU rRNA and ITS regions including 5.8 gene of the rRNA sequences. The topology was

rooted with *Rh. minuta*. Bootstrap percentages from 1,000 replications shown on the branches (value below 50% are not shown). GenBank accession numbers of the sequences are indicated after strain numbers

Seqman™ II and MegAlign™ (DNASTar Inc., UK); alignments were checked and corrected manually. Phylogenetic analysis was performed using Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0 (Tamura et al. 2007) using Maximum Parsimony analysis. Bootstrap analysis (1,000 replicates) was performed using a parsimonious heuristic search with random addition of sequences (100 replicates).

Results and discussion

Molecular phylogeny morphology: identification of the novel genus *Glaciozyma* Turchetti, Connell, Boekhout & Thomas-Hall

Fourteen yeast strains (Table 1) isolated during different sampling campaigns from cold environments in Europe (viz., Italian Alps and Apennines) and Antarctica (viz., Vestfold Hills and South Victoria Land) were grouped together due to their high rRNA identity.

In particular, according to the phylogenetic analysis of the partial 26S rRNA gene sequences (Figs. 1, 2, S1, S2), the type strain of *L. antarcticum* and all the 14 strains considered in this study and belonging to the two new species proposed, constituted a well-defined cluster forming a monophyletic clade. ITS sequence analysis supported by high bootstrap value (Figs. 1, S1, S2), confirmed the monophyly of the clade and the position as a separate lineage within class Microbotryomycetes.

The phylogenetic analysis of D1/D2 LSU rRNA sequences (Figs. 1, 2, S1, S2) linked this clade with *Kriegeria eriophori* (CBS 8387), the teleomorph of *Zymoxenoglea eriophori*, a monotypic sedge parasite with an anamorphic yeast stage (Doubles and McLaughlin 1992; Swann et al. 1999), and with *Camptobasidium hydrophilum* (CMM 8060), an aquatic hyphomycete associated with decaying leaf litter (Marvanova and Suberkropp 1990). Sequence similarity of these species with our isolates and with *L. antarcticum* had values below 95% (ranging from 93 to 95%). Additionally, physiological, morphological and ecological aspects differentiated *K. eriophori* and

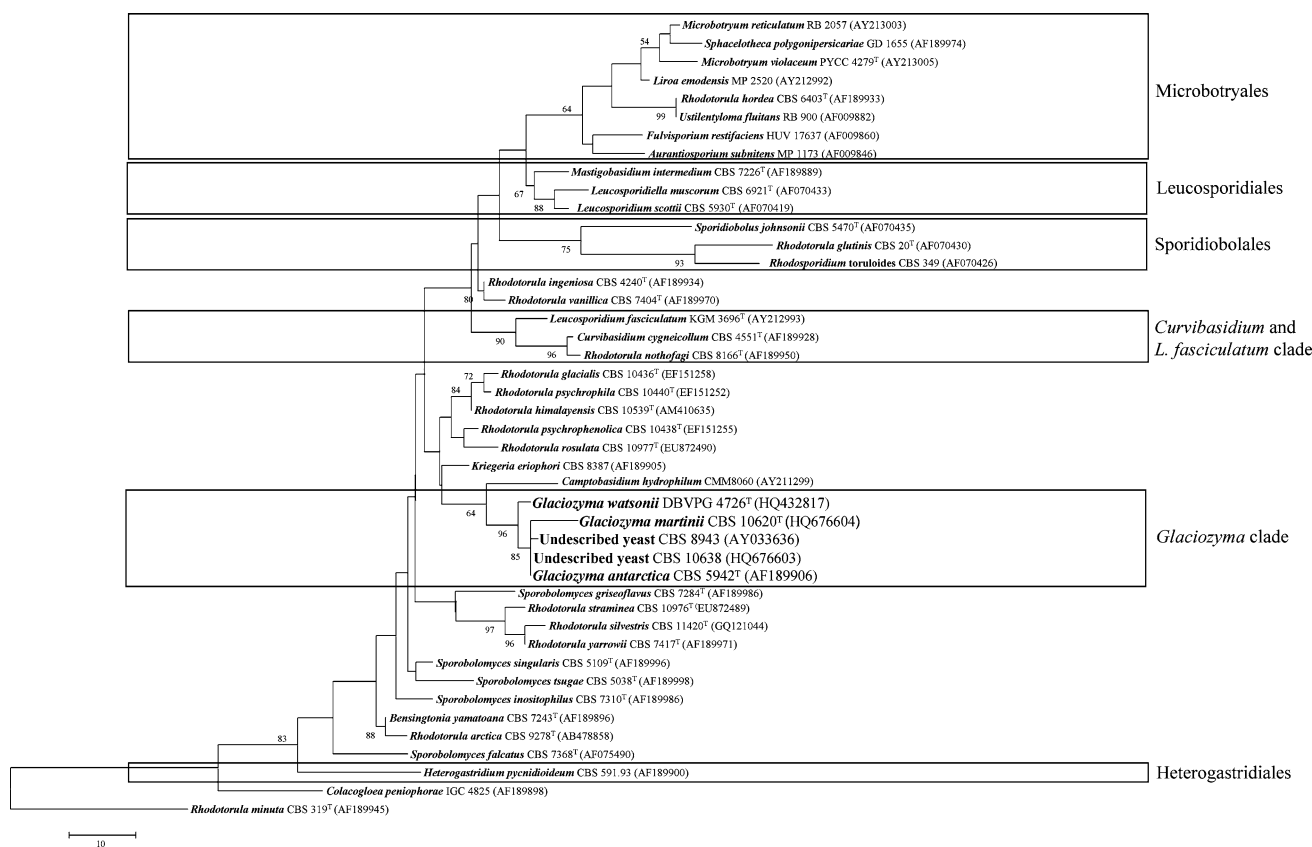


Fig. 2 Phylogenetic tree of *Glaciozyma* clade and related taxa of class Microbotryomycetes. Maximum parsimony tree of D1/D2 region of LSU rRNA sequences. Known orders are shown on the right. The topology was rooted with *Rh. minuta*. Bootstrap

percentages from 1,000 replications shown on the branches (value below 50% are not shown). GenBank accession numbers of the sequences are indicated after strain numbers

C. hydrophilum from the species belonging to the clade described in this work. According to the phylogenetic analysis described below we propose the erection of the genus *Glaciozyma* in order to accommodate the two new species described and *L. antarcticum*.

This proposal is additionally supported by the phylogenetic analysis of Scorzetti et al. (2002) where *L. antarcticum* was already reported to form a phylogenetic group with the strains CBS 8944 and CBS 8928, which belong to the two species proposed in this work.

The taxonomy of Pucciniomycotina has been revised several times during the last few years (Kirk et al. 2001; Swann et al. 2001; Weiss et al. 2004; Bauer et al. 2006) based on ultrastructural, biochemical, ecological and molecular characteristics. In 2006, Aime et al. reassessed the higher-level systematics of Pucciniomycotina combining LSU rRNA and SSU rRNA gene sequences. Pucciniomycotina comprised eight classes plus one incertae sedis group. One of the main classes is Microbotryomycetes, composed of four different orders, namely, Heterogastridiales Oberw. & R. Bauer, Leucosporidiales J.P. Samp. M.Weiss & R. Bauer, Sporidiobolales J.P. Samp. M.Weiss & R. Bauer and Microbotryales R. Bauer & Oberw. In addition, several taxa could not be ascribed to any order (Fig. 2).

Sampaio et al. (2003) emphasised the polyphyletic nature of the genus *Leucosporidium* and erected a new order (Leucosporidiales) to organise the core group of *Leucosporidium* species consisting of *L. scottii*, *L. fellii*, *L. golubevii*, *Mastigobasidium intermedium*, *Leucosporidiella (Le.) creatinivora*, *Le. fragaria*, *Le. muscorum*, *Le. yakutica*. On the contrary, *L. antarcticum* Fell, Statzell, I.L. Hunter & Phaff and *L. fasciculatum* Bab'eva & Lisichkina (Statzell-Tallman and Fell 1998; Bab'eva and Lisichkina 2000) were recognised as not being closely related to *L. scottii*, the type species of Leucosporidiales, and consequently they were not assigned to this or any other order (Sampaio et al. 2003; Sampaio 2011).

L. antarcticum was excluded from the order Leucosporidiales primarily because of its phylogenetic placement (also see Fig. 2), but also for the absence of colacosomes (previously referred to as lenticular bodies) (Kreger-van Rij and Veenhuis, 1971). These structures were observed in all the species of Leucosporidiales, and it is considered an important taxonomic characteristic common to all the species of the order (Sampaio et al. 2003). TEM of strain DBVPG 4726 (this study), did not show the presence of lenticular bodies. This observation thus confirms the taxonomic position of the genus *Glaciozyma* outside the order Leucosporidiales.

Sampaio et al. (2003, 2004, 2011), constituted the new order Leucosporidiales and underlined the polyphyletic nature of the genus *Leucosporidium*, thus suggesting that

L. antarcticum and *L. fasciculatum* should be removed from the genus. The newly proposed genus, *Glaciozyma*, includes two new species and the re-classification of *L. antarcticum* to *Glaciozyma antarctica* (*G. antarctica*). The classification of *L. antarcticum* in the new genus *Glaciozyma* constitutes another step towards the final taxonomic reorganisation of the genus *Leucosporidium*.

Molecular phylogeny and morphology: recognition of two new species

Sequences of the D1/D2 domains of the 26S rRNA gene (LSU) and those of the ITS regions of the rDNA divided the 14 strains considered in this study into two groups that correspond to two different species (Figs. 1, S1, S2). Both species were found to belong to subphylum Pucciniomycotina, class Microbotryomycetes and, more specifically, to the new genus *Glaciozyma* that clusters with *G. antarctica* with high bootstrap support. At the same time, the low intragenic DNA similarity of the considered strains (see below) supported the description of two novel species inside the *Glaciozyma* genus, namely *Glaciozyma martinii* (*G. martinii*) and *Glaciozyma watsonii* (*G. watsonii*). Representative type strains of members of the Microbotryomycetes were also considered in our analysis (Fig. 2).

LSU and ITS sequences of both species showed low intraspecific variability. Strains of *G. martinii* had similar LSU sequences (CBS 8928 varied by 2 nt, while CBS 8929 and DBVPG 4841 differed by 1 nt compared to CBS 10620^T), whereas in the ITS sequences only DBVPG 4841 showed four variable positions. Strains belonging to *G. watsonii* had identical D1/D2 LSU rRNA gene sequences, whereas two strains (DBVPG 8014 and DBVPG 4799) showed one gap in ITS sequences compared to all the other strains.

The phylogenetic analysis of D1/D2 LSU rRNA and ITS regions sequences (Figs. 1, 2, S1, S2) indicated that both new species belong to a well supported clade together with *G. antarctica* (type strain, CBS 5942^T). This clade will be referred to as *Glaciozyma* clade.

In particular, the phylogenetic analysis of D1/D2 LSU rRNA sequences showed that strains belonging to *G. watsonii* had 15–17 base pair substitutions (including two gaps) when compared to strains belonging to *G. martinii*, while seven nucleotide substitutions (98% of identity) occurred with the most closely related species *G. antarctica* CBS 5942^T. The same analysis demonstrated that *G. antarctica* (CBS 5942^T) is the closest relative of *G. martinii* differing from it by 10 to 14 substitutions, and 11 substitutions if considering the type strain CBS 10620 (98% of identity) (Figs. 1, S1, S2). Additionally, the number of nucleotide substitutions of the ITS regions were higher than those observed in D1/D2 LSU rRNA gene.

Maximum parsimony analysis of ITS regions sequences placed strains of *G. martinii* and *G. watsonii* in the same clade with *G. antarctica* (87 and 90% of identity with *G. antarctica*, respectively). They grouped in three well-separated clusters with high bootstrap values similar to the D1/D2 LSU rRNA gene analysis (Figs. 1, S1, S2).

These molecular phylogenetic data confirm that *G. martinii* and *G. watsonii* represent species distinct from *G. antarctica*. Recognition of the *Glaciozyma* species is further supported by physiological and morphological characteristics. The ability to utilise sucrose, raffinose, glycerol, 2 keto-D-gluconate, *n*-acetyl-D-glucosamine, glucuronic acid, and the maximum growth temperature, as well as the capacity to produce clamp connections, differentiate *G. martinii*, *G. watsonii* and *G. antarctica* (Table 2).

Sequences from strains originally considered *L. antarcticum* or *Leucosporidium* sp., all isolated from Antarctica (Table 1), and obtained from either the Centraalbureau voor Schimmelcultures (CBS) or from GenBank database clustered in the *Glaciozyma* clade as well (Figs. 1, S1, S2). In particular, considering the phylogenetic analysis, CBS 7009 was identified as *G. watsonii* while CBS 6581, CBS 7054 and CBS 9639 were identified as *G. martinii*.

The other five additional strains showed identical or similar (differed by 1 nt) LSU sequences to *G. antarctica*. But the analysis of the ITS regions identified two branches. One branch is formed by CBS 10638 and CBS 10639 and the other branch by CBS 10636, CBS 8939 and CBS 8943 (differing by 17–18 nt to CBS 5942^T). Whether the two clades represent distinct species or varieties of *G. antarctica* is a matter that needs further investigation through other studies such as nDNA reassociation. Here, these strains are temporarily labelled as *Glaciozyma* sp. and included in an overall *Glaciozyma antarctica* cluster.

Taxonomic part

Glaciozyma gen. nov

Latin diagnosis of *Glaciozyma* Turchetti, Connell, Thomas-Hall & Boekhout gen. nov.

Fungi Microbotryomycetum (Pucciniomycotina, Basidiomycota). In medio liquido ME cellulae vegetativae ovoidae aut longiores factae, singulae aut binae gemmationis causa sunt; hyphae et pseudohyphae in culturis maturis formantur, abundans capsula aliquoties.

Cyclus sexualis non bene cognoscitur sed omnes species ovoideas intercalares aut terminales teliosporas, cum aut sine (*G. antarctica*) fibulis, generant.

Fermentatio nulla; nitratum assimilatur; diazonium caeruleum B positivum; urea finditur; materia amyloidea iodophila non formatur. Incrementum maximum ad 20°C est.

Species typica: *Glaciozyma antarctica* (Fell, Statzell, Hunter & Phaff) Turchetti, Connell, Boekhout & Thomas-Hall.

Description of *Glaciozyma* Turchetti, Connell, Thomas-Hall & Boekhout gen. nov.

Glaciozyma (Gla.cio'zy.ma. Glaci- L. fem. n. glacies, -ei referring to ice; -zyma N.L. -zymo or -zym from Gr. fem. n. zume, referring to leaven. *Glaciozyma* N.L. fem. n. referring to yeasts isolated from ice and related cold habitat).

Belongs to subphylum Pucciniomycotina, class Microbotryomycetes. Budding yeast cells are ovoid to elongate; pseudo and true hyphae are present in mature culture and abundant capsules may be formed.

The sexual mechanisms are not well known, but intercalary and terminal teliospores are produced by all the species with or without (*G. antarctica*) clamp connections.

Glucose is not fermented. Nitrate is assimilated. DBB and urease reactions are positive. Starch-like compounds are not produced. The maximal growth temperature is 20°C.

Type species is *Glaciozyma antarctica* (Fell, Statzell, Hunter & Phaff) Turchetti, Connell, Boekhout & Thomas-Hall.

Novel combination

Glaciozyma antarctica (Fell, Statzell, Hunter & Phaff) Turchetti, Connell, Thomas-Hall & Boekhout comb. nov.

Basionym: *Leucosporidium antarcticum* Fell, Statzell, Hunter & Phaff (Fell, Statzell, Hunter and Phaff. Antonie van Leeuwenhoek 35:433–462, 1969).

Glaciozyma martinii Turchetti, Connell, Thomas-Hall & Boekhout sp. nov

Latin diagnosis of Latin diagnosis of *Glaciozyma martinii* Turchetti, Connell, Thomas-Hall & Boekhout sp. nov.

Fungus. Microbotryomycetes class (Pucciniomycotina). *Glaciozyma martinii* ex Vestfold Hills—Antarctica, ex Southern Victoria Land—Antarctica et ex Appennine Italice inventus est. In ME agar, post 7 dies, 10°C, coloniae rotundae, planae, creamae in colore, butyraceae in textura, non nitidae, cum margine integra. Post 2 menses coloniae infimo-convexae cum medio incremento (papillatae). In CM agar coloniae exiguae, planae, glabrae, nitidae et molles in textura, cum margine integra. In medio liquido ME, post 7 dies, 10°C, cellulae vegetativae subglobosae

Table 2 Physiological characteristics of the species *Glaciozyma martinii* and *Glaciozyma watsonii* and *Glaciozyma antarctica*

	<i>Glaciozyma watsonii</i> ^a	<i>Glaciozyma martinii</i> ^a	<i>Glaciozyma antarctica</i> ^b		<i>Glaciozyma watsonii</i> ^a	<i>Glaciozyma martinii</i> ^a	<i>Glaciozyma antarctica</i> ^b
Carbon compounds				Carbon compounds			
D-glucose	+	+	+	Na succinate	v	v	–
D-galactose	+	+	v	Na citrate	v	v	–
L-sorbose	w/–	v	–	Methanol	v	–/d	–
D-glucosamine	w/–	v	–	Ethanol	+	v	v
D-ribose	v	–	–	Propane 1,2 diol	+	+	nd
D-xylose	v	w/–	v	Butane 2,3 diol	v	w	nd
L-arabinose	v	w/–	–	Quinic acid	v	w	nd
D-arabinose	–	–	–	Saccharate	–	w	nd
L-rhamnose	–	–	–	Galactonic acid	–	w	nd
Sucrose	–	–	v	Hexadecane	w/–	w/–	–
Maltose	v	v	v	<i>N</i> -acetylglucosamine	+	–	–
α - α -trehalose	w/–	w/–	v	Erythritol	v	v	–
Methyl- α -D-glucoside	–	–	–	D,L-lactic acid	v	–/d	–
D-cellobiose	–	–	–	Inositol	w/–	w/–	–
Salicin	–	–	–	Nitrogen compounds			
Arbutin	+	–	nd	Nitrate	w/+	+	+
D-melibiose	–	–	–	Nitrite	–	w	+
D-lactose	–	w/–	–	Ethylamine	v	w/–	nd
D-raffinose	–	–	v	L-lysine	v	d/–	nd
D-melezitose	w/–	–	–	Cadaverine	–	–	nd
Inulin	–	–	–	Glucosamine	–	–	nd
Soluble starch	v	w/–	–	Other tests			
Glycerol	–	v	w/+	10% NaCl+5% D-glucose	v	–	–
Xylitol	–	–	v	50% D-glucose	w/–	–	–
L-arabinitol	–	–	nd	60% glucose	–	–	nd
D-sorbitol (D-glucitol)	+	w/+	v	Glucose fermentation	–	–	nd
D-mannitol	v	v	v	0.01% cycloheximide	–	–	–
Dulcitol (Galactitol)	–	–	–	0.1% cycloheximide	–	–	–
<i>myo</i> -inositol	–	+	–	Vitamine free	+	+	+
Glucono- δ -lactone	–	–	nd	Production of starch like	–	–	–
2-ketogluconate	v	+	+	Urea hydrolysis	+	+	+
5-ketogluconate	v	v	–	DBB reaction	+	+	+
D-gluconate	+	+	s	Max grow temp. (°C)	18/20	18/20	17
D-glucuronate	+	+	–				

^a Data from the present study^b Data obtained from Statzell-Tallman and Fell (1998) and Sampaio (2011)

(3.1–4.3 μm) aut ovoidae (2.7–4 μm \times 3.7–9.1 μm) sunt. Gemmationes polares. Hyphae et pseudohyphae in culturis maturis formantur. Unus vel duo vacuoli in cellulis formantur, complentes totum voluminem post 21 dies. In ME agaro, post 7 dies, CBS 10620 cellulae incapsulatae. Culturae CBS 8928 et DBVPG 4841, ubi proximae in SD et ME agaro crescunt, hyphas cum fibulis generant

afferentes ovoideas intercalares aut terminales teliosporas. Germinatio non observata est.

Proprietates biochemicae physiologicaeque in Tabula 2 describuntur. Positio phylogenetica in Figura 1 illustratur. Cultura typica CBS 10620^T in colletionie zymotica ‘Centraalbureau voor Schimmelcultures’ (CBS), Ultrajecti, Hollandia, et DBVPG 8018^T in colletionie zymotica

Industrial Yeasts Collection DBVPG, Perugia, Italia, deposita est.

Description of *Glaciozyma martinii* Turchetti, Conell, Thomas-Hall & Boekhout sp. nov.

Glaciozyma martinii (mart.ti.'ni.i. N.L. gen. masc. n. *martinii* of Martini, referring to Alessandro Martini, a yeast biologist at the University of Perugia, Italy, past director of the DBVPG collection, in whose honour the species is named).

Novel yeast species belonging to subphylum Pucciniomycotina, phylum Basidiomycota, class Microbotryomycetes.

The type strain of *G. martinii* has been deposited at the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands, and at the Industrial Yeasts Collection DBVPG (Dipartimento di Biologia Applicata, Università di Perugia, Italy) under the codes CBS 10620^T and DBVPG 8018^T, respectively.

Four strains were described in the present paper: two strains isolated from soil sampled in Lake Fletcher and Lichen Valley, Vestfold Hills area of Davis Base, Antarctica, one strain isolated from Southern Victoria Land soil, Antarctica and one strain isolated from sediment sampled from Calderone Glacier, Apennines, Italy (Table 1).

The physiological and biochemical characters of *G. martinii* are indicated in Table 2, and its phylogenetic placement is presented in Figs. 1, 2, S1 and S2.

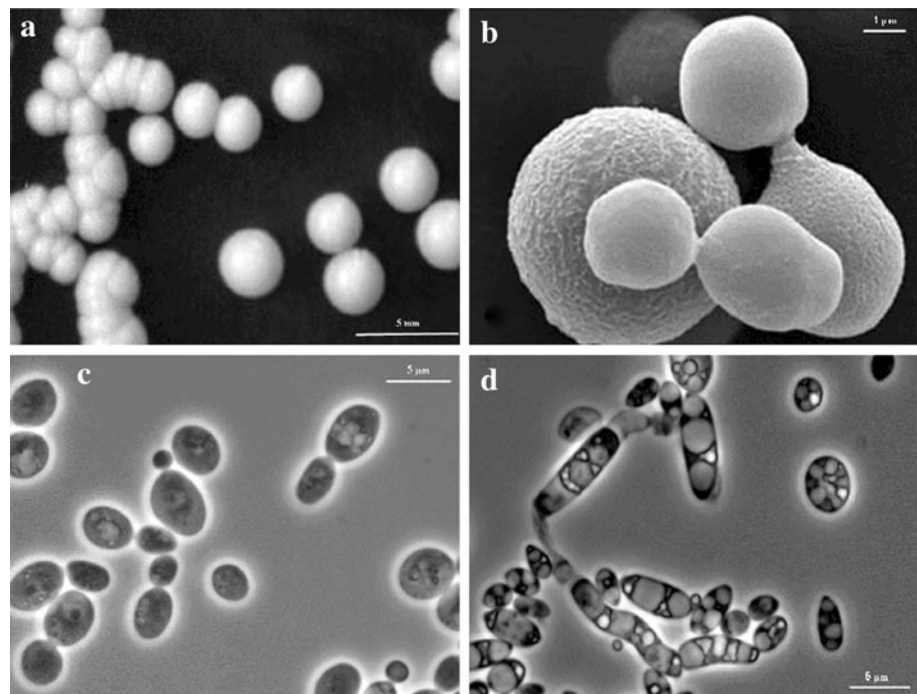
Morphology

After 1 week incubation at 10°C colonies on MEA were circular, flat, yellowish, with butyrous texture, pasty in consistency (Fig. 3); the surface was dull with the margin entire and smooth. After 8 weeks the colonies had a raised point in the middle, almost umbonate in CBS 8928, sectorized in CBS 10620^T and DBVPG 4841, with raised borders. On corn meal agar (CMA) growth was less, colonies remained smaller, flat, translucent, with mucoid texture and an entire margin. All strains showed similar morphological aspects with colonies of 0.5–1 mm diameter on MEA and 1–2 mm on CMA after 1 week at 10°C. On potato dextrose agar (PDA) and in the same conditions (incubation time and temperature) colonies were larger reaching 3 mm in diameter.

Microscopy

Growth in ME broth at 10°C after 7 days, resulted in the formation of a mucoid sediment and a slight ring on the surface of the media; cells were generally (sub)globose for CBS 8928 and CBS 8929, with a diameter of 3.1–4.3 µm, while they were ovoid to elongate for the other two strains, 2.7–4 µm × 3.7–9.1 µm. Limited elongate budding cells occurred, 8 µm × 10.6 µm (Fig. 3). Budding is polar and

Fig. 3 *Glaciozyma martinii*
a colonies of *G. martinii* CBS 8929 on MEA after 3 weeks incubation at 10°C. Round and convex colonies with a white cream colour; individual colonies show a raised dimple in the middle. Bar = 5 mm.
b Scanning electron micrograph (SEM) of *G. martinii* CBS 8929 in ME after 1 week incubation at 10°C. Polar budding cell on a denticle. Enteroblastic budding scar is visible. Bar = 1 µm.
c Polar budding cells of *G. martinii* CBS 8928 on PDA after 1 week incubation at 10°C. Bar = 5 µm. **d** Hyphae and pseudohyphae produced by *G. martinii* CBS 10620^T grown on CMA, after 3 weeks of incubation, at 10°C. Bar = 5 µm



Glaciozyma martinii

Type strain: CBS 10620 = DBVPG 8018

Origins of strains: Antarctica, Alps

Growth temperature: 10–15°C (<20°C)

generally on a denticle (Fig. 3). Hyphae and pseudohyphae were observed in CBS 10620^T when grown on ME broth, CMA and PDA (Fig. 3). One or two vacuoles were observed in almost all cells which became larger, occupying the entire volume of the cells after 3 weeks (Fig. 3). On ME after 1 week incubation, CBS 10620^T showed a large capsule when stained with Indian ink of 1–2 µm width, which is in agreement with the mucoid texture shown by this species and in particular by this strain.

After 8 weeks in ME at 10°C, budding cells were less abundant; cells occurred singly or in parent-bud pairs; vacuoles were bigger and sometimes occupied the entire volume of the cells. When grown on CMA, PDA and ME broth, CBS 10620^T generated simple but also branched hyphae.

Sexual state

On CMA strains of *G. martinii* did not produce hyphae with clamp connections or teliospores. Only two strains (CBS 8928 and DBVPG 4841) may belong to sexual compatible strains, because when streaked next to each other on SDA and MEA at 10°C, hyphae with clamp connections bearing teliospores were formed. Globose to ovoid teliospores (5–6 µm in diam.) were observed terminally and intercalary. Germination of the teliospores was induced incubating at 10°C blocks of agar containing the teliospores in dH₂O for 2 weeks and then transferring them in 2% water agar at the same temperature. Germination was not observed.

Glaciozyma watsonii Thomas-Hall, Connell, Boekhout & Turchetti sp. nov

Latin diagnosis of *Glaciozyma watsonii* Thomas-Hall, Connell, Boekhout & Turchetti sp. nov.

Fungus. Microbotryomycetes class (Pucciniomycotina). *Glaciozyma watsonii* ex Vestfold Hills—Antarctica, ex Southern Victoria Land—Antarctica et ex Alpe Italica inventus est.

In ME agar, post 7 dies, 10°C, coloniae rotundae, albidae-cremae in colore, butyraceae in primis dies et molles in textura formantes guttas, in cultura matura. Post 2 menses coloniae cum margine erosa, infimo-convexae cum medio incremento (papillatae), perforatae aliquando sunt; butyraceae sed molle et nitido tabulato contactae. In CM agar coloniae exiguae, planae, glabrae, nitidae cum margine erosa et non molles in textura. In medio liquido ME, post 7 dies, 10°C, cellulae vegetativae sub/globosae (3.5–4.5 µm) aut ovoidae (5–12 µm × 2.5–3.7 µm) sunt. Gemmationes polares. Cellulae ellipsoideae cylindratae (9–10.5 µm × 2.5–3.5 µm) aliquando sunt et unus vel duo vacuoli formantur, complentes totum voluminem cellulae. Hyphae et pseudohyphae in culturis maturis formantur.

Post 1 mensem, 10°C, culturae diversus/contrarius sexus, ubi proximae/contigae in CM agar crescunt, hyphas cum fibulis generant afferentes ovoideas intercalares aut terminales teliosporas. Teliosporae germinantes gemmantes cellulas et breves pseudohyphas procreantes; sed basidium non observatum est. Proprietates biochemicae physiologicaeque in Tabula 2 describuntur. Positio phylogenetica in Figura 1 illustratur. Cultura typica codes CBS 10986^T in collezione zymotica 'Centraalbureau voor Schimmelcultures' (CBS), Ultrajecti, Hollandia, et DBVPG 4726^T in collezione zymotica DBVPG Industrial Yeasts Collection, Perusia, Italia, deposita est.

Description of *Glaciozyma watsonii* Thomas-Hall, Connell, Boekhout & Turchetti sp. nov.

Glaciozyma watsonii (wat'son.i.i. L. gen. masc. n. *watsonii* of Watson, referring to Kenneth Watson, a yeast biologist at the University of New England, Armidale, Australia, in whose honour the species is named).

Novel yeast species belonging to subphylum Pucciniomycotina, phylum Basidiomycota, class Microbotryomycetes.

The type strain of *G. watsonii* has been deposited at the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands, and at the Industrial Yeasts Collection DBVPG (Dipartimento di Biologia Applicata, Università di Perugia, Italy) under the codes CBS 10986^T and DBVPG 4726^T, respectively.

Ten strains were studied in the present paper: three strains isolated from soil and a benthic mat of Vestfold Hills area of Davis Base, Antarctica; three strains from soil of Southern Victoria Land, Antarctica, and four strains isolated from soil samples of Forni and Sforzellina Glaciers of Italian Alps (Table 1).

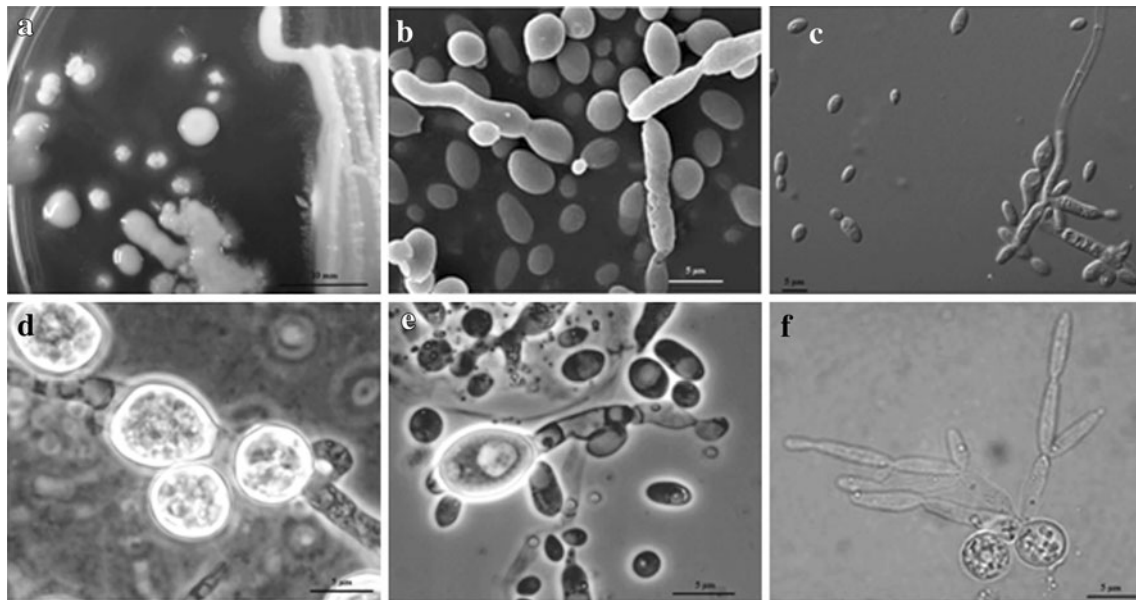
The physiological and biochemical characters of *G. watsonii* are shown in Table 2, and its phylogenetic placement is presented in Figs. 1, 2, S1 and S2.

Morphology

After 7 days at 10°C, colonies on MEA were circular, yellowish-white, with a butyrous texture turning into a mucoid and viscous texture, with drops that were formed when Petri dishes were maintained upside-down; after 8 weeks the surface was rough, wrinkled and covered by a clear mucoid and glistening layer (Fig. 4); raised in the centre, sometime with a cavity into the top, and with eroded margins that were fringed with long filaments. Colonies are 7–8 mm in diameter and the filamentous margin measures 4–6 mm beyond the body of the colonies.

On CMA colonies were whitish, butyrous, flat and not mucoid; long filaments occurred. Colonies' size was similar to those on MEA.

All the strains showed similar morphological aspects when streaked on agar media, but CBS 8940 and CBS 8944



Glaciozyma watsonii

Type strain: DBVPG 4726 = CBS 10986

Origins of strains: Antarctica, Alps

Growth temperature: 10–15°C (< 20°C)

Fig. 4 *Glaciozyma watsonii* **a** colonies of *G. watsonii* DBVPG 4726^T on CMA after 3 weeks incubation, at 10°C. The surface is rough, wrinkled and covered by a clear mucoid and glistening layer. Colonies are raised in the centre, margins are fringed with filaments. *Bar* = 10 mm. **b** Scanning electron micrograph (SEM) of *G. watsonii* CBS 8940 in ME after 1 week incubation at 10°C. Polar budding cell and pseudohyphae are shown. *Bar* = 5 μm. **c** Single cells and septate hyphae of *G. watsonii* DBVPG 4726^T on ME after 1 week incubation at 10°C. “Empty” hyphae that can be distinguished from the apical

darker part with granulose matrix. *Bar* = 5 μm. **d** Particular of intercalary teliospores with clamp connections of *G. watsonii* CBS 8940 × DBVPG 4799, after 8 weeks of incubation at 10°C, on CMA. *Bar* = 5 μm. **e** Particular of intercalary teliospores with clamp connections of *G. watsonii* CBS 8940 × DBVPG 4726^T, after 4 weeks of incubation at 10°C, on CMA. *Bar* = 5 μm. **f** Germination and yeast cells formation of two teliospores produced by *G. watsonii* CBS 8932 induced by incubated in dH₂O, at 10°C for 2 weeks and then inoculated to 2% water agar at 10°C. *Bar* = 5 μm

have a butyrous texture, and a dull surface that is not mucoid when streaked on MEA.

Microscopy

After 7 days at 10°C in ME broth, cells were variably shaped and varied from round, with a diameter of 3.5–4.5 μm, to ovoid and elongate, 5–12 μm × 2.5–3.7 μm. Budding was polar. Some cells were lemon-shaped and had large vacuoles. Occasionally elongated cells, 9–10.5 μm × 2.5–3.5 μm, occurred with two big vacuoles that occupied the greater part of the cell volume. Pseudohyphae were not observed, although in DBVPG 4726^T pseudo and true hyphae occasionally occurred.

After 8 weeks the cells maintained the same shape, with bigger size 7–15 μm × 3–4.5 μm and 1–4 vacuoles tended to occupy the entire volume of the cell. Budding cells were less abundant, whereas lemon-shaped cells became more prominent.

Pseudo and true hyphae were present when grown in ME broth. Hyphae, 1–2 μm in diameter were short, septate and

sometimes branched (Fig. 4). Usually the oldest cells of the hyphae appeared “empty” and different from the apical darker part in which granulose matrix, which can be likened to cell organelles, were microscopically observed (Fig. 4).

Sexual state

After 1 month at 10°C on CMA, streaked cultures of sexual compatible strains (CBS 8940 vs CBS 10633, CBS 10641, DBVPG 4726, DBVPG 4799, DBVPG 4802) formed hyphae with clamp connections (Fig. 4). Intercalary and terminal teliospores occurred frequently, especially in the hyphae that overgrow the margin of the streaks. Some of the strains formed teliospores when streaked individually, but clamp connections were not generated. Teliospores are globose, 5–6 μm in diameter to occasionally elongate, 5–6 μm × 8–10 μm. Teliospores were transferred to dH₂O and incubated at 10°C for 2 weeks before inducing germination by transfer to 2% water agar at 10°C. Teliospores germinated within 2 days by forming budding yeast cells and short pseudohyphae but basidia were not observed. In

some cases, short germ tubes originated from the teliospores (Fig. 4).

Final taxonomic observations

Based on D1/D2 and ITS sequences data, the genus *Leucosporidium* is currently polyphyletic, with *L. antarcticum* and *L. fasciculatum* falling outside the recently described order Leucosporidiales. Sampaio et al. (2003) recognised this problem, but could not redefine the *Leucosporidium* genus without firstly re-classifying these two species. It is, therefore, likely that these two species had to be excluded from this genus so that *Leucosporidium* could be made monophyletic within the order Leucosporidiales.

Within the new genus *Glaciozyma* three species are included that form a well defined clade: *G. martinii*, *G. watsonii* and *G. antarctica*. The re-classification of the latter species partially solves the problem related to the polyphyletic nature of the genus *Leucosporidium*. *L. fasciculatum* remains the only *Leucosporidium* species still not included in any order. The taxonomic position of *L. fasciculatum* was discussed by Sampaio et al. (2004) in relation to the description of *Curvibasidium* but it has still to be clarified. A deeper study of additional species related to *Curvibasidium* cluster will be necessary in order to describe a new genus for *L. fasciculatum* or insert it in *Curvibasidium*. The three *Glaciozyma* species are characterised by the ability to produce teliospores with or without clamp connections after mating sexually compatible strains. No basidial stage was observed (teliospores germination produced only yeasts cells or short hyphae) in any of these species. *Glaciozyma* is so described as a cross between sexual and asexual genus.

Within the *Glaciozyma* clade other strains that could not be assigned to any species and named *Glaciozyma* sp., need to be considered. CBS 8943, CBS10636, CBS 8939, CBS 10638, CBS 10639 are included in the phylogenetic analysis of Fig. 1 but they do not belong to any of the described species and their taxonomic classification remains to be defined. Currently these strains were included in an overall *Glaciozyma antarctica* cluster. LSU and ITS sequence analysis underline the monophyletic nature of the *Glaciozyma* clade and its position as a lineage within the class Microbotryomycetes. A deeper phylogenetic investigation based on polygenic DNA loci will also be necessary to further assess the higher taxonomic rank of the *Glaciozyma* clade, e.g. as a new order.

Ecological and applied implications

Glaciozyma species were isolated from cold habitats located in different parts of the world, namely Alpine and/

or Apennine glacier-associated sediments and/or Antarctic soil and Antarctic sea water. The habitat of the sampled areas with its peculiar environmental aspects was probably responsible for the selection and distribution of these species. These species show the formation of hyphae and teliospores, although the latter did not always germinate. Their ecological origin affected their physiological characteristics as all species showed an obligate psychrophilic aptitude, with a maximum growth temperature lower than 20°C.

Highly diversified microorganisms have developed adaptation strategies enabling them either to survive or to be successful in extreme environments, like true psychrophiles (Turchetti et al. 2008). These adaptations include an increased membrane fluidity, expression of cold-shock (csp) and cold-acclimatisation proteins as a response to cold-shocks, production of antifreeze proteins that protect the microbial cell against damage caused by freezing, and production of cold-active enzymes (psychrophilic enzymes or cold-enzymes). The biotechnological (and industrial) relevance of cold-enzymes from psychrophilic yeasts has been recently emphasised (Ohgiya et al. 1999; Okuyama et al. 1999; Nakagawa et al. 2002; Pazgier et al. 2003; Gomes and Steiner 2004; Krallish et al. 2006; Margesin et al. 2007, Shivaji and Prasad 2009).

In particular for *Glaciozyma* species, the presence of a cold-adapted extracellular serine proteinase produced by *G. antarctica* (cited as *L. antarcticum*) was recently observed by Turkiewicz et al. (2003), while a group of Korean researchers described the extracellular ice-binding glycoprotein produced by an Arctic psychrophilic yeast. Considering the few characteristics described in the paper (Lee et al. 2010) this strain could probably belong to *G. watsonii*.

In this context the newly described species are not only of taxonomical interest but also of biotechnological importance. The isolation, ex situ conservation of yeast strains from glacial habitats that, due to global warming, are reducing drastically, permits the discovery, description and collection of new species avoiding their possible extinction, and may also contribute to the biotech research as well as raising awareness of the great cold-adapted biodiversity and their potential for the production of new enzymes.

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References

- Aime MC, Matheny PB, Henk DA, Frieders EM, Nilsson RH, Piepenbring M, McLaughlin DJ, Szabo LJ, Begerow D, Sampaio JP, Bauer R, Weiss M, Oberwinkler F, Hibbett D (2006) An overview of the higher level classification of Pucciniomycotina based on combined analyses of nuclear large and small subunit rDNA sequences. *Mycologia* 98(6):896–905
- Bab'eva IP, Lisichkina GA (2000) A new species of psychrophilic basidiomycetous yeasts *Leucosporidium fasciculatum* sp. nov. *Mikrobiologiya* 69:801–804
- Bauer R, Oberwinkler F, Vanky K (1997) Ultrastructural markers and systematics in smut fungi and allied taxa. *Can J Bot* 75: 1237–1314
- Bauer R, Begerow D, Sampaio JP, Weiss M, Oberwinkler F (2006) The simple-septate basidiomycetes: a synopsis. *Mycol Prog* 5:41–66
- Branda E, Turchetti B, Diolaiuti G, Pecci M, Smiraglia C, Buzzini P (2010) Yeast and yeast-like diversity in the southernmost glacier of Europe (Calderone Glacier, Apennines, Italy). *FEMS Microbiol Ecol* 72:354–369
- Connell LB, Redman R, Craig S, Scorzetti G, Iszard M, Rodriguez R (2008) Diversity of soil yeasts isolated from South Victoria Land, Antarctica. *Microb Ecol* 56(3):448–459
- Connell LB, Redman R, Rodriguez R, Barrett A, Iszard M, Fonseca A (2010) *Dioszegia antarctica* sp. nov. and *Dioszegia cryoxerica* sp. nov., psychrophilic basidiomycetous yeasts from polar desert soils in Antarctica. *Int J Syst Evol Microbiol* 60(6):1466–1472
- de Garcia V, Brizzio S, Libkind D, Rosa CA, van Broock M (2010) *Wickerhamomyces patagonicus* sp. nov., an ascomycetous yeast species from Patagonia, Argentina. *Int J Syst Evol Microbiol* 60: 1693–1696
- Deming JW (2002) Psychrophiles and polar regions. *Curr Opin Microbiol* 5:301–309
- Doubles JC, McLaughlin DJ (1992) Basidial development, life history, and the anamorph of *Kriegeria eriophori*. *Mycologia* 84: 668–678
- Fell W, Statzell AC, Hunter IL, Phaff L (1969) *Leucosporidium* gen. n., the heterobasidiomycetous stage of several yeasts of the genus *Candida*. *Antonie van Leeuwenhoek* 35:433–462
- Fell J, Scorzetti G, Connell L, Craig S (2006) Biodiversity of micro-eukaryotes in Antarctic Dry Valley soils with <5% soil moisture. *Soil Biol Biochem* 38:3107–3119
- Gomes J, Steiner W (2004) The biocatalytic potential of extremophiles and extremozymes. *Food Technol Biotechnol* 42:223–235
- Hanschke R, Schauer F (1996) Improved ultrastructural preservation of yeast cells for scanning electron microscopy. *J Microscopy* 184:81–87
- Kirk PM, Cannon PF, David JC, Stalper JA (2001) Dictionary of the fungi. 9th edn. Wallingford, p 655
- Krallish I, Gonta S, Savenkova L, Bergauer P, Margesin R (2006) Phenol degradation by immobilized cold-adapted yeast strains of *Cryptococcus terreus* and *Rhodotorula creatinivora*. *Extremophiles* 10:441–449
- Kreger-van Rij NJW, Veenhuis M (1971) A comparative study of the cell wall structure of basidiomycetous and related yeasts. *J Gen Microbiol* 68:87–95
- Lee JK, Park KS, Park S, Park H, Song YH, Kang SH, Kim HJ (2010) An extracellular ice-binding glycoprotein from an Arctic psychrophilic yeast. *Cryobiology* 60:222–228
- Margesin R, Neuner G, Storey KB (2007) Cold-loving microbes, plants, and animals—fundamental and applied aspects. *Naturwissenschaften* 94:77–99
- Marvanova L, Suberkropp K (1990) *Camptobasidium hydrophilum* and its anamorph, *Crucella subtilis*: a new heterobasidiomycete from streams. *Mycologia* 82:208–217
- Müller WH, Montijn RC, Humbel BM, van Aelst AC, Boon EJ, van der Krift TP, Boekhout T (1998) Structural differences between two types of basidiomycete septal pore caps. *Microbiology* 144:1721–1730
- Nakagawa T, Yamada K, Miyaji T, Tomizuka N (2002) Cold-active pectinolytic activity of psychrophilic-basidiomycetous yeast *Cystofilobasidium capitatum* strain PPY-1. *J Biosci Bioeng* 94:175–177
- Oerlemans J, Anderson B, Hubbard A, Huybrechts Ph, Knap WH, Johannesson T, Schmeits M, Stroeven AP, van de Wal RSW, Wallinga J, Zuo Z (1998) Modelling the response of glaciers to climate warming. *Clim Dyn* 14:267–274
- Ohgiya S, Hoshino T, Okuyama H, Tanaka S, Ishizaki K (1999) In: Margesin R, Schinner F (eds) Biotechnological application of cold-adapted microorganisms. Springer, Berlin, pp 17–34
- Okuyama H, Morita N, Yumoto I (1999) In: Margesin R, Schinner F (eds) Biotechnological application of cold-adapted microorganisms Springer, Germany, pp 101–115
- Pazgier M, Turkiewicz M, Kalinowska H, Bielecki S (2003) The unique cold-adapted extracellular subtilase from psychrophilic yeast *Leucosporidium antarcticum*. *J Mol Catal B* 21:39–42
- Raspor P, Zupan J (2006) Yeasts in extreme environment. In: Peter G, Rosa C (eds) The yeast handbook biodiversity and ecophysiology of yeasts. Springer, Berlin, pp 371–417
- Sampaio JP (2011) *Leucosporidium* Fell, Statzell, Hunter & Phaff (1969). In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, 5th edn. Elsevier, Amsterdam, pp 1485–1494
- Sampaio JP, Gadanho M, Bauer R, Weiss M (2003) Taxonomic studies in the Microbotryomycetidae: *Leucosporidium golubevii* sp. nov., *Leucosporidiella* gen. nov. and the new orders Leucosporidiales and Sporidiobolales. *Mycol Prog* 2:53–68
- Sampaio JP, Golubev WI, Fell JW, Gadanho M, Golubev NW (2004) *Curvibasidium cygneicollum* gen. nov., sp. nov. and *Curvibasidium pallidicorallinum* sp. nov., novel taxa in the Microbotryomycetidae (Urediniomycetes), and their relationship with *Rhodotorula fujisanensis* and *Rhodotorula nothofagi*. *Int J Syst Evol Microbiol* 54:1401–1407
- Scorzetti G, Fell JW, Fonseca A, Statzell-Tallman A (2002) Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA region. *FEMS Yeast Res* 2:495–517
- Shivaji S, Prasad GS (2009) Antarctic yeasts: biodiversity and potential applications. In: Satyanarayana T, Kunze G (eds) Yeast biotechnology: diversity and applications. Springer Science, Berlin, pp 3–18
- Statzell-Tallman A, Fell JW (1998) *Leucosporidium* Fell, Statzell, Hunter & Phaff. In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, 4th edn. Elsevier, Amsterdam, pp 670–675
- Swann EC, Frieders EM, McLaughlin DJ (1999) *Microbotryum*, *Kriegeria* and the changing paradigm in basidiomycete classification. *Mycologia* 91:51–66
- Swann EC, Frieders EM, McLaughlin DJ (2001) Urediniomycetes. In: McLaughlin DJ, McLaughlin EG, Lemke PA (eds) Systematics and evolution. The mycota VII Part B. Springer, Berlin, pp 37–56

- Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599
- Thomas Hall SR, Turchetti B, Buzzini P, Branda E, Boekhout T, Theelen B, Watson K (2010) Cold-adapted yeasts from Antarctica and Italian Alps-description of three novel species: *Mrakia robertii* sp. nov., *Mrakia blollopis* sp. nov. and *Mrakiella niccombsii* sp. nov. *Extremophiles* 14:47–59
- Thomas-Hall SR (2004) Physiological and biochemical characterisation of antarctic yeast. Ph.D. thesis. School of Biological, Biomedical and Molecular Sciences, The University of New England, Australia
- Thomas-Hall S, Watson K (2002) *Cryptococcus nyarrowii* sp. nov., a basidiomycetous yeast from Antarctica. *Int J Syst Evol Microbiol* 52:1033–1038
- Turchetti B, Buzzini P, Goretti M, Branda E, Diolaiuti G, D'Agata C, Smiraglia C, Vaughan-Martini A (2008) Psychrophilic yeasts in glacial environments of Alpine glaciers. *FEMS Microbiol Ecol* 63:73–83
- Turkiewicz M, Pazgier M, Kalinowska H, Bielecki S (2003) A cold-adapted extracellular serine proteinase of the yeast *Leucosporidium antarcticum*. *Extremophiles* 7:435–442
- Vishniac HS (2006) Yeast biodiversity in the Antarctic. In: Peter G, Rosa C (eds) *The yeast handbook biodiversity and ecophysiology of yeasts*. Springer, Berlin, pp 419–440
- Vishniac HS, Takashima M (2010) *Rhodotorula arctica* sp. nov., a basidiomycetous yeast from Arctic soil. *Int J Syst Evol Microbiol* 60:1215–1218
- Weiss M, Bauer R, Begerow D (2004) Spotlights on heterobasidiomycetes. In: Agerer R, Piepenbring M, Blanz P (eds) *Frontiers in basidiomycete mycology*. IHW, Eching, pp 7–78
- Yarrow D (1998) Methods for the isolation and identification of yeasts. In: Kurtzman CP, Fell JW (eds) *The yeasts, a taxonomic study*, 4th edn. Elsevier, Amsterdam, pp 77–100