Whole-genome sequencing of the endemic Antarctic fungus *Antarctomyces pellizariae* reveals an ice-binding protein, a scarce set of secondary metabolites gene clusters and provides insights on Thelebolales phylogeny

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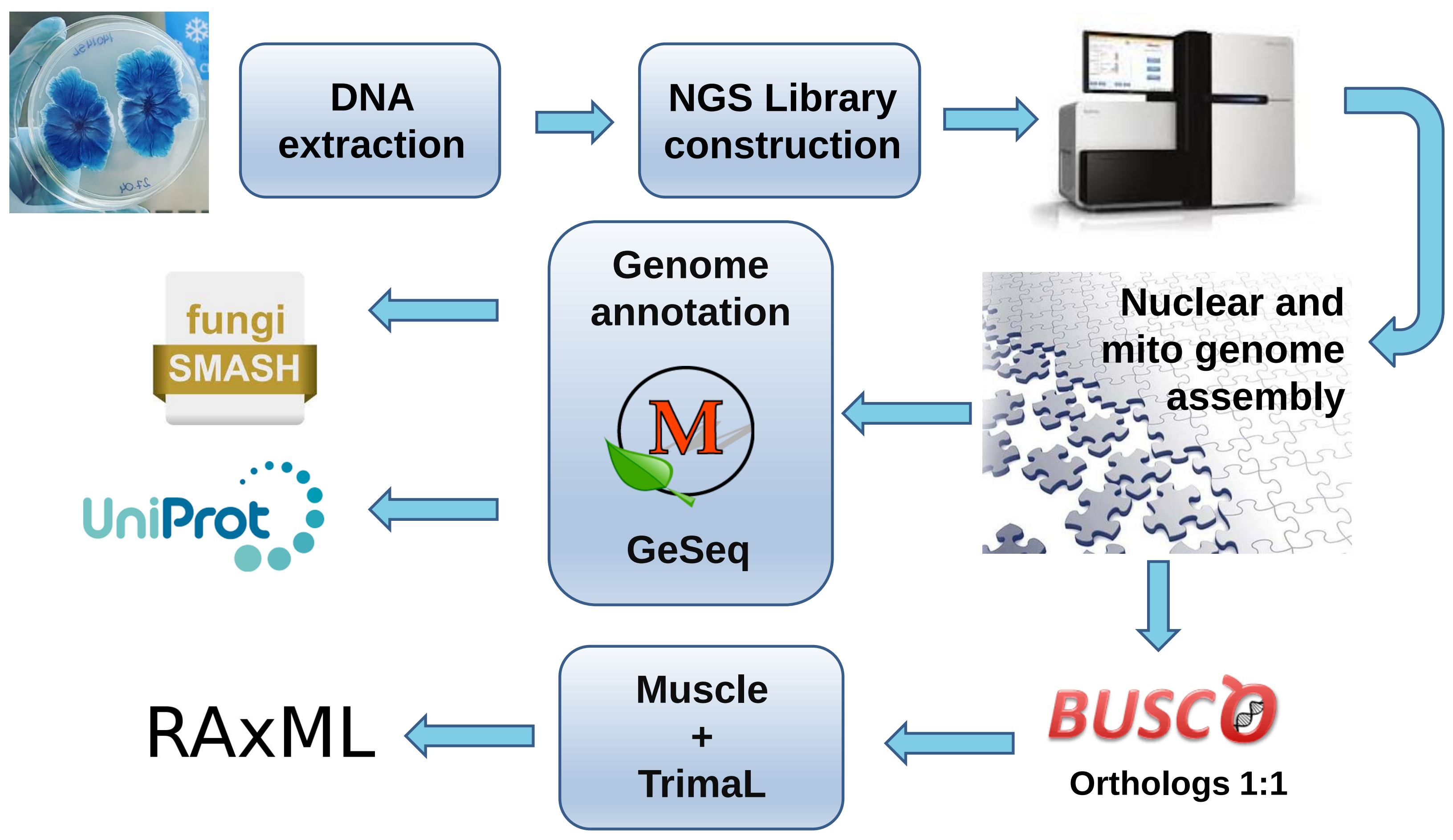
INTRODUCTION

The diverse environments found in Antarctica provide excellent opportunities to study organisms that thrive in extreme conditions. Each environment has distinct characteristics and poses different challenges to their inhabitants. A novel endemic fungal specie, *Antarctomyces pellizariae* (Ascomycota), was discovery in fresh snow samples from Robert Island during the summer of 2015. The present study sequenced and annotated the nuclear and mitochondrial genomes of A. pellizariae, and mined secondary metabolite gene clusters. One putative IBP closely resembled a bacterial AFP. The genomic information acquired herein enabled us to reconstruction the phylogenetic relationship of *A. pellizariae* with the Leotiomycetes, especially within the Thelebolales clade.

MATERIALS AND METHODS



DNA extraction

NGS Library construction

Nuclear and mito genome assembly

Genome annotation

GeSeq

fungi SMASH

UniProt

BUSCO

Orthologs 1:1

Muscle + TrimaL

RAxML

RESULTS AND DISCUSSION

Table 1
Assembly metrics and annotation data.

	General features
Genome assembled	24,217,994 bp
Number of contigs > 500 bp	395
Longest contig length	893,755 bp
Mean contig length	61,311 bp
N50	220,359 bp
L50	36
GC content	49,9%
Coded proteins predicted	8748
	Number of genes
Swissprot alignment	4907 (56.1%)
NR alignment	6961 (79.5%)
KEGG GhostKOALA alignment	3185 (36.4%)
KOG assignment	5384 (61.5%)
InterPro signature	6600 (75.4%)
TIGR signature	4272 (48.8%)
Transmembrane domain	1585 (18.1%)
Signal peptide	688 (7.8%)
Repetitive content	321,817 bp (1.33%)

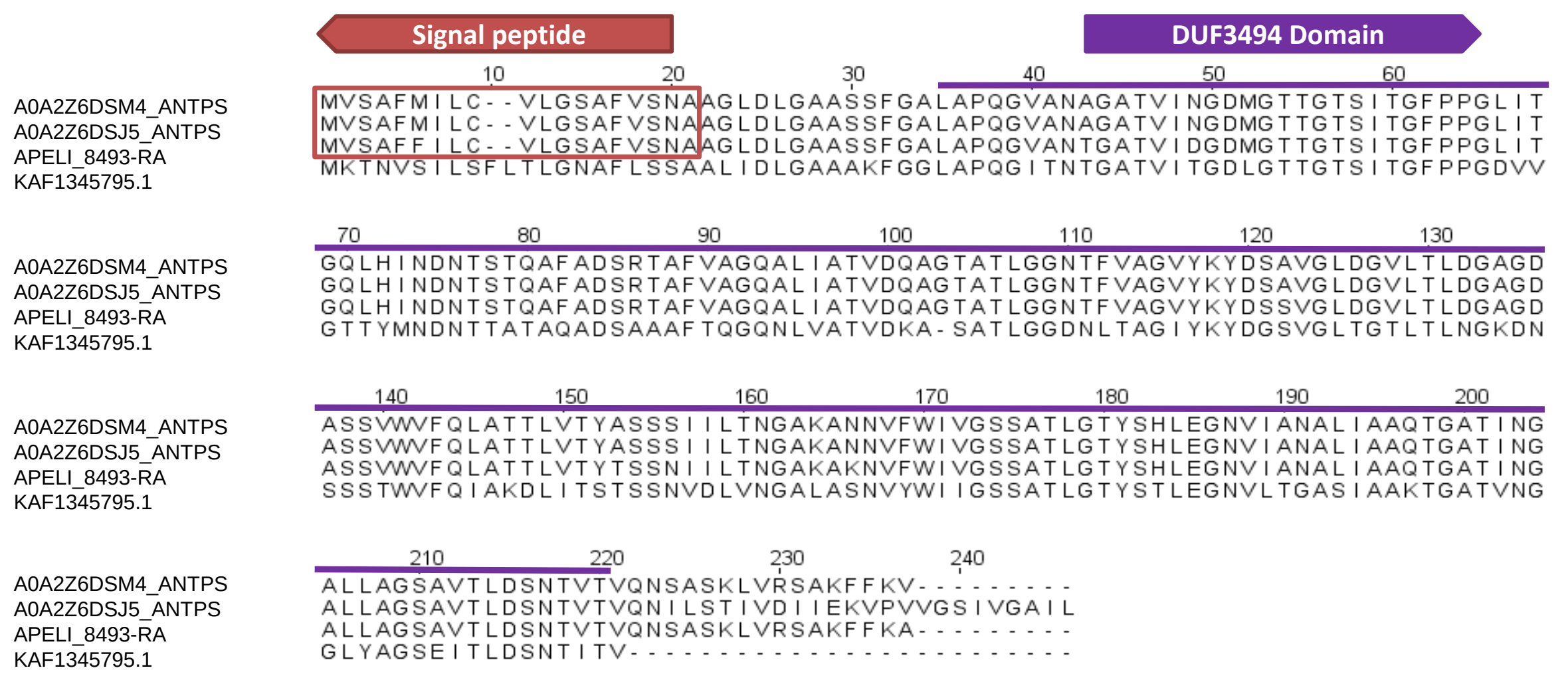


Fig. 1: Multiple alignment of putative *Antarctomyces pellizariae* IBP (APELI_8493-RA), two IBP isoforms from *Antarctomyces psychrotrophicus* (A0A2Z6DSM4 and A0A2Z6DSJ5) and hypothetical protein from *Delphinella strobiligena*. Orange box, signal peptide that is absent in KAF1345795.1; purple line DUF3494 domain.

Table 2
Secondary metabolites in fungal genomes.

Temperature tolerance	Species	Phylum	Order	Genome length (Mb)	SM clusters	Unknown clusters	
Psychrophilic	*Takashimella tepidaria*	Basidiomycota	Trichosporonales	22,37	4	3	75%
	Glaciozyma antarctica	Basidiomycota	Kriegeriales	20,03	4	3	75%
	Dioszegia crocea	Basidiomycota	Tremellales	20,59	6	6	100%
	Filobasidium wieringae	Basidiomycota	Filobasidiales	19,78	7	6	85.7%
	Antarctomyces pellizariae	**Ascomycota**	**Thelebolales**	**24,21**	**8**	**7**	**87.5%**
	Thelebolus microsporus	Ascomycota	Thelebolales	27,34	7	5	71.4%
	Thelebolus stercoreus	Ascomycota	Thelebolales	26,03	9	6	66.6%
Psychrotrophic	*Pseudogymnoascus destructans*	Ascomycota	Thelebolales	35,81	12	7	58.3%
	Pseudogymnoascus pannorum	Ascomycota	Thelebolales	30,53	20	12	60%
	Pseudogymnoascus verrucosus	Ascomycota	Thelebolales	30,17	20	14	70%
Mesophilic	*Thielavia hyrcaniae*	Ascomycota	Sordaliales	31,18	23	19	82.6%
	Thielavia appendiculata	Ascomycota	Sordaliales	32,73	23	18	78.2%
	Monilinia fructicola	Ascomycota	Helotiales	44,68	15	7	46.6%
	Monilinia laxa	Ascomycota	Helotiales	41,84	11	6	54.5%
	Botrytis cinerea	Ascomycota	Helotiales	42,63	20	15	75%
	Hyaloscypha variabilis	Ascomycota	Helotiales	55,85	25	21	84%
	Hyaloscypha bicolor	Ascomycota	Helotiales	82,38	37	28	75.6%
	Lanzia echinophila	Ascomycota	Helotiales	40,25	10	8	80%
	Blumeria graminis	Ascomycota	Erysiphales	124,48	1	1	100%
	Erysiphe necator	Ascomycota	Erysiphales	52,50	1	1	100%
	Chlorociboria aeruginascens	Ascomycota	Helotiales	33,05	13	9	69.2%
	Diplocarpon rosae	Ascomycota	Helotiales	67,78	29	25	86.2%
	Marssonina brunnea	Ascomycota	Helotiales	51,94	15	11	73.3%
	Pezicula radicicola	Ascomycota	Helotiales	62,71	63	43	68.2%
	Aspergillus nidulans	Ascomycota	Eurotiales	30,27	45	23	51.1%
Thermophilic	*Thermomyces lanuginosus*	Ascomycota	Eurotiales	19,15	16	9	56.2%
	Thermothelomyces thermophilus	Ascomycota	Sordaliales	38,74	23	17	73.9%
	Thielavia antarctica	Ascomycota	Sordaliales	40,66	13	10	76.9%
	Thielavia terrestris	Ascomycota	Sordaliales	36,91	21	15	71.4%
	Rhizomucor variabilis	Mucoromycota	Mucorales	33,03	7	6	85.7%
	Rhizomucor pusillus	Mucoromycota	Mucorales	25,59	6	5	83.3%
	Rhizomucor miehei	Mucoromycota	Mucorales	27,44	6	6	100%

The genome of A. pellizariae, subject of this study, is highlighted in bold.

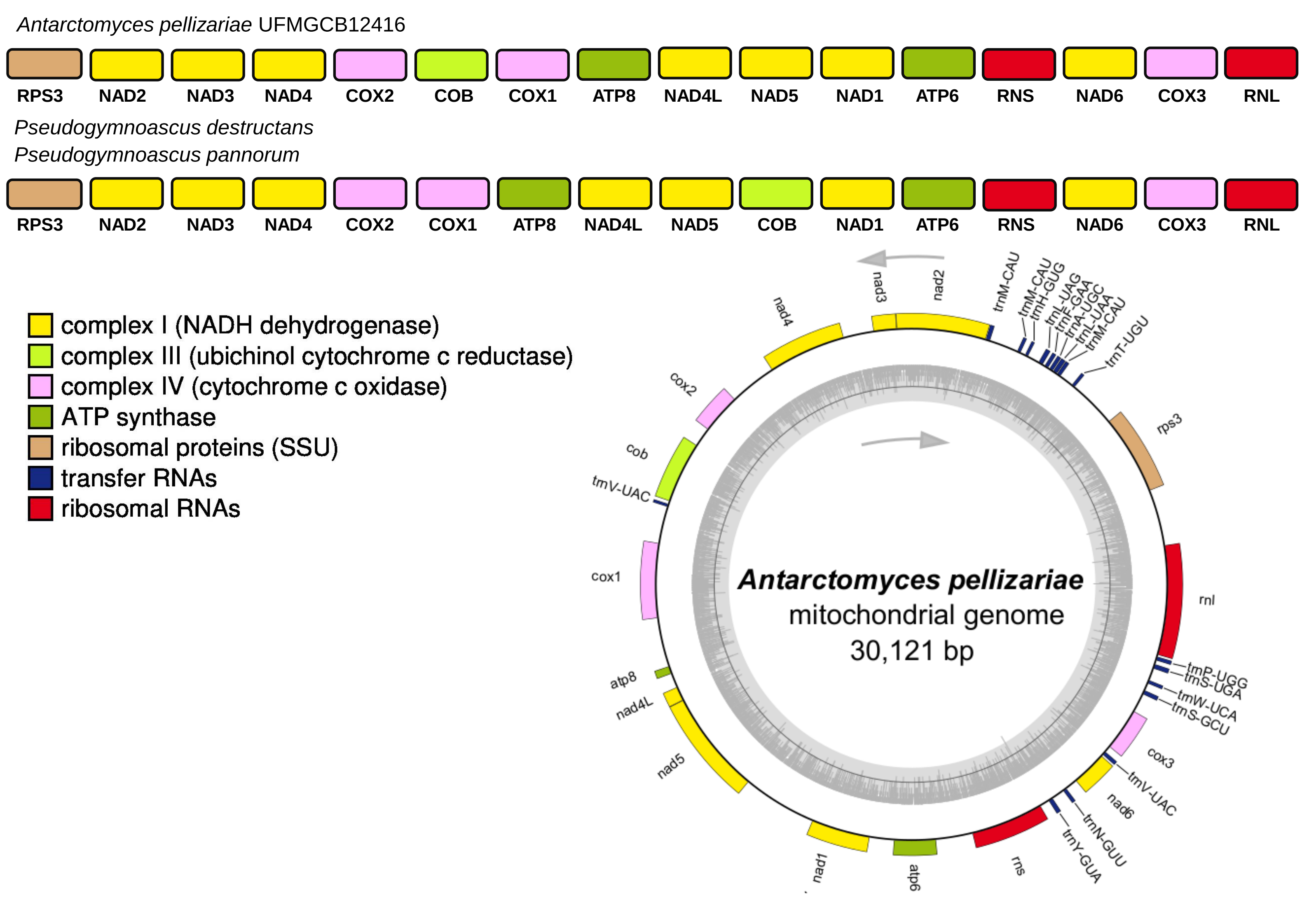


Fig. 2. Arrangement of CDS and ribosomal genes from *Antarctomyces pellizariae* UFMGCB 12416 and *Pseudogymnoascus destructans* and *P. pannorum*. Genetic map of circularized *A. pellizariae* mitochondrial genome. Colored blocks, genes according to their functional categories. Gray plot within circle shows the GC content of the mtDNA; mid line represents 50% GC.

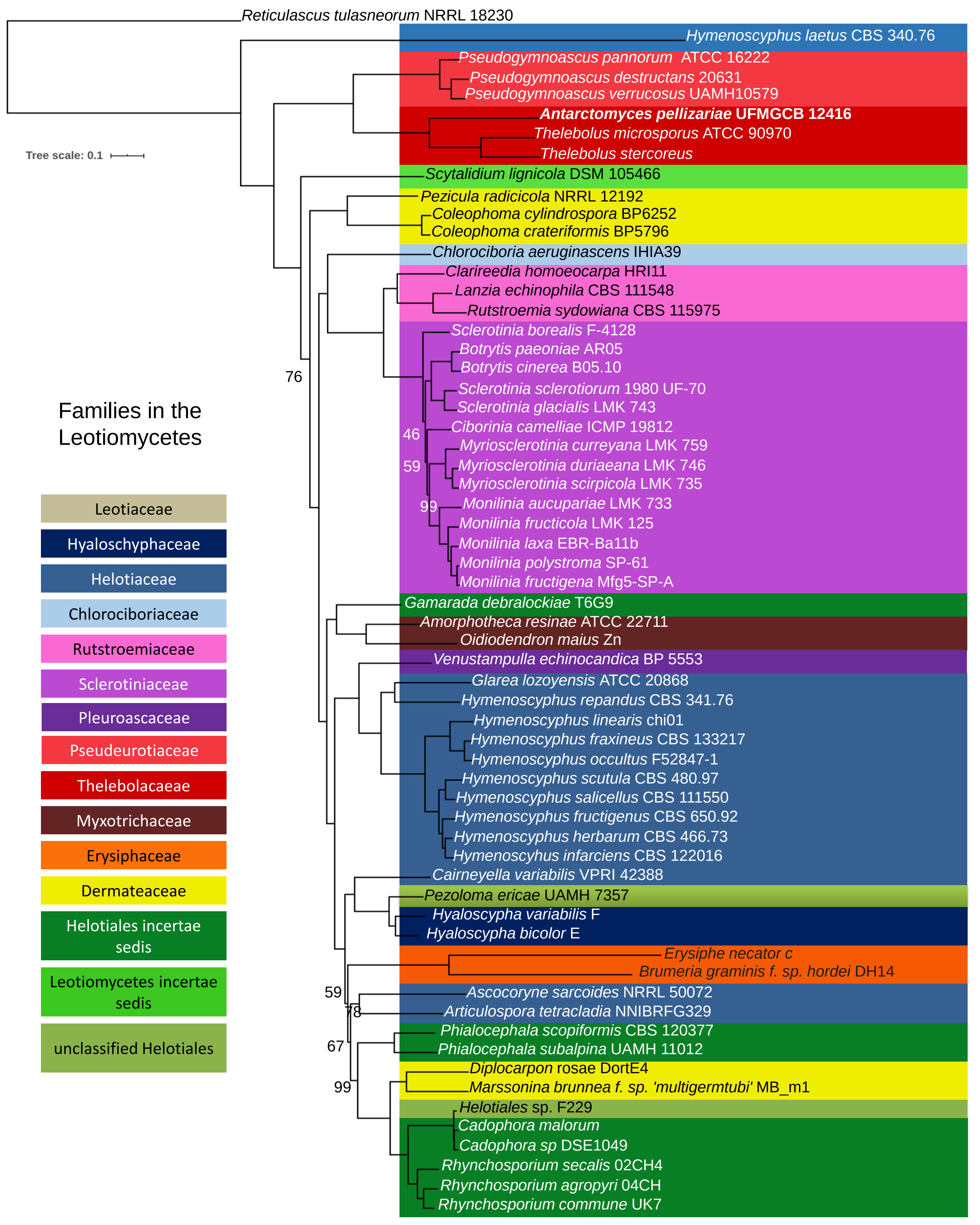


Fig. 3. Phylogenetic tree reconstructed from alignment of 137 orthologous *Antarctomyces pellizariae* UFMGCB12416 proteins compared with those of 60 other Leotiomycetes species plus *Reticulascus tulasneorum* NRRL 18230 (Sordariomycetes) as outgroups.

CONCLUSIONS

We sequenced and assembled the nuclear and mitochondrial genomes of the first known psychrophilic fungus, *A. pellizariae* (Thelebolales), isolated from Antarctic snow. We identified a putative IBP of 236 amino acids that shared sequence similarities with two IBP isoforms from *A. psychrotrophicus*, making these the only two species of Ascomycetous fungi with confirmed ability to produce AFP. Few clusters of secondary metabolite genes were identified, and this appears to be a feature of the genomes of extremophile organisms. Our phylogenetic findings confirmed that at least two families comprise the Thelebolales. However, the phylogeny can only become more reliable when high-quality genomes of other members of this class are revealed.

This will lead to a better understanding of the relationships of this clade within the Leotiomycetes.

ACKNOWLEDGEMENTS

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