# Isolation of microfungi from Arctic and Antarctic soils and their identification using ITS, LSU and SSU sequences.

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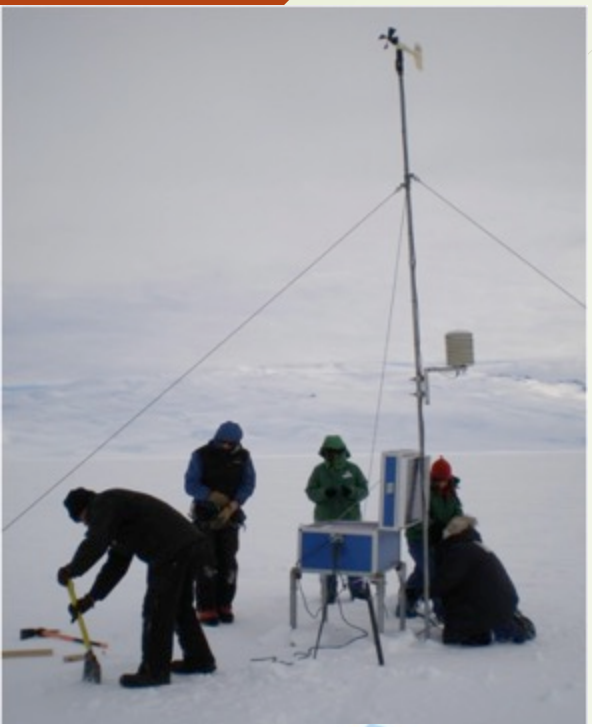
# Outline of talk

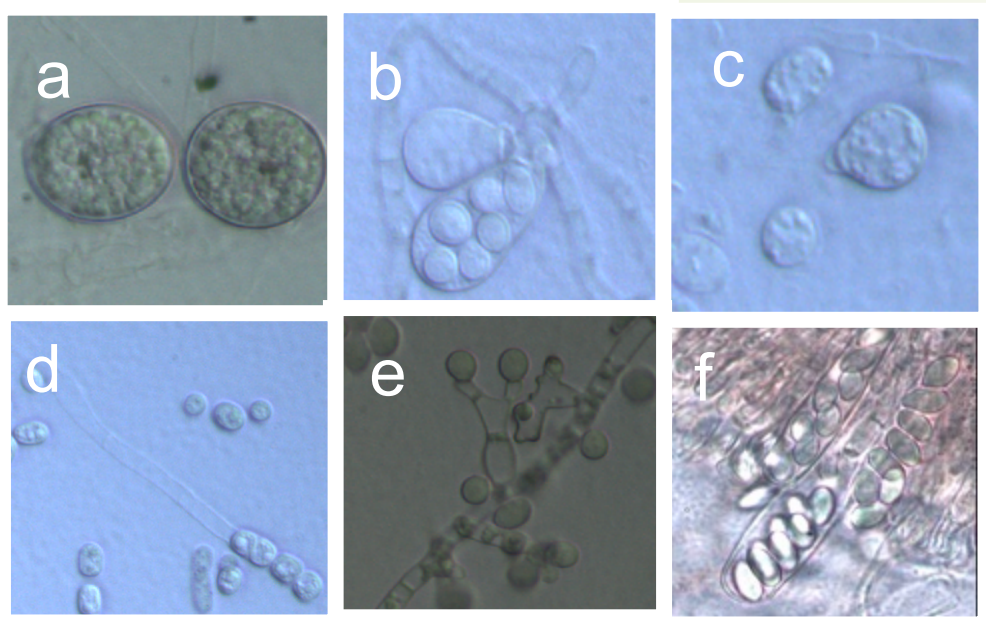
- Malaysia's involvement in polar research
- Diversity of soil microfungi from polar regions
- Barcoding of Fungi : ITS gene and other genetic markers
- This study aims to
  - Isolate microfungi from Arctic and Antarctic soil samples
  - Identify  microfungi species using ITS, LSU and SSU sequences
- Materials & Method
- Results & Discussion
- Conclusion


# Malaysia's involvement in polar research



- Malaysian Antarctic Research Programme (MARP) was initiated in November 1997.
- First Malaysian scientific expedition to Antarctica was successfully held in October 1999. Malaysia joined the ATCM in 2011.
- The National Antarctic Research Centre to coordinate the research activities of the members of the programme.
- In the year 2006, MARP has extended the interest to Arctic. At present, three scientific expeditions has been carried out in Arctic.

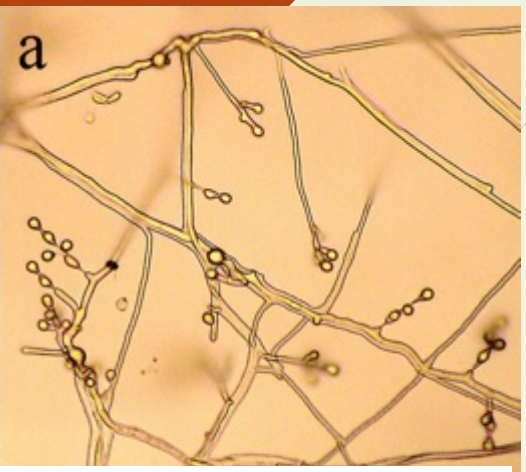

# Diversity of soil microfungi from polar



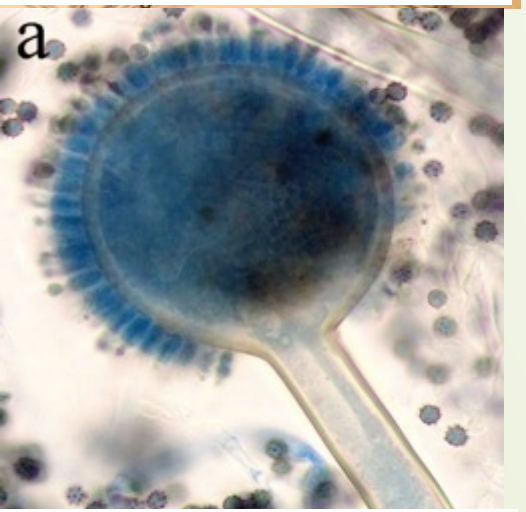

*a) Mortierella* sp. b) *Antarctomyces* sp. 4
c) *Mrakia* sp. d) Yeast sp. 16
e) *Aureobasidium* sp. f) *Thelebolus* sp.

- **Antarctica** – studies mostly in the continental rather than the maritime Antarctic.
- Easily accessible, benign locations and environmentally challenging areas such as the Victoria Land Dry Valleys (Friedmann et al. 1985), Beaufort Island (Alias et al, 2013).
- Occurrence of fungi associated with human associated habitats and artifacts have been studied extensively.
- **>80% belong to the Ascomycota**, mostly perithecial with few discomycetes

# Diversity of soil microfungi from polar regions




*Geomyces pannorum*




*Aspergillus aculeatus*

*(photos taken from Singh et al, 2012)*

- **Arctic- 2.3% of the world's fungal biota in Arctic, but few studies of fungal diversity in Arctic Soils (Singh et al, 2012)**
- **Diversity of fungi in soils of Bellsund, Svalbard, has been studied (Kurek et al., 2007), and new genera and species have been described from the region (Pang et al., 2008, 2009)**
- **Singh et al 2012 identified 19 species (14 genera) from Ny-Ålesund , Spitsbergen soils.**
- **Alias and Suhaila (2007) reported 89 microfungal taxa from the soil of Ny –Ålesund**
- **Pang *et al. (2009, 2011) reported six marine fungi isolated* from wood debris collected at Longyearbyen.**
- **Hornsund, Spitsbergen ornithogenic influenced soils showed the highest species diversity (eg. *Mortierella macrocystis, M. elongata, Mortierella sp., Cudoniella sp., Varicosporium elodeae, Beauveria bassiana, Geomyces pannorum, Penicillium sp. and Atradidymella muscivora) – (Ali et al 2014).***

# Barcoding of Fungi : ITS region and other genetic markers

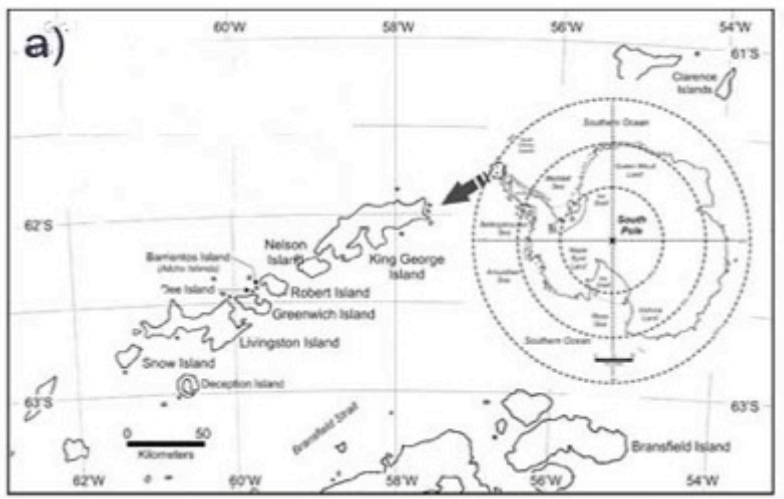
- Universal DNA barcode marker for Fungi - internal transcribed spacer (ITS) region.
- High PCR amplification and sequencing success , broadest range of fungi, defined barcode gap between inter- and intraspecific variation.
- 28S nuclear ribosomal large subunit rRNA gene (LSU)
- 18S nuclear ribosomal small subunit rRNA gene (SSU)
- Subunits of RNA polymerase II (RPB1, RPB2)
- Minichromosomemaintenance protein (MCM7)

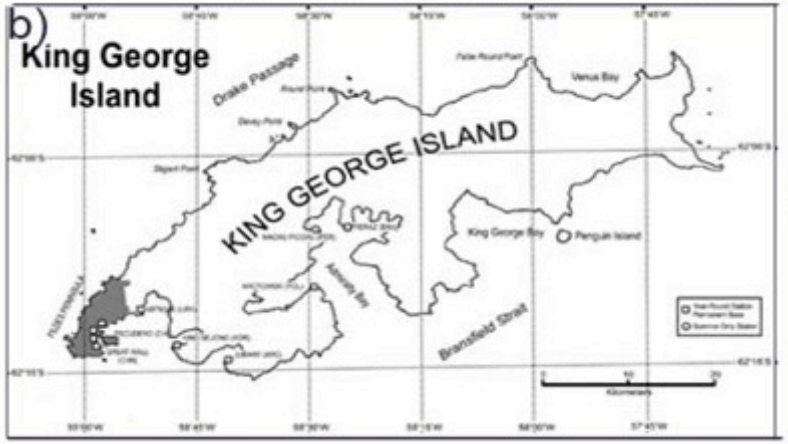
Used in fungi multigene phylogenies

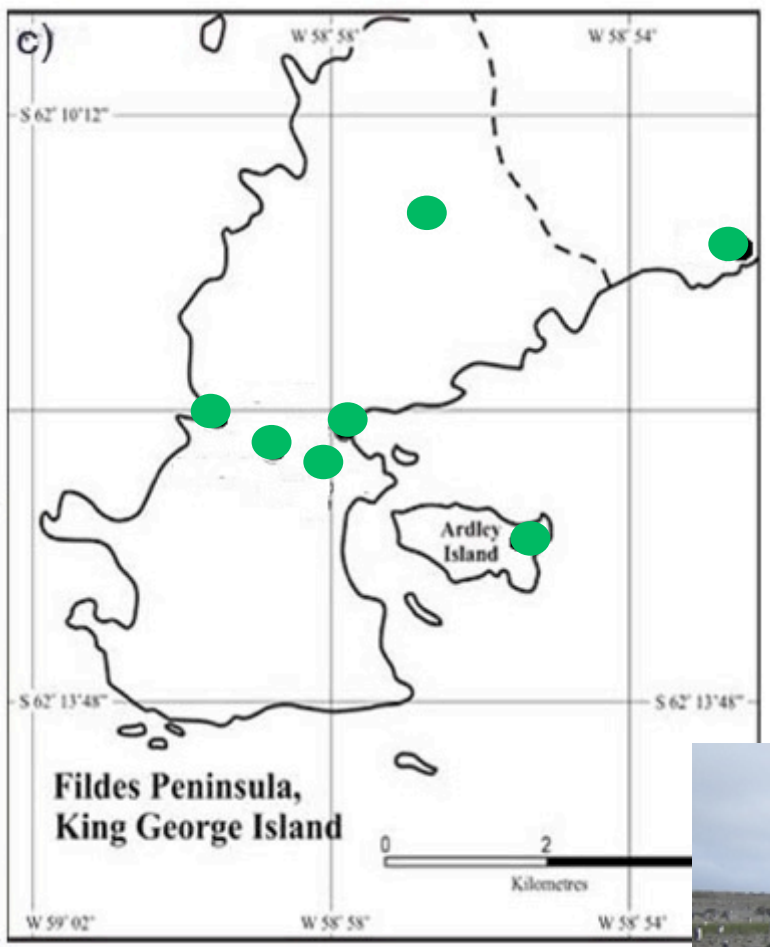
# This study aims to

- Isolate microfungi from Arctic and Antarctic soil samples
- Identify  microfungi species using ITS, LSU and SSU sequences

# Materials – Soil Samples

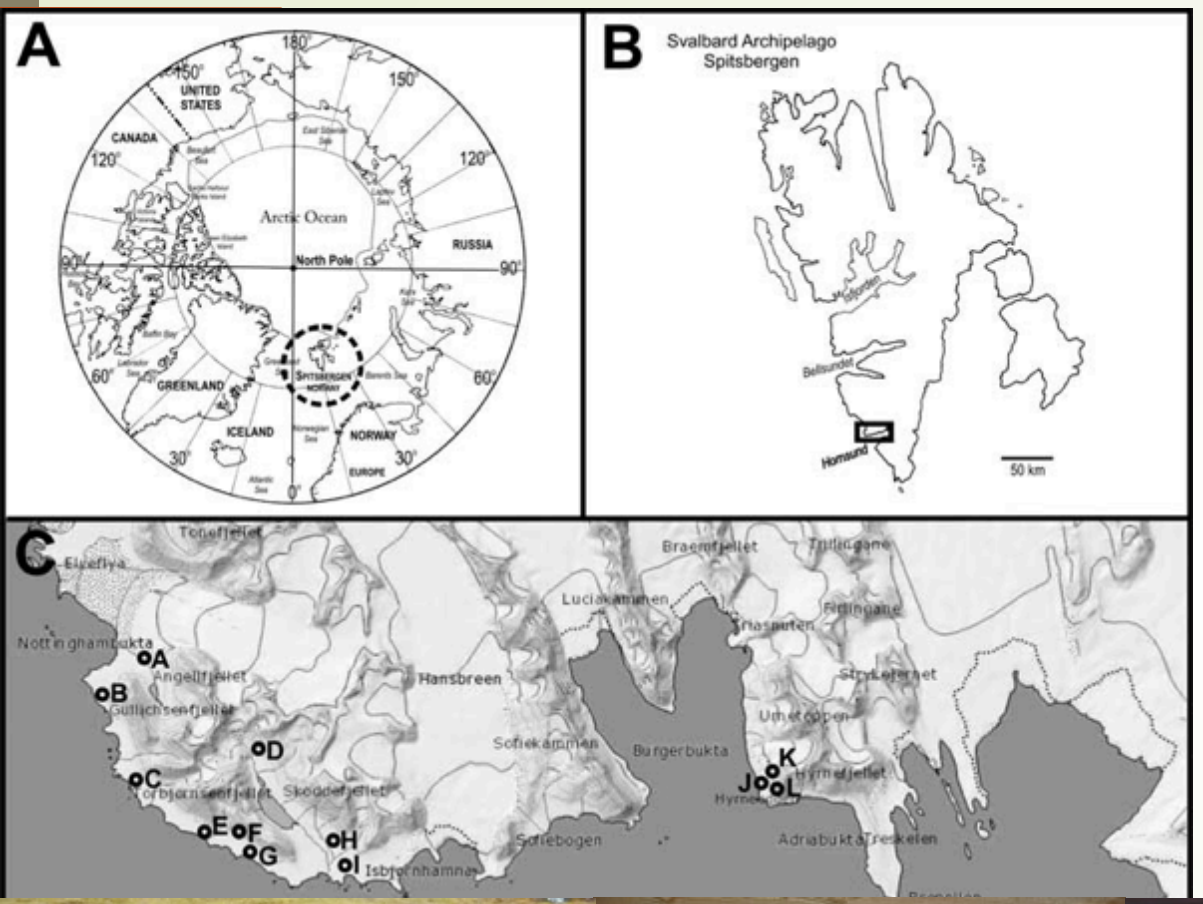



- King George Island (Antarctica)
- Austral summer 2006/2007
  - Penguin rookery
  - Pristine area
  - Human impacted area
  - Ornithogenically-influenced vegetated site

# Materials –Soil Samples




- Hornsund, Spitsbergen (Arctic)
- Boreal summer (August 2010)
- Dry and bare fellfields
- Moist moss tundra,
- Ornithogenic sites,
- Vertebrate–influenced pond shore
- Glacier foreland


# Method -Fungal isolation & Molecular Identification

Warcup soil plating technique

Incubated at 4°C and 25°C

Active growing mycelia: plated and sub-cultured onto PDA plates as individual isolates

Each isolate was identified using molecular techniques

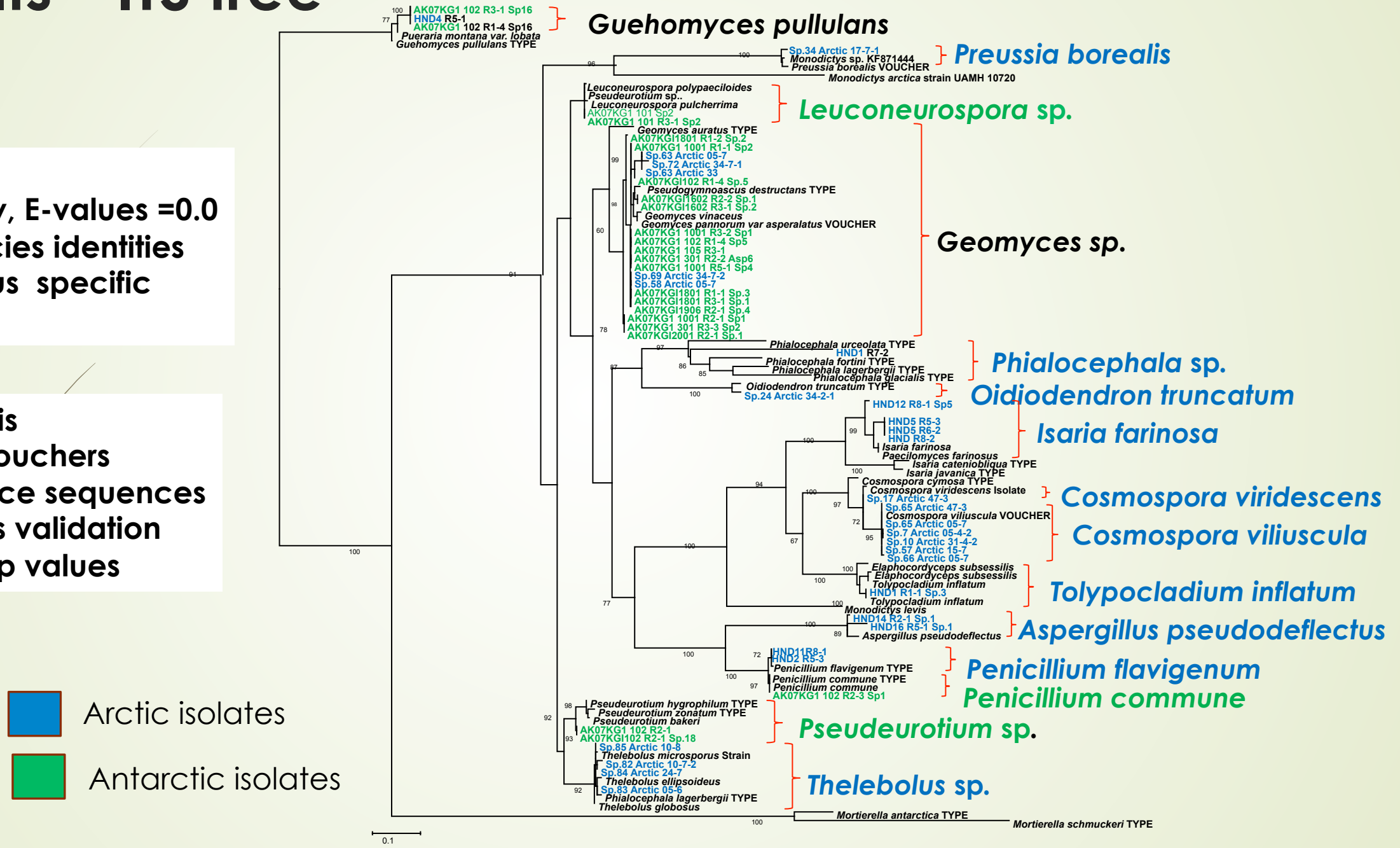
- BLAST

-Phylogenetics

# Results – ITS tree

**BLAST results**
**-> 99% identity, E-values =0.0**
**-possible species identities**
**-species/genus specific**

**RAXML analysis**
**-Many Type Vouchers**
**-Many reference sequences**
**-Better species validation**
**-High bootstrap values**




Arctic isolates

Antarctic isolates

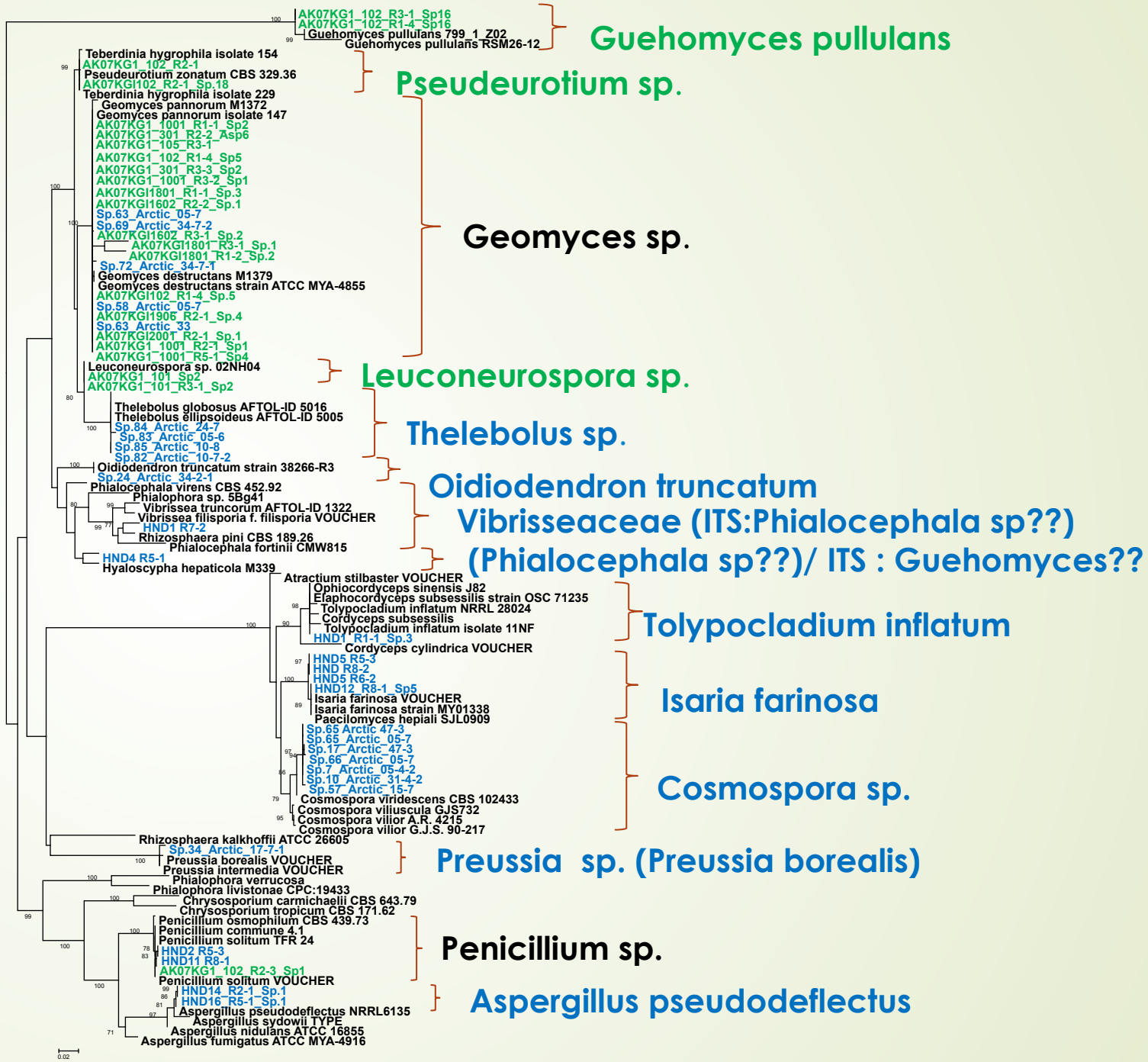
# Results – LSU tree

**BLAST results**
**-possible species identities**
**-species/genus specific**

**RAXML analysis**
**-Not many Type Vouchers**
**-Reasonably good species validation**
**-Some good bootstrap values**

Arctic isolates

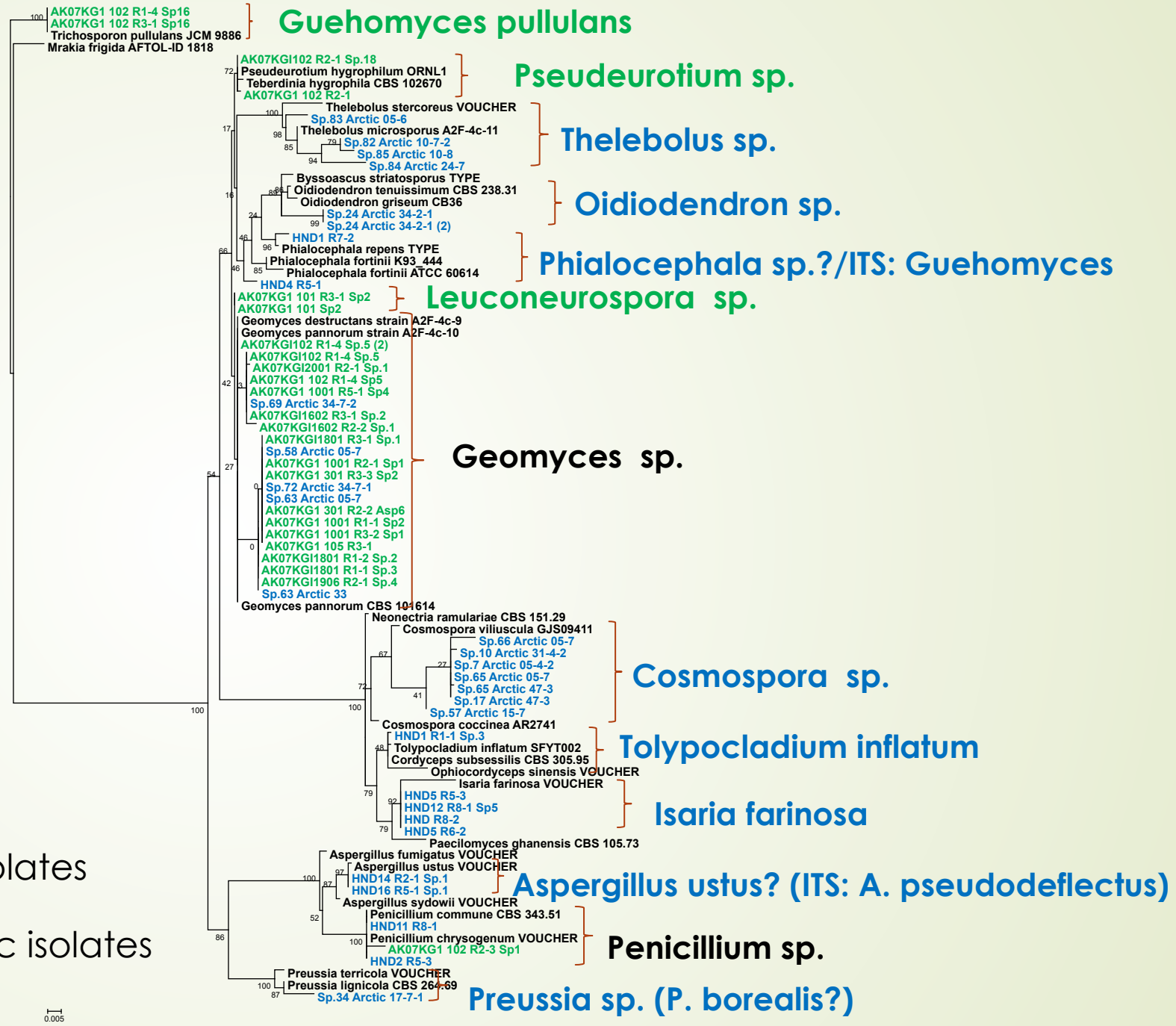
Antarctic isolates



# Results – SSU tree

**BLAST results**
**-many species/genus identities**
**-low sequence variation**

**RAXML analysis**
**-Few Type Vouchers**
**- Weak species validation**
**-Low bootstrap values**




Arctic isolates

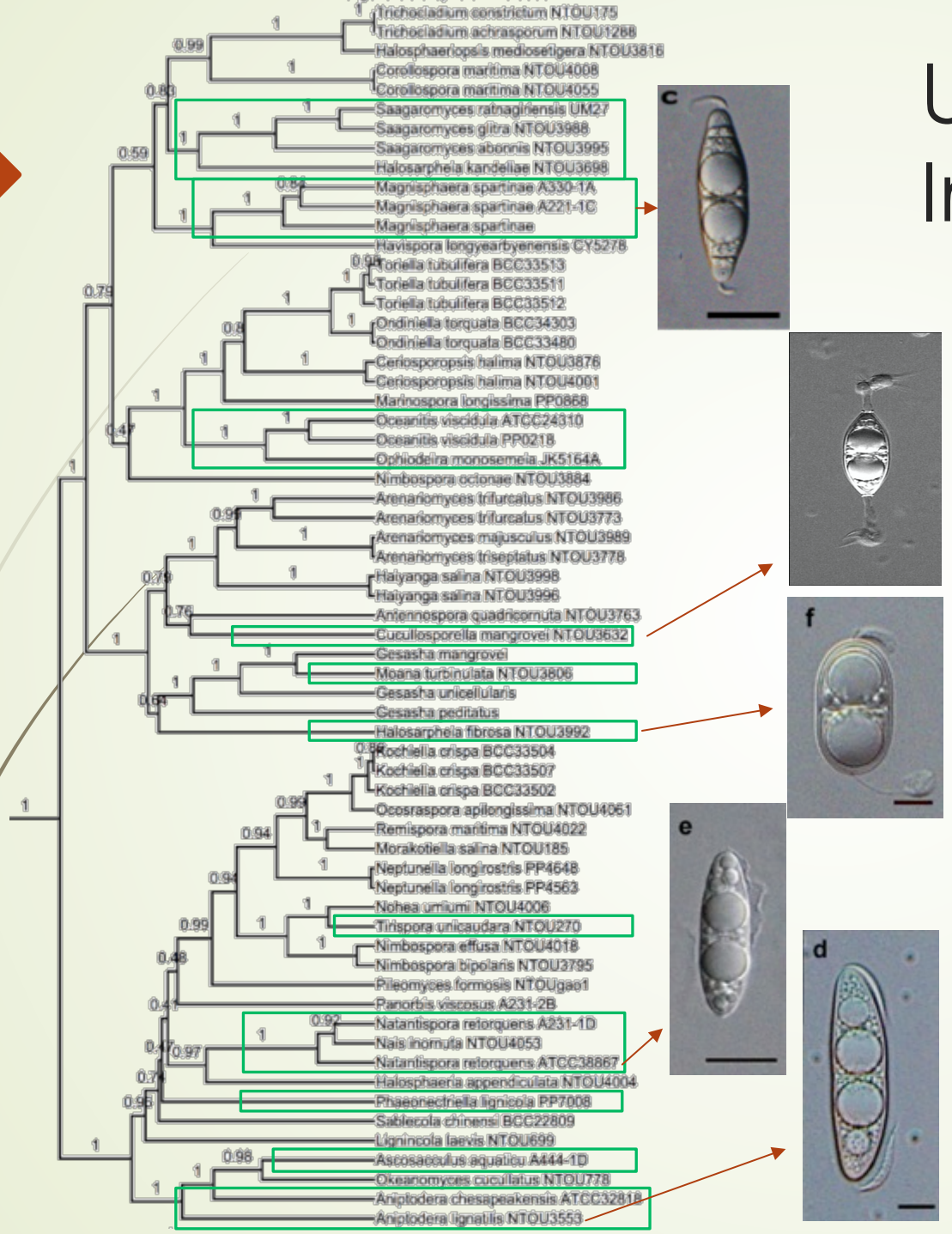
Antarctic isolates

# Discussion

- ITS region – the preferred barcoding gene in fungi
- Primary fungal barcode marker to the Consortium for the Barcode of Life (Schoch et al 2012).
- SSU – poor species level resolution
- Soil (polar) microfungi sequences still lacking in GenBank
- Studies show lower identification success can be expected in filamentous Ascomycota

# Unfurling appendages In **marine fungi**

- Taxa with unfurling ascospore appendages are polyphyletic
- A character possibly gained through **convergent evolution**
- Selection pressure for the evolution of appendages for attachment to substrata due to the scarcity of substrata in the sea
- May be an intermediate form between unappendaged ascospores to those with complex appendages




**Multigene phylogeny of marine fungi**

# Conclusion

- Vast majority of fungal species remains unknown.
- > 90% of Fungi awaits discovery from different environments
- Changes in Fungal Nomenclature
- Coordination in Field Sampling, Traditional Taxonomy & Barcoding

# THANK YOU