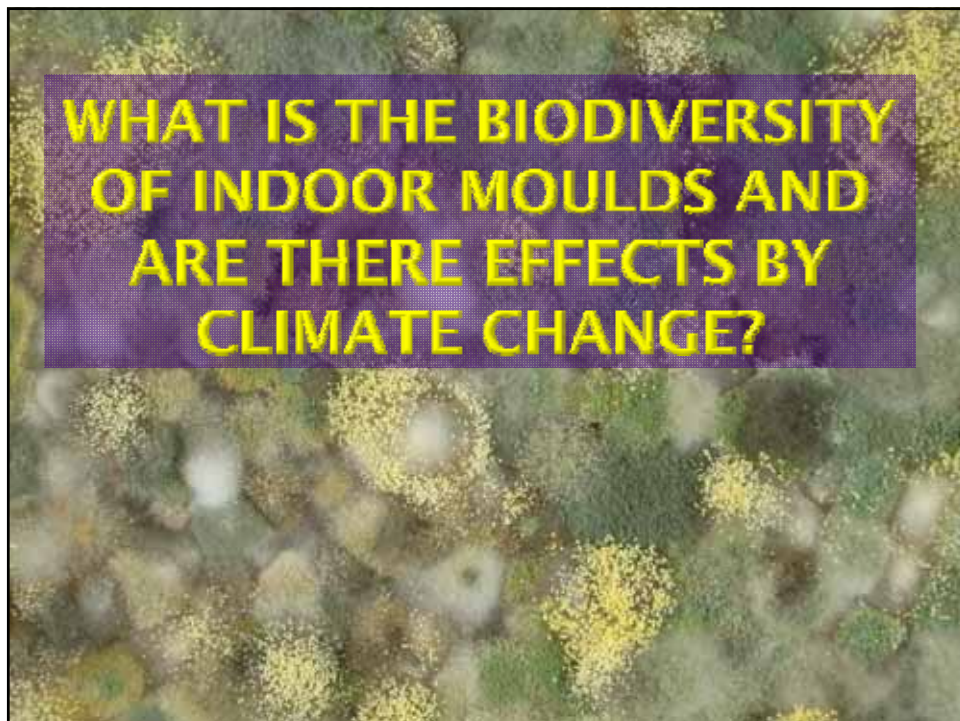
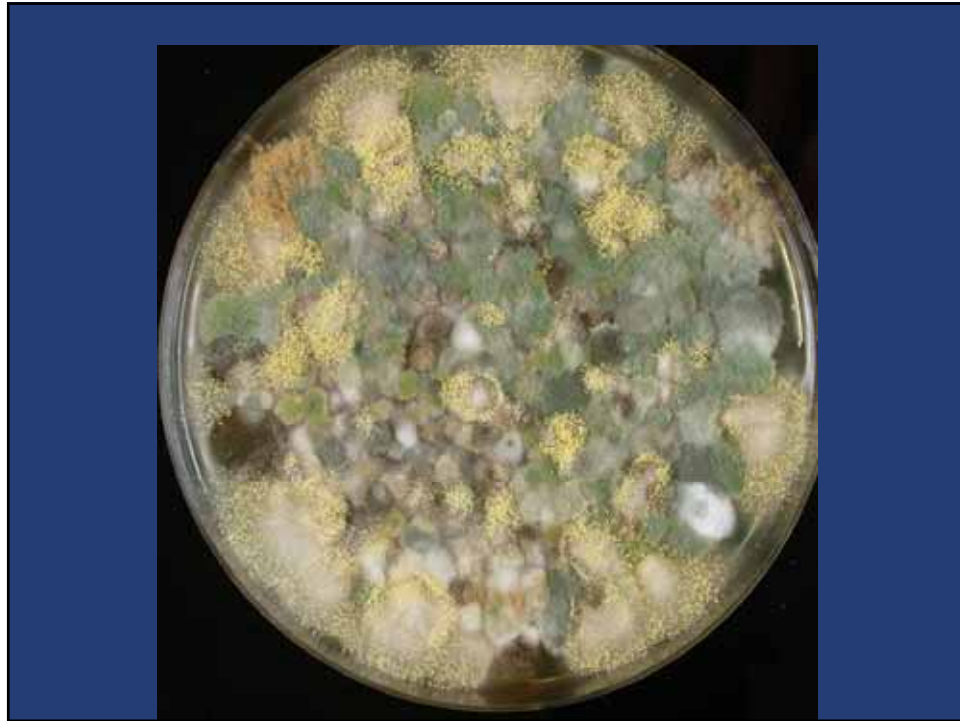


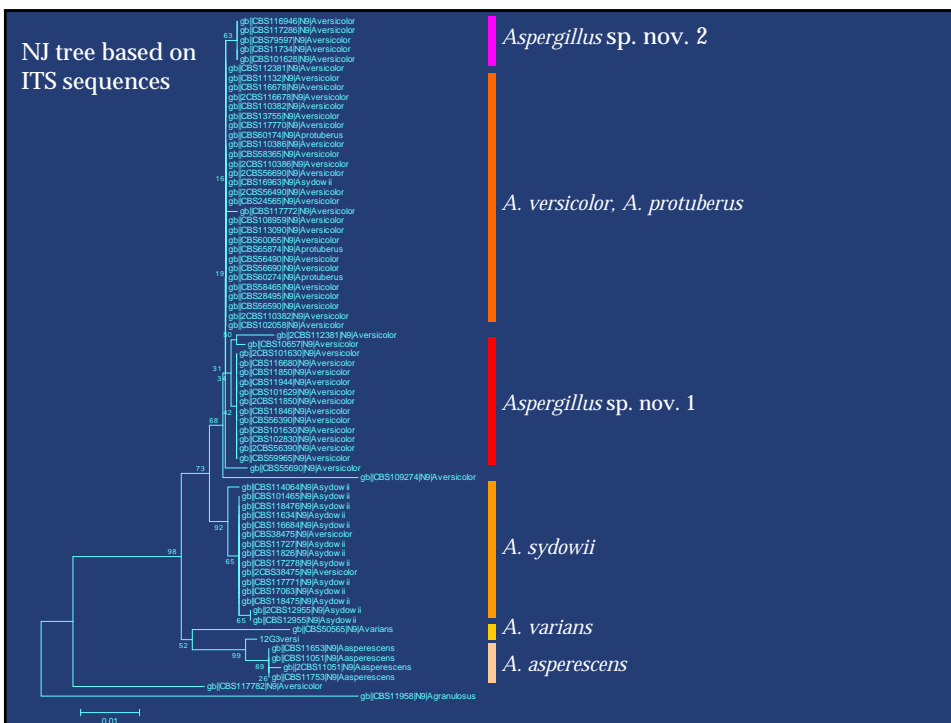
### List of fungal species occurring in indoor environments

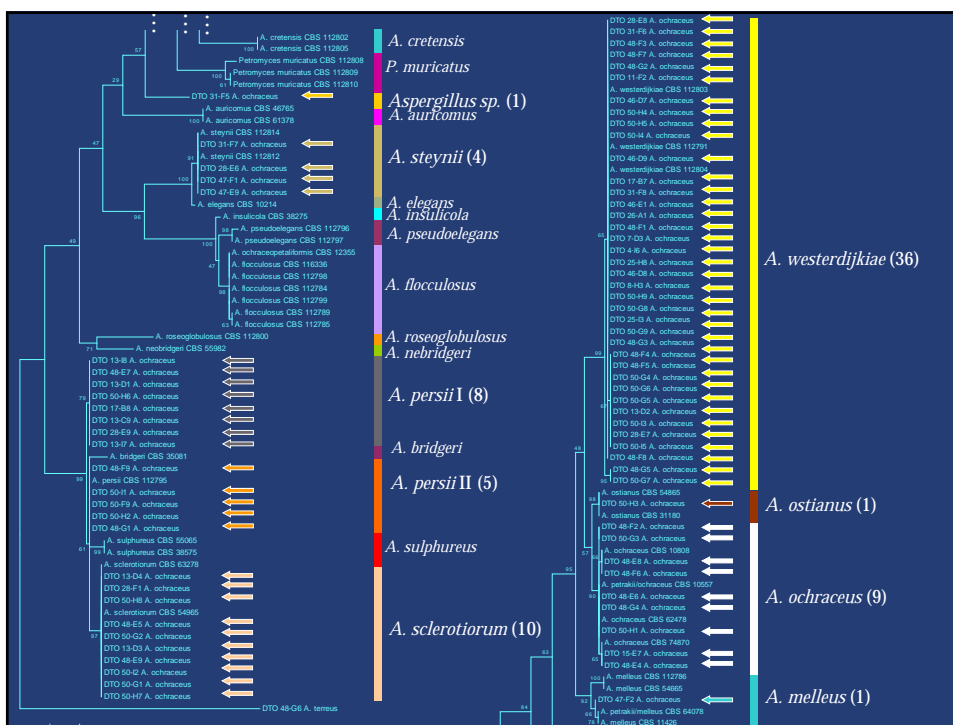
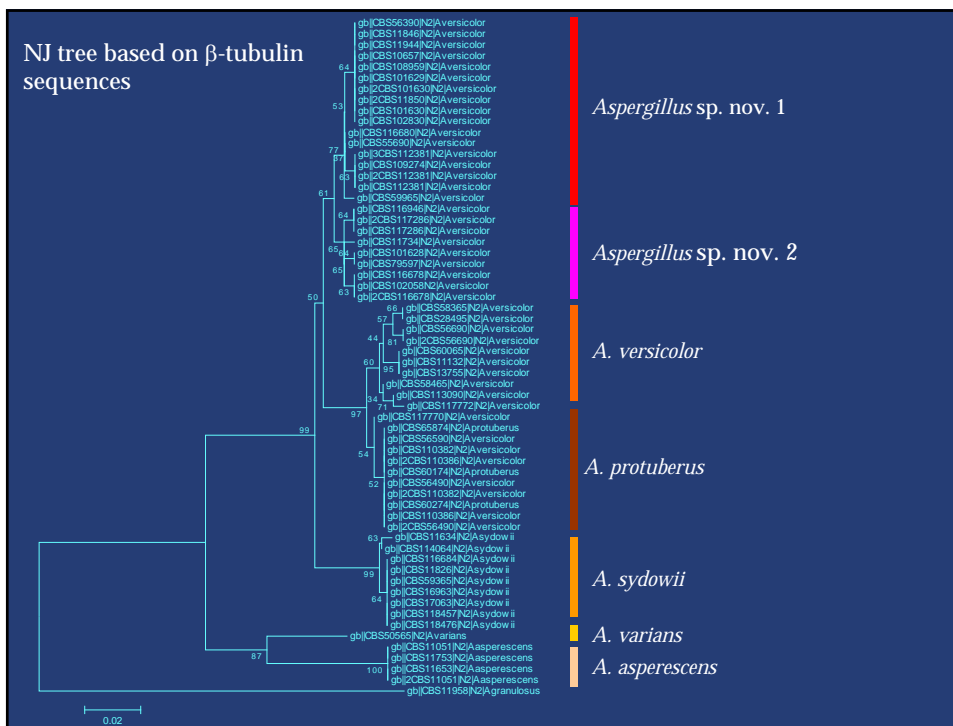
<i>Absidia corymbifera</i>	<i>Curvularia lunata</i>	<i>Penicillium olsonii</i>
<i>Acremonium murorum</i>	<i>Emericella nidulans</i>	<i>Penicillium rugulosum</i>
<i>Acremonium strictum</i>	<i>Epicoccum nigrum</i>	<i>Penicillium simplicissimum</i>
<i>Alternaria alternaria</i>	<i>Eurotium amstelodami</i>	<i>Penicillium spinulosum</i>
<i>Aspergillus candidus</i>	<i>Eurotium chevalieri</i>	<i>Penicillium variable</i>
<i>Aspergillus clavatus</i>	<i>Eurotium herbariorum</i>	<i>Phialophora fastigiata</i>
<i>Aspergillus flavus</i>	<i>Exophiala dermatitidis</i>	<i>Phialophora verrucosa</i>
<i>Aspergillus flavipes</i>	<i>Fusarium culmorum</i>	<i>Phoma glomerata</i>
<i>Aspergillus fumigatus</i>	<i>Fusarium solani</i>	<i>Phoma macrostoma</i>
<i>Aspergillus niger</i>	<i>Fusarium verticillioides</i>	<i>Pithomyces chartarum</i>
<i>Aspergillus ochraceus</i>	<i>Geomyces pannorum</i>	<i>Pyronema domesticum</i>
<i>Aspergillus penicillioides</i>	<i>Geotrichum candidum</i>	<i>Rhizopus stolonifer</i>
<i>Aspergillus restrictus</i>	<i>Memnoniella echinata</i>	<i>Rhodotorula mucilaginosa</i>
<i>Aspergillus sydowii</i>	<i>Mucor hiemalis</i>	<i>Schizophyllum commune</i>
<i>Aspergillus terreus</i>	<i>Mucor plumbeus</i>	<i>Scopulariopsis brevicaulis</i>
<i>Aspergillus versicolor</i>	<i>Mucor racemosus</i>	<i>Scopulariopsis candida</i>
<i>Aureobasidium pullulans</i>	<i>Oidiendron griseum</i>	<i>Scopulariopsis fusca</i>
<i>Botrytis cinerea</i>	<i>Oidiendron rhodogenum</i>	<i>Serpula lacrymans</i>
<i>Candida peltata</i>	<i>Paecilomyces lilacinus</i>	<i>Sistotrema brinkmanii</i>
<i>Chaetomium aureum</i>	<i>Paecilomyces variotii</i>	<i>Sporobolomyces roseus</i>
<i>Chaetomium globosum</i>	<i>Penicillium aurantiogriseum</i>	<i>Stachybotrys chartarum</i>
<i>Chaetomium indicum</i>	<i>Penicillium brevicompactum</i>	<i>Syncephalastrum racemosum</i>
<i>Chrysonillia sitophila</i>	<i>Penicillium chrysogenum</i>	<i>Trichoderma harzianum</i>
<i>Cladosporium cladosporioides</i>	<i>Penicillium citrinum</i>	<i>Trichoderma koningii</i>
<i>Cladosporium herbarum</i>	<i>Penicillium commune</i>	<i>Trichoderma viride</i>
<i>Cladosporium sphaerospermum</i>	<i>Penicillium corylophilum</i>	<i>Tritirachium oryzae</i>
<i>Clonostachys rosea</i>	<i>Penicillium expansum</i>	<i>Ulocladium chartarum</i>
<i>Coprinus cordisporus</i>	<i>Penicillium glabrum</i>	<i>Verticillium lecanii</i>
<i>Cryptococcus laurentii</i>	<i>Penicillium janthinellum</i>	<i>Wallemia sebi</i>



## New observations on the indoor mycobiota

- Some taxonomies have been refined
- In our recent surveys in the Netherlands we have encounter species which we had not seen before
- Many “new” species are Aspergilli or have a higher temperature requirement







## A new project to determine the indoor mycobiota

### A Network for the Indoor Mycobiota Barcode of Life \*

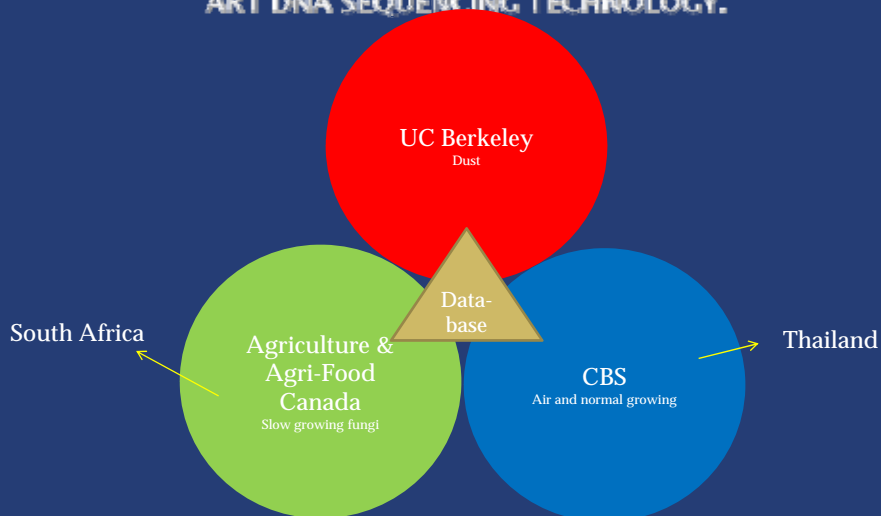
- ▣ **Tom D. Bruns** - Dept. of Plant and Microbiology, University of California at Berkeley
- ▣ **Keith A. Seifert** - University of Ottawa and Agriculture & Agri-Food Canada
- ▣ **Robert A. Samson** - CBS Fungal Diversity Centre, the Netherlands

\*Grant from the Alfred P. Sloan foundation

## The goal of the project

1. To develop a comprehensive, publicly accessible, on-line database of internal transcribed spacer (ITS) DNA barcodes (including voucher specimens, and DNA sequence chromatograms) for culturable fungi from human dwellings on a global scale.
2. To profile the complete fungal community, including species that cannot be cultured by standard techniques, using state-of-the-art high-throughput DNA sequencing technology.
3. To explore methods of obtaining voucher material (specimens or cultures) of 'nonculturable' fungi using novel techniques.
4. To use bioinformatics and genome mining tools to evaluate the potential of additional genetic loci that might serve as a second fungal barcode for indoor fungi and other ecological groups.
5. Help to develop standards for taxonomically categorizing environmental sequences.

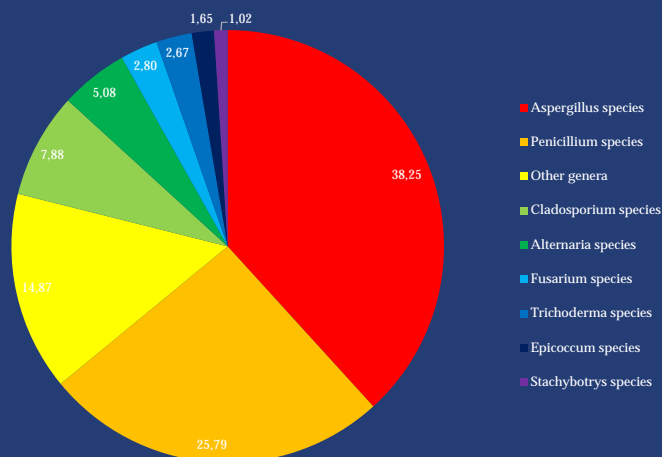
### 2: PROFILES OF THE TOTAL INDOOR FUNGAL COMMUNITY, INCLUDING SPECIES THAT CANNOT BE CULTURED BY STANDARD TECHNIQUES, OBTAINED USING STATE-OF-THE-ART DNA SEQUENCING TECHNOLOGY.

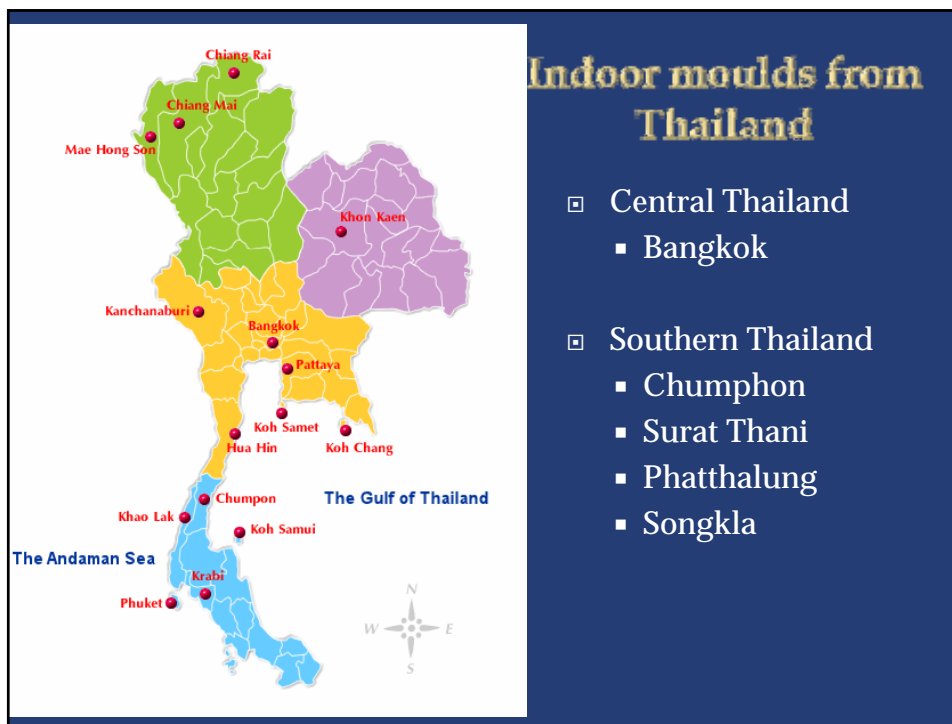


## Preliminary results

- ▣ CBS has sequenced up to now about 1600 strains originating from Europe (mainly ITS, but also calmodulin and  $\beta$ -tubulin)
- ▣ For the interpretation of the results it appeared that for many “common” genera taxonomic research is urgently needed and that correct identification is difficult
  - *Scopulariopsis*
  - *Stachybotrys*
  - *Cladosporium*

## CBS - PRELIMINARY RESULTS:





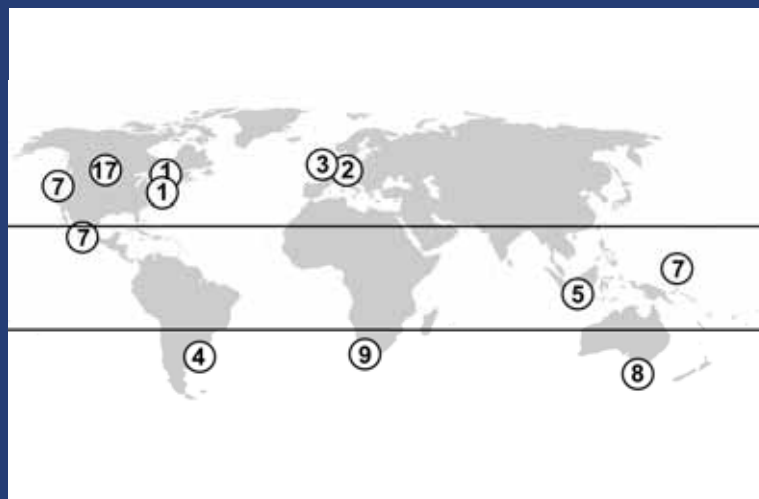
### Species found In Thailand

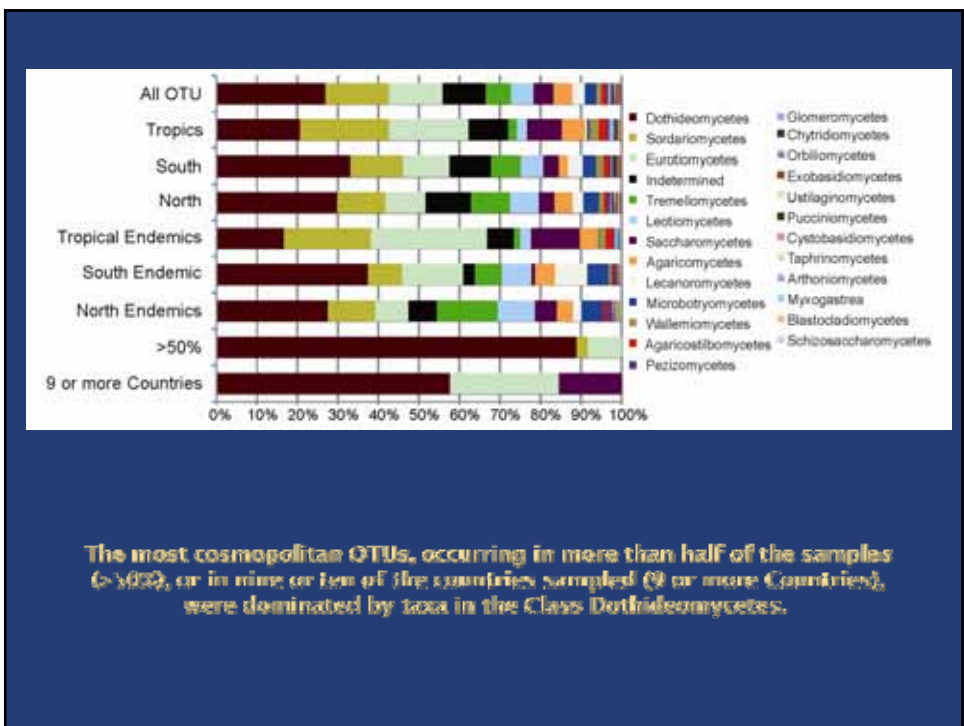
- *Aspergillus versicolor*
- *Aspergillus aculeatus*
- *Aspergillus niger*
- *Aspergillus japonicus*
- *Aspergillus oryzae*
- *Aspergillus ochraceopetaliformis*
- *Aspergillus sclerotiorum*
- *Aspergillus westerdijkiae*
- *Cladosporium cladosporioides*
- *Penicillium citrinum*
- *Penicillium steckii*
- *Chaetosartorya stromatoides*
- *Phellinus noxius*
- *Hypocreales sp.*

## Analysis indoor dust with culture-independent DNA sequencing method

- ▣ Settled dust samples from buildings on six continents was collected
- ▣ Extracted DNA was PCR amplified from two loci : a fragment containing internal transcribed spacer region 2 (ITS) and a fragment containing the D1 and D2 regions of the large subunit gene (LSU). These were pyrosequenced in multiplex using 454 Titanium technology

Dust sampling locations. All continents except Antarctica were sampled between December-March 2009. Black lines indicate Tropics of Cancer and Capricorn. Number of samples analyzed is noted inside open circles (approximate location).





### Dilution to Extinction: Fungal Isolation



- A weighed dust sample is sieved to collect 100-200  $\mu\text{m}$  particles
- It is then washed to remove excess spores
- The washed dust then undergoes serial dilution

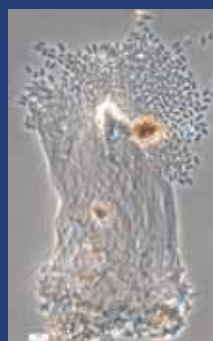
## Dilution to Extinction: Fungal Isolation



- The dilution is used to inoculate agar in microtubes
- The microtubes are incubated for 2-4 months
- Microtubes with fungi are processed for DNA sequencing and traditional agar plate culturing and morphological examination



## AGRICULTURE & AGRI-FOOD CANADA, OTTAWA



Two new species were discovered in a basement in Ontario

- 1: An obligate parasite of other microscopic fungus, *Piptocephalis* species (right)
- 2: The recently described genus of microscopic basidiomycetes, *Basidiopycnides* species (left)

*Botryosporium pulchrum*

- The biodiversity of indoor moulds is much larger than expected
- There are indications that species, which normally grow at higher temperature, are emerging in colder climates
- The occurrence of thermotolerant species might have an impact for medical mycology
- Research on indoor moulds is very relevant for the taxonomic studies
- DNA barcoding of indoor moulds will help to identify these microorganism faster and better understand their role in mouldy indoor environments