

**SECOND ANNUAL
INTERNATIONAL SCIENTIFIC WORKSHOP**

***Organismal-level population genomics
- Diagnostics of Fungal Associates -***

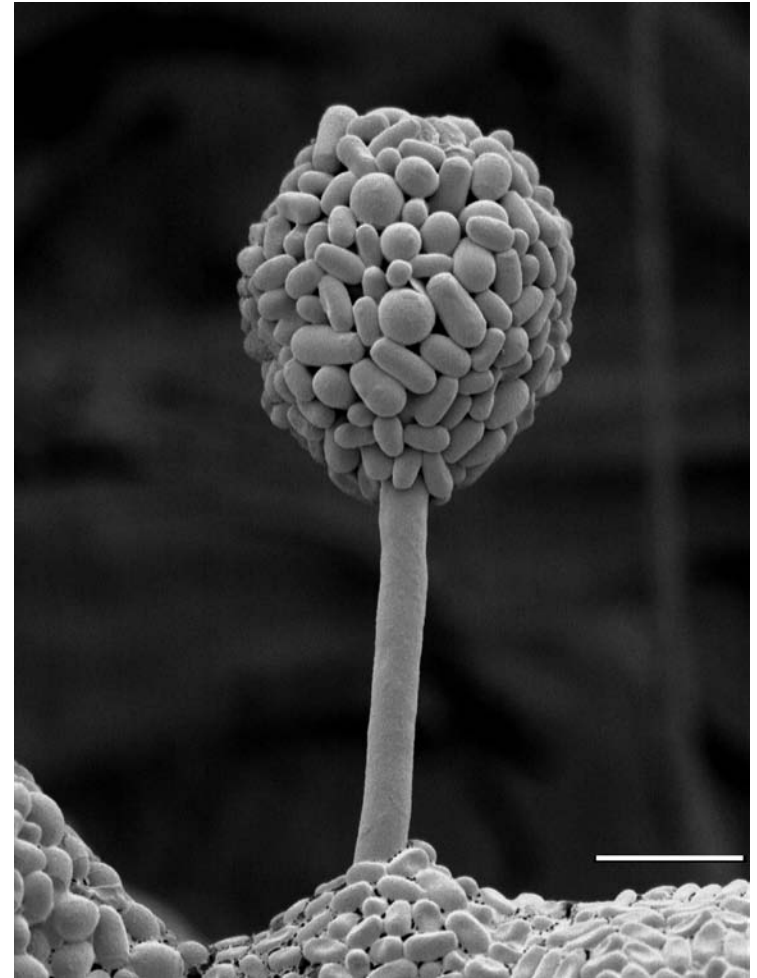
**Amanda Roe, Adrienne Rice, Janice Cooke, Felix Sperling, Dave Coltman
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Mountain pine beetle fungal associates multilocus sequence typing (MLST)

- Survey fungal strains isolated from MPB using multiple molecular markers
- Compare multilocus and single locus species diagnostics of fungal associates
- Assess morphotyping success
- Examine the success of single locus species diagnostics

MLST methods

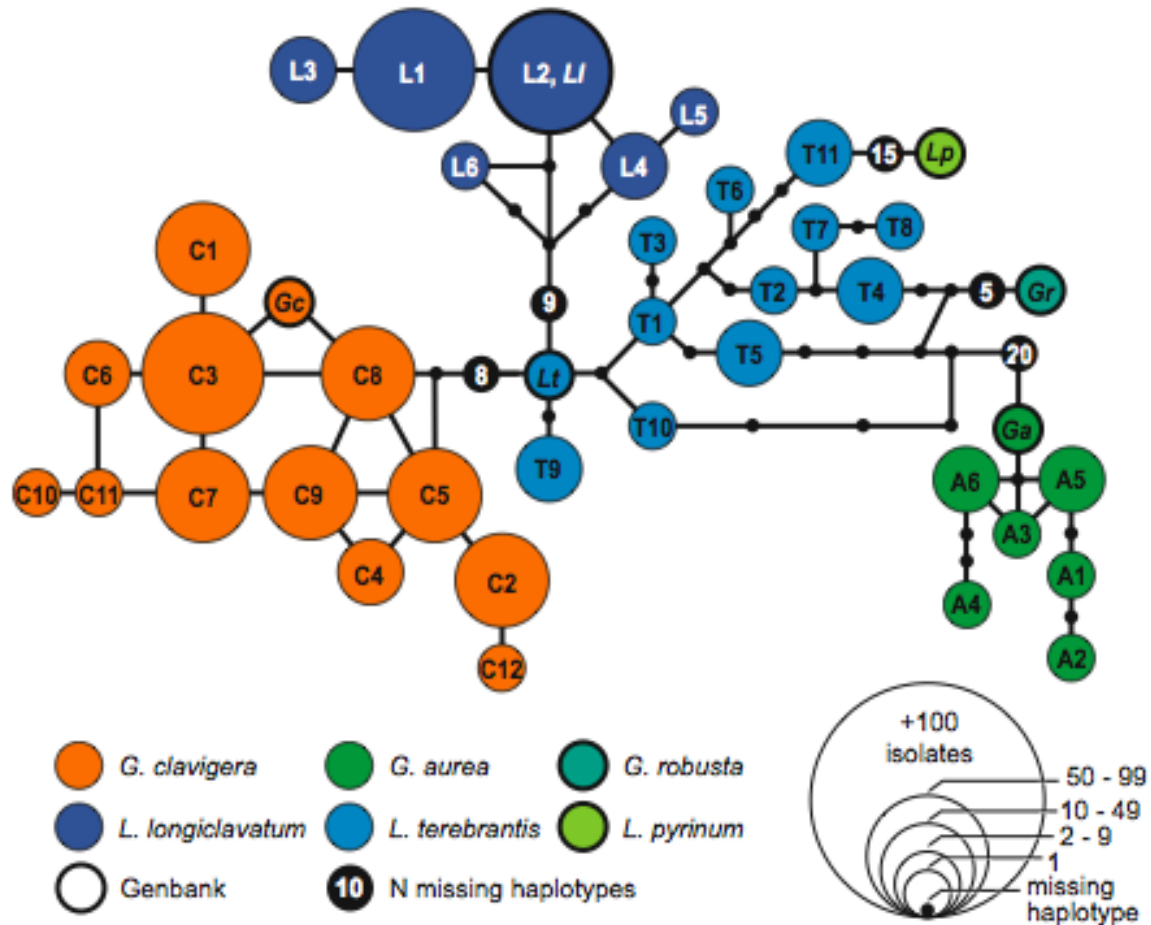
- 348 single spore isolates (SSI)
 - ~ 30 SSI per landscape
- Five molecular markers
 - Ribosomal DNA (rDNA)
 - ITS2+LSU
 - Autosomal DNA
 - elongation factor 1 alpha
 - actin
 - beta tubulin
 - Anonymous locus
 - Anonymous_contig29.6
- Sequence data compared to previously published GenBank data
- TCS vers. 1.21 and RaxML used to build haplotype networks and ML trees



A. Rice & G. Braybrook

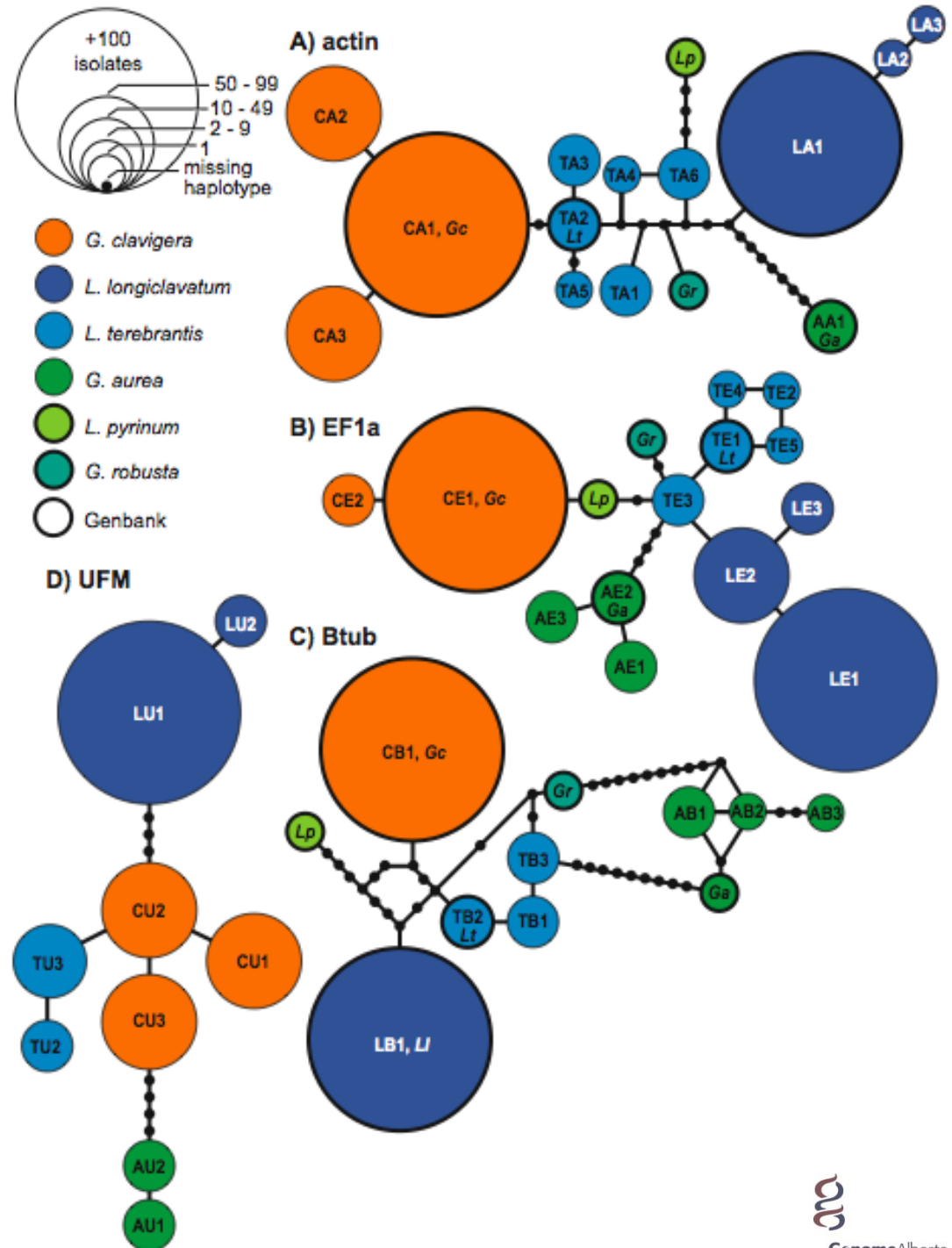
MLST results

- Morphotyping success ~75%
 - majority of strains with intermediate morphotypes were *L. longiclavatum*
 - Additional incidental species identified
- Concatenated MLST data shows clear limits between species
- Species form monophyletic groups
- No shared haplotypes



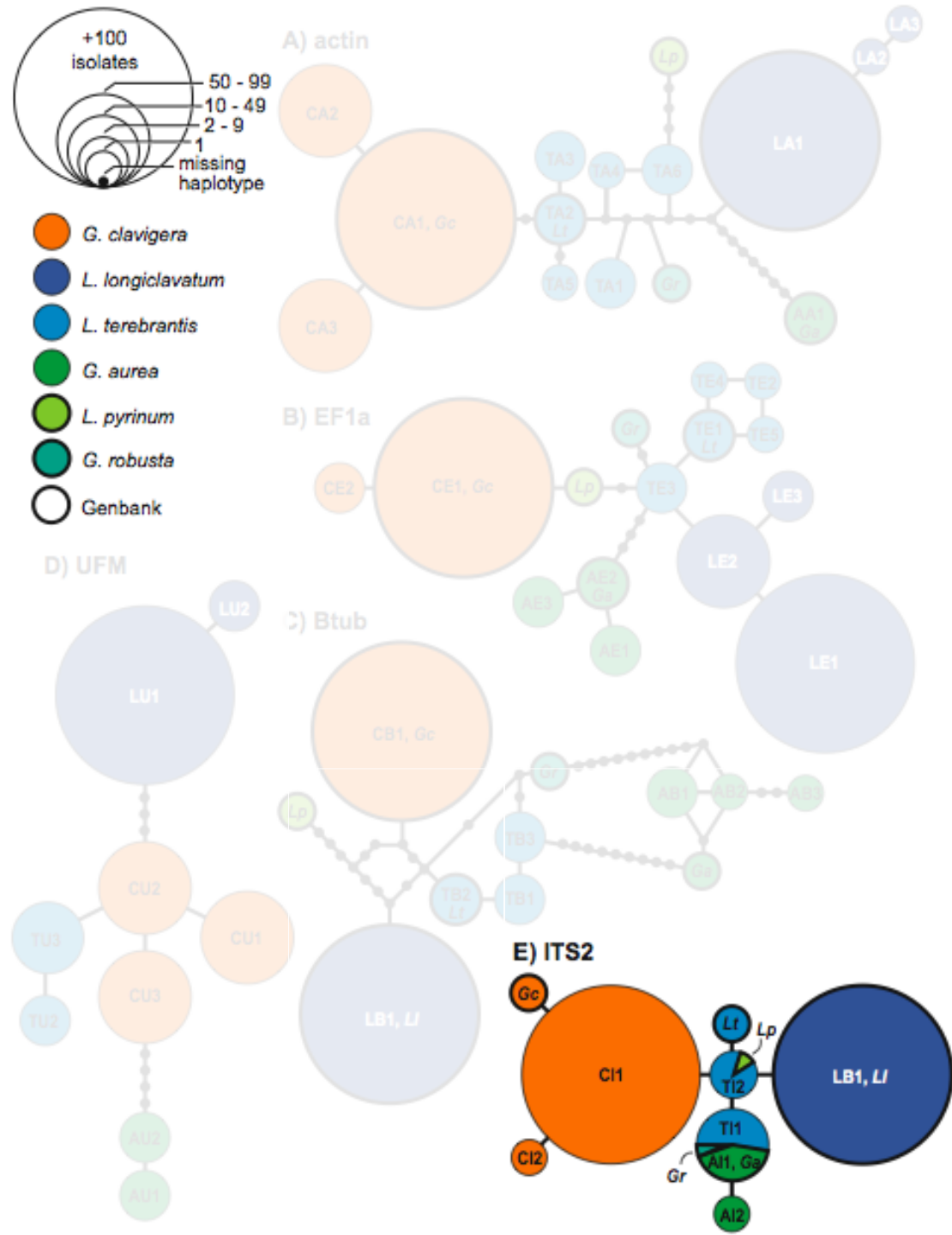
MLST results

- 4 loci show fixed haplotype differences
 - actin, EF1a, Btub, UFM
- *L. terebrantis* is paraphyletic in actin



MLST results

- 4 loci show fixed haplotype differences
 - actin, EF1a, Btub, UFM
- *L. terebrantis* is paraphyletic in actin
- rDNA less variable
- rDNA has shared haplotypes and non-monophyly of species



BARCODING FUNGI

Progress towards DNA barcoding of fungi

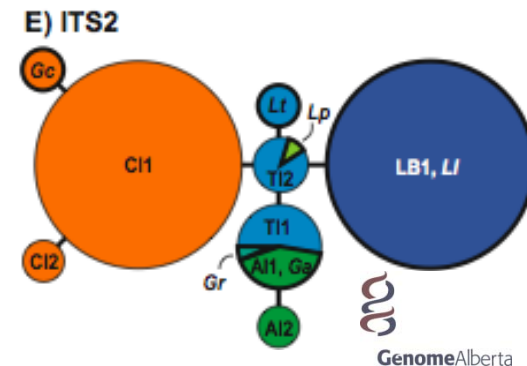
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Abstract

The use of DNA sequences for identifying fungi and fungus-like organisms predates the DNA barcoding movement by at least 10 years. A brief overview of the mycological shift from phenotypic to molecular taxonomy is provided. Exploration of the animal barcode marker, cytochrome oxidase 1, by Canadian mycologists has been fruitful for some fungi, but intron issues and lack of resolution in other taxa prevent its universal application. The momentum established by 15 years of research on the fungal nuclear ribosomal internal transcribed spacer (ITS) sequences will lead to a proposal to the Consortium for the Barcode of Life on the adoption of this marker as the fungal barcode. Existing mycological research networks should facilitate the rapid development of DNA barcoding of fungi once the marker issue is settled. Some available online fungal identification databases are briefly described.

Keywords: COX1, Fun-BOL, internal transcribed spacer (ITS), mitochondrial cytochrome oxidase 1, online identification databases.



MLST Conclusions

- Utility of individual markers variable
- Using multiple markers would improve diagnostic success, similar to results obtained by plant barcoding initiative
- MLST results provide a foundation for the development of HT-SNP diagnostics

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 PLOS ONE

Multiple Multilocus DNA Barcodes from the Plastid Genome Discriminate Plant Species Equally Well

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