

EVOLUTIONARY METABOLOMICS IN *TOLYPOCLADIUM* FUNGI TO GUIDE NATURAL PRODUCTS DISCOVERY

Richard Tehan[†], Rheannon Arvidson[‡], Joseph W. Spatafora[‡], Kerry L. McPhail[†]

[†]Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR

[‡]Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR

ABSTRACT

The fungal genus *Tolypocladium* contains several species known to produce biologically active peptide, polyketide, and alkaloid secondary metabolites (SMs). Ongoing genomic analyses of 14 different strains in the Spatafora lab indicate *Tolypocladium* as a potentially rich source of new SMs of various classes. LC/MS-based metabolomics tools were used to survey the secondary metabolomes of 11 *Tolypocladium* species, guide the discovery of new SMs, and contribute to the understanding of the evolution of peptidic SMs in *Tolypocladium*. Tandem mass spectrometry data evinced several unknown peptidic metabolites in the extracts of most species analyzed. Unique amino acid losses in the tandem mass spectra suggest that many of these metabolites are likely to be members of the peptaibiotic family of non-ribosomal peptides. These and other peptidic metabolites are being targeted for isolation, structure elucidation and pairing with their biosynthetic gene clusters (BGCs), as well as biological characterization.

INTRODUCTION

Ecological Diversification in Order Hypocreales

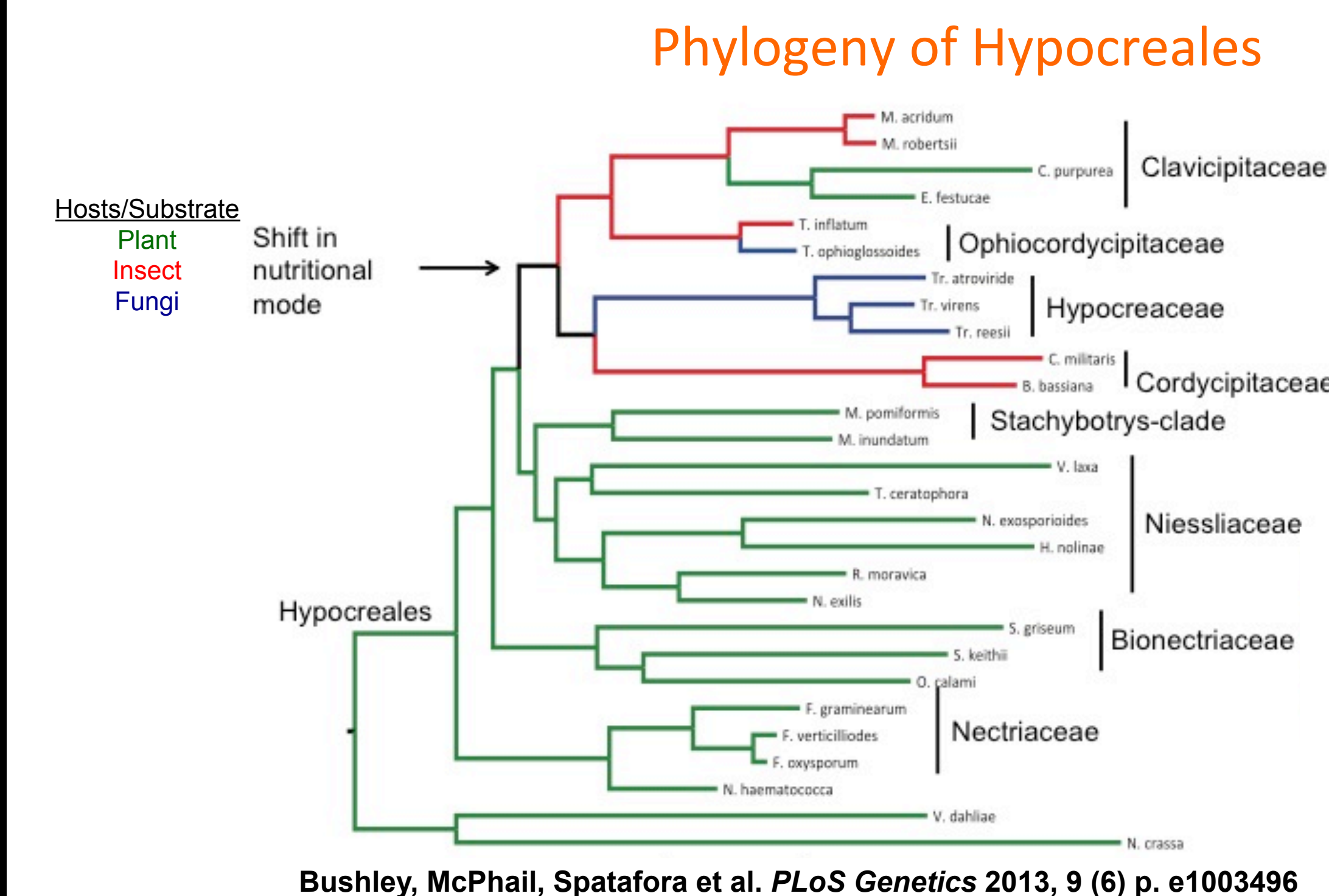


Figure 1. Evolution of the Hypocreales. The Order Hypocreales is comprised of seven major clades, uniquely defined by their ecologies. Knowledge of the phylogeny and evolution of ecologies in Hypocreales provides a predictive framework for natural product drug discovery. Shifts in nutritional mode are associated with diversification of secondary metabolism.

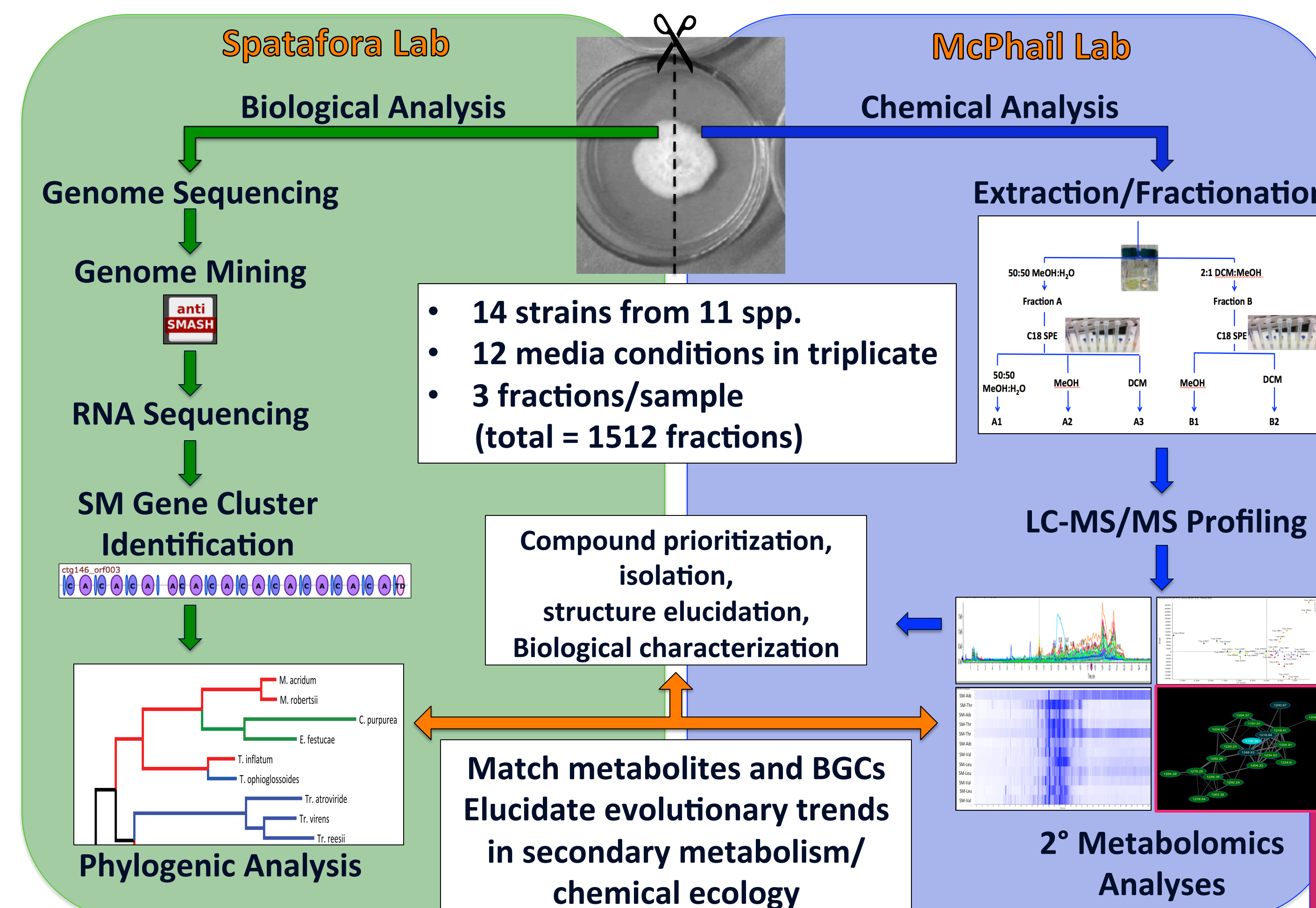
Secondary Metabolites Facilitate Nutritional Mode



- Can tracing ecological diversification guide the discovery of novel NPs with specific biological activities?
- Can secondary metabolomic profiling inform the evolutionary biology of *Tolypocladium*?

Methods

Evolutionary Metabolomics Workflow



RESULTS

Molecular Networking for Natural Product Discovery

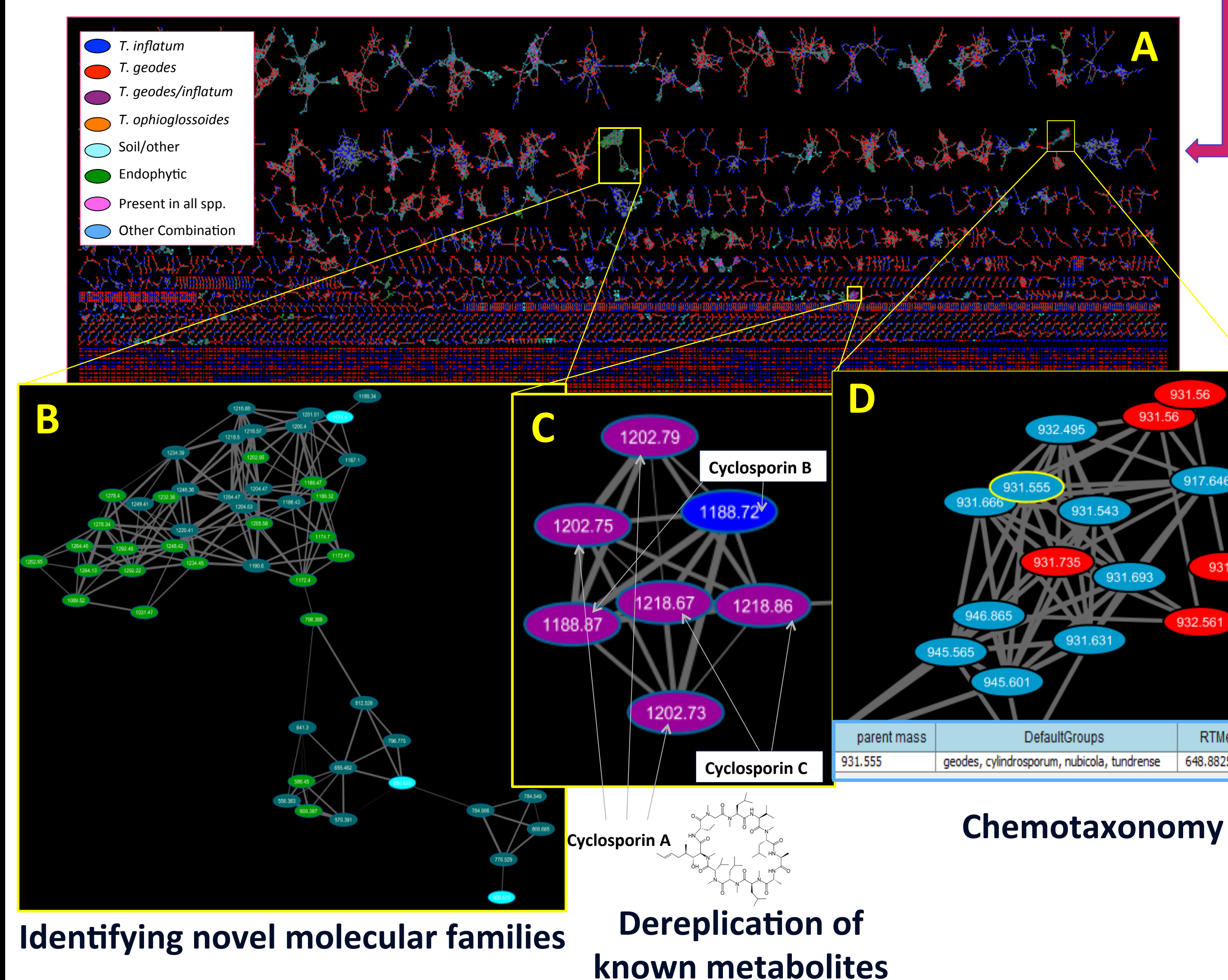


Figure 5. Molecular Networking for the discovery of new natural products. (A) A molecular network is created from LC-MS/MS data of 1008 chemical extracts of *Tolypocladium* cultures. Ion peaks with unique MS/MS fragmentation patterns are represented as a node, connected to nodes with similar MS/MS spectra, producing clusters of structurally similar molecular families. Overlaid on the network are the experimental groups, coded by color, in which each molecule is detected. (B) Molecular networking is used to identify families of molecules which are unique to particular taxa. A cluster of peptides appears to be unique to recently described endophytic *Tolypocladium* spp. (C) Molecular networking is used to dereplicate known compounds. The immunosuppressant, cyclosporine and its analogs are present in two *Tolypocladium* spp. (D) Production of an interesting compound is confined to four species.

Pursuit of New Peptaibotics

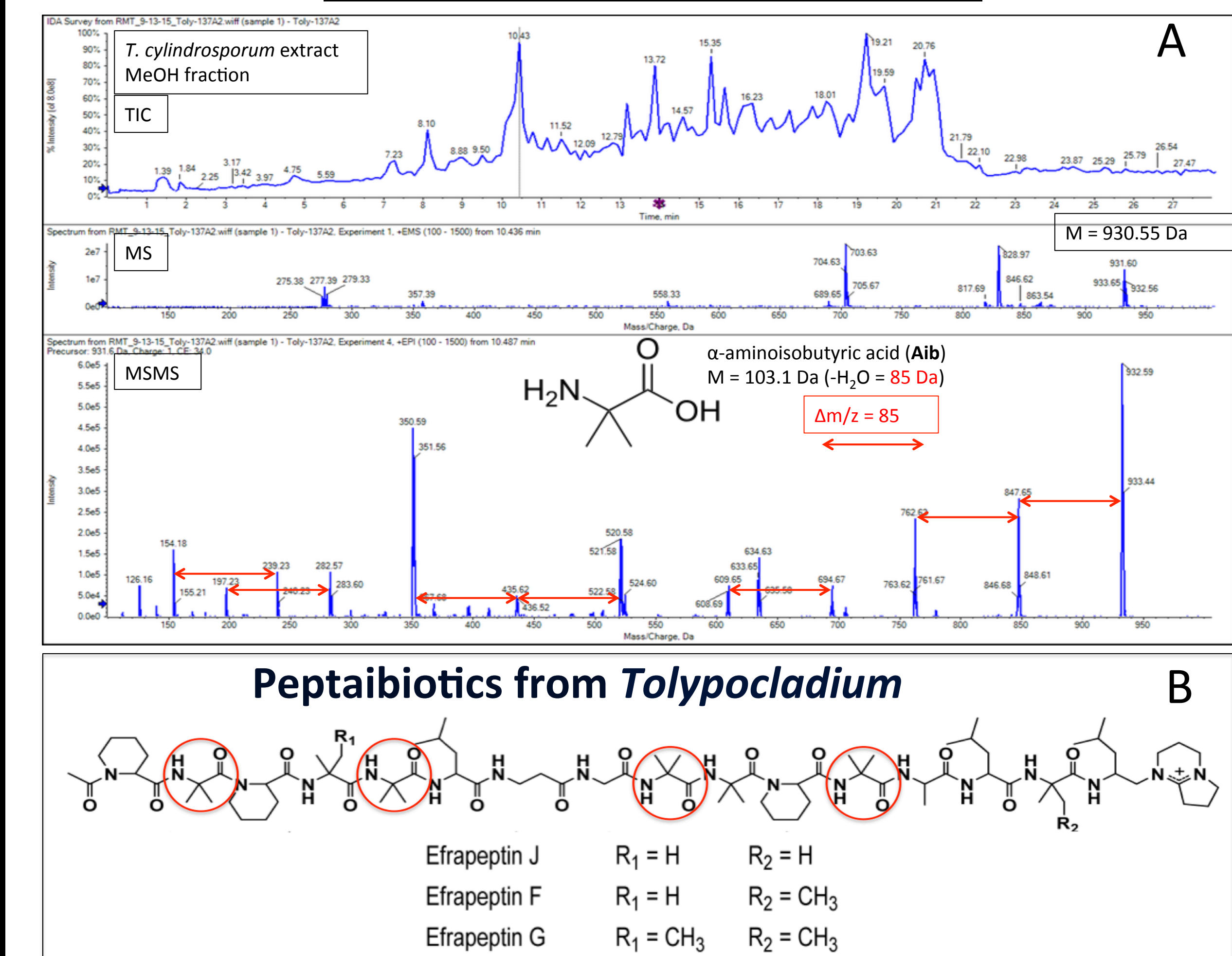


Figure 6. Peptaibol metabolites from *Tolypocladium*. (A) A new peptide in the MeOH fraction of *T. cylindrosporum*. Molecular networking (Figure 5D) revealed its presence in four *Tolypocladium* spp. Tandem MS shows α-aminoisobutyric acid (Aib) monomers, evident from losses of 85 amu. (B) Aib is characteristic of peptaibiotics, linear peptides that contain several non-proteinogenic amino acids and function as antibiotic and antifungal agents. Efraeptins are a family of peptaibiotics previously isolated from *Tolypocladium* spp. Figure reproduced from Hayakawa *et al.*²

Identifying SM Biosynthetic Gene Clusters

ctg174_orf003

Figure 7. **Identifying SM biosynthetic gene clusters.** Secondary metabolite BGCs were predicted in the genomes of five *Tolypocladium* spp. using the genome mining tools, SMURF and antiSMASH. One predicted NRPS gene cluster had homologs in four *Tolypocladium* spp. which produced a common peptide identified in the molecular network. Adenylation domains which code for non-proteinogenic amino acids and reducing domains suggest a peptaibol product.

SUMMARY

- 14 strains of 11 *Tolypocladium* spp. grown on 12 media profiled by LC-MS/MS
- New and known peptides and their BGCs were identified by combining metabolomics and genome-mining tools
- Future Work
- Large-scale culture of peptidic SM producers
- Isolation, structure elucidation, biological characterization of peptides unique to new endophytic *Tolypocladium* spp. and of peptaibiotics common to four *Tolypocladium* spp.
- Continue to mine *Tolypocladium* data, as informed by ongoing data acquisition

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