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Determination of taxonomic placement of falsely-branched taxa in soils of San Nicolas Island and reassessment of the Tolypothrichaceae

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Introduction

San Nicolas Island:

- One of the eight Channel Islands located off the coast of Southern California, United States
- Contains over 35 different species of cyanobacteria from soil crusts, which are communities of microorganisms that grow on the soil surface
- Molecular study of these cyanobacteria provides insights into the ecological and evolutionary processes that shape these unique organisms

Tolypothrichaceae:

- A well-characterized monophyletic lineage of non-attenuated, false-branching heteropolar types containing the genera *Spirirestis, Hassallia, Tolypothrix, Coleodesmium,* and *Rexia*

Molecular Techniques:

- Phylogeny of the 16S rRNA:
  - 16S rRNA: critical component of the small ribosomal subunit found in bacteria
    - Highly conserved and useful for understanding evolution in group
  - Shows the evolutionary relationships between the different taxa, with closely related cyanobacteria placed closer together on the tree and more distantly related cyanobacteria placed farther apart
- Phylogeny of the 16S-23S ITS (Internal Transcribed Spacer) region:
  - 16S-23S ITS region: segment of DNA located between the 16S and 23S rRNA genes in the ribosomal operon
  - Has become a popular target for molecular identification and phylogenetic studies of bacteria because it contains variable regions that can be used for species-specific identification and evolutionary analysis

Main objective of this study: To classify the falsely-branched taxa on San Nicolas Island as well as reassess the family Tolypothrichaceae and determine if it should be retained as presently understood, or split into two families, one for aquatic forms and one for soil forms

Methods

- Conducted microscopic analysis and imaging of San Nicolas Islands strains (see Fig. 1 for example)
- Sequenced the 16S rRNA gene and associated ITS region for all species of falsely-branching cyanobacteria in the soils of San Nicolas Island
- Compiled an alignment of the taxa currently assigned to Tolypothrichaceae, including the sequences obtained from San Nicolas Island
- Completed phylogenetic analyses of the 16S rRNA gene and ITS region for the strains of interest
- Interpreted these analyses and proposed taxonomic decisions regarding taxonomic placement of our strains

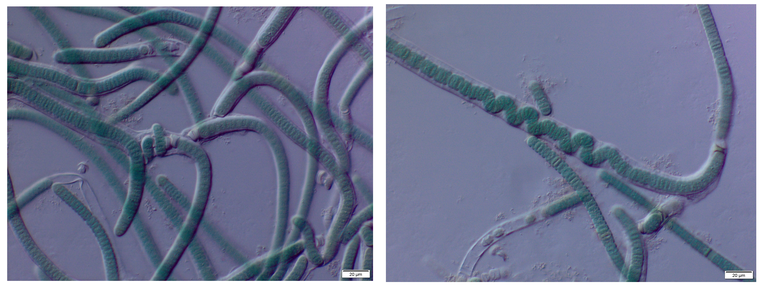

Fig. 1: Micrographs of a San Nicolas Island strain that has false-branching

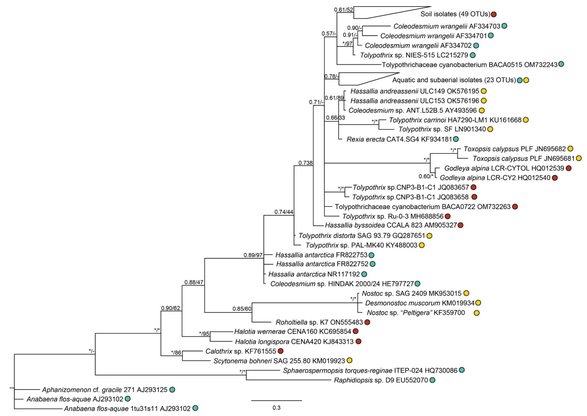

Fig. 2: Tolypothrichaceae tree with soil and aquatic groups collapsed in polygons

Fig. 3: Expanded soil clade

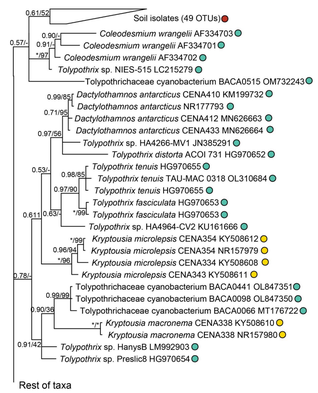

Fig. 4: Expanded aquatic/subaerial clade

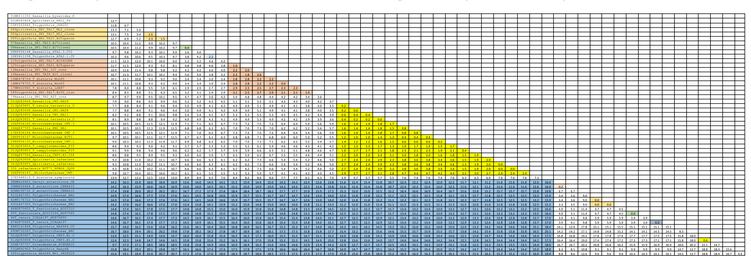

Table 1: Percent dissimilarity among ITS regions of Tolypothrichaceae. Taxa at top are from soils, taxa in same highlighting are same species. Taxa in blue are aquatic.

Results

- The phylogenetic analysis based on 16S rRNA sequences (Fig. 2) showed clear separation of the desert soil clade Tolypothrichaceae (top polygon, red circle) and the aquatic/wet subaerial clades of Tolypothrichaceae (second polygon, blue /yellow circles).
- The ecological signature of the expanded soil clade is very strong because all of the strains were from desert crusts (Fig. 3).
- The aquatic clade, when expanded, contains a mix of *Dactylothamnos, Kryptosia, Tolypothrix,* and Tolypothrichaceae cyanobacteria (Fig. 4).
- Molecular definition of cyanobacterial species has recently been achieved using a combination of phylogenetic analysis of aligned ITS regions as well as percent dissimilarity of ITS sequences (Table 1).
  - Dissimilarities below about 3.0% can be taken as evidence that strains are in the same species, while percent dissimilarities above 7% can be taken as strong evidence that strains are in different species. Dissimilarities between 3 and 7% are ambiguous, although recently values above 4 have been used to distinguish species with different ecologies or morphology as supporting evidence.
- Based on these criteria, it appears we have nine species of *Spirirestis* in the soil clade, including the type species *Spirirestis rafaelensis.* These strains need to all be reassigned to *Spirirestis* to make a monophyletic taxon.

Conclusion

The phylogenetic analysis performed in our study supports that the falsely-branched taxa on San Nicolas Island can be classified as *Spirirestis*. The phylogenetic tree also depicts that there is a clear separation between the soil and the aquatic clade. However, the family Tolypothrichaceae cannot be split into two families—one for the aquatic forms and one for the soil forms. While nine species need to be represented in *Spirirestis*, other members of the Tolypothrichaceae family clade need revision. The percent similarity of the 16S sequences for all strains in the family are very high and indicate that the Tolypothrichaceae should remain a single family.

Acknowledgements

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