# MYCOTAXON

Volume 114, pp. 395-399

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October–December 2010

## A phylogenetic study of Trechispora thelephora

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Abstract — Molecular data support the recent transfer of *Hydnodon thelephorus* to the genus *Trechispora*. These data also provide preliminary evidence that the pileate-stipitate basidiome morphology of *Trechispora thelephora* is ancestral to the resupinate morphology typical of the genus *Trechispora*. A photo, description, and line drawings of *Trechispora thelephora* are provided.

Key Words — Basidiomycota, nuclear large subunit, phylogeny

#### Introduction

*Trechispora thelephora* is a relatively common fungus that is widespread in the neotropics (Cifuentes et al. 2005, Ryvarden 2002). In spite of this and its rather striking morphology (FIG. 1), it has received relatively little attention from mycologists until recently. The basionym of *Trechispora thelephora* is *Hydnum thelephorum* (Léveillé 1844), but it was later placed in the monotypic genus *Hydnodon* (Banker 1913) where it remained for 89 years until Ryvarden (2002) proposed transferring it to the genus *Trechispora*.

Even though the micromorphology of *T. thelephora* corresponds very well to the genus *Trechispora*, the pileate-stipitate morphology of its basdiomata is unusual for this usually resupinate genus (FIG. 1a, b, c). Perhaps as a consequence of this, the nomenclatural history of *T. thelephora* is fairly complex. This has been reviewed by Cifuentes et al. (2005) and Ryvarden (2002), but molecular work would help understanding of the classification of this fungus. Our goals were to study the phylogenetics of *Trechispora thelephora*. We provide a description, photograph, and line drawings of this rarely illustrated taxon.



FIGURE 1. a) Pileate-stipitate basidiomata of *Trechispora thelephora* (UTC252606, Brad Kropp 13-Oct-02-23). Scale = 10 mm. b) Micromorphology of *Trechispora thelephora* showing section through aculeus (A), basidia (B), and basidiospores (S). Scale =  $10 \mu m$ .

### Material and methods

DNA was extracted from a basidiome of *T. thelephora* and sequence data was obtained for the portion of the nuclear large ribosomal subunit (nLSU) between primers LROR and LR5 (Moncalvo et al. 2000) and deposited in Genbank (HM104485). Sequences for additional taxa were downloaded from Genbank and aligned using ClustalX (Thompson et al. 1997). Taxon sampling included Gloeocystidiellum porosum (Berk. & M.A. Curtis) Donk, Tubulicium vermiferum (Bourdot) Jülich., and 12 members of the genus Trechispora. Tubulicium vermiferum was used as outgroup because it is sister to Trechispora according to Larsson et al. (2004). Gloeocystidiellum porosum, more distantly related to Trechispora (Larsson et al. 2004), was used to further polarize the crown group. A gap open of 5 and a gap extension of 1 for both pairwise and multiple alignment were used for the alignments. MrBayes 3.1 (Ronquist & Huelsenbeck 2003) was used to search tree space. All searches were performed using a time reversible model of evolution (Maddison 1994, Rodriguez et al. 1990) under the assumption of a discrete gamma distribution with six substitution types and some invariant sites (GTR+G+I). Posterior probabilities were approximated by sampling every hundred trees simulated using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method. All runs were conducted with eight active MCMCMC chains, heated at 0.2, and started with a neighbor-joining tree to avoid entrapment in a local minimum. All runs were



FIGURE 2. Phylogram derived from a Bayesian analysis of nLSU sequences from eleven *Trechispora* species. The phylogram has been rooted with *Gloeocystidiellum porosum*. Support measures are shown for nodes with posterior probability support of greater than 50 percent.

iterated for 1,000,000 generations. A majority consensus tree was calculated from the last 7000 trees from all runs to recover the posterior probabilities of the internal nodes using the sumt command in MrBayes. TreeView (Page 1996) was used to visualize the output from each simulation. Support measures for nodes with less than a 50% posterior probability support are not shown in Fig. 2.

Microscopical study and confirmation of the identity of our specimen was carried out using a light microscope after rehydrating sections in 10%  $\rm NH_4OH$ . Microscopical measurements were done using oil immersion at 1000× and line drawings were made with the aid of a drawing tube. The specimen from which DNA was extracted and from which the description and illustrations in FIG. 1 were made has been accessioned into the Intermountain Herbarium (UTC252606) at Utah State University.

#### Results

A BLAST search using nLSU sequence data obtained from our specimen of *Trechispora thelephora* matched other *Trechispora* sequences, supporting the proposal (Ryvarden 2002) placing the species within *Trechispora*. Results of the phylogenetic analysis (FIG. 2) of nLSU sequences from other *Trechispora* taxa also support this and provide preliminary evidence that the pileate-stipitate basidiome morphology of *T. thelephora* is ancestral to the resupinate morphology that is typical for most of the genus.

Although basidiome morphology in the *Holobasidiomycota* can apparently evolve either toward or away from complex pileate-stipitate forms, Hibbett & Binder (2002) indicate that the rate of change from resupinate toward pileate-stipitate forms exceeds the rate of change away from pileate-stipitate forms. A later study by Hibbett (2004) also supports an overall evolutionary trend in the *Holobasidiomycota* toward pileate-stipitate basidiomata, indicating that the ancestral form in this group is probably resupinate, even though results vary depending on the analytical method used.

The analysis of our sequence data with additional data from Genbank allows us to postulate that within *Trechispora* evolution has favored simplification of basidiome morphology and that the predominantly resupinate *Trechispora* basdiomata have evolved from a pileate-stipitate ancestral state. However, further work, perhaps including another pileate-stipitate species, *Trechispora gillesii* (Maas Geest.) Liberta (Liberta 1973), should be done to support this observation.

#### Trechispora thelephora (Lév.) Ryvarden, Synopsis Fung. 15: 32 (2002) FIG. 1

Basidiome pileate-stipitate, upper surface light yellow brown, glabrous or with appressed fibrils, divided into multiple irregular lobes 24–12 mm across; lower hymenial surface pinkish, lighter toward margins, finely hydnoid with teeth 1.0–0.5 mm in length, running part way down the stipe; hymenium drying soft with the subhymenial context drying hard and brittle. Stipe 5 mm wide  $\times$  20 mm tall, glabrous, concolorous with upper surface of basidiome or pallid near the base. Context pallid and not changing color when cut. Odor pleasant, fungoid. Spore print faint salmon. Hyphal system monomitic, hyphae of hymenial layer 2.0–3.5 µm wide, thin walled, with clamps, hyphae

of subhymenial context dense, with clamps, slightly thick walled (walls up to 0.5  $\mu$ m thick), 3.0–5.5  $\mu$ m wide. Basidia clavate, with four sterigmata and basal clamps, 15–23 × 5–6  $\mu$ m. Basidiospores ellipsoid, echinulate, 4.0–5.0 × 3.4–4.5  $\mu$ m.

Specimen examined — Belize. Cayo District, Las Cuevas Research Station, Brad Kropp 13-Oct-02-23 (UTC 252606).

#### Acknowledgments

We thank Dr. Karen Nakasone and Dr. Michael Castellano for reviewing our manuscript and providing valuable comments for improving the manuscript.

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