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SHORT COMMUNICATION

Transfer of two *Helicoma* species to *Troposporella* based on molecular and morphological data

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Abstract Previous molecular data demonstrated that, although *Helicoma* is polyphyletic, most species in the genus, including the type *Helicoma muelleri*, are in the *Tubeufiaceae*. Here, we use analysis of the small subunit and internal transcribed spacers (ITS) rDNA regions to show that two species, *H. monilipes* and *H. olivaceum*, are phylogenetically distant from the type, branching outside of the *Tubeufiaceae* near *Troposporella fumosa* in a basal clade of the *Dothideomycetes*. The phylogeny does not support the recent transfer of *T. fumosa* to *Helicoma*. Instead, based on the molecular evidence as well as the examination of cultures and type specimens, *Troposporella* is reinstated at the generic level, and *Helicoma monilipes* and *Helicoma olivaceum* are transferred to *Troposporella*. All three *Troposporella* species share the production of helicoid conidia borne on sporodochial conidiomata from blastic conidiogenous cells. A key to accepted species is provided.

Keywords Asexual fungi · *Hyphomycetes* · *Tubeufia*

Helicoma Corda is characterized by nonhygroscopic helically coiled conidia with thick conidial filaments (Linder 1929; Goos 1986, 1987). Previous research based on small subunit (SSU) and large subunit (LSU) rDNA showed that *Helicoma* was polyphyletic and none of the sections within the genus were monophyletic (Tsui and Berbee 2006; Tsui et al. 2006). Most *Helicoma* species clustered with *Helicomyces* and *Helicosporium* and belonged to the *Tubeufiaceae sensu stricto* (Tsui and Berbee 2006). However, two species from section *monilipes*, *Helicoma monilipes* Ellis & L.N. Johnson and *H. olivaceum* (P. Karst.) Linder, were only distantly related to the *Tubeufiaceae sensu stricto* and formed instead a basal lineage in *Dothideomycetes* (Tsui and Berbee 2006). These two species clustered with 100% bootstrap with *Troposporella fumosa* P. Karst. Further complicating the picture, *T. fumosa* had recently been transferred to *Helicoma* as *Helicoma fumosa* (P. Karst.) G.Z. Zhao, Xing Z. Liu & W.P. Wu (Zhao et al. 2007).

If *Troposporella fumosa*, *Helicoma monilipes* or *H. olivaceum* belong in *Helicoma*, they should form a monophyletic group with *H. muelleri* Corda, the type species of the genus. As a formal test of this relationship, we determined the SSU sequence for *H. muelleri* and aligned it with the sequences from fungi mentioned above and with other members of *Tubeufiaceae* and *Dothideomycetes*. We determined the internal transcribed spacers (ITS)1 and 2, including the 5.8S rDNA sequences for *T. fumosa*, *H. monilipes*, and *H. olivaceum*, to estimate the genetic distances among them. We examined the type and authentic specimens of *H. monilipes* and *H. olivaceum*, and compared their mode of conidiogenesis with *Troposporella*. Our final goal in this paper was to bring the taxonomy of *T. fumosa*, *H. monilipes*, and *H. olivaceum* into line with their phylogeny.

Cultures of *Helicoma monilipes*, *Helicoma olivaceum*, and *Troposporella fumosa* were requested from Centraalbureau voor Schimmelcultures (CBS) and Mycothèque de

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Table 1 Cultures and specimens of *Troposporella* species included in this study (Ellis 1971; Goos 1986)

	T. fumosa	*T. monilipes*	*T. olivaceum*
Culture/specimen	MUCL 15695	MUCL 19867/ FH 79123	CBS 728.83/co-type material from FH
Conidiophore (μm)	100 × 3–5	10–55 × 2–4	10–30 × 2–3
Conidia color	Golden brown	Dark brown	Yellowish brown
Width of conidial filament (μm)	3–5	3.5–5	3.5–5.5
Number of septa	7–15	7–15	3–12

l'Université catholique de Louvain (MUCL) (Table 1). They were maintained on the recommended media, and sporulation was induced as described in Tsui and Berbee (2006). Type or authentic specimens of *H. monilipes* and *H. olivaceum* were also borrowed from Farlow Herbaria, Harvard (FH) for examination (Table 1). We attempted to include the materials of *T. hyalospora* P. Rag, Rao & D. Rao in this investigation but neither specimen nor culture was available.

DNA extraction was performed as described in Tsui and Berbee (2006). The SSU genes were amplified with SL1 and CITS5, while ITS regions were amplified with primers ITS1F and ITS4 (White et al. 1990). Briefly, 12.5 μl diluted DNA was amplified in a total volume of 25 μl using puReTaq™ Ready-To-Go™ polymerase chain reaction (PCR) beads (Amersham Biosciences Corp., Piscataway, NY, USA) with 10 μl H_2O and 1.25 μl of each of the two primer pairs (10 μM). Amplification was carried out in a GeneAmp® PCR system 9700 (Applied Biosystems, Foster City, CA, USA) with 5 min of denaturation at 95°C, 35 cycles of 95°C for 20 s, 52–55°C for 30 s, and 72°C for 40 s, plus final extension at 72°C for 7 min. The PCR product was purified and the sequencing reactions were carried out using ABI PRISM® BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) (Tsui and Berbee 2006). The sequence fragments were assembled using ABI PRISM® AutoAssembler™ v.1.4 (Applied Biosystems). GenBank accession numbers of the new sequences are DQ351723–DQ351725.

The sequences were aligned with Se-Al v.1 d1 (Rambaut 1999) and analyzed using PAUP* 4.0b10 (Swofford 2003). The SSU alignment contained 58 taxa, including the sequence of *H. muelleri* (type species of *Helicoma*), and was deposited in TreeBASE (SN4534-22842). The ITS alignment contained *Troposporella fumosa*, *T. monilipes*, and *T. olivaceum*, with *Helicoon fuscosporum* Linder as outgroup (Tsui and Berbee 2006). Maximum-parsimony (MP) analyses were conducted using a heuristic search with 100 random-sequence addition replicates, tree bisection-reconnection branch-swapping algorithms, and MAXTREE set to 100. All characters were equally weighted and unordered, and gaps were treated as missing data. Bootstrap support for the branches was based on 1000 MP replicates with a single sequence addition replicate for each bootstrap replicate. A neighbor-joining (NJ) tree was also constructed using the Jukes–Cantor model as implemented in PAUP* 4.0b10, and the bootstrap support was also estimated based on 1000 replicates. A pairwise sequence comparison was performed.

Maximum-parsimony analysis of the SSU dataset resulted in a hundred equally parsimonious trees of 1076 steps (CI = 0.60, RI = 0.795, RC = 0.479) (Fig. 1). Out of 1714 characters, 1212 characters were constant and 328 were parsimony informative. A tree from neighbour joining gave the same topology. *Helicoma monilipes* and *Troposporella fumosa*, together with *H. olivaceum*, clustered together with 100% bootstrap support in *Dothideomycetes* (Fig. 1). This cluster formed a sister relationship to a clade with *Helicoon fuscosporum* and *Spilocaea oleaginea* (Castagne) S. Hughes. Molecular data also confirmed the placement of *Helicoma muelleri* in the *Tubeufiaceae s. str.* containing representatives of helicosporous fungi and closely related sexual relatives. Although Tuebufiaceae and *Troposporella* belong to the *Dothideomycetes*, they do not form a monophyletic group.

Troposporella was established to accommodate asexual fungi producing holoblastic, helicoid conidia from sporodochial conidiomata (Ellis 1971). Two former species, *T. monospora* (W.B. Kendr.) M.B. Ellis and *T. rigidospora* R.F. Castañeda & W.B. Kendr., had been transferred to *Slimacomyces* Minter and *Troposporopsis* S.R. Whitton, McKenzie & K.D. Hyde, respectively (Minter 1986; Whitton et al. 1999). Representatives of *Troposporella* share morphological similarity to *Helicoma* in having thick conidial filaments borne on macronematous conidiophores with blastic conidiogenous cells (Goos 1987). However the conidia in *Troposporella* do not secede readily, while those in *Helicoma* are borne on denticulate conidiophores and secede schizolytically (Kendrick 2003).

Although *Troposporella fumosa* (MUCL 15695) did not sporulate readily in culture and we were not able to examine the type specimen, its culture characteristics match the description of the species (Ellis 1971, 1976). Morphologically the placement of *H. monilipes* and

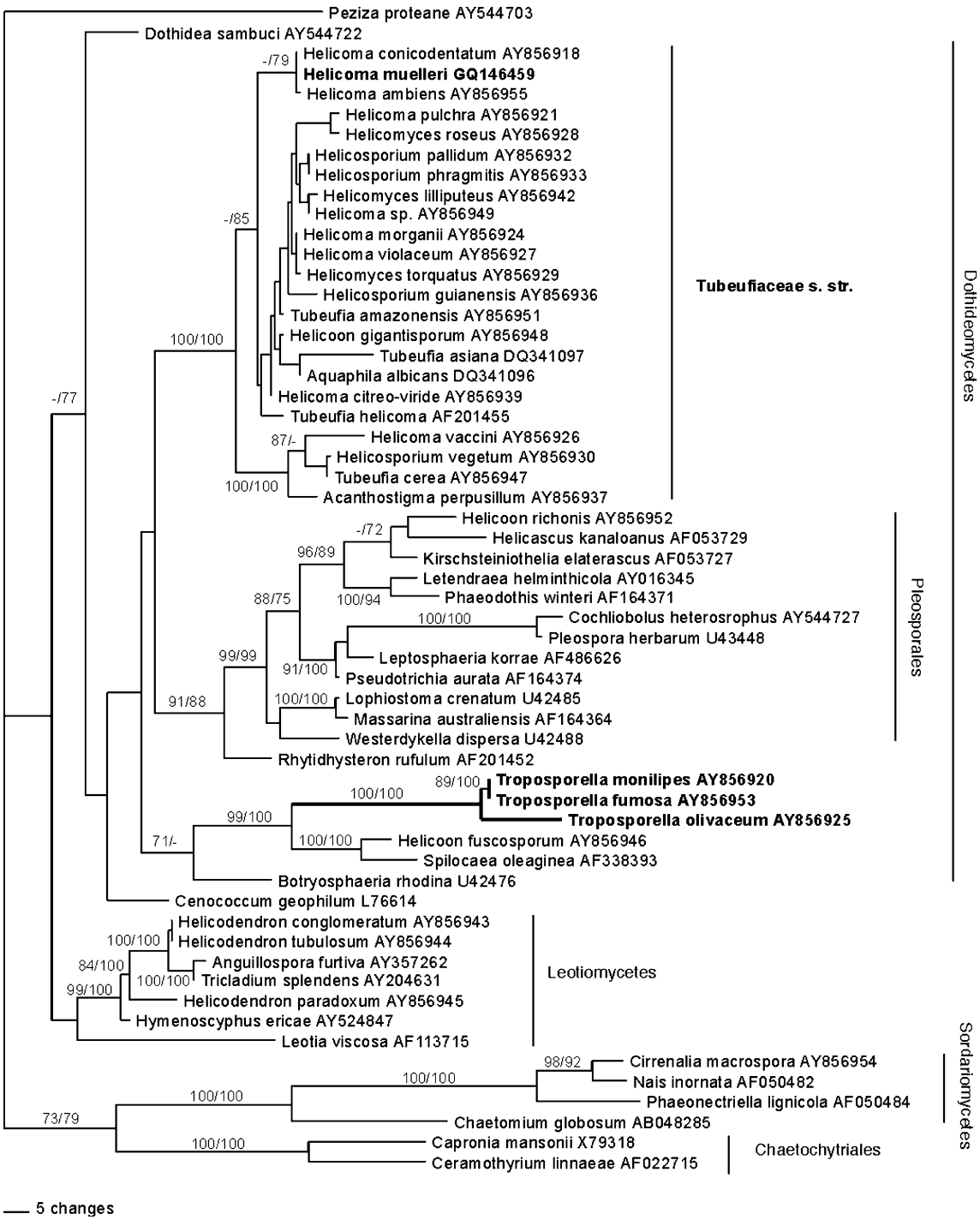
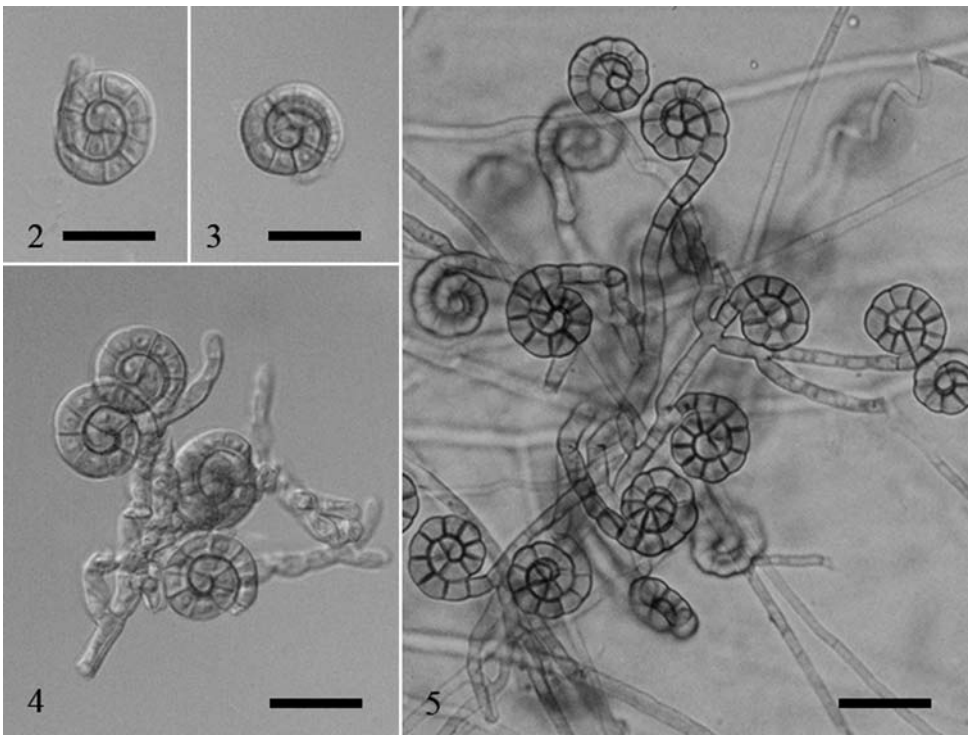


Fig. 1 A parsimony tree from ribosomal SSU data supports the transfer of *T. monilipes* and *T. olivaceum* from *Helicoma* to *Tropsporella*. *Tropsporella fumosa*, *T. monilipes*, and *T. olivaceum* are only distantly related to *Helicoma muelleri*, the *Helicoma* type species. Bootstrap percentage values (>70%) generated from 1000 replicates from MP and NJ are shown above the branches

H. olivaceum in *Tropsporella* is appropriate, as circumscribed in Ellis (1971, 1976). Both species produce macronematous, frequently branched conidiophores that form punctiform conidiomata on natural substrata. In contrast, the conidiophores of *Helicoma* are erect, unbranched, and often solitary (Goos 1986).

Taxonomy

Tropsporella is returned to generic rank and the names of type species, *Tropsporella fumosa* (Linder 1929) and a second species *T. hyalospora* (Rao and Rao 1964), are reinstated. Additional combinations include the following:



Figs. 2–5 Interference contrast micrographs of *Troposporella*. **2–3** Conidia of *T. monilipes* (FH79123). **4** Squash mount of conidia and conidiophores of *T. monilipes* (FH79123). **5** Conidia and conidiophores of *T. olivaceum* with monoblastic conidiogenous cells (CBS 728.83) *Bars* **2–4** = 20 μm, **5** = 25 μm

Troposporella monilipes (Ellis & L.N. Johnson) K.M. Tsui & Berbee **comb**. **nov**. Figs. 2–4

Basionym: *Helicoma monilipes* Ellis & L.N. Johnson, Proc. Acad. Nat. Sci. Phila. p. 376: 1894

=*Helicosporium monilipes* (Ellis & L.N. Johnson) Sacc. Syll. Fung. 11:369, 1895

Material examined. USA: Michigan, Ann Arbor, on Quercus (FH 79123—L.N. Johnson No. 666) on decayed wood, Lovell, Maine (KIRI—J. Legg).

Troposporella olivaceum (P. Karst.) K.M. Tsui & Berbee **comb**. **nov**. Fig. 5

Basionym: *Helicopsis olivaceus* P. Karst., Rev. Mycol. 11:96, 1889.

=*Helicopsis punctata* Peck. N.Y. State Mus. Bull. 167:26, 1912.

=*Helicoma olivaceum* (P. Karst.) Linder. Ann. Mo. Bot. Gard. 16:302, 1929.

Material examined. USA: on bark of *Prunus*. April 1911, Fairview, New York (FH—co-type).

Key to species of *Troposporella* based on literature (Linder 1929; Rao and Rao 1964; Goos 1986)

1. Conidiophores strongly constricted at septa, conidia golden brown, 12–16 μm in diameter, filament 4–4.5 μm thick…*T. fumosa*

1. Conidiophores smooth cylindrical, conidia different from above…2

2. Conidia subhyaline, 10–14.5 μm in diameter, filament 3–4.8 μm thick… *T. hyalospora*

2. Conidia mostly brown…3

3. Conidia yellowish brown, 11–16 μm in diameter, filaments 3.5–5.5 μm thick…*T. olivaceum*

3. Conidia dark brown, 12–19 μm in diameter, filaments 3.5–5 μm thick…*T. monilipes*

Notes. We also determined the ITS sequences for *Troposporella fumosa*, *T. monilipes*, and *T. olivaceum*; the results from BLASTN revealed no closely related taxa from *Tubeufiaceae*, confirming they are divergent from *Helicoma* representatives.

Maximum-parsimony analysis of the ITS data set containing *T. fumosa*, *T. monilipes*, and *T. olivaceum* with *Helicoon fuscosporum* as the outgroup resulted in 1 tree that required 179 steps (CI = 0.98, RI = 0.81, RC = 0.80) (not illustrated). Out of 624 characters, 469 were constant and 16 characters were parsimony informative. *Troposporella monilipes* was closest to *T. fumosa*, with 97% sequence identity through pairwise comparison of the ITS sequences. *T. olivaceum* differed from *T. fumosa* and *T. monilipes* by 9% and 7.8%, respectively.

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