Mycoscience (2007) 48:274–281
DOI 10.1007/s10267-007-0364-6

FULL PAPER

Kazuhiko Narisawa · Sarah Hambleton
Randolph S. Currah

Heteroconium chaetospira, a dark septate root endophyte allied to the Herpotrichiellaceae (Chaetothyriales) obtained from some forest soil samples in Canada using bait plants

Received: June 30, 2006 / Accepted: April 23, 2007

Abstract During an extended search in Western Canada for fungal root endophytes useful as biocontrol agents against soil-borne pathogens, we isolated *Heteroconium chaetospira*, as well as *Phialocephala fortinii* or similar taxa, from seven samples of forest soil using herbaceous seedlings of four different species (i.e., barley, Chinese cabbage, eggplant, and melon) as bait plants. Our results support a previous observation that eggplant is a particularly effective species for baiting *H. chaetospira* from soil and confirm the ability of this fungus to grow as an endophyte in the roots of axenically reared host plants. Cultural characters show that this species is similar to *P. fortinii* and other melanized fungi in the dark septate endophyte (DSE) group (e.g., *Leptodontidium orchidicola*, *P. sphaeroides*, and *Cadophora finlandica*) in that it produces darkly pigmented colonies on agar media. *Heteroconium chaetospira* differs from *P. fortinii* and other melanized members of the Leotiomycetes in the DSE group in that its conidia are fusiform and develop in blastic acropetal chains. *Heteroconium chaetospira* is phylogenetically distant from most DSE taxa because DNA sequences for the nuclear small subunit (SSU) ribosomal RNA gene (rDNA) indicate that the taxon is affiliated with the Herpotrichiellaceae of the Chaetothyriales rather than with the Leotiomycetes.

Key words *Capronia* · Dark septate endophyte (DSE) · ITS · rDNA · *Septonema* · SSU

K. Narisawa (✉)
College of Agriculture, Ibaraki University, 3-21-1 Chuo, Ami, Inashiki, Ibaraki 300-0393, Japan
Tel. +81-29-888-8667; Fax +81-29-888-8525
e-mail: kan-moc@mx.ibaraki.ac.jp

S. Hambleton
Biodiversity (Mycology and Botany), Environmental Health Program, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada

R.S. Currah
Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada

Introduction

Dark septate root endophytes (DSE) are dematiaceous fungi that occur with some regularity in the roots of apparently healthy plants, where they often form distinctive intracellular structures (e.g., microsclerotia). These fungi and their associations with roots are circumboreal in distribution (Jumpponen and Trappe 1998) but may be particularly abundant in cold- or nutrient-stressed habitats (Richard and Fortin 1974; Addy et al. 2005). When isolated in pure culture, colonies of DSE are generally nondescript, olivaceous to brown or almost black, and often lack conidia or other taxonomically distinctive characteristics. For this reason they are often left unidentified in routine surveys (Mandyam and Jumpponen 2005). Although analyses of rDNA sequences have shown that the fungi informally labeled as DSE comprise a phylogenetically diverse group of taxa, taxonomic characterization of this diversity has proceeded slowly.

Among taxa that have sporulated in culture are *Phialocephala fortinii* C.J.K. Wang & H.E. Wilcox, *P. sphaeroides* B.J. Wilson, *Cadophora finlandica* (C.J.K. Wang & H.E. Wilcox) T.C. Harr. & McNew (as "*finlandia*"), and *Leptodontidium orchidicola* Sigler & Currah. DNA sequence comparisons indicate that the four species just mentioned have affinities to teleomorph taxa within the Leotiomycetes (Jumpponen and Trappe 1998; Harrington and McNew 2003; Wilson et al. 2004). *P. fortinii* is the most commonly reported representative of this group.

Heteroconium chaetospira (Grove) M.B. Ellis (= *Septonema chaetospira* (Grove) Hughes) is a dematiaceous hyphomycete reported from wood, arthropod droppings, soil, and roots of plants collected from their natural habitats in Canada, Europe, Japan, and Russia (Hughes 1952; Ellis 1976; Domsch et al. 1980; Wilson et al. 2004) as well as from the roots of herbaceous species grown as bait plants in field soils collected in Japan (Narisawa et al. 1998, 2002). Colonial morphology resembles that of previously described species of DSE but it is distinctive in forming blastic conidia in acropetal chains. Despite its unique microscopic features,

reports of its occurrence in roots or soil are relatively uncommon, and very little is known about its distribution and phylogenetic relationships. This factor, plus its potential for use as a biocontrol agent for soil-borne diseases (Narisawa et al. 2000), prompted us to examine several sites in western Canada to obtain additional isolates of this fungus.

Our study had three objectives. The first was to isolate and identify *Heteroconium chaetospira* from western Canadian soils by using easily grown, annual species as bait plants. The second was to confirm the ability of these new isolates to colonize axenically reared host plants, and the third was to determine the phylogenetic position of the taxon and its relationship to other DSE taxa.

Materials and methods

Soil samples were collected from July to September in 1999 and 2000 from seven sites (Table 1) in Alberta and British Columbia. Each site had a mature overstory of conifers. Sites in the vicinity of Athabasca, Banff, and Jasper were dominated by *Picea glauca* (Moench) Voss, the site near Jackman Flats by *Pinus contorta* Loudon, and the site near Tofino by *Thuja plicata* D. Don. Ericaceous plants were common understory species at each location. From each site, three soil samples, approximately 200 ml each, were taken from within 20 cm of the soil surface and within 1 m of each other, placed in polyethylene bags, and stored at 4°C for up to 1 month. Soil samples from each site were combined and mixed with 2 l autoclaved potting soil (Altwin Distributors, Alberta, Canada) to prepare a composite soil for baiting fungal root endophytes (Narisawa et al. 2002). Three axenically grown seedlings of barley (*Hordeum vulgare* L. var. *hexastichon* Asch.), Chinese cabbage (*Brassica campestris* L.), eggplant (*Solanum melongena* L.) and melon (*Cucumis melo* L.) were transplanted into 200-ml pots containing the composite soil. Three replicate pots for each species were made. After 2 months, the roots of each species from each series of replicates were washed free of coarse debris and cut into 1-cm segments. Thirty segments from each species were chosen at random, washed three times in a 0.005% solution of Tween 20 three times, rinsed three times in distilled water, air-dried overnight, and then plated on nutrient agar containing 25 g corn meal (infusion form, Difco) and 15 g Bacto agar (Difco) in 1 l distilled water (Narisawa et al. 1998). Fungal isolates were identified on the basis of cultural and microscopic morphology. Cultural characteristics were further assessed based on pure cultures grown at room temperature on 6-cm Petri dishes containing oatmeal agar [OMA; 10 g oatmeal, 18 g Bacto agar (Difco), 1 g $MgSO_4 \cdot 7H_2O$, 1.5 g KH_2PO_4, and 1 g $NaNO_3$ per liter distilled water], potato dextrose agar (PDA; Difco), and half-strength cornmeal-malt-yeast extract agar [1/2 CMMY, 25 g corn meal (infusion form, Difco), 15 g Bacto agar (Difco), 10 g malt extract (Difco), 2 g yeast extract (Difco), all per 1 l distilled water]. We considered all isolates of the fungus to be similar morphologically; therefore, 25 conidia of UAMH 10312, isolated from a melon bait plant in soil from Alberta and photographed at random from slide mounts made from 6-week-old PDA cultures, were selected, measured, and the mean and standard deviations calculated. All conidia measured were free and floating unattached on the slide. Widths were measured at the widest point. Comparisons were made with herbarium material deposited in DAOM (National Mycological Herbarium, Ottawa, Canada) for *H. chaetospira* DAOM 59800 (two slide preparations made from IMI 29316c) and *Septonema chaetospira* (Grove) Hughes var. *pini* Bourch. DAOM 63760 (a dried culture of the type strain).

For inoculation studies, three axenically grown seedlings of barley, Chinese cabbage (each 3 days postgermination), eggplant, or melon (each 7 days postgermination) were aseptically introduced into 2-week-old cultures of all 43 *H. chaetospira* isolates on OMA in 6-cm Petri dishes. Seedlings were placed directly on the mycelial mat or on uninoculated media to serve as controls. They were incubated in a growth chamber at 23°C under a 16-h photoperiod (150 mol $m^{-2} s^{-2}$) for 2 weeks. After 14 days, all seedlings were transplanted to 9-cm plastic pots containing 200 ml sterile potting soil and grown in the greenhouse at 20°–25°C (approximately 180 mol $m^{-2} s^{-2}$). Two months later, roots of three randomly selected plants of each treatment were excised, washed, cut

Table 1. Frequencies for the recovery of *Heteroconium chaetospira* (HC) and *Phialocephala fortinii*-like (PF) fungi from root segments of bait plants grown in soil samples from conifer-dominated woodlands in western Canada in 1999 and 2000

Collection locations, year, and dominant overstory species	Total number of root segments (%)[a]							
	Barley		Chinese cabbage		Eggplant		Melon	
	HC	PF	HC	PF	HC	PF	HC	PF
Banff (1999): *Picea glauca*	0	0	0	6 (20)	2 (6.7)	0	2 (6.7)	4 (13)
Banff (2000): *Picea glauca*	0	0	0	1 (3.3)	6 (21)	11 (39)	0	0
Jasper (1999): *Picea glauca*	0	3 (10)	1 (3.3)	6 (20)	1 (3.3)	6 (20)	0	0
Jasper (2000): *Picea glauca*	0	0	0	9 (30)	9 (30)	7 (23)	0	3 (10)
Athabasca (2000): *Picea glauca*	3 (10)	5 (17)	4 (13)	5 (17)	2 (6.7)	7 (23)	0	13 (43)
Jackman Flats (2000): *Pinus contorta*	4 (13)	7 (23)	4 (13)	9 (30)	2 (6.7)	6 (20)	1 (3.3)	1 (3.3)
Tofino (2000): *Thuja plicata*	0	0	0	1 (3.3)	2 (6.7)	0	0	0
Total numbers of root segments yielding HC and PF	7	15	9	37	24	37	3	21

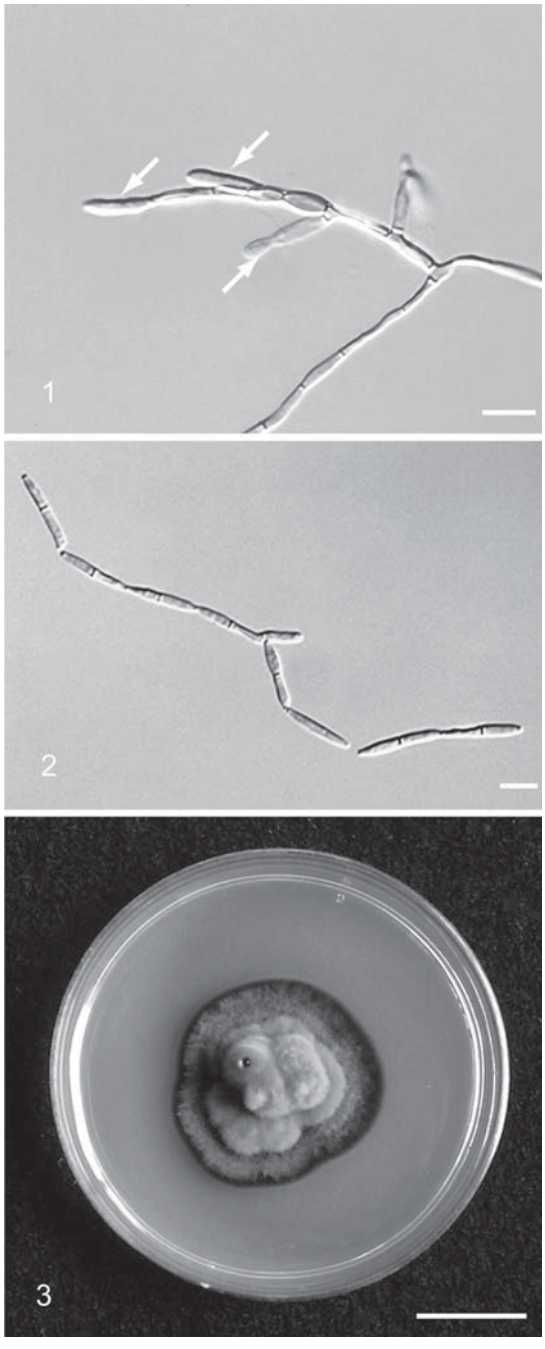
[a] Number of root segments (of 30) from which HC or PF was isolated/total number of root segments plated ×100

into 2-cm segments, stained with 0.005% cotton blue in 50% acetic acid, and observed under an Olympus BX50 microscope with UPlanFI40/0.75 and UPlanFI100/1.30 objectives.

DNA sequencing and phylogenetic analyses

Sequences for portions of the nuclear ribosomal RNA gene were determined for *L. orchidicola* (UAMH 8152), isolated from roots of *Pedicularis bracteosa*), three strains of *H. chaetospira* isolated in this study, BPM3 (deposited in the University of Alberta Microfungus Collection and Herbarium, Edmonton, Canada as UAMH 10312), BHM2, and J1HE1, and a fourth strain of *H. chaetospira* (UAMH 10418) isolated from roots of *Aralia nudicaulis* in another study from Alberta, Canada (Wilson et al. 2004). Genomic DNA was extracted from mycelium grown on PDA. DNA extraction methods, polymerase chain reaction (PCR) reaction parameters, automated sequencing protocols, and the primers used in the reactions were as outlined in Hambleton et al. (2005). To assess conspecificity of the strains of *H. chaetospira*, internal transcribed spacer (ITS) sequences were compared. To infer the phylogenetic placement of the species, small subunit (SSU) data for UAMH 8152, 10312, and 10418 were manually aligned with 68 sequences retrieved from GenBank, chosen from eight classes and one order (Mycocaliciales, *incertae sedis*) of Pezizomycotina. The alignment is based on one previously deposited in the online database TreeBASE (study accession no. S1395; Hambleton et al. 2005), with more representatives of the Chaetothyriomycetes and fewer of the Sordariomycetes included. Data for three additional DSE fungi were included: *P. fortinii*, *P. sphaeroides*, and *C. finlandica*. The outgroup consisted of three species of Taphrinomycotina or Saccharomycotina. All GenBank sequences were complete or nearly complete for the SSU region (>1600 nt), except for *Cadophora finlandica*, for which only one SSU sequence of 951 nt was available (deposited under the name *Phialophora finlandia* C.J.K. Wang & H.E. Wilcox). Classification follows Eriksson et al. (2004).

The data matrix was subjected to parsimony analysis using the heuristic search option of PAUP* version 4.0b10 (Swofford 1999) with simple stepwise addition of taxa, tree bisection-reconnection (TBR) branch swapping, and gaps treated as missing data. Bootstrap percentages used to assess support for the branching topologies were determined from 1000 resamplings of the data set using the "fast" stepwise-addition search option.



Figs. 1–3. Microscopic and cultural morphology of *Heteroconium chaetospira*, UAMH 10312 (BPM3), from the roots of melon bait plants grown in soil collected in Banff. **1, 2** Blastoconidia (*arrows*) arising from conidiophores (**1**) and typical acropetal conidial chains (**2**) of *H. chaetospira* (UAMH 10312). **3** Cultural morphology of *H. chaetospira* UAMH 10312 on potato dextrose agar (PDA) at 23°C for 4 weeks. *Bars* **1, 2** 6 µm; **3** 1.5 cm

Results

Colonies of *H. chaetospira* were detected growing from root segments of bait plants 2–3 weeks following their placement on agar media. In pure culture, colonies on all media were olivaceous-brown and powdery and grew slowly, reaching an average of 22 mm in diameter after 30 days at 23°C (see Fig. 3). Microscopic examination confirmed identifications made on the basis of colony characteristics on all three media: all cultures of *H. chaetospira* produced single acropetal chains of conidia from the apices of the conidiophores. Conidia were fusiform, sometimes slightly curved, pale brown when mature, smooth, 0–3-septate, 16–25 × 2.5–4 µm (Figs. 1, 2). Conidium measurements for UAMH 10312 were 0-septate 10–15.7 × 2.0–3.1 µm (mean ± SE = 12.4 ± 0.3

× 2.5 ± 0.1), 1-septate 11.4–18.1 × 2.3–3.4 µm (mean ± SE = 15.1 ± 0.4 × 2.9 ± 0.1), 2-septate 13.2–24.1 × 2.4–3.6 µm (mean ± SE = 17.8 ± 0.6 × 2.9 ± 0.1), 3-septate conidia 23 × 3 µm, based on only a few observations. These measurements were similar for UAMH 10418 and DAOM 63760 (ex type of *Septonema chaetospira* var. *pini*) but were consistently smaller than those for DAOM 59800 (*Heteroconium chaetospira*), which ranged from 17.5–27 × 2.2–2.7 µm for 0-septate conidia to 20.2–32.5 × 3–4 µm for 3-septate conidia.

One colony per root segment was used to establish a pure culture, resulting in the recovery of 43 isolates of *Heteroconium chaetospira* in total. Colonies resembling *P. fortinii* in growth rate and vegetative characteristics (i.e., having blistered hyphae and abundant toruloid cells) were more frequently isolated than *H. chaetospira* from soils across all four bait plant species, with the exception of the eggplant-baited soil from the Jasper site collected in 2000, from which 9 isolates of *H. chaetospira* and 7 isolates of *P. fortinii*-like fungi were obtained (see Table 1). The soil sample from the Tofino site yielded the fewest number of isolates of either *H. chaetospira* (2) or *P. fortinii*-like fungi (1).

Isolates of *H. chaetospira* were recovered from at least one bait plant species per soil sample and from each of the seven collecting sites. The detection rate varied from 0 to 9 (from 30 segments) per bait plant species per sample. Eggplant root segments yielded the most isolates (24 from 210 plated roots), with at least 1 from each site, and melon segments yielded the least (3), with only two sites represented (see Table 1). Three isolates were deposited in UAMH as living cultures: 10312 (BPM3) from a melon bait plant in soil from the Banff site (1999), and 10313 (BC1HB1) from a barley bait plant and 10314 (BC2HE2) from an eggplant bait plant, both grown in soils collected near Jackman Flats. Nine additional isolates are in the personal collection of K.N. and include J1HE1 (Jasper, 1999), BHE2 (Banff, 1999), and BC2HE2 (Jackman Flats), all from eggplant; BC1HB1, BC2HB1, BC2HB2, and BC2HB3 (Jackman Flats) from barley; and BHM2 and BPM3 (Banff, 1999) from melon.

Isolates of *P. fortinii*-like fungi, as well as *Cadophora finlandica* and *Meliniomyces variabilis* Hambleton and Sigler, were also recovered from at least one bait plant species per soil sample and from each of the seven collecting sites. The detection rate varied from 0 to 13 per bait plant species. Eggplant and Chinese cabbage root segments yielded the most isolates (37 each) with at least 1 from each site, and barley segments yielded the least (3) with only three sites represented (see Table 1). Isolates of these taxa are maintained by K.N., and more details concerning these fungi will be reported elsewhere.

Roots of plants inoculated with *H. chaetospira* had varying levels of colonization depending on isolate and plant species, but all isolates showed typical endophytic root colonization as previously reported (Narisawa et al. 1998). For example, in plants inoculated with UAMH 10312, hyphae were abundant over the root surface, including root tips, and penetrated the cells of the epidermis and exodermis as well as cells of the inner cortex. Intracellular hyphae developed in cortical cells and along the root axis up to and including root tips.

DNA sequencing and phylogenetic analyses

The ITS sequences determined for *H. chaetospira* from this study (UAMH 10312, BHM2, and J1HE1) were 543 base pairs long and 99% similar to each other, with differences among the sequences at only three positions in the ITS2 spacer region (Fig. 4). UAMH 10418, from the Wilson et al (2004) study, was more divergent but still closely related at 97% similar (Fig. 4). These data supported the identification of all four isolates as *H. chaetospira* based on morphological characters. Small subunit (SSU) sequences determined for *H. chaetospira* [UAMH 10312, 10418, and *L. orchidicola* (UAMH 8152)] were 1742 and 1715 nt, respectively. Data for the two strains of *H. chaetospira* differed only at position 585 (T for UAMH 10312, C for UAMH 10418). The GenBank accession numbers for the SSU-ITS sequences are DQ521603 (*L. orchidicola*), DQ521604 (*H. chaetospira* UAMH 10312), and DQ521605 (*H. chaetospira* UAMH 10418). The SSU data matrix comprised 71 taxa and 1745 aligned characters. Twenty-six ambiguously aligned characters were excluded. Of the remaining characters, 1109 were constant, 170 were parsimony uninformative, and 440 were parsimony informative. Parsimony analysis resulted in eight equally parsimonious trees (MPTs) of 1791 steps with a consistency index (CI) of 0.482 and a retention index (RI) of 0.735. Results of a bootstrap analysis are shown on one MPT, and branches not retained in the strict consensus of all eight trees are indicated by an asterisk (Fig. 5).

Heteroconium chaetospira grouped within the well-supported clade corresponding to the *Chaetothyriomycetes* (100% bootstrap support), while the four other DSE fungi, *L. orchidicola*, *P. fortinii*, *P. sphaeroides*, and *C. finlandica*, were allied to the Leotiomycetes. Relationships among the fungi within each of these two clades were not well resolved, although there was low support for the grouping of *H. chaetospira* with the GenBank data for *Capronia semi-immersa* (Cand. & Sulmont) Unter. & F.A. Naveau, *Cladophialophora bantiana* (Sacc.) de Hoog, Kwon-Chung & McGinnis, and *Cladophialophora devriesii* (A.A. Padhye & Ajello) de Hoog et al.

Discussion

The ability of *H. chaetospira* to colonize and grow as an endophyte in healthy roots has been demonstrated previously using Chinese cabbage (Ohki et al. 2002; Yonezawa et al. 2004; Hashiba and Narisawa 2005) and rhododendron (Usuki and Narisawa 2005); these studies were based on isolates obtained from soil samples from Japan baited with herbaceous plants. There were prior reports of this species from the roots of *Ammophila arenaria* (L.) Link collected in the UK (IMI 60911) (Domsch et al. 1980) and a smaller-

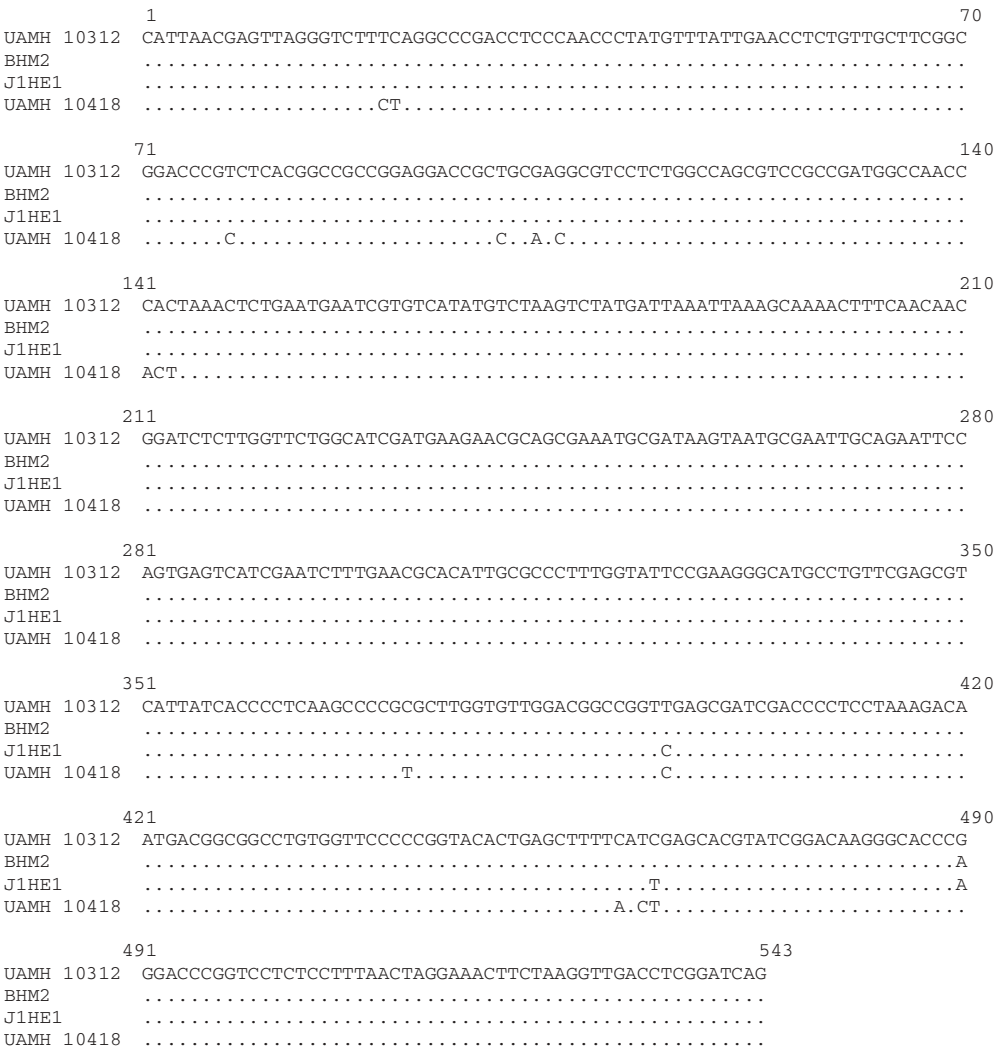


Fig. 4. The complete internal transcribed spacer (ITS) sequences determined for four isolates of *H. chaetospira* are aligned to illustrate the high level of similarity among them. A dot (.) indicates the nucleotide for the sequence in that position is identical to the reference sequence (UAMH 10312) at the same position. Nucleotide position numbers are given at the start and end of each row. The ITS1/5.8S/ITS2 regions are flanked at the 5′-end by small subunit (SSU) data (positions 1–5) and at the 3′-end by 28S data (positions 527–543)

spored variety, described by Bourchier as *Septonema chaetospira* var. *pini* (Bourchier 1961) from heartwood of living *Pinus contorta*, was reported from the roots of *Picea mariana* (Mill.) BSP. collected in Ontario (Summerbell 1989). Our isolates are identified as *S. chaetospira* var. *pini* based on comparisons with herbarium specimens of both varieties. The type of *H. chaetospira* is known from Britain and is reported to have larger conidia (20–35 × 3–4 µm), while our isolates and the variety described by Bourchier (1961), all from Canada, had a maximum conidium length of 25 and 23 µm, respectively, even for the 3-septate conidia. Bourchier (1961) noted that the conidia of *S. chaetospira* var. *pini* varied in size when the fungus grew on different substrates (i.e., 7.5–18 × 3–5 µm on malt agar and 9.5–23 × 2–3.5 µm when grown on sterilized twigs). Conidium size ranges for those grown on twigs were given for each level of septation, and they corresponded well to our data for UAMH 10312 on PDA. *S. chaetospira* var. *pini* was not formally transferred to *Heteroconium* with the type variety by Ellis (1976), and for the purposes of discussion here, our isolates will be referred to provisionally as *H. chaetospira*. *S. chaetospira* var. *pini* needs further study before a formal proposal is made for redisposition in *Heteroconium*.

Heteroconium chaetospira may be a more commonly occurring fungal endophyte in roots than previous data indicate, but the frequently reported DSE species, i.e., *P. fortinii* and *L. orchidicola*, have faster growth rates (colony diameters > 80 mm after 30 days on PDA for both *P. fortinii* and *L. orchidicola*; Currah et al. 1987) than *H. chaetospira* (up to 22 mm after 30 days on PDA), making the latter more difficult to detect and isolate in routine surveys of microfungi associated with roots. The use of annual plants as bait for *H. chaetospira* is an effective method for recovering this species from woodland soils, but species may differ in their efficacy. Narisawa et al. (2002) noted that eggplant roots seemed particularly effective as bait for *H. chaetospira* and *P. fortinii*, and our results support this observation.

The abundance of *H. chaetospira* in different habitats under natural conditions is unknown, but these data indicate the species is at least present in the humus-rich woodland soils obtained from five different locations in western Canada. Its recovery rate in this study, at an average frequency of 3.5%, is relatively high compared to previous reports in which only two isolates (0.3%) were found from similarly baited field soils collected in Japan (Narisawa et al. 1998). Ahlich et al. (1998) used bait plants (*Picea abies*

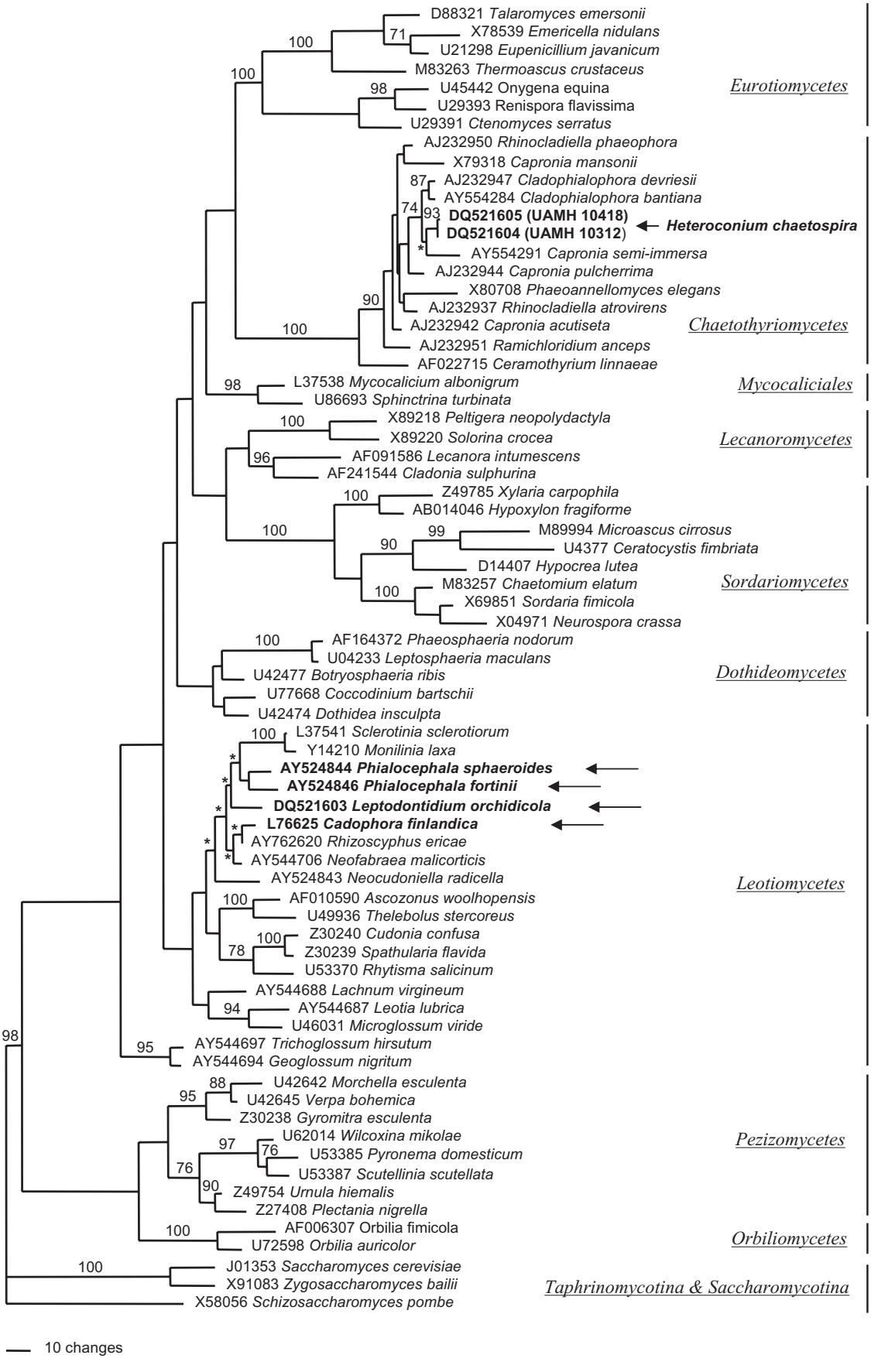


Fig. 5. One of eight equally parsimonious trees based on a heuristic search analysis of small subunit rDNA sequences showing the placement of *H. chaetospira* among the chaetothyriomycetous fungi. The analysis included data for five dark septate endophyte (DSE) (*arrows*) and 66 other fungi representing eight classes and one order (*incertae sedis*) of *Pezizomycotina* (1791 steps; CI = 0.482, RI = 0.735). Branches not retained in the strict consensus of all eight trees are indicated by an *asterisk*. Bootstrap support values over 70% from 1000 replicates of a "fast" stepwise-addition search are shown adjacent to the relevant node

(L.) Karst.) to study the distribution of root endophytic fungi in the soils of Swiss conifer stands and recovered four groups of DSE. Three groups included sporulating isolates of *P. fortinii*, but none of the groups included isolates that exhibited the slow growth rate characteristic of *H. chaetospira*. If present, *H. chaetospira* might have been obscured by the faster-growing DSE species, causing it to be missed during the preparation of pure cultures. Ahlich et al. (1998) examined their isolates microscopically and presumably would have seen the distinctive conidia of *H. chaetospira* had colonies of this fungus been visible on primary isolation plates.

Wilson et al. (2004) obtained three isolates of *H. chaetospira* directly from the roots of both herbaceous (*Aralia nudicaulis* L., two isolates) and woody (*Amelanchier alnifolia* Nutt., one isolate) perennial species. The ability of *H. chaetospira* to grow in the roots of annual bait plants and in the roots of perennial species, altogether representing six different plant families, suggests there is little host specificity, a characteristic previously noted for DSE in general (Jumpponen and Trappe 1998).

SSU rDNA sequence data indicate that our isolates of *H. chaetospira* are affiliated with the *Herpotrichiellaceae* and that a teleomorph, if found, would belong to the genus *Capronia*. Species of *Capronia* are generally observed and recorded when their minute, darkly pigmented ascocarps are found on rotting or decorticated wood, although some species have formed fruiting bodies in vitro (Untereiner 1995, 1997). Related anamorph species are morphologically diverse and are found among a variety of genera including *Cladophialophora*, *Exophiala*, *Ramichloridium*, and *Rhinocladiella*. Species of these genera inhabit a wide range of niches and have been described from many sources including plant litter, wood, soil, and vertebrate mycoses.

Bergero et al. (2003) isolated an unidentified herpotrichiellaceous endophyte from the roots of *Erica arborea* L. bait plants grown in soils obtained from a mature *Quercus* stand. Their fungus (in cultural group "Sd2") was described as producing a gray colony with a black margin. Hyphae were olivaceous brown and sometimes monilioid but did not produce conidia. In resynthesis experiments, they demonstrated that Sd2 was able to form coils typical of ericoid mycorrhizas. Allen et al. (2003) reported "*Capronia*-like fungi" in the roots of the ericaceous species *Gaultheria shallon* L. This determination was made using ITS2 sequence comparisons of DNA derived from isolates obtained from roots plated on agar media and DNA cloned directly from hyphae in root tissue. Isolates in culture did not sporulate and were not described. One isolate formed intracellular coils in the roots of axenically reared plants of *G. shallon*. Summerbell (2005) indicates that a sterile fungus from beech mycorrhizae and deposited in the Centraalbureau voor Schimmelcultures, Utrecht, Netherlands, as "*Mycelium radicis fagi*" (CBS 256.48), has affinities to *Capronia*.

Our data, and those of others (e.g., Allen et al. 2003; Bergero et al. 2003), confirm that some herpotrichiellaceous fungi occur as root endophytes, but the nature of their associations with their hosts and their precise taxonomic affiliations are still ambiguous. *Heteroconium* species have not previously been linked to *Capronia* teleomorphs, and it may be that our isolates, and others identified as *H. chaetospira*, would be more appropriately disposed in *Cladophialophora*. More isolates and a more detailed taxonomic investigation of the species of *Cladophialophora*, *Heteroconium*, and *Septonema* are required to understand their relationships and to determine the precise identity of the endophytic taxa. Isolation of these fungi in pure culture from field-collected roots could be problematical because they are easily masked by fungi that are similar in cultural morphology, e.g., dark septate endophytes such as species in the genera *Cadophora*, *Leptodontidium*, and *Phialocephala*, even though they are phylogenetically distant from these leotiomycetous anamorphs. Baiting protocols could permit the development of more complete collections of the root endophytic taxa in the Chaetothyriales and provide isolates that could be used for studies of their phylogenetic relationships and their roles as symbionts of plants.

Acknowledgments The authors are pleased to acknowledge support from the Japan Science and Technology Corporation Overseas Research Fellowship (to K.N.) and the Natural Sciences and Engineering Research Council of Canada (to R.S.C.). We also thank R.A. Shoemaker and K.A. Seifert and two anonymous reviewers for helpful comments and reviews of earlier versions of this manuscript.

References

Addy HD, Piercey MM, Currah RS (2005) Microfungal endophytes in roots. Can J Bot 83:1–13

Ahlich K, Rigling D, Holdenrieder O, Sieber TN (1998) Dark septate hyphomycetes in Swiss conifer forest soils surveyed using Norway-spruce seedlings as bait. Soil Biol Biochem 30: 1069–1075

Allen TR, Millar T, Berch SM, Berbee ML (2003) Culturing and direct DNA extraction find different fungi from the same ericoid mycorrhizal roots. New Phytol 160:255–272

Bergero R, Girlanda M, Bello F, Luppi A, Perotto S (2003) Soil persistence and biodiversity of ericoid mycorrhizal fungi in the absence of the host plant in a Mediterranean ecosystem. Mycorrhiza 13:69–75

Bourchier RJ (1961) Two new imperfect fungi from the heartwood of living lodgepole pine. Can J Bot 39:1781–1784

Currah RS, Sigler L, Hambleton S (1987) New records and new taxa of fungi from the mycorrhizae of terrestrial orchids of Alberta. Can J Bot 65:2473–2482

Domsch KH, Gams W, Anderson T-H (1980) Compendium of soil fungi. Academic Press, London (reprinted 1993 by IHW-Verlag, Eching, Germany, with supplementary material)

Ellis MB (1976) More dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew

Eriksson OE, Baral H-O, Currah RS, Hansen K, Kurtzman CP, Rambold G, Laessøe, T (eds) (2004) Outline of Ascomycota—2004. Myconet 10:1–99

Hambleton S, Nickerson NL, Seifert KA (2005) *Leohumicola*, a new genus of heat-resistant hyphomycetes. Stud Mycol 53:29–52

Harrington TC, McNew DL (2003) Phylogenetic analysis places the *Phialophora*-like anamorph genus *Cadophora* in the Helotiales. Mycotaxon 87:141–151

Hashiba T, Narisawa K (2005) The development and endophytic nature of the fungus *Heteroconium chaetospira*. FEMS Microbiol Lett 252:191–196

Hughes SJ (1952) Four species of *Septonema*. Naturalist (Lond) 840:7–12

Jumpponen AT, Trappe JM (1998) Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. New Phytol 140:295–310

Mandayam K, Jumpponen A (2005) Seeking the elusive function of the root-colonising dark septate endophyte fungi. Stud Mycol 53:173–189

Narisawa K, Tokumasu S, Hashiba T (1998) Suppression of clubroot formation in Chinese cabbage by the root endophytic fungus, *Heteroconium chaetospira*. Plant Pathol 47:210–216

Narisawa K, Ohki T, Hashiba T (2000) Suppression of clubroot and *Verticillium* yellows in Chinese cabbage in the field by the endophytic fungus, *Heteroconium chaetospira*. Plant Pathol 49:141–146

Narisawa K, Kawamata H, Currah RS, Hashiba T (2002) Suppression of *Verticillium* wilt in eggplant by some fungal root endophytes. Eur J Plant Pathol 108:103–109

Ohki T, Masuya H, Yonezawa M, Usuki F, Narisawa K, Hashiba T (2002) Colonization process of the root endophytic fungus *Heteroconium chaetospira* in roots of Chinese cabbage. Mycoscience 43:191–194

Richard C, Fortin J-A (1974) Distribution géographique, écologie, physiologie, pathogenicité et sporulation du *Mycelium radicis atrovirens*. Phytoprotection 55:67–88

Summerbell RC (1989) Microfungi associated with the mycorrhizal mantle of and adjacent habitats within the rhizosphere of black spruce in boreal Canada. Can J Bot 67:1085–1095

Summerbell RC (2005) From Lamarckian fertilizers to fungal castles: recapturing the pre-1985 literature on endophytic and saprotrophic fungi associated with ectomycorrhizal root systems. Stud Mycol 53:191–256

Swofford DL (1999) PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer Associates, Sunderland, MA

Untereiner WA (1995) Fruiting studies in species of *Capronia* (Herpotrichiellaceae). Antonie Leeuwenhoek 68:3–17

Untereiner WA (1997) Taxonomy of selected members of the ascomycete genus *Capronia* with notes on anamorph-teleomorph connections. Mycologia 89:120–131

Usuki F, Narisawa K (2005) Formation of ericoid mycorrhizal structures by the root endophytic fungus *Heteroconium chaetospira* with *Rhododendron obtusum* var. *kaempferi*. Mycorrhiza 15:61–64

Wilson BJ, Addy HD, Tsuneda A, Hambleton S, Currah RS (2004) *Phialocephala sphaeroides*, sp. nov., a new species among the dark septate endophytes (DSE) from a boreal wetland in Canada. Can J Bot 82:607–617

Yonezawa M, Usuki F, Narisawa K, Takahashi J, Hashiba T (2004) Anatomical study on the interaction between the root endophytic fungus *Heteroconium chaetospira* and Chinese cabbage. Mycoscience 45:367–371