Molecular systematics of E-strain mycorrhizal fungi: *Wilcoxina* and its relationship to *Tricharina* (Pezizales)

Keith N. Egger

Abstract: Nuclear-encoded ribosomal RNA gene sequences (rDNA) spanning 107 base pairs at the 3′ end of the 18S gene, the 5′ internal transcribed spacer region (ITS1), and across divergent domain D1 near the 5′ end of the 28S gene were analyzed to infer a phylogeny for taxa of the E-strain mycorrhizal fungal genus *Wilcoxina* and to determine their relationship to representatives of the genus *Tricharina*. The phylogeny suggests that *Wilcoxina* and *Tricharina*, although related, should be maintained as separate genera. *Wilcoxina* taxa formed a distinct group that exhibited interspecific variation of 37.6% in the ITS1 region. *Wilcoxina alaskana* was the most distant taxon, which is consistent with its growth on an unusual substrate (rotting wood). It remains to be confirmed that this taxon is mycorrhizal. A cryptic *Wilcoxina* species, known only from root isolates, was found. Sequence analysis of the ITS1 region distinguished two varieties of *Wilcoxina mikolae*: var. *mikolae* and var. *tetraspora*. Among the taxa of *Tricharina* examined there was 31.2% variation in the ITS1 region. The most divergent taxon in the *Tricharina* group was *Tricharina praecox*, although sequence analysis was unable to distinguish the varieties described within this species. The remaining taxa in *Tricharina* formed a tight group with only 10.2% interspecific divergence in the ITS1 region. There is sequence evidence that at least two taxa are included in *Tricharina gilva* as presently delineated. As the sole report of mycorrhiza formation in *Tricharina* is shown to be based upon a misidentification, it therefore appears that only *Wilcoxina* taxa are mycorrhizal.

Key words: E-strain, *Wilcoxina*, *Tricharina*, mycorrhiza, ribosomal DNA, phylogeny.

Résumé : Le but poursuivi était de déduire une phylogénie pour les taxons du groupe mycorhizien E-strain du genre *Wilcoxina* et de déterminer leur relation avec des représentants du genre *Tricharina*, à partir de marqueurs moléculaires. À cette fin, l'auteur a analysé des séquences de gènes de l'ARN ribosomal nucléaire couvrant 107 paires de bases à l'extrémité 3′ du gène 18S, la région 5′ de l'espaceur interne transcrit (ITS1), et le domaine divergent D1 près de l'extrémité 5′ du gène 28S. La phylogénie obtenue suggère que les *Wilcoxina* et les *Tricharina*, bien qu'apparentés, doivent être maintenus dans des genres distincts. Le taxon *Wilcoxina* forme un groupe distinct qui montre des variations interspécifiques de 37,6% dans la région ITS1. Le *W. alaskana* est le taxon le plus distant ce qui est congruent avec sa croissance sur un substrat inhabituel (bois pourri). Il reste à confirmer si ce taxon est mycorhizien ou non. L'auteur a trouvé une espèce de *Wilcoxina* peu fréquente, connue seulement à partir d'isolats racinaires. L'analyse séquentielle de la région ITS1 permet de distinguer deux variétés du *Wilcoxina mikolae* : var. *mikolae* et var. *tetraspora*. Parmi les taxons de *Tricharina* examinés, on observe 31,2% de variation dans la région ITS1. Le taxon le plus divergent chez les *Tricharina* est le *Tricharina praecox*, bien que l'analyse séquentielle ne puisse pas discerner les variétés décrites chez cette espèce. Les autres taxons du genre *Tricharina* forment un groupe étroit avec seulement 10,2% de divergence dans la région ITS. Les séquences démontrent qu'au moins deux taxons sont inclus dans le *T. gilva* tel que présentement défini. L'auteur démontre que le seul rapport concernant la formation de mycorhizes par un *Tricharina* est basé sur une mauvaise identification, et que conséquemment seul les taxons de *Wilcoxina* sont mycorhiziens.

Mots clés : E-strain, *Wilcoxina*, *Tricharina*, mycorhize, ADN ribosomal, phylogénie.
[Traduit par la rédaction]

Introduction

Laiho and Mikola (1964) first used the term E-strain for the mycobiont responsible for forming ectendomycorrhizae on pine in Finnish nurseries. This mycorrhizal type is characterized by a thin mantle and Hartig-net hyphae that penetrate root cortical cells on some hosts (Scales and Peterson 1991*a*, 1991*b*). E-strain mycorrhizae are widely distributed on coniferous and deciduous hosts in tree nurseries, burned sites, and disturbed forest sites in Finland (Mikola 1965), the United States (Laiho 1965; Wilcox et al. 1974, 1983; Yang and Wilcox 1984), Canada (Danielson 1991; Danielson et al. 1983, 1984), the United Kingdom (Thomas et al. 1983), and Kenya (Ivory and Pearce 1991).

The taxonomic position of the fungus that formed E-strain mycorrhizae was unclear to the original researchers because sexual fruiting bodies were not observed (see Mikola 1965).

Received July 6, 1995.

K.N. Egger.[1] Department of Biology, Memorial University of Newfoundland, St. John's, NF A1B 3X9, Canada.

[1] Present address: Natural Resources and Environmental Studies, University of Northern British Columbia, Prince George, BC V2N 4Z9, Canada.

An analysis of cultural characteristics of the E-strain fungus led Danielson (1982) to postulate that it was an ascomycete belonging to the order Pezizales. This was confirmed by Yang and Wilcox (1984) who described the sexual stage of the fungus as *Tricharina mikolae*. Later, Yang and Korf (1985*b*) did a taxonomic study of *Tricharina* and delineated a new genus, *Wilcoxina*, for taxa that form, or were suspected to form, E-strain type mycorrhizae.

Wilcoxina differs from *Tricharina* in a number of characters. Apothecia of *Tricharina* species tend to be partially sunken into the substrate, hairs are limited to the margins, and ascospores fill the ascus, whereas in *Wilcoxina* ascocarps are sessile on the surface of the substrate, hairs extend to the base of the apothecium, and ascospores tend to be positioned in the upper half of the ascus (Yang and Korf 1985*b*). They differ also in ascospore germination and culture characteristics. Rehydrated spores of *Tricharina* are reported to germinate quickly and produce rapidly elongating germ tubes, while spores of *Wilcoxina* may take up to 2 weeks to germinate and often produce intercalary chlamydospores (Yang and Korf 1985*a*, 1985*b*). Some species of *Wilcoxina* (see Egger et al. 1991) also produce thick-walled terminal chlamydospores that have been referred to *Complexipes* (Walker 1979). *Tricharina* species produce anamorphs in *Ascorhizoctonia* (Yang and Korf 1985*a*; Yang and Kristiansen 1989).

Yang and Korf (1985*b*) also noted differences between the genera in habitat and ecology. *Tricharina* species are often found on disturbed or burnt soil and were hypothesized to be saprobic, while *Wilcoxina* species (with the exception of *Wilcoxina alaskana*, which has not been isolated in culture) are known to be mycorrhizal (Yang and Korf 1985*b*). It is reported in the literature that *Tricharina gilva* forms mycorrhizal associations (Ingleby et al. 1990), but results of the present study show that this report was based upon a misidentification.

In previous papers (Egger and Fortin 1990; Egger et al. 1991), we showed that cultural characteristics and DNA restriction fragment variation in nuclear and mitochondrial ribosomal RNA genes could be used to elucidate the taxonomy and population structure of *Wilcoxina* taxa. In this paper, I look more closely at taxonomic concepts and phylogenetic relationships of *Wilcoxina* and *Tricharina* taxa inferred from analysis of ribosomal DNA sequences.

Materials and methods

Nucleic acids were extracted as described in Egger and Fortin (1990) from cultures or dried ascocarp tissue of *Sphaerosporella brunnea* (Alb. & Schwein.) Svrček & Kubička, *Tricharina ascophanoides* (Boud.) Yang & Korf, *Tricharina gilva* (Boud. in Cooke) Eckblad, *Tricharina groenlandica* Dissing, Yang & Korf, *Tricharina ochroleuca* (Bres.) Eckblad, *Tricharina praecox* (P. Karst.) Dennis var. *cretea* (Cooke) Yang & Korf, *T. praecox* var. *intermedia* Egger, Yang & Korf, *T. praecox* var. *praecox*, *W. alaskana* Kempton, Yang & Korf, *Wilcoxina mikolae* (Yang & Wilcox) Yang & Korf var. *mikolae* Yang & Korf, *Wilcoxina mikolae* var. *tetraspora* Wilcox, Yang & Korf, and *Wilcoxina rehmii* Yang & Korf. Distribution of voucher specimens and original cultures is documented in Yang and Korf (1985*a*, 1985*b*), Yang and Kristiansen (1989), Egger and Fortin (1990), and Egger et al. (1991). Source of isolates, taxonomic placement, and associated information is summarized in Table 1.

Primers ITS9mun and ITS10mun were used to amplify a region of the ribosomal DNA containing 107 base pairs (bp) at the 3′ end of the small subunit gene (18S) and the internal transcribed spacer 1 (ITS1), and primers NL5mun and NL6Amun were used to amplify a portion of the large subunit gene (28S). Primer sequences and their approximate annealing position are given in Egger (1995). ITS9mun/ITS10mun fragments were amplified and sequenced as described in Egger and Sigler (1993). NL5mun/NL6Amun fragments were amplified as described above except that the annealing temperature was 46 or 48°C and the total number of cycles was 32. Following amplification, the NL5mun/NL6Amun products were purified using Wizard PCR Prep (Promega, Madison, Wis.) columns following the protocol suggested by the manufacturer. An additional change was that the NL5mun/NL6Amun products were cycle-sequenced using a PRISM DyeDeoxy sequencing kit (Perkin-Elmer: Applied Biosystems, Norwalk, Conn.), then purified before loading on the ABI 373A automated sequencer by running the sample through a Fine Sephadex (Pharmacia LKB, Piscataway, N.J.) column rather than a series of isopropanol precipitations. In all cases, sequences were obtained from both strands and corrected by comparing forward and reverse orientations.

Sequences were aligned manually for the complete set of isolates listed in Table 1, using *S. brunnea* as the outgroup taxon. Several outgroup taxa were initially tried, but *Sphaerosporella* was chosen because it gave the most reliable alignment across the ITS1 region (see Fig 1). A subset of isolates were then subjected to phylogenetic analysis using PAUP 3.1 (Swofford 1993). Isolates not included in the phylogenetic analysis will be discussed in relation to intraspecific variation within *Wilcoxina* species.

Results

Three regions were aligned, the 18S, ITS1, and 28S, consisting of 107, 187, and 342 bp, respectively. The 18S region was most highly conserved, differing at 12 of 107 positions. The 28S region was moderately variable, differing at 56 of 343 positions. These regions yielded relatively unambiguous alignments (available from the author on request). The most variable region was the ITS1, which differed at 126 of 187 positions. This region is often difficult to align across different genera or distantly related species because of its variability and rapid rate of saturation (Berbee et al. 1995). Despite the presence of numerous insertion or deletion events (indels), it was possible to align the ITS1 across the three genera, and indels were mostly conserved within species or across species within genera (Fig. 1).

Several different alignments were analyzed. First, data were run for the complete nucleotide data set, using the heuristic search option of PAUP with random stepwise addition of taxa and 10 replications for each search. Because of alignment ambiguities in the ITS1 region, some nucleotides were eliminated from the analysis (shown in lower case in Fig. 1). Gaps were treated as missing data. A bootstrap analysis (Felsenstein 1985) was then done using 1000 replicates of the heuristics search with default options to estimate confidence limits on branches. Second, transversions were weighted 2:1 over transitions and the analysis repeated. The topology of the tree remained unchanged with weighting except that *T. praecox*, whose position was previously unresolved, was grouped with the rest of the *Tricharina* taxa. Bootstrap values on the major branches also increased. In the third analysis, transversions were weighted 2:1 over transitions and, following the suggestion of Bruns et al. (1992), the data were recoded to minimize ambiguities. Ambiguous

Table 1. Fungal isolates examined, including host, substrate, geographical information, and GenBank accession numbers.

No.*	Name†	Host	Substrate	Origin	Ref.‡	GenBank No.§
KNE 2116	*S. brunnea*	Unknown	Burnt soil	Mt. Ste. Anne, Que.		U38587, U38586
KNE 1126	*T. praecox* var. *intermedia*	Unknown	Burnt soil	Christina Lake, B.C.	1, 2	U38625, NS
KNE 1132	*T. praecox* var. *intermedia*	Unknown	Burnt soil	Nelson, B.C.	1, 2	U38585, U38584
CSY 19	*W. mikolae* var. *mikolae*	*Pinus resinosa*	Nursery soil	Oregon, U.S.A.	1, 2	U38561, U38560
CSY 50	*W. mikolae* var. *tetraspora*	*Pinus resinosa*	Nursery soil	New York, U.S.A.	1, 2	U38563, U38562
CSY 57	*W. mikolae* var. *tetraspora*	*Pinus resinosa*	Nursery soil	New York, U.S.A.	1, 2	U38626, NS
CSY 85	*W. rehmii*	Unknown	Soil	Norway	1, 2	U38565, U38564
CSY 98	*T. gilva*	Unknown	Sandy clay	Norway	1, 2	U38573, U38572
CSY 99	*T. gilva*	Unknown	Loose silt	Norway	1, 2	U38575, U38574
CSY 100	*W. rehmii*	?*Pinus* sp.	Soil	Spain	1, 2	U38567, U38566
CSY 102	*T. ochroleuca*	Unknown	Sandy soil	Norway	1, 2	U38579, U38578
CSY 103	*T. praecox* var. *praecox*	Unknown	Burnt soil	Norway	1, 2	U38627, NS
CSY 104	*T. groenlandica*	Unknown	Clay soil	Greenland	1, 2	U38577, U38576
CSY 106	*T. ochroleuca*	Unknown	Sandy soil	Greenland	1, 2	U38628, NS
CSY 107	*T. gilva*	Unknown	Sand	Greenland	1, 2	U38629, NS
CSY 123	*T. ascophanoides*	Unknown	Burnt paper	Norway	3	U38581, U38580
PEK 6-5-63#4	*W. alaskana*	Unknown	Rotting wood	Alaska, U.S.A.	2	U38583, U38582
RMD 947	'*W. mikolae*'	*Picea glauca*	Mine spoil	Luscar, Alta.	4	U38630, NS
RMD 977	'*W. mikolae*'	*Picea glauca*	Mine spoil	Luscar, Alta.	4	U38631, NS
RMD 2136	'*Wilcoxina* sp. nov.'	*Pinus banksiana*	Tailings or peat	Canmore, Alta.	5	U38569, U38568
RMD 2144	'*W. mikolae*'	*Picea glauca*	Mine spoil	Luscar, Alta.	5	U38571, U38570
RMD 2147	'*W. rehmii*'	*Picea glauca*	Spoil or peat	Luscar or Canmore, Alta.	5	U38632, NS
RMD 2154	'*W. mikolae*'	*Picea glauca*	Mine spoil	Luscar, Alta.	5	U38633, NS
RMD 2338	'*W. rehmii*'	*Pinus banksiana*	Mineral soil	Bitumont, Alta.	6	U38634, NS
RMD 2394	'*W. mikolae*'	*Pinus banksiana*	Soil or peat	Ft. McMurray, Alta.	7	U38635, NS
BDG-MISS	'*W. mikolae*'	?*Pinus resinosa*	Nursery soil	Miss., U.S.A.	8	U38636, NS
BDG-WV	'*W. mikolae*'	?*Pinus resinosa*	Nursery soil	W.Va., U.S.A.	8	U38637, NS
E-57	'*W. mikolae*'	*Picea abies*	Nursery	Finland	9	U38638, NS
NSW 6254	'*W. mikolae*'	Unknown	Bare soil	Oreg., U.S.A.		U38639, NS

*Specimens were obtained by the following: KNE, K.N. Egger; CSY, C.S. Yang; PEK, P.E. Kempton; RMD, R.M. Danielson; BDG, H.E. Wilcox; NSW, N.S. Weber.

†Names in single quotes are E-strain root isolates identified by ribosomal DNA sequences and (or) restriction patterns rather than by examination of ascocarps.

‡1, Yang and Korf 1985*a*; 2, Yang and Korf 1985*b*; 3, Yang and Kristiansen 1989; 4, Danielson 1982; 5, Danielson 1991; 6, Danielson et al. 1983; 7, Danielson and Visser 1989; 8, Wilcox et al. 1983; 9, Laiho 1965.

§GenBank accession numbers are for the combined SSU–ITS1 sequence and the LSU sequence, respectively. NS indicates that the region was not sequenced.

positions were coded as N (A, C, G, or T) and missing data (gaps) were counted as a fifth character state by setting the gapmode option in PAUP. To eliminate the problem of large gaps being counted as multiple independent events, gaps were coded as a single gap character with the remainder of the gap coded as N. This method extracts information contained in indel regions by (*i*) by assuming that shared indels are phylogenetically informative and (*ii*) by including in the analysis regions opposite gaps that are aligned for groups of taxa that would otherwise be treated as missing data. The recoding did not change the overall topology of the tree but did resolve the position of *T. groenlandica* and *T. ochroleuca*, which was previously unresolved. This analysis resulted in two most parsimonious trees of length 363. A bootstrap analysis (1000 replications) was also run on the recoded data, and the resulting consensus tree is shown in Fig. 2. The bootstrap consensus tree was identical to the consensus of the two most parsimonious trees, except that branches uniting *W. mikolae*, *Wilcoxina* sp.nov., and *W. rehmii* collapsed to a trichotomy in the bootstrap analysis.

Taxa within the *Wilcoxina* clade exhibited 37.6% variation in the ITS1 region among seven isolates representing four species (one species with two varieties). *Wilcoxina alaskana* was the most divergent taxon, which corresponds to habitat and possibly ecological differences. *Wilcoxina mikolae* was well supported by the analysis, as was *W. rehmii*. Anomalous isolates RMD 2136 and 2141 in Egger et al. (1991) are clearly shown by the DNA sequence data to be a distinct species of *Wilcoxina*. Although the phylogenetic analysis was unable to resolve its position in relation to *W. mikolae* and *W. rehmii*, this species differs in the ITS1 region at 21 sites (11.3% divergence) from *W. mikolae* and at 38 sites (20.4%). from *W. rehmii*.

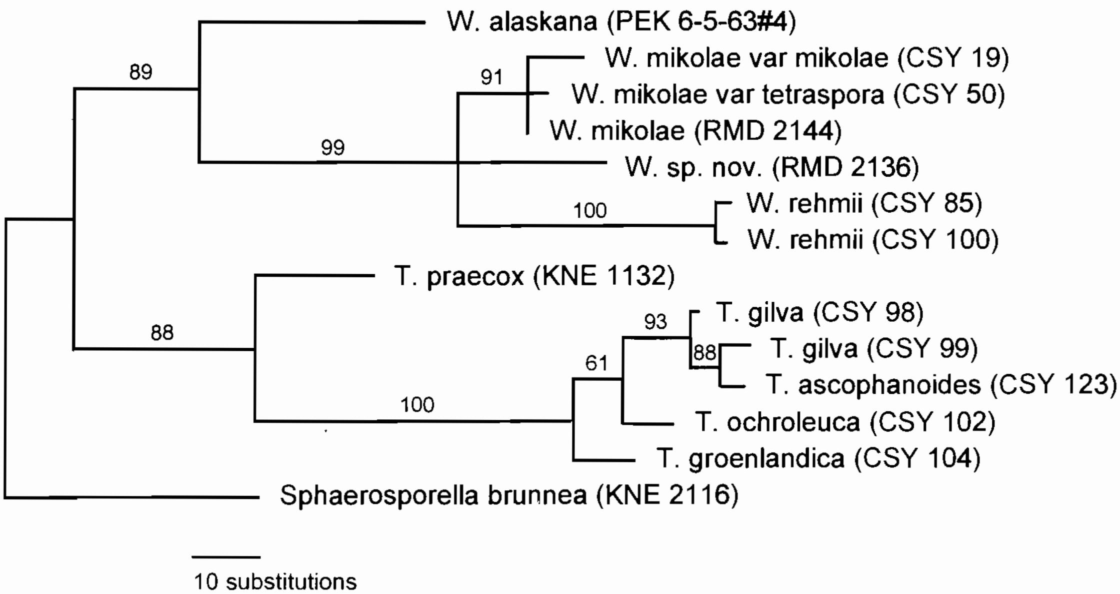
Yang and Korf (1985*b*) described two varieties of *W. mikolae*, *W. mikolae* var. *mikolae* and *W. mikolae* var. *tetraspora*, the latter distinguished by presence of numerous four-spored asci. DNA sequencing of the ITS1 region of 12 *W. mikolae* isolates representing the two varieties as well as isolates obtained from roots, revealed intraspecific variation of 6.4%. Of the 11 variable sites, 5 represented single

Fig. 1. Alignment of ITS1 sequences. Numbering indicates the position of the nucleotide in the right hand column. Nucleotides in lower case were excluded from the analysis due to alignment ambiguities (see text).

```
                                                                                                          94 bp]
PEK 6-5-63#4 .....T-..TGCATATA.ATT--.T.TA..CACT.AACATA......C..C.A.......G...............A.GTA-..-A.AC.T.T.
CSY 19       ....A.-..TG..TATA.ACTTCAT.TA.CAAC...TT--A......T........C...G............A...AG.A-..-A...T..T.
CSY 50       ....A.-..TG..TATA.ATTTCAT.TA.CAAC...TT--A......T........C...G............A...AG.C-..-A...T..T.
RMD 2144     ....A.-..TG..TATA.ATTTCAT.TA.CAAC...TT--A......T........C...G............A...AG.C-..-A...T..T.
RMD 2136     ..C.C.-..TG..TATA.ATTTCAT.TA.CAAC..CAT--A......T..C.A...C...G............A....G.C-..-A.C.T..T.
CSY 85       ......T..-...TATA.ATT.T.T.TA.CGAC.AAAT--AT.....T....A...C...G............A...AG.C-..-A.ACAT.T.
CSY 100      ......T..-...TATA.ATT.T.T.TA.CGAC.AAAT--AT.....T....A...C.C.G............A...AG.C-..-A.ACAT.T.
KNE 1132     ......A...T..---AT..T.T.-.AA...AC-..TTC-A.....-..........................ACCG..-...A......T.
CSY 98       C.....A...C..---.T..TT..-.AA...GC-A.TTC-A.....C-........C..................CCG..-...AT.....T.
CSY 99       C.....A...C..---.T.......-.AA...GC-A.TTTCA.....C-........C..G...............CTG..-...AT.....T.
CSY 104      C.....A...T..---AT..T.C.-.AA...GC-A.TTTCA.....C-........C..................CTG..-C..AT...T.T.
CSY 102      C.....A...T..---.T..TTC.-.AA...GC-A.TTC-A.....C-........C..C...............CCG..-...AT.....T.
CSY 123      C.....A...C..---.T...T..-.AA...GC-A.TTTCA.....C-........C..................CTG..-...A......T.
KNE 2116     AAAGTA-ATAAGT---GCTCCCAGCGCGTATTATGT-----AACCCA-TCTGTGTATCTTACCTGTTGCTTCCGTGGGTCGGTGG-CTTCGGCC

                                                                                                         187 bp]
PEK 6-5-63#4 .---..T..TT..-------cacagatga.at.a.tctt..........a-.....T...TTCA...AAAT..C...TC..........ATC..
CSY 19       ..-..T.GTG..------gcaaatctcaaga.a.tc....t..a....ca.ca..-....TCA..T--ATC.....TCT.........-.C..
CSY 50       ..-..T.GTG..------gtaaatcccaag..a.tc....t..a....ca.ca.TA....TCA..T--ATC....TTCT.........-.C..
RMD 2144     ..-..T.GTG..------gtaaatcccaag..a.tc...at..a....ca.ca..A....TCA..T--ATC.....TCT.........-.C..
RMD 2136     ..-..T.G.G..------gtgaatccca.ga.a.tt....t.......aa.c-..T...TTCA..T--ATC.....TCT.........-.C..
CSY 85       ..-..TGGGG..------taacatcccgag..a.cct..gt..a....aa.ca.-GT...TC...--AATC.....TC..........T.A..
CSY 100      ..-..TGGGG..------ttacatcccgag..a.cct..gt..a....aa.ca.-GT...TC...--AATC.....TC..........T.A..
KNE 1132     C.C..A.GCTG.acttcggtacgggtccaga.t.ccggtg...a....a-..........TC....--AT..C...TCT.........-.C..
CSY 98       C.C...CTTGC.----------------.ga.t.ccgg...........---...-.CG....TCA...--AT.TC...TC..........-.C..
CSY 99       C.C...CTCGCC----------------.ga.t.ccgg...........---...-.CA....TCA...--AT.TC...TC..........-.C..
CSY 104      C.C...GTCGCA----------------.ga.t.ccgg...........---...-.CA...TTC....--AT.TC...TC..........-.C..
CSY 102      C.C...C.TGC.----------------.ga.t.ccgg...........---...-.CA...TTC....--AT.TC...TC..........-.C..
CSY 123      C.C...CTTTCC----------------.ga.t.ccgg...........---...-.CA....TCA...--AT.TC...TC..........-.C..
KNE 2116     AC-TCCTCAAAT---------------gcggggaacccacgggaggt-ctacATCAAACCTTTGC--TCTCAATGATATCTGTCTGA-ATTG
```

Fig. 2. Bootstrap consensus tree from 1000 heuristic analyses of the recoded (see text) complete nucleotide data set (18S, ITS1, and 28S). Numbers above lines indicate bootstrap percentages supporting that branch. Branch lengths are proportional to the number of substitutions, which can be determined from the scale bar.



substitutions that were not found in more than one isolate. The six remaining sites distinguished the reference isolate of *W. mikolae* var. *mikolae* (CSY 19) from *W. mikolae* var. *tetraspora* (CSY 50, CSY 57), supporting the hypothesis of Yang and Korf (1985*b*) that these two varieties are distinct. These varieties were not distinguishable by restriction analysis (Egger and Fortin 1990; Egger et al. 1991). The four isolates of *W. rehmii* sequenced exhibited only 1.8% intraspecific variation in the ITS1 region.

The *Tricharina* clade exhibited 31.2% variation in the

ITS1 region among five species (one with three varieties). *Tricharina praecox* is quite distinct from other *Tricharina* taxa, varying at 42 positions (22.5%) and containing three indels not present in other *Tricharina* taxa. Intraspecific variation within *T. praecox* was low. Despite the fact that I was only able to obtain a reliable sequence from *T. praecox* var. *praecox* and *T. praecox* var. *intermedia*, representing two of the three varieties described by Yang and Korf (1985*b*), only a single site varied among the three isolates.

Other *Tricharina* taxa form a tight clade with low levels of divergence among taxa, suggesting a recent radiation with several speciation events. Only 19 positions varied among seven isolates representing four species (10.2% divergence). Given the relatively low bootstrap value, and that *T. groenlandica* was only resolved from *T. ochroleuca* in the recoded data set, the exact branching order of these two taxa is unresolved. They differ from *T. ascophanoides* at 14 positions (7.5%) and 9 positions (4.8%), respectively.

Two distinct ITS1 sequences were found among three isolates referred to *T. gilva* by Yang and Korf (1985*b*). CSY 99 and CSY 107 shared one sequence, but CSY 98 differed at seven positions (3.8%) from this consensus sequence. This is a greater degree of divergence than between CSY 99 – CSY 107 and *T. ascophanoides* (five positions, 2.7%).

Discussion

Two clusters containing taxa of *Wilcoxina* and *Tricharina* were clearly distinguished in the phylogenetic analysis, supporting the decision by Yang and Korf (1985*b*) to segregate *Wilcoxina* from *Tricharina*.

The most divergent taxon in the *Wilcoxina* group, *W. alaskana*, is found on rotting wood and has not been conclusively shown to form mycorrhizae. Although growth of *W. alaskana* on rotting wood does not preclude ability to form mycorrhizae (Yang and Korf 1985*b*), given its divergent position, future studies should endeavor to confirm that it is mycorrhizal. This species was considered to be a synonym of *Wilcoxina sequoia* (Phill.) T. Schum. by Schumacher (1988). However, the author notes that Yang and Korf (1985*b*) also examined the type and arrived at a different conclusion. The substrate of *W. sequoia* is similar to *W. alaskana*, in that both occur on woody materials, but *W. alaskana* is described on rotting wood, while *W. sequoia* was found on bark and foliage of the giant sequoia. I was unable to obtain material of the type for sequencing, so the question of synonymy of these taxa is unresolved.

The sequence analysis supports previous studies that showed that *W. rehmii* could be distinguished from *W. mikolae* on the basis of ascocarp morphology (Yang and Korf 1985*b*), culture morphology (Yang and Korf 1985*a*; Egger and Fortin 1990), and restriction fragment patterns (Egger and Fortin 1990; Egger et al. 1991). This study also revealed a new species of *Wilcoxina*. At present this taxon is known only from culture, where it resembles *W. mikolae* in cultural morphology (Egger and Fortin 1990; Egger et al. 1991). I refer to it here as *Wilcoxina* sp. nov. It will be described as a distinct species once a fruiting collection is found.

Based upon mitochondrial as well as nuclear ribosomal RNA variation, Egger et al. (1991) suggested that *W. mikolae* var. *tetraspora* may have resulted from hybridization between *W. mikolae* and *W. rehmii*. Of the 26 sites that varied between *W. mikolae* var. *mikolae* and *W. rehmii*, *W. mikolae* var. *tetraspora* shared 21 with var. *mikolae* and 5 with *W. rehmii*. While this does not preclude a hybrid origin for *W. mikolae* var. *tetraspora*, there is no evidence of recent hybridization, such as intragenomic variation among tandem repeats in *W. mikolae* var. *tetraspora*. Whether hybridization and introgression occurred in the past cannot be determined from these data.

The root isolates from Alberta matched *W. mikolae* var. *tetraspora* at five of the six variable sites, the other variable site being shared with *W. mikolae* var. *mikolae*. This indicates that the Alberta isolates are more similar to var. *tetraspora*, and they likely represent the same taxon. Egger et al. (1991) suggested that there may be divergence among *W. mikolae* isolates based on host preference, as indicated both by molecular differences in mitochondrial DNA RFLP patterns and by morphological differences (isolates from spruce lacked the terminal chlamydospores commonly found in *W. mikolae* isolates from pine). There were no polymorphisms that uniquely identified isolates on spruce. Therefore, this region does not provide evidence to support the hypothesis that there is population differentiation among *W. mikolae* isolates on the basis of host preference. However, mitochondrial genes are generally more variable than their nuclear counterparts and thus may be more sensitive to host variation. Alternatively, the differences in mtDNA may have been a geographical sampling artifact, since isolates on the same host were often isolated from the same sample site or region. The question of host specialization will need to be studied further.

Tricharina praecox was the most divergent species within the *Tricharina* group. Although the branch uniting *T. praecox* with the remaining *Tricharina* taxa was unresolved in the first analysis, bootstrap support for this branch was 69% when transversions were weighted 2:1 over transitions and increased to 88% when the data were recoded. This indicates that a large proportion of the substitutions uniting *T. praecox* to the main *Tricharina* clade were transversions and that many were contained in indel regions. There is good support for *T. praecox* belonging to *Tricharina*, but given the deep divergence of this taxon, addition of further taxa may change this conclusion.

The low level of sequence divergence among *T. praecox* isolates does not allow molecular differentiation of distinct varieties in *T. praecox*. Either the varieties have not been separate sufficiently long to diverge in this region, or morphological variants result from differential phenotypic expression. Further research using other markers will be required to determine if the morphological varieties described by Yang and Korf (1985*b*) represent distinct subpopulations or sibling species, rather than phenotypic plasticity.

Two distinct sequence variants were found among the three isolates of *T. gilva* examined. Yang and Korf (1985*b*) acknowledged the possibility of more than one taxon in *T. gilva* when they placed *Peziza fimbriata* Quél. in synonymy. They noted that there were differences among the types: *P. fimbriata* possessed longer hairs (up to 400 μm) with 5–8 septa, while *T. gilva* has shorter hairs (up to 220 μm) with 5 or fewer septa. However, they did not feel these differences were sufficiently consistent to recognize

two taxa. The molecular sequence data suggest that Yang and Korf (1985*b*) did incorporate two taxa in *T. gilva*. However, it is not known if the two sequences obtained in this study correspond to these two species, and if so which sequence corresponds to which name. Resolution of this question will require a re-examination of the types, possibly accompanied by DNA sequence analysis, although given the age of the type specimens recovery of satisfactory DNA may be difficult.

Habitat preferences and ecological niche vary among *Wilcoxina* and *Tricharina* taxa (see Egger et al. 1991). *Wilcoxina* sp.nov., *W. mikolae*, and *W. rehmii* are mycorrhizal species that are associated with disturbance. *Wilcoxina mikolae* is common on highly disturbed sites with low organic matter, such as burned sites or coal mine spoils, and is the E-strain most commonly found in nurseries and greenhouses. *Wilcoxina rehmii* also occurs on disturbed sites but tends to be found on unburned soils with higher organic matter or where soils have been amended with peat. *Wilcoxina* sp.nov., although similar in culture morphology to *W. mikolae*, has only been found on oil-sands tailings that were amended with peat. *Wilcoxina alaskana* may be mycorrhizal, but given its distance from other *Wilcoxina* taxa and its unusual substrate (rotting wood), this should be verified.

Yang and Korf (1985*b*) suggested that *Tricharina* species are saprotrophs that are often associated with disturbed sites. *Tricharina praecox* is found on burned sites and is likely either saprotrophic or weakly parasitic (Egger and Paden 1986). There is one report of mycorrhiza formation by *T. gilva* (Ingleby et al. 1990). I obtained dried ascocarp tissue of that collection from the senior author of the publication and amplified and sequenced the ITS1 region. The sequence obtained was identical to the E-strain isolate E-57, now known to be *W. mikolae*, indicating that this report is based upon a misidentification. The taxonomist who identified the collection as *T. gilva* was aware of the possibility that it was conspecific with *W. mikolae* (as *T. mikolae*), but in the absence of voucher material decided to use the name *T. gilva*. Therefore, mycorrhiza formation has only been conclusively demonstrated in *Wilcoxina* and there is no evidence of mycorrhiza formation in *Tricharina*.

Acknowledgements

I thank C.S. Yang, H. Wilcox, R.Danielson, N.S. Weber, and K. Ingleby for generously providing cultures or specimens. Technical assistance was provided by G. Osmond and Q. Baldwin. Funding was provided by the Natural Sciences and Engineering Research Council of Canada.

References

Berbee, M.L., Yoshimura, A., Sugiyama, J., and Taylor, J.W. 1995. Is *Penicillium* monophyletic? An evaluation of phylogeny in the family Trichocomaceae from 18S, 5.8S and ITS ribosomal DNA sequence data. Mycologia, **87**: 210–222.

Bruns, T.D., Vilgalys, R., Barns, S.M., Gonzalez, D., Hibbett, D.S., Lane, D.J., Simon, L., Stickel, S., Szaro, T.M., Weisburg, G., and Sogin, M.L. 1992. Evolutionary relationships within the fungi: analyses of nuclear small subunit rRNA sequences. Mol. Phylogenet. Evol. **1**: 231–241.

Danielson, R.M. 1982. Taxonomic affinities and criteria for identification of the common ectendomycorrhizal symbiont of pines. Can. J. Bot. **60**: 7–18.

Danielson, R.M. 1991. Temporal changes and effects of amendments on the occurrence of sheathing (ecto-) mycorrhizas of conifers growing in oil sands tailings and coal spoil. Agric. Ecosyst. Environ. **35**: 261–281.

Danielson, R.M., and Visser, S. 1989. Host response to inoculation and behaviour of introduced and indigenous ectomycorrhizal fungi of jack pine grown on oil-sands tailings. Can. J. For. Res. **19**: 1412–1421.

Danielson, R.M., Visser, S., and Parkinson, D. 1983. Microbial activity and mycorrhizal potential of four overburden types used in the reclamation of extracted oil sands. Can. J. Soil Sci. **63**: 363–375.

Danielson, R.M., Zak, J.C., and Parkinson, D. 1984. Mycorrhizal inoculum in a peat deposit formed under a white spruce stand in Alberta. Can. J. Bot. **63**: 2557–2560.

Egger, K.N. 1995. Molecular analysis of ectomycorrhizal fungal communities. Can. J. Bot. **73**(Suppl. 1): S1415–S1422.

Egger, K.N., and Fortin, J.A. 1990. Identification of taxa of E-strain mycorrhizal fungi by restriction fragment analysis. Can. J. Bot. **68**: 1482–1488.

Egger, K.N., and Paden, J.W. 1986. Biotrophic associations between lodgepole pine seedlings and post-fire ascomycetes (Pezizales) in monoxenic culture. Can. J. Bot. **64**: 2719–2725.

Egger, K.N., and Sigler, L. 1993. Relatedness of the ericoid endophytes *Scytalidium vaccinii* and *Hymenoscyphus ericae* inferred from analysis of ribosomal DNA. Mycologia, **85**: 219–230.

Egger, K.N., Danielson, R.M., and Fortin, J.A. 1991. Taxonomy and population structure of E-strain mycorrhizal fungi inferred from ribosomal and mitochondrial DNA polymorphisms. Mycol. Res. **95**: 866–872.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution, **39**: 783–791.

Ingleby, K., Mason, P.A., Last, F.T., and Fleming, L.V. 1990. Identification of ectomycorrhizas. Institute of Terrestrial Ecology Research Publication No. 5, Edinburgh Research Station, Scotland.

Ivory, M.H., and Pearce, R.B. 1991. *Wilcoxina mikolae* newly identified as a mycorrhizal fungus on pines in Africa. Mycol. Res. **95**: 250–253.

Laiho, O. 1965. Further studies on the ectendotrophic mycorrhiza. Acta For. Fenn. **79**(3): 1–35.

Laiho, O., and Mikola, P. 1964. Studies on the effect of some eradicants on mycorrhizal development in forest nurseries. Acta For. Fenn. **77**: 1–33.

Mikola, P. 1965. Studies on the ectendotrophic mycorrhiza of pine. Acta For. Fenn. **79**(2): 1–56.

Scales, P.F., and Peterson, R.L. 1991*a*. Structure and development of *Pinus banksiana* – *Wilcoxina* ectendomycorrhizae. Can. J. Bot. **69**: 2135–2148.

Scales, P.F., and Peterson, R.L. 1991*b*. Structure of ectomycorrhizae formed by *Wilcoxina mikolae* var. *mikolae* with *Picea mariana* and *Betula alleghaniensis*. Can. J. Bot. **69**: 2149–2157.

Schumacher, T. 1988. The *Scutellinia* battle; the lost, missing and dead. Mycotaxon, **33**: 149–189.

Swofford, D. 1993. PAUP: phylogenetic analysis using parsimony, version 3.1. Illinois Natural History Survey, Champaign, Ill.

Thomas, G.W., Rogers, D., and Jackson, R.M. 1983. Changes in the mycorrhizal status of Sitka spruce following outplanting. Plant Soil, **71**: 319–323.

Walker, C. 1979. *Complexipes moniliformis*: a new genus and species tentatively placed in the Endogonaceae. Mycotaxon, **10**: 99–104.

Wilcox, H.E., Ganmore-Neumann, R., and Wang, C.J.K. 1974. Characteristics of two fungi producing ectendomycorrhizae in *Pinus resinosa*. Can. J. Bot. **52**: 2279–2282.

Wilcox, H.E., Yang, C.S., and LoBuglio, K. 1983. Responses of

pine roots to E-strain ectendomycorrhizal fungi. Plant Soil, **71**: 293–297.

Yang, C.S., and Korf, R.P. 1985*a*. *Ascorhizoctonia* gen. nov. and *Complexipes* emend., Two genera for anamorphs of species assigned to *Tricharina* (Discomycetes). Mycotaxon, **23**: 457–481.

Yang, C.S., and Korf, R.P. 1985*b*. A monograph of the genus *Tricharina* and of a new segregate genus, *Wilcoxina* (Pezizales). Mycotaxon, **24**: 467–531.

Yang, C.S., and Kristiansen, R. 1989. *Ascorhizoctonia ascophanoides* sp. nov.: anamorph of *Tricharina ascophanoides*. Mycotaxon, **35**: 313–316.

Yang, C.S., and Wilcox, H.E. 1984. An E-strain ectendomycorrhiza formed by a new species, *Tricharina mikolae*. Mycologia, **76**: 675–684.