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Phylogenetics of *Trichaptum* Based on Mitochondrial Small Subunit rDNA Sequences

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To study the phylogenetic relationships of species of *Trichaptum* and to infer intraspecific divergence of *T. abietinum*, partial mitochondrial small subunit rDNA sequences were determined. Six strains of *T. abietinum*, two of *T. biforme*, and one of *T. fusco-violaceum* were examined. Parsimony and distance analyses showed that each *Trichaptum* species forms a distinct group and that *T. abietinum* consists of two or more subgroups. Strains from North America were distantly related to one another but the European strain formed an independent group with three Korean strains, suggesting the possibility that Korean taxa may be phylogenetically closer to European taxa than to North American taxa.

Key words: Geographic variation, mt SSU rDNA, phylogeny, *Trichaptum*

Trichaptum Murrill (Polyporaceae, Aphyllophorales) is a well-known genus of cosmopolitan polyporoid wood-rotting fungi. Until recently, this genus has been called by the name *Hirschioporus*. Because some of its species are active decomposers of balsam firs killed by spruce budworms (14), of coniferous slash of Douglas firs and pines (1) and of pine trees killed by polluted air and recreational impact (9), they are known to be important in forest ecology and economy. The genus is identified by the general appearance of a purplish to violet pore surface and by the paling to buff or pale brown color with age and on drying. Members of the genus also have typical cylindrical spores, fusiform to clavate cystidia, and dimitic hyphal systems with skeletal hyphae dominating.

The parenthosome in the dolipore apparatus of *Trichaptum* is imperforate, a character which, until now, has only been seen in polyporoid fungi of the family Hymenochaetaceae so far (6, 16, 17). In accordance with this fact, recent molecular data support that *Trichaptum* and this family are closely related (7, 8, 11). *Trichaptum* species generally look similar except for three hymenophoral forms: poroid, irpicoid, or lamellate. Therefore, this has been the source of some controversy in terms of species designation. While some authors have considered these three forms to be too similar to separate believing them as varieties of a single species, others have treated them as three different species. Macrae (13) and Magasi (14) reported that the three forms of *Trichaptum* were incompatible with one another and could be treated as three different species. Quite recently, their results were confirmed again using molecular data of ribosomal DNA sequences (10, 11).

The poroid form of *Trichaptum, T. abietinum* is a first invader on newly fallen logs of conifers such as *Abies, Larix, Pinus* and *Taxus* (with rare exceptions of hardwoods), and frequently covers them with hundreds of small basidiocarps (1, 6, 13, 17). Its basidiocarps are usually effused-reflexed but sometimes sessile or resupinate. Its pilei are solitary or imbricate and have gray hirsute upper surface. Furthermore, *T. abietinum* has dimitic hyphal systems with abundant cystidia in the hymenium and its spores are cylindric to allantoid, hyaline, and smooth. While *T. biforme*, also formerly called *T. pergamenum*, is much similar to *T. abietinum*, it occurs only on hardwoods. The irpicoid form is called *T. fusco-violaceum* and the lamellate form is called *T. laricinum* (6, 17).

Interestingly, *T. abietinum* has been reported to consist of three intercompatible groups from cultural studies (13, 14), so it is treated as a species complex. Two groups from North America were found to be incompatible with each other, and both were partially compatible with a third group from Europe. Incompatibility between the two groups from North America was not correlated either with host or geographic range. From these reports, it

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can be assumed that the *T. abietinum* complex consists of several biological or geographic species. However, there has been no additional researches on this species complex.

To study phylogenetics of the genus *Trichaptum* and to infer an evolution of the *T. abietinum* complex, partial mitochondrial small subunit rDNA (mt SSU rDNA) sequences were used. Mitochondrial DNA is known to evolve about 10 times faster than nuclear DNA, and it has been used in intrageneric or interspecific phylogenetic analyses (2, 3, 4, 8). Therefore, mitochondrial DNA is appropriate to study phylogenetic relationships among lower taxa rather than among higher taxa. In this study, mitochondrial small subunit rDNA sequences were used mainly to infer the phylogenetic grouping of strains within *T. abietinum* and to compare phylogenetic relationships between them.

Materials and Methods

Sources and DNA isolation

Strains and specimens of *Trichaptum* used in this study are listed in Table 1. To obtain total DNA, a modificaion of the method described by Lecellier *et al.* (12) was used. In the case of cultured strains, mycelium grown on the cellophane disc was recovered into an Eppendorf tube using a spatula. As for the collected specimens, dried pieces of fungal specimens from the Seoul National University Fungal Collection (SFC) were used directly for DNA extraction. A total of 600 µl extraction buffer [100 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0), 100 mM NaCl and 2% SDS)] was added to about 300 mg of dried piece. The sample was frozen for 30 sec in liquid nitrogen and thawed for 60 sec in a 70°C incubator. This process was repeated 3 to 5 times until a thorough breakage of cells was achieved. Extracted DNA was purified with a phenol-chloroform extraction and precipitated with 1 volume of isopropanol. The pellet was resuspended in 50 µl of sterile DDW and then used as a template for PCR amplification.

Table 1. List of *Trichaptum* strains with their collection numbers and localities used in this study

Species name	Collection number	Locality
T. abietinum	DAOM 73811	Gustrow, East Germany
T. abietinum	DAOM 72245A	Quebec, Canada
T. abietinum	FPL-8973[a]	-
T. abietinum	SFC 950815-17	Uleung-do, Korea
T. abietinum	SFC 960608-11	Mt. Myeongsung, Korea
T. abietinum	SFC 961028-11	Mt. Chiak, Korea
T. biforme	HHB-7316-sp	Wisconsin, USA
T. pergamenum (=*T. biforme*)	CBS 324.29	Canada
T. fusco-violaceum	DAOM 53127	Holt, Aust-Agder, Norway

[a] FPL-8973 isolate from USDA Forest Products Laboratory, Madison, Wisconsin, was sequenced by D.S. Hibbett (8).

PCR amplification and DNA cloning

About 700 bps from a portion of mitochondrial rDNA were amplified, using the primers MS1 and MS2 (21). DNA amplifications were performed in a reaction mixture containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 2 mM $MgCl_2$, 0.2 mM dATP, 0.2 mM dCTP, 0.2 mM dGTP, and 0.2 mM dTTP, 1 µl of each primer (25 mM), 15 µl of BSA (0.15%), 1/10 or 1/100 diluted template DNA, and 5 units of *Taq* DNA polymerase. The total volume was adjusted to 50 µl. Thirty cycles of the following reaction sequence were performed: 60 sec at 94°C for denaturation, 60 sec at 50°C for annealing, and 60 sec at 72°C for extension with 1 sec extended each cycle, followed by a final extension for 20 min at 72°C. Amplified products were electrophoresed on a 0.7% agarose gel, detected with EtBr staining under UV light, and were purified by the LMP agar elution method (18). Purified DNAs were cloned into T-vectors. To reduce PCR errors, several clones were acquired from each strain.

DNA Sequencing

The dideoxy chain termination procedure was used to sequence cloned DNAs, using either MS1 and MS2 primers or vector primers and an internal primer MS4 (3). Sequenase version 2.0 (United States Biochemical) was used and reaction conditions were according to supplier's instructions.

Data analyses

Sequences acquired from eight strains of three *Trichaptum* species were aligned with the one of *T. abietinum* FPL-8973, the only other sequence of *Trichaptum*, retrieved from the GenBank database using a multiple alignment program CLUSTAL W (20). The alignment was visually optimized and gaps were treated as missing data. A maximum parsimonious tree was obtained using the exhaustive option of PAUP 3.1.1. (19). Support for the phylogenetic grouping was obtained by bootstrap analysis using 100 replications with random addition input orders of sequences during each branch-and-bound search. Maximum sequence divergence was also determined using PAUP 3.1.1. (19). And another phylogenetic tree was also produced and bootstrap analysis was executed by the UPGMA method of the PHYLIP 3.5c package (5).

Results and Discussions

The MS1 and MS2 primer combination amplified a single product which was about 700 bp long in size when resolved on a 0.7% agarose gel. Partial mt SSU rDNA sequences were determined to be between 498 and 620 bps in length for eight *Trichaptum* species. Variations in sequences ranged from single base pair changes to multiple deletions or insertions. Two multiple deleterious regions were unique for two strains of *T. biforme*. Thus, sequence sizes of *T. biforme* were somewhat shorter than those of *T. abietinum*. When these sequences were aligned with the one accessed from the GenBank, they resulted in an alignment of 597 sites. 522 visually optimized sites (Fig. 1) were then submitted for computer analysis. Computed

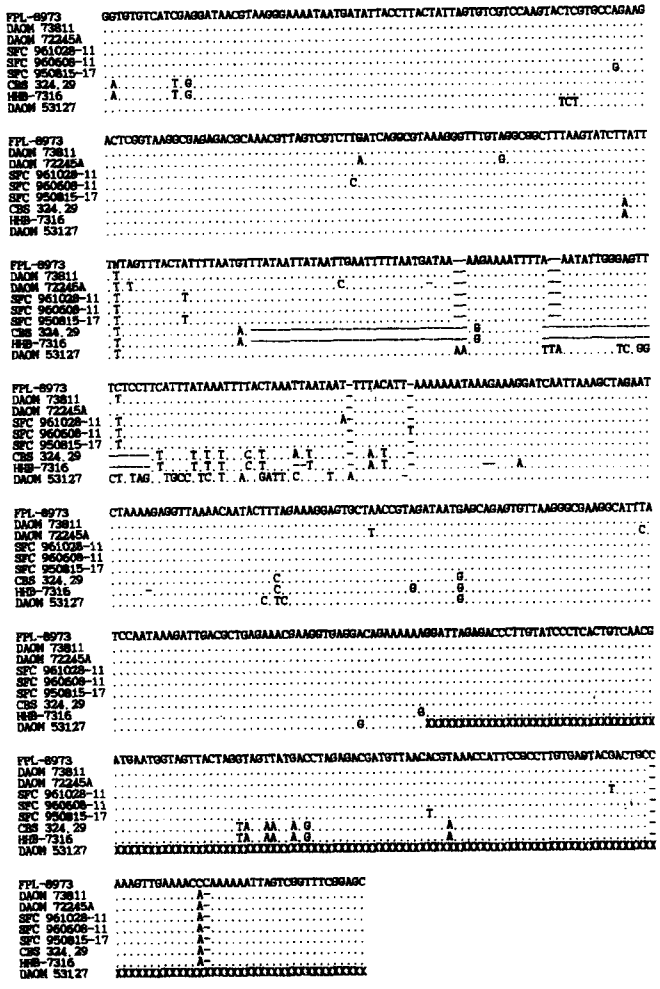

Fig. 1. Alignment of mitochondrial small subunit rDNA sequences of *Trichaptum* strains used in this study. Visually optimized sequences resulted in an alignment of 522 sites. Dots correspond to identical sites with those of *T. abietinum* FPL-8973 and bars indicate gaps. X's are undetermined sites.

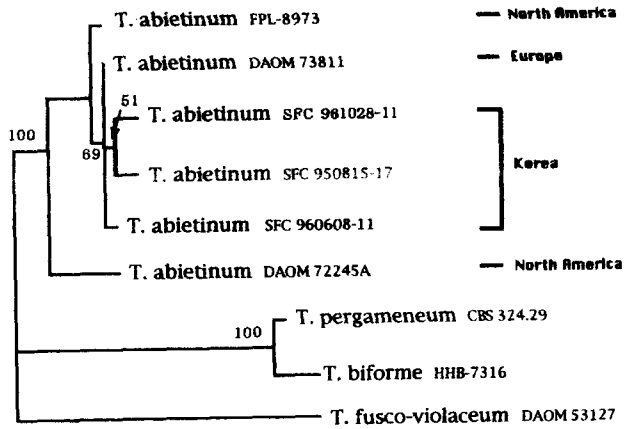

Fig. 2. Phylogram constructed using the parsimony analysis with the exhaustive search of PAUP 3.1.1. (19) with midpoint rooting. The tree has 72 nucleotide substitutions, a consistency index of 1.000, and a retention index of 1.000. Bootstrap values are given on branches supported by more than 50%.

pairwise distances to evaluate nucleotide substitutions between aligned sequences were generated in a matrix.

From this data set, a most parsimonious tree (MPT) and a UPGMA tree were generated. The parsimonious tree (Fig. 2) was produced by midpoint rooting using the exhaustive search option of PAUP 3.1.1. (19) and was 72 steps in length. The other phylogenetic tree (Fig. 3) was produced by the UPGMA method of PHYLIP 3.5c (5) and came out similar to the MPT. Phylogenetic analyses showed that three species of *Trichaptum* form distinct separate groups and that *T. abietinum* consists of at least two or more subgroups. Both phylogenetic trees indicated that *T. abietinum* is monophyletic and that *T. biforme* and *T. pergamenum* (a

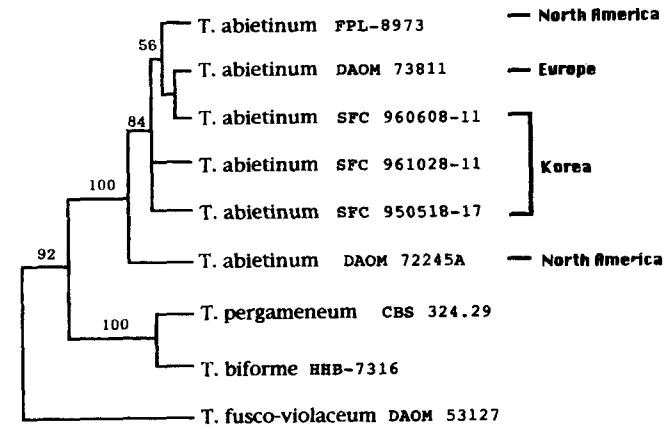

Fig. 3. Phylogenetic tree generated by the UPGMA method of PHYLIP 3.5c package (5). Bootstrap values are given above branches supported by more than 50%.

synonym of *T. biforme*) were another monophyletic group (both clades supported by 100% bootstrap values in both trees). In addition, strains of *T. abietinum* except DAOM 72245A were grouped into one cluster. The facts that the genus *Trichaptum* is monophyletic, that several hymenophoral forms of *Trichaptum* are distinct species, and that there are more than two subgroups in *T. abietinum* had been proven by previous ultrastructural, cultural, and molecular phylogenetic studies (10, 11, 13, 14, 15). This was again confirmed by the present study based on mt SSU rDNA.

Mating tests of previous studies (13, 14) and molecular analyses of nuclear small subunit rDNA and the ITS region (11) showed that *T. abietinum* might be subdivided into at least two groups, possibly either subspecies or varieties. In the trees of Figs. 2 and 3, it is interesting that two strains from North America (FPL-8973 and DAOM 72245A) are distantly related. One strain FPL-8973 groups with European and Korean strains and the other strain DAOM 72245A forms an outgroup to the rest of *T. abietinum*. In the UPGMA tree, the bootstrap value supporting the clade consisting of *T. abietinum* strains except DAOM 72245A is 84%, which is rather high, although it is less than 50% in the parsimonious tree. These results partly support previous findings by incompatibility tests that there are two or more mating groups (biological species) within *T. abietinum* (13, 14). That is, two incompatible groups from North America were partially compatible with the third one from Europe. Macrae's results (13) actually showed that North American DAOM 72245A and European DAOM 73811 are partially compatible. Furthermore, there is a chance for North American FPL-8973 and European DAOM 73811 to be partially compatible, but there are no known incompatibility that have been tests tried between other strains included in this study.

In the parsimony analysis of Fig. 2, the European DAOM 73811 made an independent clade with three Korean strains (bootstrap value 69%) separately from North American FPL-8973. In the UPGMA tree of Fig. 3, European, North American, and Korean strains were grouped together, but its bootstrap value was 56% and proved to be too low to support a particular clade robustly. Fig. 2 suggests a good possibility that European isolates, if more strains are added to DAOM 73811, have a potential to form an independent group with Korean strains. As Europe and Asia consist of a united Eurasian Continent, it is biogeographically predictable that Korean and East Asian taxa are phylogenetically closer to European taxa rather than to North American taxa.

So far, no phylogenetic relationships of fungal taxa including *T. abietinum* strains from East Asia have been properly studied in relation to other continental taxa. It is somewhat inferable that there are some distinctions in Korean strains compared with other geographic strains like North American DAOM 72245A. However, aligned sequences of Fig. 1 and pairwise distances show that mitochondrial small subunit rDNA sequences are too conserved to detect exact intraspecific relationships. Thus, it is necessary that more divergent genes or spacer regions like internal transcribed spacers be searched and compared together. In addition, more sufficient molecular data and incompatibility tests are required for a comprehensive conclusion on phylogenetic relationships of *T. abietinum* strains.

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