

# ITS Sequence Comparison of Germplasm Resources Of *Auricularia polytricha*

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**Abstract:** The internal transcribed spacer (ITS) region in rRNA gene of *Auricularia polytricha* was cloned and sequenced, and the sequences were compared with those of some common species of *Auricularia*. The results showed that 6 strains among tested strains, except Teda with different origins, had the same length of ITS2. There were base variances in the ITS region of rRNA gene among the tested strains, and with 11 variant loci in ITS region. Compared with other 4 species from the Gene Bank, ITS1 and ITS2 sequences of *A. polytricha* varied in great extent. According to the analysis of distance matrix, the genetic relationship between strains and the related known species from systematic dendrogram, ITS sequence analysis supports the traditional classification of *Auricularia* based on morphology.

## Introduction

rRNA is one of the most ancient molecules in cells. With the functional and evolutionary homology, rRNA is the living fossil of some research, including the origin of life, the early evolution and molecular phylogeny. The most conservative area of rDNA is 18S, 5.8S, 28S rRNA genes. Spacer in rRNA genes is the fastest evolution area<sup>[1]</sup>, while ITS is a moderate conservative area, which is characterized by intraspecific relatively consistency and interspecific obviously difference<sup>[2]</sup>.

In the study of molecular identification and phylogeny in ectomycorrhizal fungi, such as *Tuber*, *Laccaria*, *Hebeloma*, *Tricholoma bakamatsutake*, the ITS section have a common application<sup>[3]</sup>. But the reports have been seldom in other higher fungus. In this paper, the ITS sequence of 7 strains *A. polytricha* rDNA were studied to reveal the genetic variations in different strains, which also could provide molecular evidence for *A. polytricha* identification and phylogeny.

## Materials and Methods

**Tested Materials.** The fruiting bodies of seven *A. polytricha* strains with morphological differences were selected as the test material, including two wild isolates, five cultivated strains (Table 1).

**Total DNA Extraction.** The total DNA was extracted by CTAB (Cetyltrimethyl Ammonium Bromide)<sup>[1]</sup>.

Table 1 Tested strains of *A. polytricha*

No.	Abbreviation	Strain name	Source	Note
1	A.pap	AP7	Sichuan Academy of Agricultural Sciences	Wild poplar
2	A.pzj	Hybrid 1#	Edible Fungi Center of jinning Shangdong	
3	A.pxs	Xiaoshan 3#	Edible Fungi Farm of Sichuan	
4	A.ptd	Teda	Edible Fungi Farm of Sichuan	
5	A.pte	Taier 319	Jiangsu Institute of Edible Fungi	
6	A.pml	M 1	Hebei Institute of Microbiology	
7	A.pel	AP059	Wolong Nature Reserve of Sichuan	Wild in Wolong

**ITS Fragments of PCR Amplification.** Fungi universal primers ITS1/ITS2 and ITS3/ITS4 were used to specifically amplify the segments of ITS region (ITS1, 5.8S rDNA and ITS2) in the PCR reaction (1 min initial denaturation at 94°C, and 36 cycles of 1 min denaturation at 94°C, 1 min at 54°C for annealing, 1.5 min at 72°C for extension, and a final extension at 72 °C for 10 min). The PCR products were examined by 1.4% agarose gel electrophoresis. After electrophoresis, the target fragments were purified by 3s Spin Agar Gel Purification Kit.

**Cloning and Sequencing of ITS Fragment.** Connect the purified PCR products to Japanese Takara's PMD-18T Vector, transform into E.coli competent cell JM109, screen by the AMP plate with X-gal IPTC, pick white colonies for culture, extract plasmid and test by PCR. After cloned strains successfully, two-way sequencing were done by Beijing Huadazhong Biological Technology Co. Limited of China, and the whole sequence was spliced by DNAMAN.

**Analysis of ITS Sequence.** The ITS sequence was automatic alignment by DNAMAN program. The published ITS sequence of *Tremell fuciformis* (*T.fuc*), *Auricularia auricular-judae* (*A.aur*), *Auricularia delicata* (*A.del*), *Auricularia fuscossuccinea* (*A.fus*) and *Auricularia mesenterica* (*A.mes*) were downloaded from NCBI Database. Analysis phylogenetic relationships of above mentioned strains. The range of ITS1 and IST2 were determined by the scope of *A. auricular-judae*. Data computing and phylogenetic trees constructing was carried out by DNAMAN software. *T. fuciformis* was the classified outgroup.

## Results

**Variation Analysis of ITS Sequence.** The ITS sequences of 7 tested *A. polytricha* strains and other 4 species belonging to *Auricularia*, including *A. auricular-judae*, *A. delicata*, *A. fuscossuccinea* and *A. mesenterica*, and *T. fuciformis* were downloaded from Gene Bank (Figure 1).

The lengths of the ITS sequences were in the range of 502-599 bp for all of the strains tested. The shortest was that of *T. fuciformis* (502 bp), and the longest was that *A. polytricha* M1 (599 bp). Difference in the length of the ITS sequence was found in all strains. The length of the ITS sequence ranged from 532 to 599 bp for the *A. polytricha* strains. All the strains were the same in length of the 5.8 S rRNA genes (165 bp), except that *T. fuciformis* had a missing site. The lengths of the IST1 sequences were in the range of 163-230 bp. The shortest length of IST1 sequences was

found in Hybrid 1#, and the longest was found in M1. Taier 319 and Teda were the same in length of ITS1(280 bp). The IST1 sequence of *T. fuciformis* was 182 bp in length. The lengths of ITS2 sequences were in the range of 163-210 bp. The shortest length of IST2 sequences was found in *T. fuciformis*, and the longest length of IST2 sequences was found in *A. polytricha*. All tested strains of *A. polytricha* were the same length of ITS2(201 bp) except Teda(209 bp). Other 4 species of *Auricularia* were different length of IST2 sequences, namely *A. auricular-judae*(207 bp), *A. delicata*(183 bp), *A. Fuscosuccinea*(181 bp) and *A. mesenterica*(178 bp). Exclude insertions and deletions, the ITS region of all tested *A. polytricha* strains were 11 variant loci. 5 were found in IST1 region and 6 were found in ITS2 region. In figure 1, significant difference is found in three base sites. At the 168th base site, the base of Taier 319 and Teda are G, the other 5 are A. At the 532th base site, the base of Taier 319 and Teda are C, AP059 is missing, and the other 4 strains are T. At the 552th base site, the variation is the same as the 532th site.

Strains 1

70

T.fuc	TCCGTAGGTG	AACCTGCGGA	AGGATCATT	GAGATTA	?????????	?????????	?????????	?????????
A.aur	?????????	?-?-?-?-?-?	-?-?-?-?-?A	A-?-?-?-?CTYG	GGGTCGTCC	CCCAAACCTC	ATTCAGCTGT	
A.del	?????????	?????????	?????????	?????????-?CTG	GGCCCTTACC	TGCCCGTTTG	TTCAGCTGT	
A.fuc	?????????	?????????	?????????	????????-?CTG	GGCCCTTACC	TGCCCAYTCT	ATTCAGCTGT	
A.mes	?????????	?????????	?????????	?????????	?-?-?-?-?CCAGC	TCTGCCTTTT	CCTCAGCTGT	
A.pap	?????????	?-G-?-?-?-?	-?-?-?-?-?A	A-?-?-?-?TAG	GGCTTTTAA?	??CCCGATCG	??TTCAGCTGT	
A.pzj	?????????	?????????	?????????	?????????	?????????	?????????	?????????	
A.pxs	?????????	?-G-?-?-?-?	-?-?-?-?-?A	A-?-?-?-?TTG	GGCTTTTAA?	??CCCGATCG	?TTCAGCTGT	
A.ptd	-?-?-?-?-?	-G-?-?-?-?	-?-?-?-?-?A	A-?-?-?-?TTG	GGCTTTTAA?	??CCCGATCG	??TTCAGCTGT	
A.pte	-?-?-?-?-?	-G-?-?-?-?	-?-?-?-?-?A	A-?-?-?-?TTG	GGCTTTTAA?	??CCCGATCG	?TTCAGCTGT	
A.pml	-?-?-?-?-?	-G-?-?-?-?	-?-?-?-?-?A	A-?-?-?-?TTG	GGCTTTTAA?	??CCCGATCG	?TTCAGCTGT	
A.pel	?????????	?-G-?-?-?-?	-?-?-?-?-?A	A-?-?-?-?TTG	GGCTTTTAA?	??CCCGATCG	??TTCAGCTGT	

Strains 71

140

T.fuc	?????????	?????????CA	CCGGGCCGTG	AGGCCCTTCC	AAACACCTGT	GCACATCGGA	CC??GCGCCC	
A.aur	GCGCTTC???	?-?-?GGCTG--	-GCT-GA?C?	GAAAATC--?	?-?-?-?-?-?	----C-TTTC	GGTC--GTT	
A.del	GCGCTAA???	?-?-?CGCTG--	-GCT-GA-CT	CAA-ATC--?	?-?-?-?-?-?	----C-TTTC	GGTT--G-T	
A.fuc	GCGCTTC???	?-?-?GGCTG--	-GCT-GA TG?	CAA-A-C--?	?-?-?-?-?-?	----CATTTC	GGTT--G--	
A.mes	GCGCTTA???	?-?-?GGCTG--	-GCT-T??G?	GAA-AAC--?	?-?-?-?-?-?	----C-TTTC	GGTT--G--	
A.pap	GCGCCCTTCA	CAGGGATG--	-GCT-GA?G?	CAAGA-CC-?	?-?-?-?-?-?	----C-TTTC	GGTT--G-T	
A.pzj	GCGCCATCA	CAGGGATG--	-GCT-GA?G?	CAAGA-CC-?	?-?-?-?-?-?	----C-TTTC	GGTT--G-T	
A.pxs	GCGCCCTTCA	CAGGGATG--	-GCT-GA?G?	CAAGA-CC-?	?-?-?-?-?-?	----C-TTTC	GGTT--G-T	
A.ptd	GCGCCCTTCA	CAGGGATG--	-GCT-GA?G?	CAAGA-CC-?	?-?-?-?-?-?	----C-TTTC	GGTT--G-T	
A.pte	GCGCCCTTCA	CAGGGATG--	-GCT-GA?G?	CAAGA-CC-?	?-?-?-?-?-?	----C-TTTC	GGTT--G-T	
A.pml	GCGCCCTTCA	CAGGGATG--	-GCT-GA?G?	CAAGA-CC-?	?-?-?-?-?-?C	-----TTTC	GGTT--G-T	
A.pel	GCGCCCTTCA	CAGGGATG--	-GCT-GA?G?	CAAGA-CC-?	?-?-?-?-?-?	----C-TTTC	GGTT--G-T	

Strains 141

210

T.fuc	CCGGGCCGGG	CCGCCTTAC	ACAAACATAT	?????GTCAAGA	ACGTAATGCA	T??CAT??AA	CATGAAACAA	
A.aur	T--C?-CTT	-CG--T---	-TGA-AC--C	AAAA?GTCC-	GAA-GTGATC	-AAA CGAT-?	A-A-T-----	
A.del	T--T-GCT-	----T----A	-TGA-AC--C	TCA--TC--	-T--C--TAT	AAA-TA??T-	A-A-T-----	
A.fuc	TT--C-GCT-	----?-----A	-TGA-ACC-C	TCA--TC--	-T--C-ACA-	AACT--??--	A-A-T-----	
A.mes	T-T-T-CTT	-CG--T---	-TGC-ACC-C	AATCGGICT-	GAA-GTGTAT	AAAAC-AT-?	?-A-T-----	
A.pap	T--T-GCT-	---T----A	-TGC-ACA-C	TCA--TC--	-T--T-ACA-	AAC--??--	A-A-T-----	
A.pzj	T--T-GCT-	---T----A	-TGC-ACA-C	TCA--TC--	-T--T-ACA-	AAC--??--	A-A-T-----	
A.pxs	T--T-GCT-	---T----A	-TGC-ACA-C	TCA--TC--	-T--T-ACA-	AAC--??--	A-A-T-----	
A.ptd	T--T-GCT-	---T----A	-TGC-ACG-C	TCA--TC--	-T--T-ACA-	AAC--??--	A-A-T-----	
A.pte	T--T-GCT-	---T----A	-TGC-ACG-C	TCA--TC--	-T--T-ACA-	AAC--??--	A-A-T-----	
A.pml	T--T-GCT-	---T----A	-TGC-ACA-C	TCA--TC--	-T-CT-ACA-	AAC--AA--	A-A-T-----	
A.pel	T--T-GCT-	---T----A	-TGC-ACA-C	TCA--TC--	-T-CT-ACA-	AAC--AA--	A-A-T-----	

Strains	211	5.8S rDNA →						280
T.fuc	CTTTCAACAA	CGGATCTCTT	GGCTCTCGCA	TCGATGAAGA	ACGCAGCGAA	TTGCGAAAAG	TAATGTGAAT	
A.aur	-----	-----	-----	-----	-----	A-----T---	-----	
A.del	-----	-----	-----	-----	-----	A-----T---	-----	
A.fuc	-----	-----	-----	-----	-----	A-----T---	-----	
A.mes	-----	-----	-----	-----	-----	A-----T---	-----	
A.pap	-----	-----	-----	-----	-----	A-----T---	-----	
A.pzj	-----	-----	-----	-----	-----	A-----T---	-----	
A.pxs	-----G	-----	-----	-----	-----	A-----T---	-----	
A.ptd	-----	-----	-----	-----	-----	A-----T---	-----	
A.pte	-----	-----	-----	-----	-----	A-----T---	-----	
A.pml	-----	-----	-----	-----	-----	A-----T---	-----	
A.pel	----C-----	-----	-----	-----	-----	A-----T---	-----	
Strains	281							350
T.fuc	TGCAGAATTC	AGTGAATCAT	CGAATCTTTG	AACGCACCTT	GCGCCTTTTG	GTATCCGAA	AGGCATGCCT	
A.aur	-----	-----	-----	-----	---TCC---	-----ATG	GA-----	
A.del	-----	-----	-----	-----T---	---TCC---	-----ATG	GA-----	
A.fuc	-----	-----	-----	-----T---	---TCC---	-----ATG	GA-----	
A.mes	-----	-----	-----	-----T---	---TCC---	---C---ATG	GA---A---	
A.pap	-----	-----	-----	-----T---	---TCC---	-----ATG	GA-----	
A.pzj	-----	-----	-----	-----T---	---TCC---	-----ATG	GA-----	
A.pxs	-----	-----	-----	-----T---	---TCC---	-----ATG	GA-----	
A.ptd	-----	-----	-----	-----T---	---TCC---	-----ATG	GA-----	
A.pte	-----	-----	-----	-----T---	---TCC---	-----ATG	GA-----	
A.pml	-----	-----	-----	-----T---	---TCC---	-----ATG	GA-----	
A.pel	-----	-----	-----	-----T---	---TCC---	-----ATG	GA-----	
Strains	351	ITS2 →						420
T.fuc	GTTTGAGTGT	CATGTAGACT	CA <sub>2</sub> ACCCCC	GGGTTTCTGA	CCCGGCGGTG	TTGGATTTGG	GCCCTGCCTC	
A.aur	-----	--C---A--C	-TC---T-G	C-A-G-AAC-	GT--C-C-C <sub>2</sub>	GGT-GAC-T-	-A--GTG-CG	
A.del	-----	--C---A--C	-TC---TTG	C-A-G-AAC-	GT--CA-CGC	GGT-GA--T-	-A-TGTG-CG	
A.fuc	-----	--C---A--C	-TC---TTG	C-A-G-AAC-	GT--CACCGC	GGT-GA--T-	-A-TGTG-CG	
A.mes	-----	--C---A--C	-TC---T-G	C-A-G-AAC-	GT--TCC-C <sub>2</sub>	GGT-GAC-T-	-A--GTG-CG	
A.pap	-----	--C---A--C	-TC---TTG	C-A-G-AAC-	GT--C-C-- <sub>2</sub>	GGT-GAC-T-	-A-TGTG-CG	
A.pzj	-----	--C---A--C	-TC---TTG	C-A-G-AAC-	GT--C-C-- <sub>2</sub>	GGT-GAC-T-	-A-TGTG-CG	
A.pxs	-----	--C---A--C	-TC---TTG	C-A-G-AAC-	GT--C-C-- <sub>2</sub>	GGT-GAC-T-	-A-TGTG-CG	
A.ptd	-----	--C---A--C	-TC---TTG	C-A-G-AAC-	GT--C-C-- <sub>2</sub>	GGT-GAC-T-	-A-TGTG-CG	
A.pte	-----	--C---A--C	-TC---TTG	C-A-G-AAC-	GT--C-C-- <sub>2</sub>	GGT-GAC-T-	-A-TGTG-CG	
A.pml	-----	--C---A--C	-TC---TTG	C-A-G-AAC-	GT--CTC-- <sub>2</sub>	GGT-GAC-T-	-A-TGTG-CG	
A.pel	-----	--C---A--C	-TC---TTG	C-A-G-AAC-	GT--C-C-- <sub>2</sub>	GGT-GAC-T-	-A-TGTG-CG	
Strains	421							490
T.fuc	TCCTGGCTGG	CCTT <sub>2</sub> AAA	TGCCITAGTG	GTTTACGCA	GACGTCGTAA	GTTACGCTG	GACTGTGGGC	
A.aur	-AA- <sub>2</sub> CGGCT	-G-CITG---	---A---CT	-GCG-TTTT-	--GTG-TGGG	?????????	---G---T-A	
A.del	-GA-TCGGCT	-G-CITG---	---A---CT	-GCG-TTTT-	--GTG-TGGG	?????????	---G---T-A	
A.fuc	-AAC <sub>2</sub> CGGCT	-G-CITG---	---A---CT	-GCG-TTTT-	--GTG-TGGG	?????????	---G---T-A	

A.fuc	- AAC ?CGGCT	- G - CTTG ---	--- A --- CT	- GCG - TTTT -	- - GTG-TGGG	?????????-	--- G --- T - A
A.mes	- AG - ?TGGCT	- G - CTTG ---	--- A --- CT	- G - G - TTTT -	- - GTG-TGGG	?????????-	--- G --- T - A
A.pap	- AAC ?TGGCT	- G - CTTG ---	--- A --- CT	- GCG - TTTT -	- - GTG-TGGG	?????????-	--- G --- T - A
A.pzj	- AAC ?CGGCT	- G - CTTG ---	--- A --- CT	- GCG - TTTT -	- - GTG-TGGG	?????????-	--- G --- T - A
A.pxs	- AAC ?CGGCT	- G - CTTG ---	--- A --- CT	- GCG - TTTT -	- - GTG-TGGG	?????????-	--- G --- T - A
A.ptd	- AAC ?CGGCT	- G - CTTG ---	--- A --- CT	- GCG - TTTT -	- - GTG-TGGG	?????????-	--- G --- T - A
A.pte	- AAC ?CGGCT	- G - CTTG ---	--- A --- CT	- GCG - TTTT -	- - GTG-TGGG	?????????-	--- G --- T - A
A.pml	- AAC ?CGGCT	- G - CTTG ---	--- A --- CT	- GCG - TTTT -	- - GTG-TGGG	?????????-	--- G --- T - A
A.pel	- AAC ?AGGCT	- G - CTTG ---	--- A --- CT	- GCG - TTTT -	- - GTG-TGGG	?????????-	- AAC ?AGGCT
Strains	491				↓		↓ 560
T.fuc	CGCTCACAAC	CCCCCTAC?	!!!!!!!!!!!!	!!!!!!!!!!!!	!!!!!!!!!!!!	!!!!!!!!!!!!	!!!!!!!!!!!!
A.aur	TAA - T - TCTG	- G - AA - GC	CCTGGGCCTC	TTCAGCGGCG	CTGCTTACAG	TTCAGCGGCG	CTGCTTACAG
A.del	TAA - T - TCTG	- G - AA - ?GC	CCTGGGCCTC	TTCAGCGGTG	CTGCTTATAG	TTCAGCGGTG	CTGCTTATAG
A.fuc	TAA - T - TCTG	- G - AA - ?GC	CCTGGGCCTC	TTCAGCGGTG	CTGCTTACAG	TTCAGCGGTG	CTGCTTACAG
A.mes	TAA - T - TCTG	- G - AA - ?GC	CCTGGGCCTC	TTCAGCGGTG	CCGCTTACAG	TTCAGCGGTG	CCGCTTACAG
A.pap	TAA - T - TCTG	- G - AA - ?GC	CTTAGGCCTC	TTTAGCGGTG	CTGCTTACAG	TTTAGCGGTG	CTGCTTACAG
A.pzj	TAA - T - TCTG	- G - AA - ?GC	CTTAGGCCTC	TTCAGCGGTG	CTGCTTACAG	TTCAGCGGTG	CTGCTTACAG
A.pxs	TAA - T - TCTG	- G - AA - ?GC	CTTAGGCCTC	TTCAGCGGTG	CTGCTTACAG	TTCAGCGGTG	CTGCTTACAG
A.ptd	TAA - T - TCTG	- G - AA - ?GC	CTTAGGCCTC	TTCAGCGGTG	CCGCTTACAG	TTCAGCGGTG	CCGCTTACAG
A.pte	TAA - T - TCTG	- G - AA - ?GC	CTTAGGCCTC	TTCAGCGGTG	CCGCTTACAG	TTCAGCGGTG	CCGCTTACAG
A.pml	TAA - T - TCTG	- G - AA - ?GC	CTTAGGCCTC	TTCAGCGGTG	CTGCTTACAG	TTCAGCGGTG	CTGCTTACAG
A.pel	- G - CTTG ---	--- A --- CT	- GCG - TTTT	- - GTG - TGGG	?????????-	TTCAGCGGTG	CTGCTTACAG
Strains	561						630
T.fuc	!!!!!!!!!!!!	!!!!!!!!!!!!	???? TTTTGC	ACTCTGGCCT	CAAATCAGGT	AGGGCTACCC	GCTGAACCTA
A.aur	CCGTCCCTCG	TGG??ACAA	CTAT --- AAA	G - T -----	-----	--- A -----	-----
A.del	CCGTCCCTCY	GCGGACAAC	YTTT ??? - AAA	G - T -----	-----	--- A??????	!!!!!!!!!!!!
A.fuc	TCGTCCCTCT	GCGGACAAC	AATA - C - AAA	G - T -----	-----	--????????	!!!!!!!!!!!!
A.mes	TCGTCCCAG	AGG??ACAA	CGTA - C - AAA	G - T -----	-----	--????????	!!!!!!!!!!!!
A.pap	CCGTCCCTCTR	GTGGACACAT	TATT --- AAA	G - T -----	-----	--- A -----	----- GT ---
A.pzj	CCGTCCCTCTR	GTGGACACAT	TATT --- AAA	G - T -----	-----	--- A -----	----- GT ---
A.pxs	CCGTCCCTCTR	GTGGACACAT	TATT --- AAA	G - T -----	-----	--- A -----	----- GT ---
A.ptd	CCGTCCCTCTR	GTGGACACAT	TATT --- AAA	G - T -----	-----	--- A -----	----- ? GT ---
A.pte	CCGTCCCTCTR	GTGGACACAT	TATT --- AAA	G - T -----	-----	--- A -----	----- GT ---
A.pml	CCGTCCCTCTR	GTGGACACAT	TATT --- AAA	G - T -----	-----	--- A -----	----- GT ---
A.pel	CCGTCCCTCTR	GTGGACACAT	TATT --- AAA	G - T -----	-----	--- A -----	----- GT ---
Strains	631	640					
T.fuc	AGCATATCAA	T					
A.aur	-----	?					
A.del	!!!!!!!!!!!!	?					
A.fuc	!!!!!!!!!!!!	?					
A.mes	!!!!!!!!!!!!	?					
A.pap	-----	-					
A.pzj	-----	-					
A.pxs	-----	-					

**Genetic Distance Analysis.** The genetic distances of all strains based on ITS sequence were calculated by DNAMAN Software. In table 2, the genetic distances of all strains range from 0.002 to 0.351, and average is 0.110. The genetic distances of all species in *Auricularia* range from 0.002 to 0.301, and average is 0.0739. The genetic distances of all *A. polytricha* strains range from 0.002 to 0.301, and average is 0.0361. The genetic distances of family is greater than genus, and genus is greater than species, indicating uniformity between traditional classification and ITS classification, and also confirming the reasonableness of the traditional classification.

Table 2 Genetic distance matrix based on ITS sequence

Strains	A. pml	A. pte	A. ptd	A. pxs	A. pzj	A. pap	A. aur	A. del	A. fus	A. mes	T. fuc
A. pte	0.013										
A. ptd	0.012	0.002									
A. pxs	0.010	0.007	0.005								
A. pzj	0.011	0.008	0.006	0.004							
A. pap	0.014	0.010	0.009	0.007	0.006						
A. aur	0.129	0.121	0.121	0.121	0.112	0.122					
A. del	0.088	0.080	0.082	0.082	0.061	0.086	0.132				
A. fus	0.074	0.067	0.069	0.069	0.050	0.073	0.138	0.063			
A. mes	0.156	0.146	0.146	0.150	0.134	0.148	0.097	0.143	0.138		
T. fuc	0.298	0.293	0.294	0.302	0.310	0.300	0.312	0.333	0.338	0.351	
A. pel	0.014	0.014	0.012	0.010	0.009	0.007	0.126	0.076	0.076	0.156	0.301

**Phylogenetic Analyses.** Figure 2 is the phylogenetic tree of *Auricularia* based on ITS sequence, described by DNAMAN and *T.fuciformis* was classified outgroup. From the figure, at less than 94% supporting strength, 11 strains in ingroup are divided into five groups. At 99% supporting strength, a separate branch is formed in the 7 strains of *A. polytricha* in the A group, with 12 bp base differences of ITS sequences, distinguishing from other species.

At 86% supporting strength, all species of *Auricularia* are gotten together, showing their phylogenetic relationships. With 95% supporting strength for the border, each species is divided in to single branch, namely group A~E for *A. polytricha*, *A. delicate*, *A. fuscosuccinea*, *A. auricular-judae* and *A. mesenterica*, indicating that ITS sequence analysis supports species of *Auricularia* divided by morphological characteristics.

All *A. Polytricha* strains including two wild strains are subsumed into group A, showing their close genetic relationship, and matching their morphological characteristics. Teda and Taier 319, Hybrid 1# and Xiaoshang 3# are clustered together. The reasons may be a strain with two names. Variations is found in two wild strains (AP7 and AP49) and AP57 *A. auricular-judae*, *A. delicate*, *A. fuscosuccinea* and *A. mesenterica* have been reported that their ITS sequence exist some interspecific variability<sup>[1]</sup>.

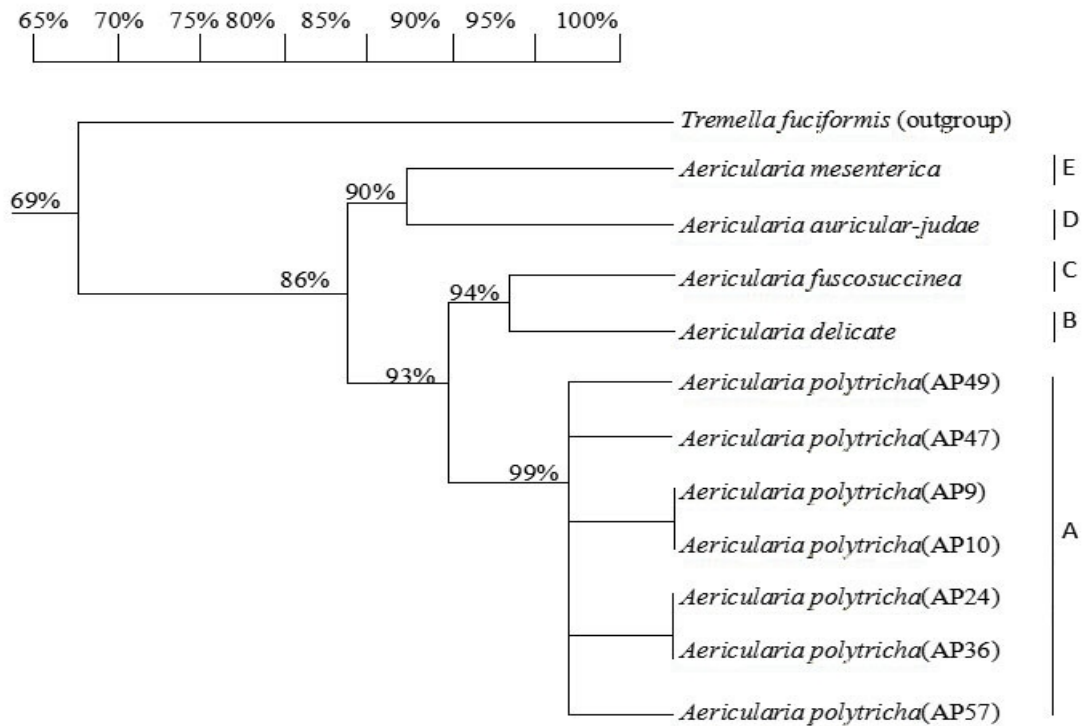


Fig. 2 Phylogenetic tree based on ITS region sequence alignment

## Discussion

Ultrastructure plays an important role in the phylogenetic classification of Basidiomycetes. For a long time, the main classified indicators based on the type of basidium covered the authentic *Auricularia* phylogeny status<sup>[4]</sup>. Basidium type was considered to be an important indicator to define higher taxa. On the basis of basidium type, *Introductory Mycology* divided basidiomycetes into three subclasses that are *Holobasidiomycetidae*, *Phragmobasidiomycetidae* and *Teliomycetidae*<sup>[5]</sup>. Along the classification system, fungi, with heterobasidium divided into four cells by diaphragm or mediastinum, compose *Phragmobasidiomycetidae*. In this subclass, *Tremellales* and *Auriculariales* are made up of saprophytic bacteria. *Tremellales* is characterized by heterobasidium divided into four cells by mediastinum. *Phlogiotis* spp, some species of *Exidia* spp. and *Tremella* spp. are part of *Tremellales*. The members of *Auriculariales* are characterized by heterobasidium and divided into four cells by diaphragm. However, according to *The Macrofungi in China* and some traditional classification, *Phlogiotis* spp. *Exidia* spp. and *Auricularia* are filed under *Auriculariales*. Further, *P. helvelloides*, *E. glandulosa* and *E. recisa* are filed under *Auricularia*<sup>[6]</sup>. The classification is based on the idea that fungal basidiocarp is glia to the deme. *Dacrymycetales*, *Tremellales* and *Auriculariales* are included in gelatinous fungus. But the *Dacrymycetales*'s basidiocarp has no diaphragm, and some species of *Eichlerilla incarnate* are classified in *Tremellaceae*.

Bandoni, combining microscopic morphology with ultrastructure, ecology and embryology data, come up with a selective classification opinion for *Tremellales* and *Auriculariales*' division<sup>[7]</sup>. He put the *Auriculariales*' species with simple septal pore out, so *Auriculariales* include basidiocarp with diaphragm or mediastinum, hyphae haploid phase, continuous bunghole overlap. This classification is supported by rRNA and rDNA sequence analysis. So *Auriculariales*, except *Auricularia*, also include some species under *Tremellales* classified by Martin, and *Tremellales* just



include species with septal pore complex and hyphae haploid phase<sup>[8]</sup>.

*Exidia* and *Auricularia* have different basidium type. *Auricularia* has diaphragm. Some species of *Exidia* and *Exidia glandulosa* Fr. have mediastinum. *Auricularia* Fr and *Exidia* Fr. are similar in morphology, fruiting body anatomy, pigments accumulation and the stage of non-morphological. Wei M, who took advantage of ITS sequence analysis to study the phylogenetic relationship of *A. auricula* and related taxa of it. The result support that *Exidia*, *Auricularia* and some species in *E. deglubens* are coexist in *Auriculariales*, distinguishing tremellales, which include *Tremella*, *Sirobasidium magnum*, and *Filobasidiella neoforman*<sup>[4]</sup>.

Mycologist put forward many classification systems<sup>[3]</sup>, but no one is the academic consensus. McGuire pointed out: because of lacking connected information on inconspicuous characteristics, we may not be making sure the real relationship in *Auriculariales*. Taking advantage of ITS sequencing and other molecular biology techniques, combining with morphology, microstructure and ultrastructure can make phylogenetic status of *Auriculariales* more objective and make the relationship with related taxa *Auriculariales* closer to the actual<sup>[9]</sup>.

Fungal rDNA is conservative, but IST on rDNA evolves quickly. In same genus, different strains may have variation. Interspecific variation of ITS sequence in *A. auricula-judae*, *A. delicata*, *A. fuscusuccinea* and *A. mesenterica* have been reported<sup>[1]</sup>. For the first time, PCR amplification, cloning and sequencing to the ITS region gene segments of *A. auricula-judae* are successfully carried out. The results showed: variation is found in the ITS sequences of seven strains, and nucleotide variation range from 1-4 bp. Combining sequence from Gene Bank, comparative analysis is carried out, the finding *A. polytricha*, *A. auriculajudae*, *A. delicata*, *A. fuscusuccinea* and *A. mesenterica*'s ITS sequence variation is greater than the intraspecific variation of *A. polytricha*.

Finally, the results of ITS sequence analysis support the traditional classification of *Auricularia* and *Tremella*, so do the taxonomy of species in *Auricularia* and the separation of strains in species.

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