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Partial 25S rDNA Sequences and Phylogenetic Relationships of *Cantharellus*, *Craterellus* and *Gomphus**

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Abstract: Based on the analyses of the obtained sequences and the sequences determined by predecessors from other 14 representative species of 10 basidiomycete orders and 1 zygomycete order, phylogenetic trees of partial 25S rDNA sequences are constructed in which the outgroup is represented by the zygomycete *Mucor racemosus*. Judging from the 40 trees constructed with Fitch method of Phylip program and the consensus tree, *Cantharellus* and *Craterellus* are the most homogeneous. *Gomphus* is also closely related to them. The cantharelloid fungi appear comparatively closely related with the true agarics, but they seem to have a relatively separated evolutionary line among them; it is still regarded as apt that *Craterellus* and *Cantharellus* are gathered into a family Cantharellaceae, and treated the cantharelloid fungi are treated as an independent order Cantharellales.

Keywords: *Cantharellus*, *Craterellus*, *Gomphus*, 25S rDNA, phylogeny

Classification number: Q 949.329, Q 751

1 Introduction

Cantharellus Adans. & Fr. (1821), *Craterellus* Pers. (1825) and *Gomphus* Pers. ex Gray (1821) are 3 genera of the so-called cantharelloid fungi. They are a group of cosmopolitan fleshy homobasidiomycetes, and some of them are of economical significance. Mycologists are interested not only in their edible or medicinal values, but also more often in their naturally phylogenetic positions. Traditional mycologists generally taxonomically treated them according to the morphological, anatomical and microscopic analyses, but they have some phylogenetically "indefinite" or "intermediate" morphological characters. The fleshy and agaroid fruitbodies are similar to those of the true agarics, but their hymenophore is not typically lamellate, and the long and slender basidia are remindful of those of clavarioid fungi or *Hydnum* of Aphyllophorales and even those of heterobasidiata auriculariaceae fungi.

Since the differences of the consideration emphasis and the shortages of sufficient evidences, different phylogenetic placements and hypotheses about their evolutionary history and relationships to other basidiomycetes have long been highly controversial. Most mycologists agree that *Cantharellus* and

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Craterellus compose a family Cantharellaceae Schroet (1988), while *Gomphus* is grouped in Gomphaceae Donk (1961) with several other genera, and the two families belong to Order Cantharellales Gaeumann, but Jülich^[1] established an order Gomphales separated from Cantharellales. Some mycotaxonomists consider them as the primitive and key group of all Homobasidiomycetes^[2], and even consider that they derived from auriculariaceous fungi, the other taxa of Homobasidiomycetes derived from them or their allies through several main evolutionary lines^[1]; while other mycotaxonomists insist that they might have closer relationship with clavarioid fungi^[2,3], or considered them closely related to *Hudnum* and placed them in Aphyllophorales (Donk, 1964); the active and noted British mycologist D.N. Pegler and some others prefer to treat them as an order Cantharellales and place the order phylogenetically after Aphyllophorales and in front of the rest agaric orders. In Ainsworth's system^[4], the order is also placed in the primitive position within holobasidiomycetes.

Recently, biological molecular systematics techniques have been extensively applied in fungal taxonomic and phylogenetic studies. The results of the recent studies are quite delighting. As to cantharelloid fungi, foreign experts have begun to applied molecular biological techniques to their systematic studies. Feibelman et al.^[5], for instance, published a paper of "Length Variation in the Internal Transcribed Spacer of Ribosomal DNA in *Chanterelles*". Another recent study^[6] with molecular method on auriculariaceous basidiomycetes has implied that auriculariaceous fungi have been developing along its own evolutionary line, it is improbable that the Homobasidiomycetes derived from auriculariaceous fungi. Therefore, the hypothesized "affinity" between auriculariaceous fungi and cantharelloid fungi becomes more inconceivable, the relationships between cantharelloid fungi and other homobasidiomycetes should be reconsidered. While a more recent study^[7], in which the nuclear and mitochondrial small subunit rDNA of *Cantharellus tubaeformis* (Bull.) Fr. are examined, suggests that the chanterelle is closer to *Hydnum* and *Clavulina* of Aphyllophorales and is relatively independent from other "true agarics (euagarics)" of the gilled mushrooms.

The present study was to further investigate its phylogenetic relationships with the molecular systematic techniques. Since 25S rDNA contains both slowly and rapidly evolving domains, potentially representing a wide-spectrum indicator, partial sequences of 25S rDNA of the type species of these 3 genera, *Cantharellus cibarius* Fr., *Craterellus cornucopioides* (L.) Pers. and *Gomphus floccosus* (Schw.) Sing., are determined in this study; and the obtained sequences are compared with the homologous sequences determined by predecessors from 13 representative species of 10 different basidiomycete orders, phylogenetic trees are constructed in which the outgroup is represented by a zygomycete *Mucor racemosus* Fres., and their phylogenetic positions are discussed.

2 Materials and methods

Fungal specimens: *Cantharellus cibarius*, was collected from Jianfengling Nature Reserve at alt. 800 ~ 850 m, Hainan Province, China on 20th May, 1992, duplicate specimens (HMIGD 14516) were preserved in the Herbarium of Microbiology Institute of Guangdong (HMIGD). *Craterellus cornucopioides* and *Gomphus floccosus* were collected from Linzhi County at alt. 3 000 m, Tibet, China on 20th August, 1997, duplicate specimens (HMIGD 17860 and HMIGD 17861) are also preserved in the Herbarium.

DNA extraction, PCR amplification and sequencing; the test methods are the same those as in reference [8].

Sequence alignment and analysis; sequences are manually aligned and gaps are introduced into the sequences to increase their aligned similarity. Phylogenies are estimated from the distance matrices us-

ing Fitch-Margoliash (Fitch Program) with global rearrangements. Bootstrapping is performed using the Seqboot Consense programs with 40 replicates. The maximum likelihood trees are constructed with a ratio 2.0 of transtion to transversion.

Tab. 1 Species analyzed, their ordinal position, DNA source, taxa abbreviation and Genbank accession number

Species	Order	Abbr	Genbank accession
<i>Amanita muscaria</i> (L. & Fr.) Pers.	Amanitales	Aman	unsubmitted
<i>Auricularia auricula-judae</i> (Bull. & Fr.) Schroet.	Auriculariales	Auri	L20278
<i>Cantharellus cibarius</i> Fr.	Cantharellales	Cant	unsubmitted
<i>Craterellus cornucopioides</i> (L.) Pers.	Cantharellales	Crat	unsubmitted
<i>Ganoderma tsugae</i> Murr.	Aphyllophorales	Gano	X78778
<i>Gomphus floccosus</i> (Schw.) Sing	Gomphales (or; Cantharellales)	Gomp	Unsubmitted
<i>Helicobasidium mompa</i> Tanaka	Auriculariales	Hbas	L20281
<i>Helicogloea variabilis</i> Wells	Auriculariales	Hglo	L20282
<i>Lactarius corrugis</i> Peck	Russulales	Lact	U11919
<i>Phyllporus rhodoxanthus</i> (Schw.) Bres.	Boletales	Phyl	U11925
<i>Pleurotus cornucopiae</i> Paulet & Fr.	Aphyllophorales	Pleu	U04135
<i>Puccinia graninis</i> Pers.	Pucciniales	Pucc	L08728
<i>Septobasidium caestianum</i> Bres.	Septobasidiales	Sept	L20289
<i>Tilletia caries</i> (DC.) Tul.	Tilletiales	Till	L20285
<i>Tricholoma vernaticum</i>	Tricholomatales	Tric	U76461
<i>Ustilago hordei</i> (Pers.) Lagerh.	Ustilaginales	Usti	L20286
<i>Mucor racemosus</i> Fres.	Mucorales	Mucr	M26190

3 Results and discussion

More than 700 base pairs of each species fragment are determined and about 260 bps of the sequences of the fragments are selected and compared with the homologous sequences determined by the predecessors from 13 representative species of 10 basidiomycete orders. Among the compared sequence fragments, *Craterellus cornucopioides* possesses the longest one with 265 bps, and *Cantharellus cibarius* has the second longest one with 258 bps, they have stronger similarities and more inserted bps; while the others have 214 to 241 bps, e. g. *Gomphus floccosus* has 240 bps. The segments of Cantharellaceae species have more base inserts than those of other orders although they still have most of the common molecular similarities of Homobasidiomycetes. This interesting situation is similar to the examination result by Feibelman et al^[5] that *Cantharellus cibarius* group had the longest ITS-1 region among their tested fungi. It is still unknown if the longer DNA sequences in the taxa of Cantharellaceae have any more phylogenetic implication.

Based on the analyses of the obtained sequences, phylogenetic trees of partial 25S rDNA genes are constructed with the methods of Maximum Likelihood and Fitch, in which 16 basidiomycetes are compared and the outgroup is represented by a zygomycete *Mucor racemosus*, indicating the phylogenetic positions of the studied cantharelloid fungi and their relationships with some other main relative fungal orders. Although the positions of them are not constantly stable in all the trees, many of them are quite similar and they have shown that some of the results are close to the traditional morphological analysis. In these 40 trees, *Craterellus* and *Cantharellus* are usually (70%) gathered in a same branch, and the branch in most cases is separated from the group of the rest homobasidiomycetes; they mostly go to the group of Homobasidiomycetes. *Gomphus* is often (45%) in the position neighbouring to either of

the 2 species of Cantharellaceae, much more often than to any other compared taxon. Most of the trees, in which all the homobasidiomycetes gather in a big group, possess the above characters (see representative trees A, B, C and D in Fig. 1).

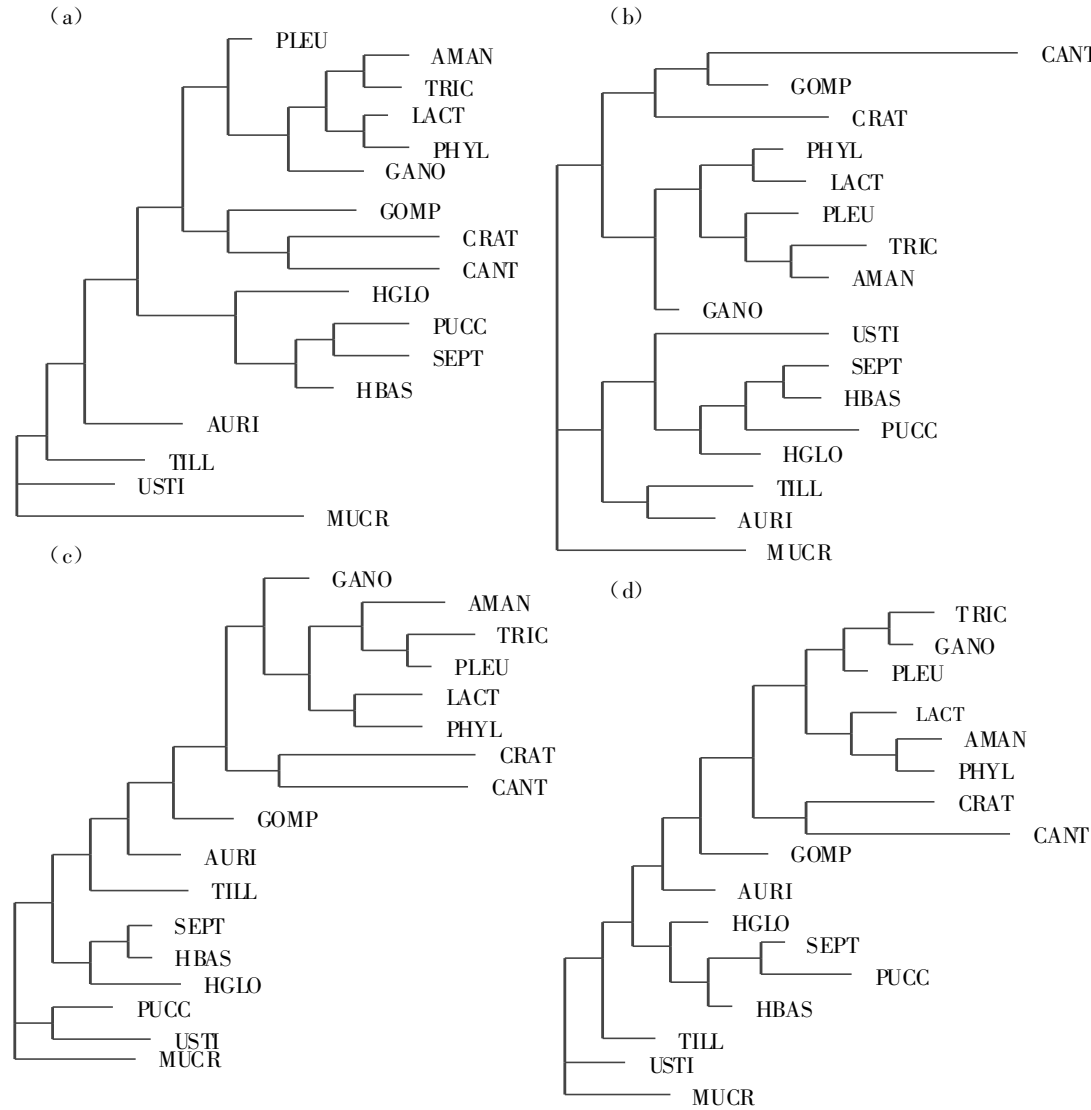


Fig 1 The phylogenetic trees constructed based on the correction values of nucleotide difference of 25S rRNA 5' terminal region with Fitch method (Phylip version 3.5) 40 random input order MUCR as outgroup; Four representative trees are presented

According to the above results, it can be seen that *Cantharellus* and *Craterellus* are the most homogenous and are apt to be gathered as a natural family Cantharellaceae. *Gomphus* seems to be closely related to them. Gaumann's creation of order Cantharellales (1966) including Gomphaceae is still an appropriate treatment, although some mycologists still insist on splitting an order Gomphales from Cantharellales^[2]. Cantharelloid fungi probably have a relatively independent evolutionary lineage from any other true agarics. The above results more or less agree with the traditional morphological analysis. It is worthy being further studied, however, if Cantharellales should be phylogenetically placed in the position between Aphyllophorales and the true agaric orders. Evidences can not prove the hypothe-

sis of Jülich that cantharelloid fungi were derived from auriculariaceous fungi, the other taxa of Homobasidiomycetes derived from them or their allies; but we know that Cantharellales has closer relationship with Homobasidiomycetes than with the heterobasidiomycetes (i. e. the auriculariaceous fungi with transversely septate metabasidia in this study, including Septobasidiales, Pucciniales and Auriculariales).

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鸡油菌、喇叭菌和钉菇的 25S rDNA 部分序列及其系统学关系

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摘 要: 从分子系统学角度出发, 对鸡油菌属(*Cantharellus*)、喇叭菌属(*Craterellus*)和钉菇属(*Gomphus*)的模式种的 25S rDNA 部分序列进行了测定, 并与另外 10 目的 13 个担子菌和 1 个接合菌的 25S rDNA 序列进行比较分析, 以接合菌总状毛霉(*Mucor racemosus*)为外类群构建出这些担子菌的部分 25S rDNA 序列的系统树图, 探讨它们 3 属之间及它们与其他担子菌的关系及其系统学上的意义。认为鸡油菌与同科的喇叭菌关系最为亲近, 而钉菇属看来也应该归入鸡油菌目 Cantharellales 中, 这与传统方法的分析结果较为一致; 虽然鸡油菌类真菌又与各肉质的伞菌类群的关系相对较密切, 但它应该有其相对独立的进化路线, 将这一类真菌上升至目一级的分类阶元是比较恰当的; 但钉菇目 Gomphales 上升到目的水平是否恰当则有待更多的数据去证明。

关键词: 鸡油菌属 *Cantharellus*, 喇叭菌属 *Craterellus*, 钉菇属 *Gomphus*, 25S rDNA, 系统学

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