



An Asian edible mushroom, *Macrocybe gigantea*: its distribution and ITS-rDNA based phylogeny

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Abstract

An updated phylogeny of *Macrocybe*, a rare pantropical genus, is presented, and new insights regarding the distribution of *M. gigantea* are given. At the end of last century, this genus was segregated from *Tricholoma* on morphological and molecular data yet it is still being treated under *Tricholoma*. In the phylogenetic analysis based on ITS-rDNA, we observed the monophyletic lineage of *Macrocybe* from the closely related genera *Calocybe* and *Tricholoma*. Six collections of *M. gigantea*, a syntype, re-collected and sequenced from Pakistan, clustered with Chinese and Indian collections which are available in GenBank under *Tricholoma giganteum*, an older name. The purpose of this work is to support *Macrocybe* as a separate genus from *Tricholoma* and *Calocybe* using ITS-rDNA based phylogeny.

Key words – *Calocybe* – macro fungi – Siderophilous granulation – *Tricholoma*

Introduction

Macrocybe Pegler & Lodge accommodates those tricholomatoid species which have large, fleshy, white, cream to grayish ochraceous, convex, umbonate to depressed basidiomata. Microscopically, these lack siderophilous granulation in the basidia, cheilocystidia, and are not ectomycorrhizal (Pegler et al. 1998). Previously, the members of *Macrocybe* were treated in *Tricholoma* sect. *Leucorigida* Singer (1986), but the type specimen of this section belongs to *Calocybe* (Pegler et al. 1998). *Macrocybe* and *Calocybe* are very closely related genera as both have conspicuous, saprophytic fruiting bodies but differ in that the former lacks siderophilous granulation, and its members are distributed in tropical or subtropical areas. This granulation pattern is the main difference between tribus *Tricholomatae*, *Lyophyllae* and *Macrocybe* is treated in former tribus.

Macrocybe has been treated in *Tricholoma* until Pegler et al. (1998) segregated *Macrocybe* from *Tricholoma* and ranked it to a genus using morphological and molecularly distinct characteristics. Species of *Macrocybe* are saprophytic, large, with clamped hyphae, while those of *Tricholoma* have clampless hyphae and are obligatory ectomycorrhizal. Pegler et al. (1998) also conducted molecular analysis based on the larger sub-unit of ribosomal DNA. This analysis also reflected the phenotypic segregation at the genetic level. In their phylogenetic analysis, *Macrocybe* separated from *Tricholoma* and showed close relationship with *Calocybe*, *Ca. gambosa*. Pegler et al. (1998) included seven species mostly from Asia and syntypes of *M. gigantea* from India and Pakistan in their study.

Macrocybe gigantea is a common edible mushroom in India and many closely related species are given the same name (Prakasam et al. 2011). Since 1998, *Tricholoma giganteum* has been replaced with *M. gigantea* but the former name is still being used especially in the submission of sequences to GenBank. Not much literature after Pegler et al. (1998) is available using the name *Macrocybe* which is a very confusing situation. This was not the first work for the introduction of a new genus, as Moncalvo et al. (1993) had proposed the idea of an independent lineage for *T. giganteum* based on their molecular analysis.

In this paper, we made six different collections of *M. gigantea* from Pakistan and described them morphologically and molecularly. We present for the first time the phylogenetic analysis of *Macrocybe* using the ITS regions of rDNA. This differs from Pegler et al. (1998) where they described the genus using LSU and from a limited number of collections.

The purpose of this work is to re-introduce the work of Pegler et al. (1998) and increase the knowledge concerning the genus *Macrocybe* and *M. gigantea*, an Indian species.

Materials and Methods

All the basidiomata were carefully dug up and photographed in the field. Material was characterized morpho-anatomically and molecularly. For microscopic observation, sections were stained with Congo Red and Melzer's reagent. Each of 25 basidiospores and 20 basidia were measured. The following abbreviation is used: Q for length-width ratio of basidiospores. Drawings were made using a camera lucida attached to a compound microscope. Dried specimens were deposited in the LAH Herbarium, Department of Botany, University of the Punjab, Lahore.

The protocol of Extract-N-Amp (XNAP-2) (Sigma, St. Louis, MO, USA) was followed. Dried material of the basidiomata (approx. 1 mg) were put into small PCR tubes and 10 µl of extraction solution was added. These tubes were first incubated at 65°C for 10 min then at 94°C for 10 min. After that, 10 µl of dilution solution (XNAP-2) was added and the tubes were left for one hour at room temperature. ITS regions of rDNA were amplified using the universal primer pair ITS1F and ITS4 (White et al. 1990, Gardes and Bruns 1993). PCR was performed in 25 µL reaction volume following the protocol given by Gardes and Bruns (1993). The PCR product of the ITS-amplified region containing ITS1, 5.8S and ITS2 was directly sequenced in both directions using the same pair of amplification primers (Macrogen, Korea).

All available ITS sequences of *Tricholoma giganteum* (*Macrocybe gigantea*) and its closely related genera *Tricholoma* and *Calocybe* were downloaded from GenBank. *Tricholomopsis flammula* was used as outgroup. The newly generated sequences and those which were retrieved from GenBank were aligned using the ClustalW program of Molecular Evolutionary Genetics Analysis (MEGA) software (Tamura et al. 2011). Following Dentinger et al. (2011) to get complete ITS sequences, all sequences were trimmed with the conserved motifs 5'-(...GAT) CATT— and —GACCT (CAAA...)-3' and the alignment portion between them was included in the analysis. Gaps were treated as data for construction of phylogeny. Phylogenetic analysis was determined following Razaq et al. (2012). The dataset of a total of 33 sequences was locally analyzed by a maximum likelihood (ML) method with MEGA 5.1 version and one hundred ML bootstraps were performed.

The newly produced nucleotide sequences were submitted to the European Molecular Biology Laboratory (EMBL) database and are available in GenBank. Numbers are listed with the collections and in Fig1.

Results

Molecular characterization and phylogenetic analysis

The ribosomal DNA especially ITS1, ITS2 and 5.8S parts of rDNA were amplified successfully from six (6) Pakistani collections using Polymerase Chain Reaction (PCR). This reaction produced fragments of approximately 750bp using the universal fungal primer pair,



Fig. 1 – Phylogenetic relationships of Pakistani collections of *Macrocybe gigantea* with other closely related genera based on Maximum Likelihood analysis of ITS- rDNA sequences. Bootstrap values >50, based on 100 replicates are shown at each node. The analysis involved 33 sequences, with *Tricholomopsis flammula* as the outgroup.

ITS1&ITS4. Extraction of DNA and amplification of targeted regions from freshly collected and old collections were equally successful. An initial BLAST analysis of nucleotide sequences revealed that the Pakistani collections matched best with *Tricholoma giganteum* (JN006792, EU051917, JX041888, JX193694, JX068527 etc.,).

In the phylogenetic analysis based on ITS regions of ribosomal DNA, the three major clades were formed: *Macrocybe*, *Tricholoma* and *Calocybe*. The *Macrocybe* and *Tricholoma* clades are

sister clades to each other and all collections of *M. gigantea* (six from Pakistan, six from China and India) showed a monophyletic relationship. The Pakistani sequences showed more intraspecificity than the Chinese and Indian ones and these were recovered in a separate sub-clade. The Pakistani sequences clustered together distinct from the rest of the isolates suggesting geographical isolation. Two more Indian sequences, (bold branches, Fig1) representing *T. giganteum* and *Calocybe indica*, are also species of *Macrocybe* and these are closely related to *M. gigantea*.

The *Tricholoma* clade also has the species that are mycorrhizal and all these species have a monophyletic relationship. *Macrocybe* has a close evolutionary relationship with *Tricholoma* rather than *Calocybe* in spite of the morphological and life mode differences. All European species of *Calocybe* clustered in two sub-clades of the *Calocybe* clade, yet these are monophyletic in origin. Although *Calocybe* and *Macrocybe* are both saprophytes and phenotypically confusing, still there is no close similarity in their DNAs. The presence/absence of siderophilous granulation appears to be a more significant feature than ecology and morphology. All these three monophyletic clades had significant statistical values.

Taxonomy

Macrocybe gigantea (Masse) Pegler & Lodgel. Mycologia, 1998

Fig. 2

Pileus 10–30 cm across, convex to flat, white to grayish white, paler towards margin, glabrous and silky smooth, margin entire and incurved, often cracking. Lamellae notched, crowded, grayish white straw yellow, many tiers of lamellulae. Stipe central, 15–50 × 4–8 cm, solid, concolorous with pileus, fibrillose. Basidiospores 5–6.5×3.5–4.5 μm average 5.5×3.8 μm Q=1.32–1.51, ovate to ellipsoidal, thin walled, hyaline, smooth. Basidia, four spored, av. 27.5×7 μm, narrowly clavate to sub-cylindrical, light brown to hyaline, oil droplets prominent, basal clamp connections present. Cystidia absent. Lamellar edges fertile. Hymenophoral trama regular, made up of thin-walled parallel hyphae. Pileipellis a cutis of narrow hyphae 4.2–7.5 μm (av = 5.5 μm) in diameter, hyaline in 5% KOH, clamp connections present. Spore print white. Odour and taste not recorded.

Material Examined – Pakistan, Punjab, Lahore, 217 m a.s.l., gregarious, on decayed woods of *Dalbergia sisoo*, 23 August 2010, A. Razaq (LAH 230840); A.N Khalid, 2012, 2013.

Discussion

Singer (1986), morphologically, segregated the large-sized mushrooms of *Tricholoma* into a new sect. *Leucorigida* but he overlooked the siderophilous granulation character. Later on, Pegler et al. (1998) realized the need to separate this section into a new genus, *Macrocybe*. They also confirmed their hypothesis by molecular analysis using large sub unit (LSU) of rDNA. In the present work, the distinct lineage of *T. giganteum* has been re-evaluated using ITS regions of rDNA and our findings are in accordance with the previous studies (Pegler et al. 1998, Moncalvo et al. 1993, 2002, Hofstetter et al. 2002). Mycologists from China and India, are still hesitant to include their collections in *Macrocybe* (Prakasam et al. 2011, GenBank sequences data).

In our phylogenetic analysis, *Macrocybe* separated from *Tricholoma* and *Calocybe* distinctly showing independent origin. Moncalvo et al. (2002) in their LSU analysis recovered these three clades in different sub-clades: *Calocybe* in Lyophylleae group, *Tricholoma* in tricholomatoid group, while *Macrocybe* was in the callistosporioid group. According to the work of Moncalvo et al. (2002), *Macrocybe* is closer to *Entoloma* than *Tricholoma* or *Calocybe*. Morphologically however, *Calocybe* has a close resemblance to *Macrocybe* and in India the larger size and white coloration of both genera are confusing and lead to misidentification. In the present phylogenetic analysis, one sequence of *Calocybe indica* (JN874408) was retrieved from GenBank which is misidentified and represents possibly a *Macrocybe* sp. Hofstetter et al. (2002) reported *Calocybe* as different from other closely related genera using ITS, LSU and mt SSU (mitochondrial small-sub unit). All these studies showed the significance of siderophilous granulation, the idea followed by Pegler et al. (1998).

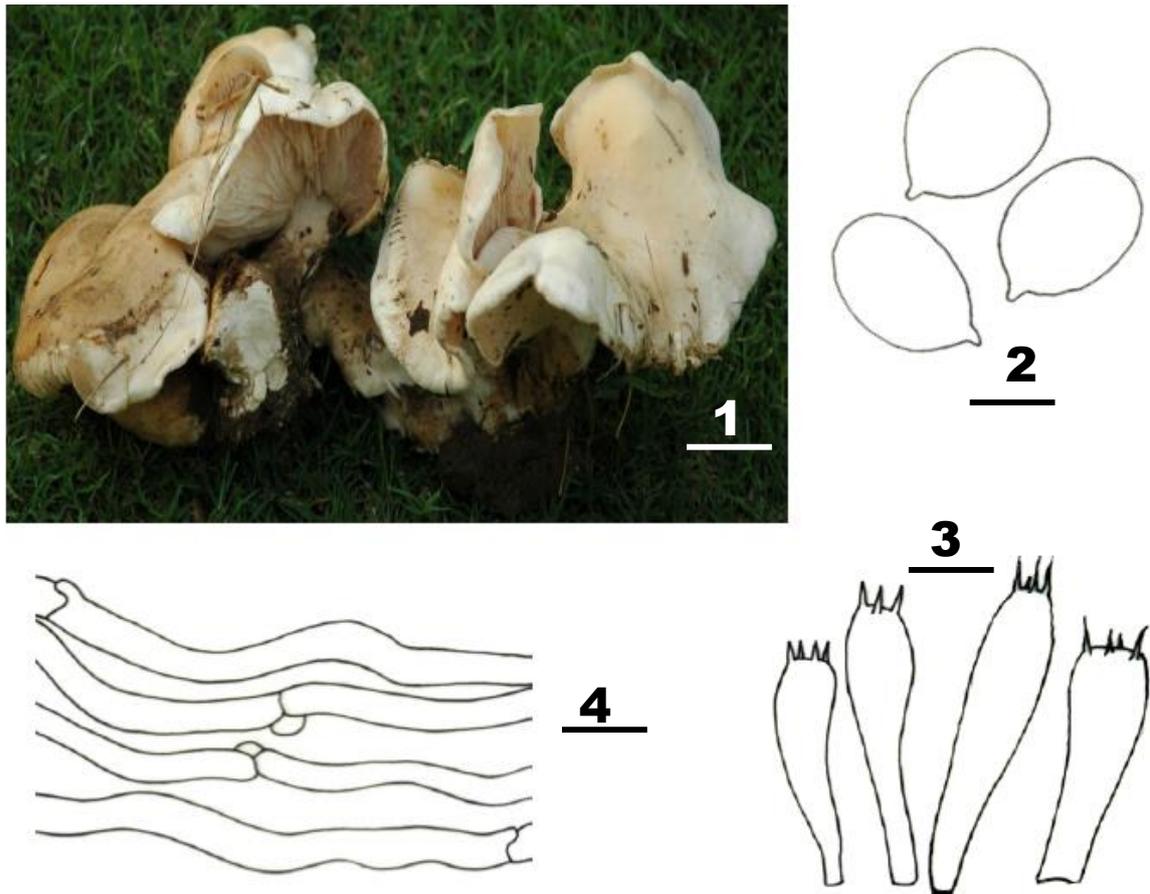


Fig. 2 – *Macrocybe gigantea*. 1. Basidiomata. 2. Basidiospores. 3. Basidia 4. Hyphae of the pileipellis. Scale bars: 1 = 1 cm, 2 = 3.45 μ m, 3 = 6.25 μ m, 4 = 21.25 μ m.

The Pakistani collections of *M. gigantea* were separated from the rest of the collections from China and India in the same clade (Fig. 1, clade *Macrocybe*) showing the distinct genetic makeup as these share the same ecological and climatic zone. This species has been reported from the West Bengal plains of India where the maximum temperature reaches 39°C while in the Pakistani plains this temperature reaches up to 50°C. West Bengal is famous for its edible larger-sized mushrooms especially *M. gigantea* and *M. lobayensis* (R. Heim) Pegler & Lodge (Chakravarty & Sarkar 1982). This problem of misidentification has been reflected in our phylogenetic analysis of some collections as *T. giganteum* (HM120872) clustered with *Ca. indica* and both these species represent a different *Macrocybe* sp. or possibly *M. lobayensis* on the basis of sequence origin. To date, *M. gigantea* is distributed only in Asian countries: China, India, Nepal and Pakistan and there is no other report of this species from the western Hemisphere.

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