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A New Record of Genus *Craterellus*, Edible Basidiomycotous Fungus from Pakistan

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Abstract: Basidiomata of a *Craterellus* sp. were collected from Oaks forest, Swat, Pakistan. Based on morphology and molecular phylogeny, the specimens were identified as *Craterellus cinereus.* This is first record of occurrence of this genus from Pakistan.
Keywords: Chanterelle, edible, ITS, *Quercus* species.

INTRODUCTION

The Cantharellaceae is an important family of ectomycorrhizal fungi, and popularly known as chanterelles. Several species (*Cantharellus cibarius, Craterellus tubaeformis* and *Hydnum repandum)* in the family are highly prized mushrooms. They have important nutrition value due to high level of proteins, lipids, minerals, vitamins, and neutraceuticals [1]. The chanterelle hymenium can be smooth, wrinkled, veined, or ridged, but never forms bladelike gills. Most chanterelles have spore bearing ridges that typically extend from the edge of the cap well down the tapered stipe. In a recent classification, the genera *Craterellus* along with *Cantharellus*, *Hydnum*, *Clavulina*, *Multiclavula*, *Sistotrema* and *Membranomyces* form one clade that is characterized by stichic basidia [2], variable septal pore morphology and ectomycorrhizal mode of nutrition [3].

The genus *Craterellus* is characterized by its small, funnel- shaped fruiting bodies with a hollow stipe that may also be highly reduced. It includes several edible forms of nutritive values. *Craterellus* is represented by 20 species [4]. From Pakistan, this is first report of occurrence of this genus.

One of these is *Craterellus cinereus* that is edible worldwide [5]. *Craterellus cinereus*, a new record from Swat, Pakistan is described, illustrated and discussed. The specimens were identified on the basis of morphological characters as well as molecular datasets based on the internal transcribed spacer (ITS) of nrDNA.

MATERIALS & METHODS

Sampling Site

The specimens were collected during a field investigation of ectomycorrhizal communities associated with Oaks forests, Toa in Swat, KPK, and Pakistan. The forest is located at 34°54′00″N 72°39′00″E at 850–2350 m elevation. The climate of the area has very prolonged cold winter with short summer. The annual rainfall is approximately 1415.9 mm.

Morphological and anatomical analyses

Specimen was photographed in field using a Nikon D70S digital camera. Odor and color change upon bruising were recorded at the time of collection, then wrapped in aluminium foil and kept separately in a collection box to avoid mixing or crushing. Specimens were dried by fan heater, sealed in plastic bag, and deposited in Lahore Herbarium, Department of Botany, University of the Punjab, Lahore, Pakistan (LAH35239). Colors were designated by mColorMeter.

For detailed anatomical examination, tissues from lamellae, pileipellis and stipitipellis were mounted on glass slides and observed in Phloxine (1%) for better contrast, Melzer's reagent for amyloid basidiospore ornamentation, and KOH (5%) for colored hyphae using a Meiji Techno MX4300H microscope. Dimensions were determined for basidiospores, basidia and other elements from basidiomata under the light microscope equipped with a camera lucida.

DNA extraction, amplification and sequencing

Genomic DNA was extracted from gill tissue using a modified CTAB method [6]. ITS region was amplified by the primer pairs ITS1F/ITS4B. All PCR products were evaluated for successful amplification using SYBR Green and 1.5% agarose gels with TAE buffer for gel electrophoresis. Amplicons were prepared for sequencing via enzymatic purification using

Exonuclease I and Shrimp Alkaline Phosphatase enzymes [7]. Purified products were sequenced by the University of Florida's Interdisciplinary Center for Biotechnology Research (http://www.biotech.ufl.edu/). Sequence chromatograms were trimmed, edited, and assembled using Sequencer 4.1 (GeneCodes, Ann Arbor, MI). DNA sequences generated form this study is deposited in GenBank (MF374488 & MF374489).

Molecular phylogenetic analysis

Consensus sequences generated in BioEdit software were BLAST searched at NCBI (http://www.ncbi.nlm.nih.gov/). Sequences with closest match were selected from GenBank to reconstruct phylogeny. Published sequences of the most closely related species were also included in the final data set. Multiple sequences were aligned using online MUSCLE by EMBL-EBI (http://www.ebi.ac.uk/Tools/msa/muscle/). A maximum likelihood tree was inferred for each alignment using RAxML-HPC2 v 8.1.11 [8] with a GTR + gamma model of nucleotide substitution. One thousand bootstrap iterations were performed with rapid bootstrapping. Significant support was considered to be ≥70%. All phylogenetic analyses were performed on the CIPRES Portal v. 3.1. [9]. The phylogeny from ML analysis was displayed with FigTree 1.4.2 [10] and exported to Adobe Illustrator.

RESULTS

Craterellus cinereus (Pers.) Pers., *Mycol. eur. (Erlanga)* 2: 6 (1825) Fig.1&2 Basidiomata large sized. Pileus 1.5–4.5 cm, grayish to brown (9.1YR 5.1/0.6) with black (0.9B 1.8/0.5) fibrillose or squamose, infundibuliform, shallowly depressed at center, dry. Margins black (0.9B 1.8/0.5), striate, slightly incurved, wavy, wrinkled. Hymenophore gray (7BG 3.7/0.1), decurrent, folded, interveined, consisting of longitudinal ridges with prominent forking. Stipe 3–5 cm long, 0.3–1 cm in width, dark gray to black (4.3B 0.9/0.3), central, equal, hollow, tapering downward slightly, more or less curved, compressed, fibrillose. Basidiospores (7.3–) 7.9–9.4 (–9.6) × (4.3–) 4.5–5.8 (–7) µm, av.L = 7.0 µm, av.W = 5.2 µm, Q = 1.34, light yellow in 5% KOH, ellipsoid to broadly ellipsoid, smooth, nonamyloid, guttulate. Basidia 42–55 × 5.8–7 µm, clavate, some sub cylindrical, 5–6 spored (a few are 4), strigmata long (up to 5–6 µm). Basidioles abundant. Hymenophoral Trama light yellow 5% in KOH, interwoven hyphae, branched. Clamps absent. Pileipellis 3.6–12 µm wide, hyaline to faint yellow in KOH, septate, branched, cylindrical hyphae, thin to thick walled hyphae, brownish yellow encrustations on some hyphae. Stipepellis 3.2–8.0 µm wide, light yellow in KOH, branched, septate. Clamp Connection absent.

Material examined

Pakistan, Khyber Pakhtunkhwa Province, Swat District, Toa, 2800 m.a.s.l., on soil under *Quercus incana* Roxb., Arooj Naseer & Abdul Nasir Khalid. July 15, 2015, AST12 (LAH35239).

Fig-1: Morphology of *Craterellus cinereus.* A–B. Basidiomata. A & B. LAH35239. Scale Bar = 1.65 cm.

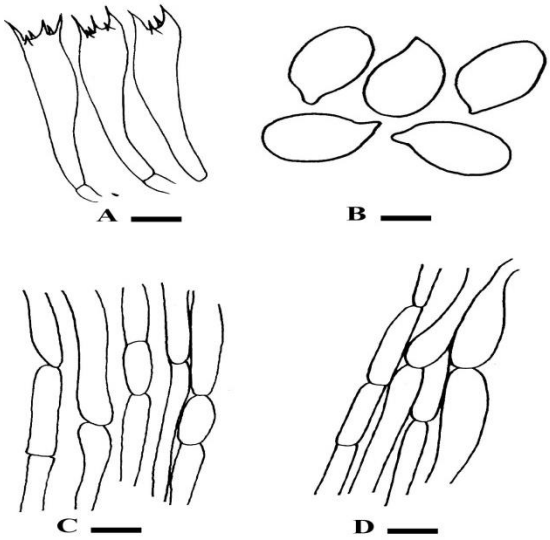

Fig-2: Anatomy of *Craterellus cinereus*. A–D. A. Basida; B. Basodiospores; C. Pileipellis; D. Stipitipellis; Bars; A = 10.85 µm; B = 3.93 µm; C = 2.95 µm; D = 1.5 µm.

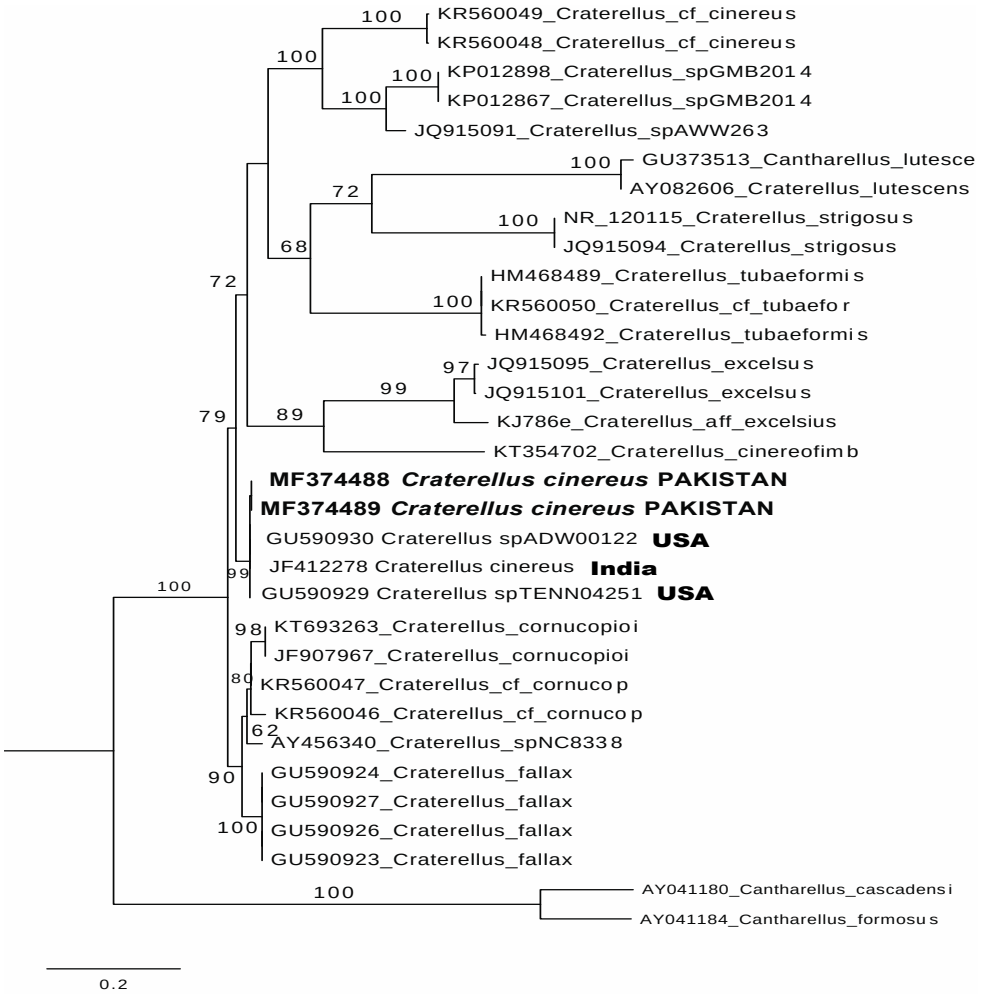

Fig-3: Midpoint-rooted maximum likelihood phylogram of *Craterellus cinereus* based on ITS ribosomal DNA as generated with RAxML with 1000 bootstrap iterations. Bolded lettering refers to sequences generated in this study. Bolded country names show sequences *Craterellus cinereus* from other parts of World

Molecular phylogenetic characterization

Sequencing of the PCR products of ITS region of *Craterellus cinereus* yielded fragments of 695–723 base pair by using of ITS1F and ITS4B primers. Consensus sequence of 668 and 675 base pairs were obtained by trimming the motifs and BLAST searched at NCBI. These sequences showed 99% identity to sequences from India (JF412278) with 100% query cover and same identity to sequence from USA (GU590930) with 99% query cover and 0.0 E values.

For phylogenetic tree, ITS sequences from closely related taxa were retrieved from the GenBank. *Cantharellus formosus* Corner (AY041184) and *Cantharellus cascadensis* Dunham, O'Dell & R. Molina (AY041180) were added in the final alignment file to root the tree. The sequences generated from LAH35239 clustered with the similar taxa from India with 99% bootstrap.

DISCUSSION

The *Craterellus cinereus* is distinguished by its olive gray to blackish brown basidioma with forked ridged hymenophore and absence of clamp connections. Its pileus surface is made up of cylindrical hyphae, upright and protruding beyond surface. Its cells are usually short and hyphae are finely encrusted. The spores are ellipsoid and basidia are mostly 6–spored. The sequences generated during this study clustered with other sequences of *Craterellus cinereus* reported from India and USA.

From American collection, it differs by slightly smaller basidia. However, there are also some morphological differences between Indian and Pakistani collections. *Craterellus cinereus* from Pakistan is large in size as pileus is 4.5 cm in diameter as compared to Indian collection which is 3.5 cm in diameter. Stipe is also thinner being 3–5 × 0.3–1 cm as compared to Indian fruiting bodies that is 1.5–4.5 × 1.0–1.5 cm in diameter, and more darker in color becoming blackish in color. *Craterellus cinereus* has been reported in association with *Pinus roxburghii* and *P. wallichiana* in India. In this study it has been observed under canopy of *Quercus incana* in pure Oaks forests, Swat, KPK, and Pakistan.

This is first report of *Craterellus cinereus* from Pakistan on molecular basis. Our *Craterellus cinereus* collection represents an addition to the mycobiota of Pakistan and a first report of the genus from Pakistan.

REFERENCES

1. Pilz, D., Norvell, L., Danell, E., & Molina, R. (2003). *Ecology and management of commercially harvested chanterelle mushrooms*. Gen. Tech. Rep PNW-GTR-576. Portland, OR: US Department of Agriculture, Forest Service, Pacific Northwest Research Station.
2. Pine, E., Hibbett, D.S., & Donoghue, M.J. (1999). Phylogenetic relationships of cantharelloid and clavarioid Homobasidiomycetes based on mito-chondrial and nuclear rDNA sequences. Mycologia, 91(6): 944–963
3. Hibbett, D.S., Bauer, R., Binder, M., Giachini, A.J., Hosaka, K., Justo, A., Larsson, E., Larsson, K.H., Lawrey, J.D., Miettinen, O., Nagy, L.G., Nilsson, R.H., Weiss, M. & Thorn, R.G. (2014). Agaricomycetes. In: McLaughlin DJ, Spatafora JW (eds) The mycota VII part a. Springer-Verlag, Berlin, pp. 373–412.
4. Kirk, P.M., Cannon, P.F., Minter, D.W., & Stalpers, J.A. (2008). Dictionary of the fungi. 10th edn. CAB International: Wallingford, U.K. 640 p.
5. Soothill, E., & Fairhurst, A. (1978). The new field guide to fungi. Michael Joseph, London.
6. Gardes, M., & Bruns, T.D. (1993). ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. Mol. Ecol, 2: 113–118.
7. Werle, E., Schneider, C., Renner, M., Völker, M., & Fiehn, W. (1994). Convenient single-step, one tube purification of PCR products for direct sequencing. Nucleic Acids Res., 22: 4354–4355.
8. Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312-1313.
9. Miller, M.A., Pfeiffer, W., & Schwartz, T. (2010). Creating the CIPRES science gateway for inference of large phylogenetic trees. In: Proceedings of the 1st conference extreme science and engineering discovery environment, pp. 1–8.
10. Rambaut, A., Suchard, M.A, Xie. D., & Drummond A.J. (2014). Tracer v1.6. Available at http://beast.bio.ed.ac.uk/Tracer [Verified May 2014].