

***Morchella crassipes* (Vent.) Pers.: First Record from Aravalli Mountain Range, Rajasthan Based on ITS Sequence Analysis**

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(Submitted on September 13, 2023; Accepted on September 29, 2023)

ABSTRACT

An ascomycetes fungus *Morchella* is well well-known, highly priced delicious edible macrofungi. It was first recorded from the Aravalli Mountain Range Forest, Rajasthan. An internal transcribed spacer (ITS) sequence was used for the delineation and identification of *Morchella* species that were confirmed as *M. crassipes*. Over-exploitation of *M. crassipes* for culinary and selling purposes from rare occurring sites of Aravalli Mountain Range Forest imposes a serious threat of destruction and disappearance of the species in the emerging area.

Keywords: Aravalli Mountain, Rajasthan, *Morchella crassipes*, Phenetic approaches, DNA barcoding, Phylogenetic analysis.

INTRODUCTION

Morchella is one of the most highly-valued wild edible macrofungi among ascomycetous apothecial fungi. The genus *Morchella* is distributed worldwide and attained popularity as the most expensive edible mushroom due to its medicinal and nutritional values (Wani *et al.*, 2023). According to the Index Fungorum, a total of 352 records (including species, subspecies, and varieties) of *Morchella* are listed (<http://www.indexfungorum.org/names/names.asp>). However, 28 species of *Morchella* with their worldwide distribution (Hawksworth *et al.*, 1995) and 36 species of morels from wide-ranging biome of the world (Krick *et al.*, 2008) have been reported. From India, only 13 species of *Morchella* viz., *M. angusticeps* Peck, *M. conica* Pers, *M. crassipes* (Vent.) Pers., *M. deliciosa* Fr., *M. elata* Fr., *M. esculenta* (L.) Pers., *M. hybrida* (Sow.) Pers., *M. kaiba-bensis* Beuj, T. A. Clem. and T. J. Baroni, *M. rotunda* (Pers.: Fr.) Boudier, *M. semilibera* DC, *M. tomentosa* M. Kuo., *M. tridentina* Bres., and *M. vulgaris* (Pers.) Gray, are known so far from higher altitudes, hilly landform with low temperature microclimate regions (Singh *et al.*, 2004; Kanwal *et al.*, 2010; Kumar and Sharma, 2011; Kumar and Sharma, 2013; Kotwal *et al.*, 2014; Dorjey *et al.*, 2019; Wani *et al.*, 2023). Nevertheless, as per available records, *Morchella* has not been reported from dry deciduous climatic condition of Aravalli Mountain range forest except a single report without any species delineation of *Morchella* by Paliwal *et al.*, 2013. The Aravalli Mountain Range, one of the world's oldest mountain range that originated in the Precambrian period (Roy, 1990), features tropical

deciduous forest and a rich diversity of flora, including various fungal species.

Field trips were undertaken to collect macro-fungi from Mount Abu wild life sanctuary, Kumbhalgarh wild life sanctuary and Sitamata wild life sanctuary of Aravalli Mountain Forest region during July, 2020 to June, 2022. During the survey, very rare specimens of *Morchella* were observed in the Mount Abu wild life sanctuary. The photography, latitude and longitude were digitally recorded. The morphological characters such as shape, size, colour, growing habitat and other dimension of the fruiting bodies were recorded under natural condition. The collected fruiting bodies were brought to the laboratory. The shape and size of ascus; shape size, and colour of ascospores with other characteristic features were examined under compound microscope and observations were recorded.

Taxonomic features revealed saprotrophic apothecial occurring as individual or groups (**Figure 1**). The pileus was brown in colour, sub-globous, elongated, narrowed at the apex and was in 3.0-5.5 × 1.5-2.5 cm in size having irregular honeycomb pits. The honey combed pits of about 2×1 cm have thin ridges and depressions on the surface due to unequal growth of the hymenial surface. Stipe was 2.5-3.0 × 1.5-2.5 cm in size, light yellowish-white (cream) in colour, smooth, hollow, swollen at the base and narrow at top while the ascus was cylindrical, translucent and had eight ascospores. The paraphyses were filamentous and hyaline and ascospores were 15-20 × 7-10 µm in size, elliptical, smooth and translucent. The available literatures, monographs, and identification keys were

used for confirmation of *Morchella* species (Jandaik and Sharma, 1995; Sa *et al.*, 2022; Wani *et al.*, 2023). Surprisingly, the observed macroscopic and

microscopic characteristics overlapped with those of known reported species, making it challenging to conclusively distinguish *Morchella* species.



Figure 1: Ascocarp of *Morchella crassipes* growing in natural habitat.

Identification of *Morchella* species based on classical taxonomy is very difficult due to high morphological complexity and plasticity of apothecium colour and shape. Differences in macroscopic (colour, shape, and size of apothecium) and microscopic (ascus and ascospores) characteristics of *Morchella* have also been observed at different developmental stages. Besides these, morphology and color of *Morchella* species are highly variable (Du *et al.*, 2014). The plasticity and polymorphic nature of macroscopic and microscopic features of apothecial ascocarps depend on type of soil, intermittent rainfall, temperature, surrounding vegetation, shadows of tree canopy, obstacle during ascocarps growth and other

climatic factors that created misperception during taxonomic study. Therefore, molecular characterization is required for delineation of *Morchella* species (Sa *et al.*, 2022; Wani *et al.*, 2023).

In the present study, both the morphological approaches and rRNA gene (ITS-5.8S gene sequences) were employed for identification, delineation and description of species. For this purpose, the total genomic DNA was extracted from the preserved specimen by using of DNA isolation kit (MB 505; Hi media laboratories, Mumbai, India). PCR amplification was done using ITS-1 and ITS-4

primers (White *et al.*, 1990) with some modifications. PCR-amplified products were observed in agarose gel with DNA ladder for quantification of amplified ITS-5.8S gene under UV-illumination gel documentation system. Sequencing of amplified product was done using Big dye terminator (on ABI Prism DNA Sequencer). The quality of sequence was checked with the sequence scanner software. NCBI Blast was done to find out maximum similarities with previously authenticated species of *Morchella*. The sequences were then submitted to NCBI (National Centre for Biotechnological Information) to obtain the GenBank accession number. Phylogenetic analysis was carried out using MEGA-XI (Tamura *et al.*, 2021) and the nucleotide sequences were aligned employing the ClustalW software. The phylogenetic tree analysis was done using the neighbor-joining method (Saitou and Nei, 1987) with 1000 bootstraps

replicates. ITS-5.8S gene sequences of 13 species reported from India were taken from the NCBI database for construction of phylogenetic tree to compare the similarities and distinctiveness of collected *Morchella* specimens.

The results of PCR amplified ITS-5.8S region of rRNA gene revealed a size of 1118 base pairs (bp). The sequences exhibited maximum (99.73%) similarity with *M. crassipes* (GQ228466) upon NCBI Blast search confirming that the specimen under present study was *M. crassipes*. The sequence was submitted to NCBI GenBank and GenBank accession number OR441012 was obtained. The phylogenetic tree revealed different clusters, indicating genetic diversity among *Morchella* species (**Figure 2**). The gene sequence closely aligned with *M. crassipes* sequences, confirming the specimen's identity as *M. crassipes* (**Figure 2**).

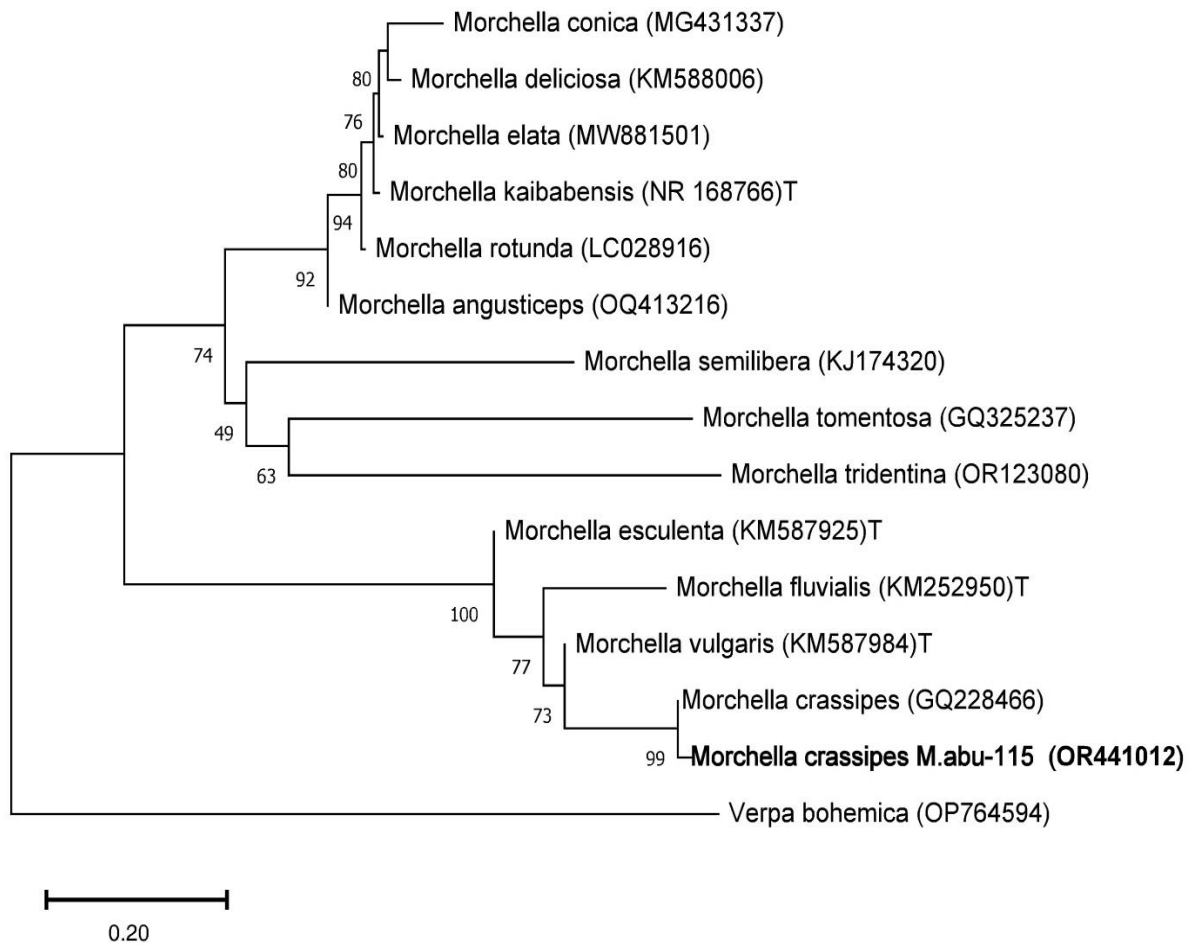


Figure 2: Phylogenetic tree of ITS-5.8S gene sequences of *Morchella crassipes* strain M.abu-115 along with other species of *Morchella* found in India using the Neighbor-Joining method. GenBank accession numbers are given in parentheses. T, represents sequence from type stains.

According to available records and literature, the study reports identification and documentation of *M. crassipes* for the first time from Aravalli Mountain range forest (Bilgrami *et al.*, 1979; 1981; 1991; Jamaluddin *et al.*, 2004; Manoharachary *et al.*, 2022). *M. crassipes* have several nutraceutical and pharmaceutical potentials. Therefore, over exploitation of it for culinary and selling purpose from rare occurring site of Aravalli Mountain range forest impose serious threat of destruction and disappearance of *M. crassipes* in the emerging area.

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