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Macrocybe sardoa (Callistosporiaceae, Agaricales) a new record from India

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A large basidioma collected from Tungabhadra riverbank near Holalur village in Karnataka has been identified based on morphological characters and molecular phylogenetic studies as *Macrocybe sardoa*, a new record from India. Also, *Macrocybe crassa* collected from Hosagunda, a patch of Central Western Ghats of India was compared with previous collections. The generated sequence data from the nuclear ribosomal internal transcribed spacer and nuclear ribosomal large subunit region were deposited in the NCBI GenBank database and a combined dataset is used to determine the phylogenetic relationship within the Callistosporoid clade. Morphological descriptions, photographic illustrations of Indian collection and phylogeny of Callistosporiaceae members are provided.

Key words : Agaricomycetes, Central Western Ghats, morpho-molecular, phylogeny, taxonomy

INTRODUCTION

Species of the genus Macrocybe Pegler & Lodge, produce large, fleshy basidiomata and often grow in large caespitose clusters. Some are edible after removing the toxic compounds by cooking. Pegler *et al*. (1998) segregated Macrocybe from Tricholoma and ranked as genus with distinct morphological and molecular characteristics. The genus consists of species that are of non-ectomycorrhizal lifestyle, saprophytic, large basidiomes with clamped hyphae. Considering the morphological and molecular characteristics Moncalvo *et al.* (2002) confirmed that the genera Macrocybe, Callistosporium and Pleurocollybia constitute the Callistosporoid clade.

Recently, Vizzini *et al.* (2020) proposed the new family Callistosporiaceae to classify most genera that were previously considered as related with Catathelasma; i.e. *Anupama*, *Callistosporium*, *Guyanagarika*, *Macrocybe*, *Pseudolaccaria*, and *Xerophorus*.

In India 5 species viz. *M. crassa*, *M. gigantea*, *M. lobayensis*, *M. pachymeres*, and *M. titans* have been reported till date (Manimohan *et al.* 2007, Mohanan 2011). However, as pointed by Vizzini *et al*. (2020) the Indian collection of *M. titans* (Farook *et al.* 2013) represent *M. crassa*. The present study aims to report a new occurrence record of the *Macrocybe sardoa* from Western Ghats region of India.

MATERIALS AND METHODS

Collection and morphological studies

The specimens of Macrocybe spp. were found during our field survey in dry deciduous forests of central Western Ghats region of Karnataka. The specimens were photographed in situ using a Nikon D5600 Digital SLR camera. Macro-morphological characters are recorded in the field using a field key designed by Atri *et al*. (2017). We removed debris using soft brush and collected the specimen with care. The microscopic characters were studied by mounting the fresh tissues on 5% KOH stained with Pholxine B under Olympus CH20i light microscope. The specimens were dried and preserved in Department of Botany, Kuvempu University for further characterization.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from fresh basidiomata using the modified CTAB method

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(Kantharaja and Krishnappa 2020). The nuclear ribosomal internal transcribed spacer (nrITS) and nuclear ribosomal DNA large subunit (nrLSU) were amplified using the primer pairs ITS1 – ITS4 (White *et al.* 1990) and LROR – LR5 (Vilgalys and Hester 1990) respectively. The modified protocols of Kantharaja and Krishnappa (2020) were followed for PCR amplification and sequencing. The newly generated sequences were aligned and consensus sequences were generated using BioEdit v.7.2.5 (Hall, 1999). The consensus sequences were used for BLAST search on the NCBI GenBank nucleotide database (https://www.ncbi.nlm.nih.gov/) to know the sequence similarity and distance tree results and the identified sequences are deposited in the GenBank.

Sequence alignment and phylogenetic analysis:

The dataset of 32 combined sequences (32 nrITS and 32 nrLSU) of which 2 are derived from the present study and 30 sequences retrieved from NCBI GenBank database according to the specimen identifier with respect to sequence similarity and based on the published literature (Vizzini *et al*. 2020) (Table. 1). The dataset used to assess the alignment confidence score for each residue pair under MAFFT (Katoh *et al*. 2019) on GUIDANCE (Sela *et al*. 2015) webserver (http://guidance.tau.ac.il). The alignment output was is used to conduct the phylogenetic reconstruction with Maximum Likelihood (ML) analysis using RAxML HPC2 on XSEDE with 1000 bootstrap replications and Bayesian Analysis (BA) was performed with MrBayes on XSEDE for one million generations using the GTR+G model as suggested by jModelTest v.2.1.10 (Darriba *et al*. 2012) at CIPRESS Science Gateway (Miller *et. al*. 2010) The Bayesian posterior probabilities were calculated and the trees were viewed and edited in FigTree v.1.4.4 (Rambaut, 2009).

RESULTS AND DISCUSSION

Phylogenetic analyses

The final RAxML tree of dataset comprising 32 combined nrITS and nrLSU sequences of 14 species belonging to Callistosporoid clade and as an outgroup *Collybia brunneola* is selected according to previous work by Vizzini *et al*. (2020). The Maximum Likelihood analysis of combined sequences of nrITS and nrLSU region consisted of 812 distinct alignment patterns. The best tree (Fig. 1) found with final ML optimization likelihood score of -8921.310950.

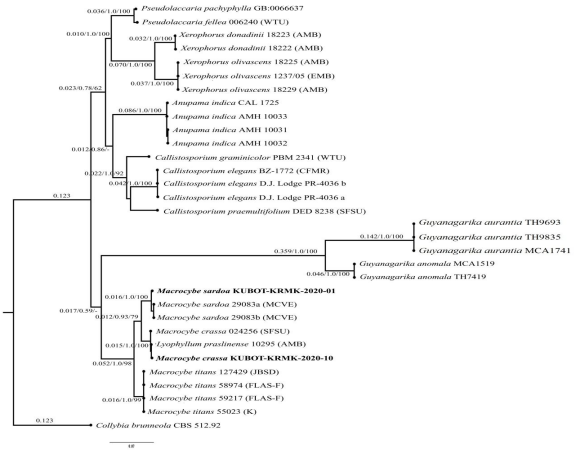

Fig. 1 : RAxML tree of Macrocybe spp. based on Maximum Likelihood analysis of combined sequence dataset of nrITS and nrITS and nrL.SU sequence by GTRGAMMA model with Collybia brunneola as outgroup showing branch length (BL). Bayesian poslerior probability values (PP>0.5) and Bootstrap support (BS>50) as BLPP/BS)

The sequences of newly recorded *Macrocybe* sp., specimen (KUBOT-KRMK-2020-01) showed highest similarity with the sequences of originally described specimen of *M. sardoa* [29083a (MCVE) and 29083b (MCVE)]. Phylogenetically recovered in a well-supported clade with bootstrap support (BS– 100%) and Bayesian posterior probabilities (PP– 1.0). Furthermore, Indian specimen of *Macrocybe crassa* (KUBOT-KRMK-2020-10) is found clustered with specimens of *Macrocybe crassa* from Thailand [024256 (SFSU)] and type specimen of *Lyophyllum parslinense* [10295 (AMB)] downloaded from GenBank with strong support. The phylogenetic reconstruction by Maximum Likelihood analysis illustrates a well explained ancestral relationship in the genera of Callistosporiaceae.

Taxonomy

Macrocybe sardoa Vizzini, Consiglio, M. Marchetti, Fungal Diversity 101: 247 (2020) Fig. 2. MycoBank number: 831403.

Pileus 18.2 – 45.6 (52.1) cm in diam. hemispherical to convex with incurved edges at first (Fig. 2-A), then broadly convex to flat with decurved margin slightly exceeding the lamellae. Surface pure white when young, becoming pale yellow at the center afterwards, not hygrophanous, glabrous, smooth when young, and cracking concentrically showing

Table 1: Details of Specimen sequences used in the phylogenetic analysis.

Species	Voucher/ isolate no.	Country	GenBank Accession No.	
			ITS	LSU
Anupama indica	CAL 1725	India	MH989587	MH989583
Anupama indica	AMH 10031	India	MH989588	MH989584
Anupama indica	AMH 10032	India	MH989589	MH989585
Anupama indica	AMH 10033	India	MH989590	MH989586
Callistosporium elegans	D.J. Lodge PR-4036 a	Puerto Rico	MN017513	MN017454
Callistosporium elegans	D.J. Lodge PR-4036 b	Puerto Rico	MN017514	MN017455
Callistosporium elegans	BZ-1772 (CFMR)	Belize	MN017512	MN017453
Callistosporium praemultifolium	DED 8238 (SFSU)	Sao Tome and Principe	MN017524	MN017464
Callistosporium graminicolor	PBM 2341 (WTU)	USA	DQ484065	AY745702
Collybia brunneola	CBS 512.92	USA	MH862373	MH874036
Guyanagarika anomala	TH7419	Guyana	KX092096	KX092110
Guyanagarika anomala	MCA1519	Guyana	KX092095	KX092109
Guyanagarika aurantia	TH9835	Guyana	KX092079	KX092099
Guyanagarika aurantia	TH9693	Guyana	KX092078	KX092098
Guyanagarika aurantia	MCA1741	Guyana	KX092073	KX092097
Lyophyllum praslinense	10295 (AMB)	Seychelles	MN017539	MN017479
Macrocybe crassa	**KUBOT-KRMK-2020-10**	**India**	**MT883354**	**MT883286**
Macrocybe crassa	024256 (SFSU)	Thailand	MN017540	MN017480
Macrocybe sardoa	**KUBOT-KRMK-2020-01**	**India**	**MT880333**	**MT879639**
Macrocybe sardoa	29083b (MCVE)	Italy	MN017543	MN017482
Macrocybe sardoa	29083a (MCVE)	Italy	MN017542	MN017481
Macrocybe titans	59217 (FLAS-F)	USA	MN017546	MN017485
Macrocybe titans	58974 (FLAS-F)	USA	MN017545	MN017484
Macrocybe titans	55023 (K)	Puerto Rico	MN017544	MN017483
Macrocybe titans	127429 (JBSD)	Dominican Republic	MN017547	MN017486
Pseudolaccaria fellea	006240 (WTU)	USA	MN017549	MN017487
Pseudolaccaria pachyphylla	GB:0066637	Sweden	KU058504	KU058542
Xerophorus donadinii	18223 (AMB)	Italy	MN017552	MN017491
Xerophorus donadinii	18222 (AMB)	Italy	MN017551	MN017490
Xerophorus olivascens	18229 (AMB)	Italy	MN017560	MN017498
Xerophorus olivascens	18225 (AMB)	Italy	MN017557	MN017495
Xerophorus olivascens	1237/05 (EMB)	Italy	MN017555	MN017493

the underlying white context (Fig. 2-B). *Lamellae* sinuate to adnate, 2.5 – 3.2 cm broad, crowded, lamellulae numerous or frequent, whitish at first then pale greyish to creamy pink. *Context* 3.8–4.9 cm broad, white, firm, not changing on bruising. *Stipe* 21.0 – 24.5 (-25.8) × 9.5 – 13.5 (-14.2) cm, cylindrical to clavate, tapering towards base (Fig. 2-C), sometimes flattened, concolorous with pileus at first then greyish scales more prominent on maturity, compact and solid. Context compact, firm, white, not changing on exposure. *Annulus* absent. Odor pleasant and sweetish to unpleasant taste mild. *Spore* print white.

Basidiospores 5.2 – 8.0 × 4.6 – 5.1 µm (n = 25, Lm =5.1 µm, Wm = 4.9 µm, Q = 1.0 - 1.2, Qm = 1.04) broadly ellipsoid (Fig. 2-D, F), smooth, hyaline in KOH, inamyloid. *Lamellar trama* parallel, *Basidia* 25.5 - 42 × 6.5 - 8.2 µm clavate, tetrasterigmatic with sterigmata about 5.2 – 8.5 µm long (Fig. 2-E, G), *Pseudocheilocystidia* 18.5 – 32 × 3.2 – 5.8 ìm, fusiform to narrowly lageniform (Fig. 2-H), thin walled, scattered, abundant. *Pseudopleurocystidia* 36 – 52 × 6.8 – 13.2 µm, scattered, fusiform, smooth, sometimes septate. *Lamellar trama* 1.5 – 5.2 µm wide, subregular to regular with cylindrical hyphae. *Pileipellis* a cutis 35 - 55 µm broad with loosely interwoven hyphae. *Clamp connections* frequent.

Known distribution – originally described from Sardinia, Italy and India might be the second place of occurrence.

Material examined – India, Karnataka, Shivamogga district, Shivamogga taluk, Holalur village (14.026528N, 75.676639E), under coconut tree, 02 June 2020, Kantharaja R, KU-BOT-KRMK-2020-01.

Notes – *Macrocybe sardoa* is a species with large tricholomatoid, caespitose basidiomata and is first described from Sardinia, Italy by Vizzini *et al*. (2020) after the claims of Cappai *et al.* (2016) considering the collection as a first report of *Macrocybe titans* to the country. Our collection match well with the original description, except for the variation in size of pileus which is 15-20 cm broader compared to Italian collection, may be due to strong environmental and nutritional factors. The species if found in caespitose cluster under a coconut tree which is also a member of Arecaceae and confirms substrate preference as of the type specimen 29083 (MCVE).

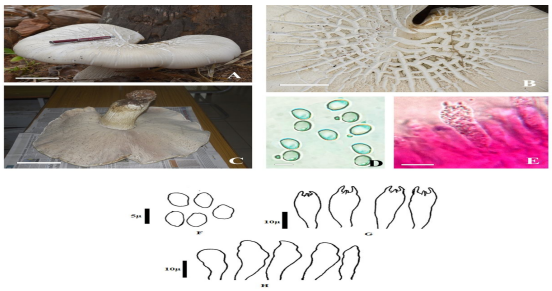

Fig. 2: *Macrocybe sardoa* -A. Basidiocarp under *Cocos nucifera* tree; B. Pileus chowing concentric cracks; C. Lamellae and stipe; D & F. Basidiospores ; E & G. Basidia. H. Pseudocheilocystidia. (Scale Bars; A-C: 5cm, D&F: 5µm, E,G &H: 10µm

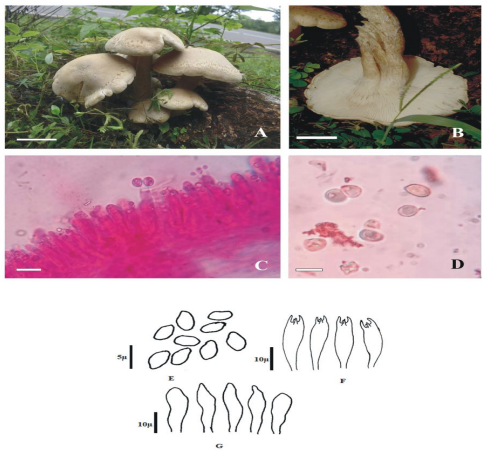

Fig. 3: *Macrocybe crassa*; A. Basidiomes on wood log; B. Lamellae and stripe; C.Lamellar edge; D-E. Basidiospores; F. Basidia; G. Pseudocheilocystidia.(Scale Bars; A-B: 5 cm, C: 10 µm,D-E:5µm, F-G:10µm).

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