

**Research Article**

## ***HYMENOCHAETE CONCHATA* (HYMENOCHAETACEAE), A NEW RECORD FOR INDIAN MYCOBIOTA**

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### **ABSTRACT**

During repeated mycological forays to Rajmahal hills, a widely distributed, host specific, resupinate to effused-reflexed basidiomata of *Hymenochaete conchata* were collected. In the present communication this species is reported for the first time from India with morphological description along with the illustrations and nrITS-based phylogenetic analysis. Comparison with the allied species is also discussed.

**Keywords:** *Hymenochaetaceae*, *Jharkhand*, *Molecular Phylogeny*, *Taxonomy*

### **INTRODUCTION**

Traditionally, genus *Hymenochaete* is assigned to such xanthochoric taxa which are characterized by: coriaceous to hard stipitate to resupinate basidiomata; hymenial surface even or rarely granular to hydroid; monomitic hyphal system; presence of setae; hyaline basidiospores; and presence or absence of cystidioles (Patouillard, 1900; Burt 1918; Corner 1948 & 1991; Cunningham, 1957; Donk, 1964; Jülich 1981; Parmasto 2001; Ryvarden, 2004; Dai, 2010; He & Dai, 2012). Interestingly, diverse range of basidiomata (stipitate to substipitate, pileate to effused-reflexed to resupinate corticoid forms) amongst the members of this genus bear less taxonomic value while correlated with the molecular phylogenetic studies (Wagner & Fischer, 2002; He & Dai, 2012; He & Li, 2013; Baltazar *et al.*, 2014; Parmasto *et al.*, 2014). But, micromorphological features, like presence or absence of structures like ‘cortex’, ‘context’ and ‘setigerous layers’ in combination are fundamental basis to place them either under the sections *Hymenochaete* (all three layers present), *Fultochaete* (context and setigerous layer present), *Gymnochaete* (setigerous layer present) or *Paragymnochaete* (setigerous layer and cortex present) (Léger, 1998; Wagner & Fischer, 2002). Rajmahal hills in Jharkhand (Figure 1) are one of the oldest mountain ranges of the world spreading over the area of about 3000 square Km (O’Malley, 1910).

Its rich wealth of flora, fauna, mycobiota and fossils are now under immense anthropogenic pressure and diminishing rapidly. Unfortunately, in terms of macrofungi, this area remains unexplored after the work of Kurz (Curry, 1874), Bodding (1925–1940), and Panigrahi (1966).

After a long gap recently, Botanical Survey of India (BSI) the premiere institute working on systematics of plant and fungi have undertaken the thorough macrofungal explorations in order to fulfill the gap area. Since 2013 one of the authors (MEH) repeatedly collected *Hymenochaete* specimens (from *Shorea robusta* Gaertn. f. dominated patches of these hilly areas) which after thorough studies (morphotaxonomy and nrITS sequence based phylogenetic analysis) appeared as *Hymenochaete conchata* Zhou (originally reported from Thailand), which falls under section *Hymenochaete*.

This species does not match with any of the previously reported 38 valid taxa of *Hymenochaete* from India (Bilgrami *et al.*, 1991; Sorbhoy *et al.*, 1996; Sharma, 1995 & 2012; Jamaluddin *et al.*, 2004; De, 2008; Ranadive, 2013; Tiwari *et al.*, 2013; Kaur *et al.*, 2015; Sharma & Mishra, 2015).

Macro- and micromorphological details coupled with the illustrations and a phylogenetic estimation based on ITS sequence data are presented.

## Research Article

### MATERIALS AND METHODS

#### Morphology

Macromorphological/field characterization was undertaken with the fresh basidiomata. Field-photographs of these basidiomata and its habitat were captured with the aid of Sony Cyber shot DSC-RX100. Colour codes and terms (mostly) follow Methuen Handbook of Colour (Kornerup & Wanscher, 1978). GPS data were recorded with the help of Garmin *etrax* 30 device. In the laboratory, macromorphological characters were again observed both from the fresh and dry materials with the help of a stereo-zoom dissecting microscope Olympus SZ51. Micromorphological characters were recorded and microphotographs were taken with the help of a light microscope Olympus CX 41 from the free hand sections of the dry materials stained in a mixture of 5% KOH and phloxin and then mounted with 30% glycerol. Drawings of Micromorphological features were prepared with the help of drawing attachment tube attached to Olympus CX 41 compound microscope. Sections were also mounted in Cotton blue and Melzer's reagent separately.

Measurements for the spores were noted based on that of twenty randomly chosen basidiospores. Spore-measurement and Quotient indicating length-width ratio ( $Q = L/W$ ) are presented as minimum–(mean)–maximum. Herbarium name follows Holmgren *et al.*, (1990). Distributional map is prepared with the help of Arc GIS software.

#### DNA Isolation, PCR and Sequencing

Genomic DNA was isolated from 100 mg of dry basidiome with the help of InstaGene™ Matrix Genomic DNA isolation kit (Biorad, USA) following the manufacturer's instructions. The nrITS gene region was amplified by using primer pairs: ITS1F and ITS4B (White *et al.*, 1990). PCR-amplification was performed on a thermal cycler (Eppendorf, Germany) programmed for 2 mins at 94°C, followed by 35 cycles of 45 secs at 94°C, 1 min at 55°C, 1 min at 72°C and a final stage of 10 mins at 72°C. The PCR products were duly purified using the QIAquick PCR Purification Kit (QIAGEN, Germany). Both the strands of the PCR fragments were sequenced on the 3730xl DNA Analyzer (Applied Biosystems, USA) using the amplifying primers.

The DNA sequence of the reverse strand was edited with Sequence Navigator version 1.0.1 (Applied Biosystems). The final consensus sequence was deposited at GenBank to procure the accession number (MF373838).

#### Phylogenetic Analysis

Phylogenetic analysis based on ITS sequence data was carried out to establish the phylogenetic placement of our isolated taxon. Reference sequences and outgroup were selected from the relevant literature (Pan & Zhou, 2016), BLAST (Altschul *et al.*, 1997) search and from public databases like GenBank (Clark *et al.*, 2016) and Unite (Kõljalg *et al.*, 2013). All sequences were aligned with MAFFT v. 7 (Katoh & Standley, 2013). No manual editing was done within the alignment. Phylogram was generated from maximum likelihood (ML) method based on the Kimura 2-parameter model (Kimura, 1980). Evolutionary analysis was conducted in MEGA6 (Tamura *et al.*, 2013). One-thousand bootstrap replicates were analyzed to obtain the nodal support values. The European collection of *Fomitopsis pinicola* and Brazilian collection of *Trametes villosa* were chosen as outgroup taxa.

### RESULTS AND DISCUSSION

#### Results

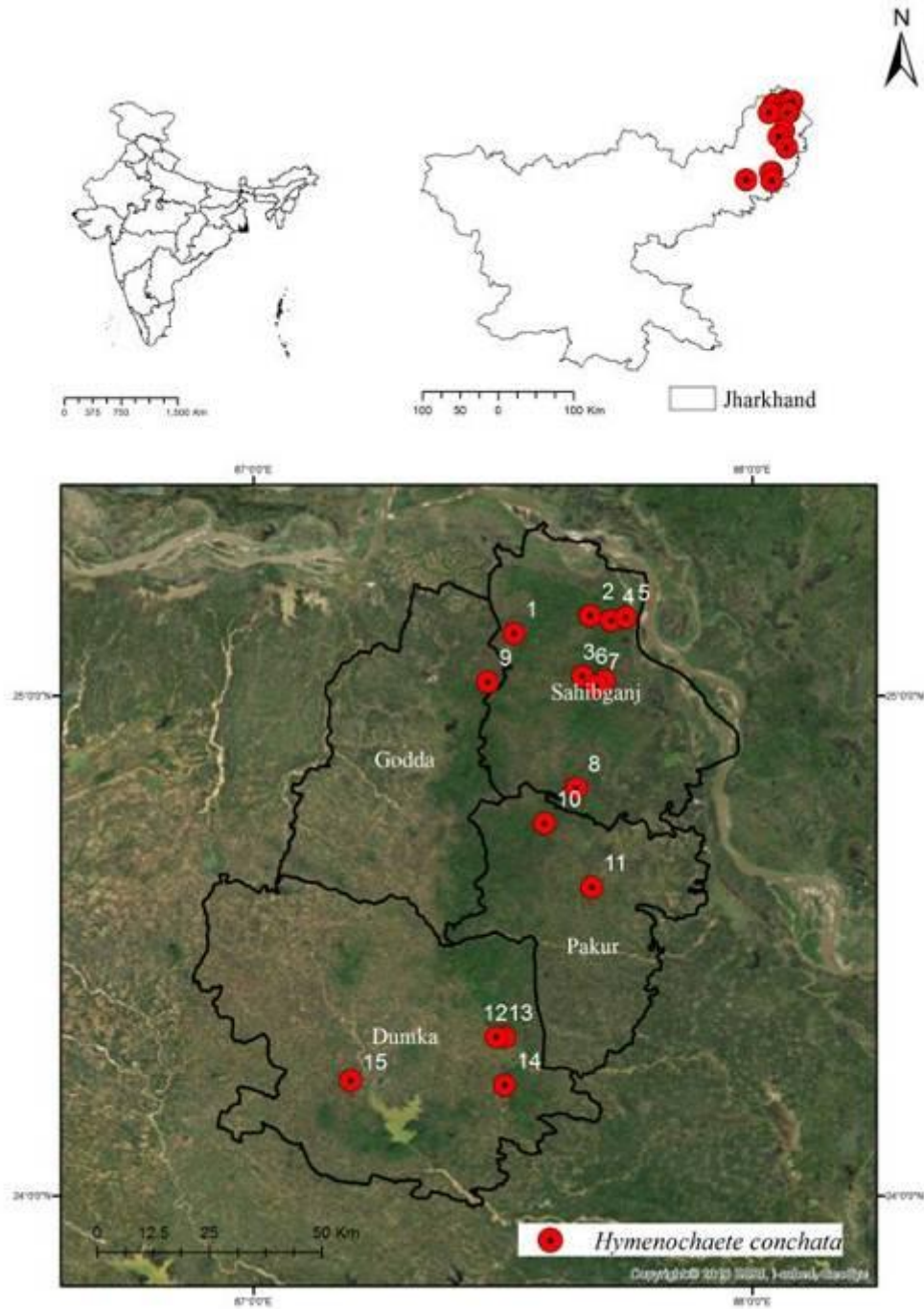
##### Phylogenetic Inference

Present phylogenetic analysis with 54 sequences (including present Indian specimen) has resolved the genus *Hymenochaete*. Our Indian specimen MEH-70150 (*H. conchata*) is nested amongst few Asian species of this genus being sister to two Thai specimens (GenBank accession numbers KX258959 and KX258960) of *H. conchata* with strong statistical support (100% bootstrap). The phylogram is presented in Figure 2.

##### Taxonomy

*Hymenochaete conchata* L.W. Zhou, Phytotaxa **273**(3): 202 (2016) Figure 3 & 4

**Research Article**

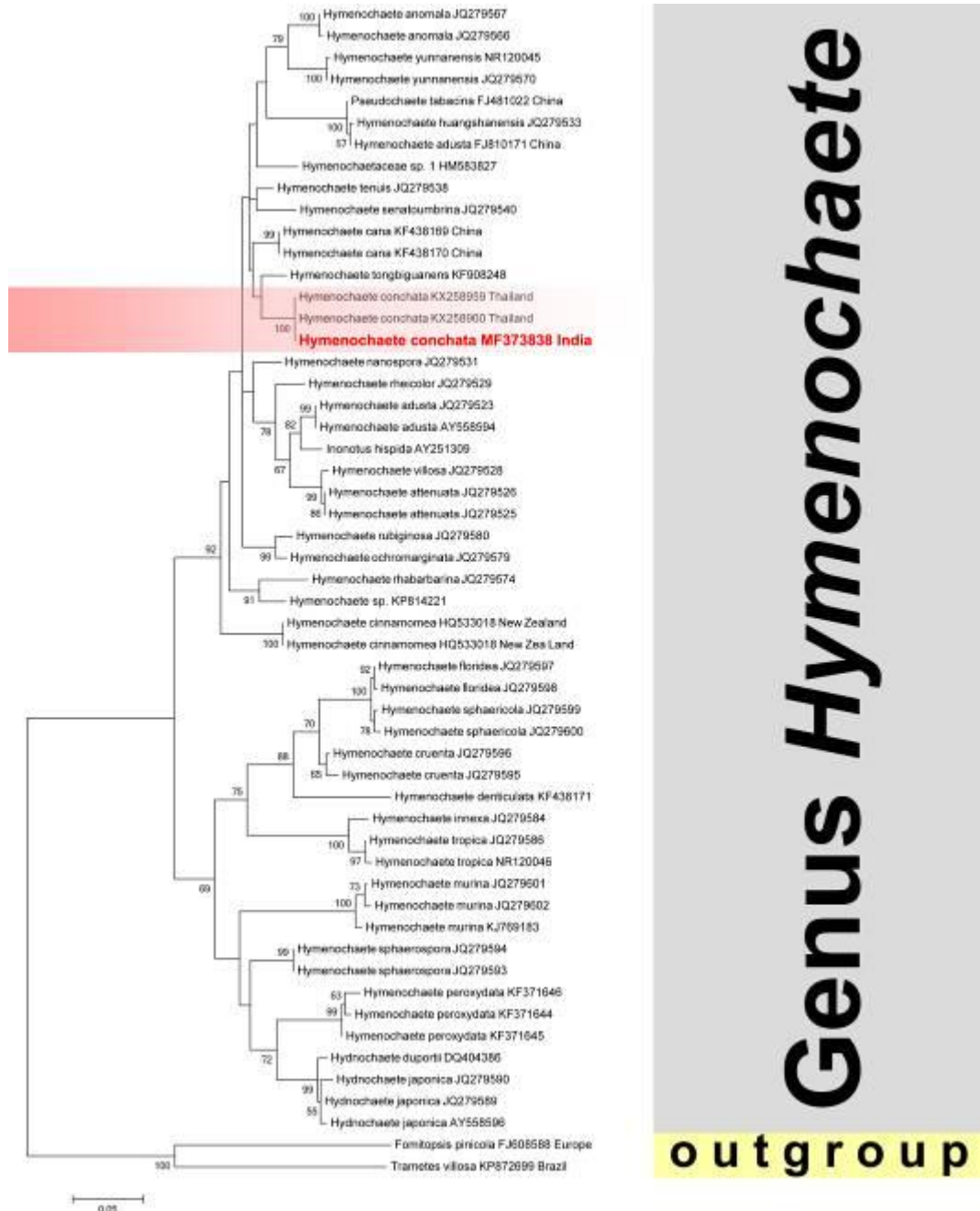


**Figure 1: Distributional Map of *Hymenochaete conchata* Zhao in Rajmahal Hills, Jharkhand, India.**

*Basidiomata* effused up to 10–500 × 5–200 mm in longest and widest dimension on host, annual, lignicolous, crusty, initially arise as small round patch with golden brown margin then effuses all the direction to form resupinate to effused-reflexed to pileate shape, leathery when fresh, hard and brittle on drying. *Pileus* 5–55 × 5–30 mm, 0.4–0.9 mm thick, applanate, conchate, often imbricate, laterally confluent; pilear surface villose tomentose when fresh, becoming glabrous towards maturity, zonate,

**Research Article**

reddish blond to light brown (5C4–6D5) in young parts gradually becomes olive brown to raw umber (4F8–5F8) to almost charcoal black in older part. *Margin* 0.5–2 mm wide, sterile, acute, entire, wavy, golden yellow (5B7) when young, deep orange (5A8) when old. *Hymenophore* smooth to weakly zonate, occasional island like papillae present, grayish blue to dull blue (20D4–21D5) to mouse grey. *Context* 0.3–0.8 mm thick wide near base, tough waxy to more or less hard while sectioning, duplex due to presence of setigerous layer, upper part reddish blond to light brown (5C4–6D5) lower part light brown to brown (6D5–6E5).



**Figure 2: Phylogeny of MEH-70144 (Indian collection of *Hymenochaete conchata*, is in Bold and Red Font) inferred from Maximum Likelihood analysis of ITS Sequences Using MEGA 6.0.**



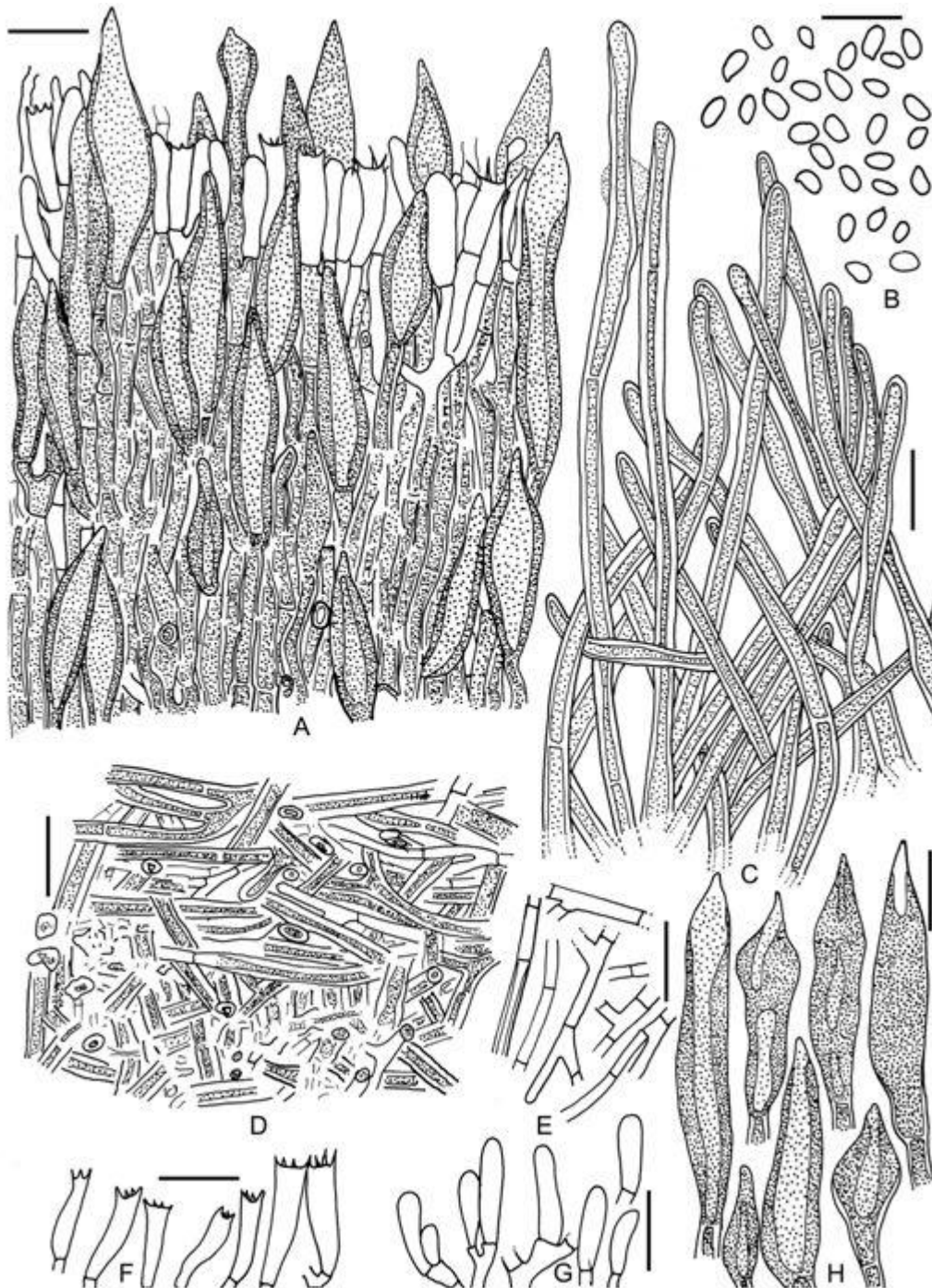
### Research Article

*Hyphal System* monomitic; generative hyphae simple septate, thin to distinctly thick-walled, rarely branched, smooth (mostly) but sometimes slightly crystalline on the stratified hymenium and tomentum, hyaline to dark brown in KOH, acyanophilic, inamyloid. *Pileipellis* trichoderm; hyphae 2–4  $\mu\text{m}$  wide, erect, sometimes interwoven, moderate to distinctly thick-walled (0.5–1  $\mu\text{m}$  thick), rarely septate (septate at long interval). *Context* hyphae 1.5–4  $\mu\text{m}$  wide, highly interwoven, thin (infrequent, hyaline)– to thick-walled (dominating, dark brown,), three distinct layer cortex tomentum and hyphal layer can be seen. *Stratified hymenium* 60–80  $\mu\text{m}$  wide, hyphae 1.5–4  $\mu\text{m}$  wide, more or less parallel, compactly arranged along with setae, thin–(infrequent, hyaline) to thick-walled (wall 0.5–1  $\mu\text{m}$  thick, dominating, dark brown) septate at long interval; setae 20–55  $\times$  4–9  $\mu\text{m}$ , abundant, subulate, thick-walled, lumen narrow to wide, smooth, without any crystalline sheath, up to 25  $\mu\text{m}$  projecting beyond hymenium, dark brown; cystidia and cystidioles absent but few hyaline thin-walled hyaphe projecting into the hymenium along with setae; basidia 10–15  $\times$  2.5–3.5  $\mu\text{m}$ , septate at base, 4-sterigmate, sterigmata 1–3  $\mu\text{m}$  long, thin-walled, hyaline, weakly cyanophilic; basidioles 8–14  $\times$  2–3  $\mu\text{m}$ , septate at base, thin-walled, hyaline, weakly cyanophilic. *Basidiospores* 2.5–(3.4)–4  $\times$  1.5–(1.69)–2.7  $\mu\text{m}$ , Q = 1.4–(1.69)–2.23, oblong ellipsoid, thin-walled, smooth, hyaline, acyanophilic to weakly cyanophilic, inamyloid.



**Figure 3:** *Hymenochaete conchata* (MEH-70144); **A:** Habitat; **B & C:** Habit; **D:** Setigerous layer near Hymenophore; **E–I** Different shapes and size of setae; **J:** Pileipellis; **K:** Basidiospores; Scale bars: **C** = 10 mm; **D–K** = 10  $\mu\text{m}$ .

**Research Article**



**Figure 4: Hymenochaete conchata (MEH-70144); A: Section of basidiomata showing Hymenophore and setigerous layer; B: Basidiospores; C: Pileipellis; D: Section showing juncture of context and cortex region; E: Thin-walled generative hyphae; F: Basidia; G: Basidioles; H: Size and shape variation in setae; Scale bars: A–H = 10 µm**

*Specimens examined:* INDIA; Jharkhand, Rajmahal hills, Sahibganj district, Mandro block, near Mandro Fossil Park, alt. 142 m, 25°07'31.3"N 87°31'22.3"E, on the dead part of living *Shorea robusta* Gaertn. f. branch, 20 August 2013, M.E. Hembrom, MEH-66038; *ibid.*, Sahibganj district, Borio block, Pir-Baba Kairasol forest area, alt. 126 m, 25°09'41.7"N 87°40'31.9"E, on the dead part of living *S. robusta* branch,



### Research Article

24 August 2013, M.E. Hembrom, MEH-66076; *ibid.*, Borio block, Dhogada-paharia burial ground forest, alt. 110 m, 25°02'23.7"N 87°39'35.8"E, on the living *S. robusta* branch, 08 November 2016, M.E. Hembrom, MEH-70157; *ibid.*, Taljhari block, Karanpurato village forest toward Gogi, alt. 61 m, 25°09'02.9"N 87°43'02.3"E, on the dead stump of *S. robusta*, 06 November 2016, M.E. Hembrom, MEH-70144; *ibid.*, Taljhari block, Karanpurato Chapaldumri, alt. 72 m, 25°09'27.9"N 87°44'50.2"E, on the dead stump of *S. robusta*, 06 November 2016, M.E. Hembrom, MEH-70150; *ibid.*, Taljhari block, Brindaban Joshkuti reserve forest, alt. 63 m, 25°01'52.1"N 87°42'16.5"E, on the dead part of living *S. robusta* branch, 31 August 2013, M.E. Hembrom, MEH-66143; *ibid.*, Taljhari block, Tinpahar, Gutibeda, alt. 79 m, 25°01'15.1"N 87°41'15.5"E, on the living *S. robusta* branch, 31 August 2013, M.E. Hembrom, MEH-66160; *ibid.*, Pathna block, Barharwa, Pandan-Bhitta and surrounding forest, alt. 104 m, 24°48'57.0"N 87°38'53.7"E, on the dead part of living *S. robusta* branch, 8 September 2013, M.E. Hembrom, MEH-66205; *ibid.*, Godda district, Boarijore block, Mangra Dahar-Langi and surroundings, alt. 136 m, 25°01'43.0"N 87°28'13.8"E, on the dead part of living *S. robusta* stump, 01 September 2013, M.E. Hembrom, MEH-66163; *ibid.*, Pakur district, Litipara block, Sathiya to Sathiyapahar forest area, alt. 225 m, 24°44'44.3"N 87°35'03.8"E, on the dead part of living *S. robusta* branch, 02 September 2014, M.E. Hembrom, MEH-66281; *ibid.*, Pakur district, Hiranpur block, Talpahari to Tugutola forest area, alt. 94 m, 24°37'02.6"N 87°40'45.2"E, on the dead part of living *S. robusta* branch, 22 August 2014, M.E. Hembrom, MEH-66330; *ibid.*, Dumka district, Kathikund block, Kanhaidih reserve forest, alt. 132 m, 24°19'04.2"N 87°29'14.3"E, on the dead part of living *S. robusta* branch, 18 September 2015, M.E. Hembrom, MEH-69905; *ibid.*, Dumka district, Sikaripara block, Karakata forest area, alt. 241 m, 24°13'19.0"N 87°30'16.2"E, on the living *S. robusta* branch, 23 October 2015, M.E. Hembrom, MEH-69956; *ibid.*, Dumka district, Maslia block, Doman Pahari, towards Dumka, alt. 177 m, 24°13'53.2"N 87°11'42.1"E, on the living *S. robusta* trunk, 26 September 2015, M.E. Hembrom, MEH-69975.

**Notes:** Common, exclusively on dead branches, stumps and living tree of *Shorea robusta* throughout the study areas. Morphologically, present Indian materials are on conformity with Thai collections of *Hymenochaete conchata* (Pan and Zhou, 2016). Moreover, genetically the Indian collections are undoubtedly very close (99% identity under 94% query coverage in BLAST search) to the Thai collections (GenBank nos. KX258959 & KX258960) showing the conspecificity amongst them.

Three, species of *Hymenochaete* earlier reported (although their phylogenetic position has not yet been verified) from India viz. *H. ochromarginata* P.H.B. Talbot, *H. rubiginosa* (Dicks.) Lév. and *H. villosa* (Lév.) Bres. are morphologically similar to *H. conchata*.

Presence of duplex context with cortex and a dense setigerous layer make *H. ochromarginata* (originally reported from South Africa) and *H. rubiginosa* (originally reported from Great Britain) (Job, 1987) morphologically close to the present species. But, colliculose, coriaceous, bistre, basidiomata and longer setae [up to 70 µm long as mentioned by Job (1987)] and larger of basidiospores [ $6 \times 3.5$  µm as mentioned by Rattan (1972) and  $5-7 \times 3-4.5$  µm as mentioned by Sharma (1995)] in *H. rubiginosa* immediately distinguishes it from the present species. Similarly, the presence of slightly, larger basidiospores separates *H. ochromarginata* [oblong-elliptical shaped  $3-4 \times 2-3$  µm long as mentioned by Job (1987)] and *H. villosa* [elliptical, apiculate,  $3.5-4 \times 2-2.5$  µm as stated by Cunningham (1957)] from *H. conchata*. Moreover, these three extralimital species are genetically distant (as evident in Figure 1) from *H. conchata*.

One more Asian species, *Hymenochaete tongbiguanensis* T.X. Zhou & L.Z. Zhao (originally reported from China) is also placed close to *H. conchata* but morphologically, the former is entirely different because its adnate papery thin resupinate basidiomata (as image shown in [www.mycobank.org](http://www.mycobank.org)). Further, microscopically denticulate setae (Pan and Zhou, 2016) is quite distinct in *H. tongbiguanensis*.

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### Research Article

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### Research Article

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