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A New Record of *Hypoxylon carneum* Petch from India, based on morphological and molecular characteristics

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The genus *Hypoxylon* is highly diversified, with species exhibiting saprophytic, symbiotic, or moderately parasitic habitat. A five-year survey conducted in Arunachal Pradesh, North-East India, resulted in the collection of fungi belonging to the families Hypoxylaceae and Xylariaceae, including six species of *Hypoxylon*. Among these, *Hypoxylon carneum* Petch is reported as a new record for India, based on morphological characters and molecular phylogenetic analysis. The teleomorphic features (stromata, perithecia, asci, and ascospores) of the specimens were examined and compared with other valid *Hypoxylon* species. Phylogenetic analysis using Maximum Likelihood (ML) of the internal transcribed spacer (ITS) regions of rDNA confirmed the identification. Globally, the genus *Hypoxylo*n comprises approximately 232 species, of which 36 were previously reported from India. This study increases the known number of *Hypoxylon* species in India, contributing to the understanding of fungal diversity in the region.

Keywords : Fungi, Hypoxylaceae, Phylogeny, Sordariomycetes

INTRODUCTION

The genus *Hypoxylon* Bull., the largest within Hypoxylaceae, comprises 232 species globally, with 95 having molecular sequence data. It belongs to Hypoxylaceae (Xylariales, Xylariomycetidae, Sordariomycetes), previously classified under Xylariaceae. Multigene phylogenetic studies by Wendt *et al.* (2018) redefined *Hypoxylon* within Hypoxylaceae, a family characterized by *Nodulisporium*-like anamorphs (Hyde *et al*., 2020). Traditionally, species were identified by stromatal and ascospore morphology (Miller, 1961), later supplemented with stromatal pigments and asexual morphs (Ju and Rogers, 1996). Modern taxonomy integrates morphological and molecular data for species delineation. *Hypoxylon* species are primarily saprophytic or endophytic in angiosperms to others, contributing to cellulose and lignin decomposition and providing ecological benefits such as pathogen resistance. Some species act as facultative parasites, and their secondary metabolites facilitate species segregation (Helaly *et al*., 2018). While *Hypoxylon* is globally distributed, it is most diverse in tropical and subtropical regions.

Arunachal Pradesh, a biodiversity hotspot in northeastern India, features diverse vegetation across altitudinal gradients, from tropical rainforests to alpine shrubs. Its unique ecosystem and rich biodiversity, including endemic fungi, underscore its importance for ecological studies (Deb *et al.* 2008; Dutta and Singh, 2023 a,b,c; Singh *et al.* 2024).

MATERIALS AND METHODS

Specimens were examined macroscopically and microscopically, with morphological features observed using a stereo zoom microscope (Zeiss Stemi58). Slides were prepared for compound

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microscopy (Zeiss A1) to study stromatal structures, including ascomata, perithecia, ostioles, asci, paraphyses, periphyses, and ascospores. A 10% KOH test was performed on stromatic tissues (Ju and Rogers, 1996), with color descriptions following Rayner (1970). The ascal apical apparatus was determined using Melzer's reagent. Measurements of stromata (n=10), ascomata (n=10), and ascospores (n=40) were taken in water, with mean values included in descriptions.

The specimens are deposited at ARUN (Arunachal Pradesh, India), with the isotype stored at ARFR, Rajiv Gandhi University. Photographs were processed using Adobe Photoshop. DNA was extracted using the CTAB method, and the ITS region of rDNA was amplified with primers ITS1 and ITS4. PCR conditions were: initial denaturation at 95°C for 3 min, 35 cycles of 94°C for 40 sec, 55.8°C for 45 sec, and 72°C for 1 min, followed by a final extension at 72°C for 10 min (White *et al.* 1990). The ITS1 and ITS4 consensus sequence is submitted to NCBI, obtaining a GenBank accession number.

Molecular Phylogenetic analysis

In this study, for the phylogenetic analysis limited nrITS sequences of different species including the newly generated sequence, were used for data analysis. Phylogenetic analysis was performed using the nrITS dataset, which comprised the newly generated sequence along with sequences retrieved through BLAST searches of previously published data. The nrITS dataset was aligned using MAFFT (Katoh *et al.* 2019). Maximum likelihood (ML) phylogenetic analysis based on the nrITS sequences was conducted using MEGA 11 software (Tamura *et al*., 2013). Nodal support values were estimated with 1,000 bootstrap (BS) replicates, and bootstrap support values (mostly >50%) from the ML analysis are displayed above at the nodes.

RESULTS AND DISCUSSION

Taxonomy

***Hypoxylon carneum* Petch, Ann. R. bot. Gdns Peradeniya 8: 157 (1924). Fig. 1**
GenBank No: PQ069709

Distribution: Worldwide (GBIF)
Habitat: Saprophytic, growing on tree twigs
Teliomorph: Stromata effused–pulvinate, plane, 0.7–5.0 cm long, 0.3–1.3 cm broad and 0.6–0.7 mm thick, surface chestnut (40); brown, black or light reddish brown granules immediately beneath surface and between perithecia, tissue below the perithecial layer thin, 0.2–0.4 mm.,10% KOH extractable pigments olivaceous green; Ostioles depressed below the stromatal surface; Perithecia cylindrical to hemispherical,0.4–0.5×0.2–0.3 mm diam.; Asci 92–160 µm long, 6.5–8.0 µm broad, with spore–bearing parts 67–83 µm long and stipe 25–57 µm long, apical apparatus bluing in Melzer's reagent, discoid, 0.8–1.0 µm high × 2.2–2.5 µm broad; Ascospores light brown to brown, unicellular, ellipsoid, nearly equilateral, with narrowly rounded ends, 8.0–11.5 × 4.5–5.0 µm, with straight germ slit up to spore length, perispore smooth, dehiscent or indehiscent in 10% KOH, epispore smooth.

Specimen examined: India, Arunachal Pradesh, Papum Pare district, Doimukh, Sub-tropical forest (27º08'57"N 093º46'11"E; 205 msl), On dead tree twigs, 10 January 2024, coll. G. Dutta. ARUN F-0091; ARFR-822

Notes: Morphologically *Hypoxylon carneum* is very similar to *H. hongheensis* but different in respect to positioning of ostioles which is positioned at the level of the stromata surface in the former whereas depressed in the latter species. Additional difference between them are dark orange vs. olivaceous green KOH-extractable pigments; cylindrical to hemispherical (0.4–0.5 × 0.2– 0.3 mm diam.) vs. spherical (0.3–0.45 × 0.3–0.5 mm diam.) perithecia; and straight germ slit vs. no germ slit (Yang *et al.* 2022). Moreover, both species are comparatively similar in having effused-pulvinate, superficial stromata with a reddish-brown to brown surface, and a peridium composed of brown, thick-walled cells of textura angularis. The asci measure 92–160 × 6.5–8.0 µm, with spore-bearing parts 67–83 µm long and stipes 25–57 µm long; the apical apparatus is discoid (0.8–1.0 µm high × 2.2–2.5 µm broad) and turns blue in Melzer's reagent. In contrast, *H. hongheensis* has 8-spored, unitunicate, cylindrical, hyaline asci measuring 67–102 × 7–11 µm, apically rounded and

Table 1: ITS sequences with GenBank accession number used in this study and newly recorded species sequences are marked in bold

Species	GenBank accession No.	Origin	Reference
Hypoxylon carneum	KY610400.1	France	Wendt *et al*. 2018
Hypoxylon carneum	PQ069709	India	This Study
H. cercidicola	KC968908.1	France	Wendt *et al*., 2018; Kuhnert *et al*., 2014
H. crocopeplum	KC968907.1	France	Wendt *et al*., 2018; Kuhnert *et al*., 2014
H. fendleri	KF234421.1	French Guiana	Wendt *et al*., 2018; Kuhnert *et al*., 2014
H. fendleri	FM209440.1	Thailand	Suwannasai *et al*. 2013
H. fragiforme	KC477229.1	Germany	Wendt *et al*. 2018
H. fuscum	KY610401.1	France	Wendt *et al*. 2018
H. griseobrunneum	KY610402.1	India	Wendt *et al*. 2018; Kuhnert *et al*. 2014
H. guilanense	MT214997.1	Iran	Pourmoghaddam *et al*. 2020
H. haematostroma	KC968911.1	Martinique	Wendt *et al*. 2018; Kuhnert *et al*. 2014
H. howeanum	AM749928.1	Germany	Wendt *et al*. 2018; Kuhnert *et al*. 2014
H. hypomiltum	KY610403.1	Guadeloupe	Wendt *et al*. 2018
H. investiens	KC968925.1	Malaysia	Wendt *et al*. 2018; Kuhnert *et al*. 2014
H. lateripigmentum	KC968933.1	Martinique	Wendt *et al*. 2018; Kuhnert *et al*. 2014
H. lenormandii	KC968943.1	Ecuador	Wendt *et al*. 2018; Kuhnert *et al*. 2014
H. lenormandii	DQ223745.1	Thailand	Suwannasai *et al*. 2005
H.lenormandii	OR237613.1	China	From BlastN analysis
H. lenormandii	DQ223746.1	Thailand	Suwannasai *et al*. 2005
H.lenormandii	KM610294.1	Thailand	Kuhnert *et al*. 2014
H. lenormandii	PQ069716	India	From BlastN analysis
H. mangrovei	NR_166288.1	China	Zeng *et al*. 2022
H. mangrovei	OQ726608.1	Indonesia	From BlastN analysis
H. musceum	KC968926.1	Guadeloupe	Wendt *et al*. 2018; Kuhnert *et al*. 2014
H. olivaceopigmentum	MK287530.1	USA	Sir *et al*. 2019
H. papillatum	KC968919.1	USA	Wendt *et al*. 2018; Kuhnert *et al*. 2014
H. perforatum	KY610391.1	France	Wendt *et al*. 2018; Kuhnert *et al*. 2014
H. petriniae	KY610405.1	France	Wendt *et al*. 2018; Kuhnert *et al*. 2014
H.pilgerianum	KY610412.1	Martinique	Wendt *et al*. 2018
H. porphyreum	KC968921.1	France	Wendt *et al*. 2018; Kuhnert *et al*. 2014
H. pulicicidum	JX183075.1	Martinique	Wendt *et al*. 2018; Kuhnert *et al*. 2014
H. rickii	KC968932.1	Martinique	Wendt *et al*. 2018; Kuhnert *et al*. 2014
H. rubiginosum	KC477232.1	Germany	Wendt *et al*. 2018; Kuhnert *et al*. 2014
H. rubiginosum	MT214998.1	Iran	Pourmoghaddam *et al*. 2020
H. samuelsii	KC968916.1	Guadeloupe	Wendt *et al*. 2018; Kuhnert *et al*. 2014
H. sublenormandii	ON178674.1	China	From BlastN analysis

(contd. Table 1)

H. texense	MK287536.1	USA	Sir *et al*. 2019
H. ticinense	JQ009317.1	France	Wendt *et al*. 2018
H. trugodes	KF234422.1	Sri Lanka	Wendt *et al*. 2018; Kuhnert *et al*. 2014
H. vogesiacum	KC968920.1	France	Wendt *et al*. 2018; Kuhnert *et al*. 2014
H. olivaceopigmentum	MK287530.1	USA	Sir *et al*. 2019

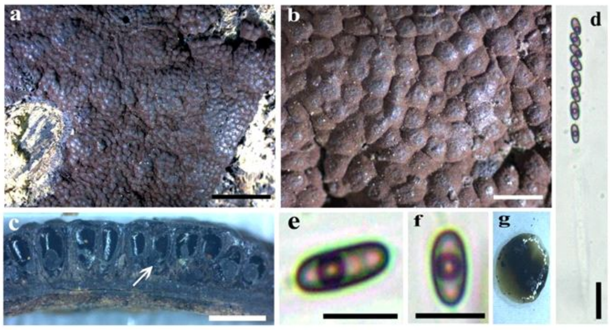

Fig. 1: ***Hypoxylon carneum*** (a) Stromata on host, (b) Stromata surface, (c) Perithecia, (d) Ascus, (e, f) Ascospores shape with oil globule, (g)10% KOH colour test; Scale bars: (a) 4 mm, (b) 1 mm, (c) 0.5 mm, (d) 20 µm, (e, f) 10 µm.

moderately pedicellate with pedicels 16–22 µm long, and a 'J+' apical apparatus. Ascospores are light brown to brown, unicellular, ellipsoid, nearly equilateral with narrowly rounded ends, measuring 8.0–11.5 × 4.5–5.0 µm, with a straight germ slit extending the full length of the spore; the perispore is smooth and dehiscent or indehiscent in 10% KOH, and the epispore is also smooth. Additionally, *H. hongheensis* ascospores 9–11 × 4.5–5.5 µm, uniseriate, crescent to somewhat hemispherical, initially hyaline to yellow and becoming brown or black when mature, usually containing 1–2 guttules, aseptate, rounded at the ends, and lacking a germ slit, gelatinous sheath, or appendages. (Yang *et al.*, 2022).

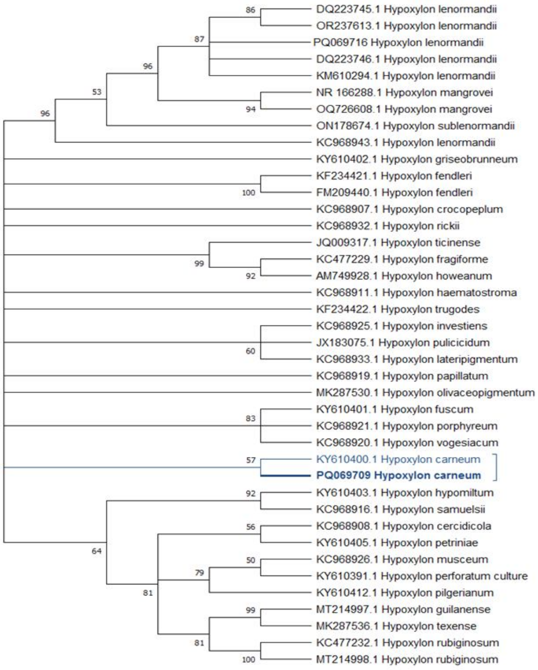

Fig.2: Phylogenetic tree resulting from ITS-rDNA sequences of *Hypoxylon carneum*; The evolutionary history was inferred by applying maximum likelihood in RAxML-HPC2, Bootstrap support values (>50%).

The phylogenetic tree of *Hypoxylon carneum*, Fig. 2 revealed four distinct clades (I, II, III, IV). Clade III comprised only two strains of *Hypoxylon carneum* with 57% bootstrap support: PQ069709, the presently isolated strain, and KY610400 from Germany. Clade I grouped with the *Hypoxylon lenormandii* cluster, while Clades II and IV included other globally distributed *Hypoxylon* species. Based on phylogenetic analysis, the tree, supported by strong bootstrap values, indicates that all clades of *Hypoxylon* species, including the analyzed strain, share similarities with globally identified *Hypoxylon* species.

CONCLUSION

The genus *Hypoxylon* demonstrates significant diversity, encompassing species with saprophytic, symbiotic, and moderately parasitic lifestyles. Through a five-year survey of fungal species in Arunachal Pradesh, North-East India, six species of *Hypoxylon* are identified, including *Hypoxylon carneum* Petch, which is recorded for the first time in India. The identification is validated using morphological examinations and molecular phylogenetic analysis of ITS rDNA regions. With this study, the number of *Hypoxylon* species reported from India has increased to 37, enhancing the understanding of fungal diversity in the region and underscoring the importance of exploring biodiversity hotspots Arunachal Pradesh.

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DECLARATION

Conflict of interest. Authors declare no conflict of interest

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