ORIGINAL RESEARCH PAPER

Botany

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF RARE MEDICINAL POLYPORE INONOTUS HISPIDUS (BULL.: FR.) P. KARST.(APHYLLOPHORALES:HYMENOCHAETACEAE) FROM KOLHAPUR DISTRICT (MS), INDIA

KEY WORDS:
Hymenochaetaceae, Inonotus, Medicinal polypores, nSSU, Taxonomy

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ABSTRACT

Fungi that are edible and therapeutic play an essential part in human food and traditional medicine. *Inonotus hispidus* (Bull.) P. Karst. (Hymenochaetaceae) is a well-known edible and medicinal mushroom with a long history as a health food and ancient folk medicine in Europe and East Asian countries, especially China. It is characterized by annual, orange rust to fulvous basidiocarp, irregular to polygonal pores, monomitc hyphal system with hyphal setae, ventricose to subulate setae, subglobose to broadly ellipsoid spores and abundant Chlamydospores. During study of the polyporoid fungi from Kolhapur district some interesting specimens were collected. Detailed taxonomic study of the collected specimens identified them as Inonotus hispidus, with a new distribution record to Maharashtra state. Taxonomic account of the species is presented.

INTRODUCTION

Inonotus P. Karst. was erected by Karsten in 1879 to accommodate pileate polypores with colored spores, and was emended by Donk (1933) to include more species with the features of both colored spores and a rusty brown fibrous context. The genus Inonotus was traditionally distinguished from the genus Phellinus Quel. based on the discrepancy of hyphal system and consistent features of basidiocarp, namely Inonotus is monomitic and annual, while Phellinus is dimitic and perennial (Fiasson & Niemela, 1984; Larsen & Cobb-Poulle, 1990; Ryvarden, 1991). Microscopically, Inonotus usually has a mono-mitic hyphal system, simple-septate hyphae, and colorless or yellowish to brownish basidiospores. Based on 28S rDNA-based phylogenetic analyses, Wagner and Fischer (2002) subdivided Phellinus s.l. and Inonotus s.l. into the genera Inonotus s.s., Phellinus s.s., Aurificaria D.A. Reid, Fomitiporella Murrill, Fomitiporia Murrill, Fulvifomes Murrill, Fuscoporia Murrill, Inocutis Fiasson & Niemela, Inonotopsis Parmasto, Mensularia L. Ibiza, Onnia P. Karst., Phylloporia Murrill, Porodaedalea Murrill and Pseudoinonotus T. Wagner & M. Fisch. However, the Inonotus s.s. sensu Wagner and Fischer is still a broad sense of the genus. Wagner and Fischer (2002) and Wu et al. (2022) regarded Inonotus as polyphyletic group. Many studies have been conducted to revise the generic concept of Inonotus based on morphological and molecular phylogenetic evidence, resulting in many species being segregated to other genera, such as Cylindrosporus L.W. Zhou, Flaviporellus Murrill, Inonotus sensu stricto, Inocutis Fias-son & Niemelä, Inonotopsis Parmasto, Mensu-laria Lázaro Ibiza, Nothonotus Y. C. Dai, F. Wu, L. W. Zhou, Vlasák & B. K. Cui, Onnia P. Karst, Pachynotus Y. C. Dai, F. Wu, L.W. Zhou & B. K. Cui, Pseudoinonotus T. Wagner & M. Fischer, Rigidonotus Y. C. Dai, F. Wu, L. W. Zhou, Vlasák & B. K. Cui, Sanghuangporus Sheng H. Wu, L. W. Zhou & Y. C. Dai, and Tropicoporus L. W. Zhou, Y. C. Dai & Sheng H. Wu (Wagner and Fischer 2002; Tian et al. 2013; Vlasák et al. 2013; Zhou et al. 2016, Wu et al. 2022).

Materials & methods

Morphological studies

Fruit bodies were collected from forest Botanical garden, Rajaram College, Kolhapur during the monsoon seasons. Macroscopic characters of fresh specimens were recorded. Microscopic observations were made on materials stained using aqueous solutions of 3% phloxine B and 1% congo red, and mounted in 5% aqueous KOH. Reaction of the basidiospores on treatment with Melzer's reagent and cotton blue was noted. All collections examined are deposited at the Herbarium of Department of Botany, Rajaram College, Kolhapur. Current taxonomic names and systematic positions of taxa used in this study are according to the MycoBank database http://www.mycobank.org accessed on 02 June 2025.

Phylogenetic studies

For DNA extraction from the specimens (Y24V4P12), NucleoSpin® Plant II kit (Macherey-Nagel 2014, Germany) was used. PCR amplification of nSSU gene region was carried out using the primers NS 1F and NS 4R. Sequencing was done using BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA). DNA sequencing was outsourced to Paeon Laboratories Kolhapur, Maharashtra, India. BioEdit sequence alignment editor ver. 7.2.5 (Hall, T.A. 1999) was used to check the quality of the freshly generated sequencing data and for editing the sequences where needed. The newly generated nSSU sequences were deposited in the GenBank database (www.ncbi.nlm.nih.gov) with accession numbers (PV731356). Sequence similarity assessments were conducted using BLAST search in NCBI's GenBank nucleotide database (https://blast.ncbi.nlm.nih.gov/). The newly generated sequences and those taken from GenBank were aligned using MEGA 7 (Kumar et al. 2016). Polyporus tricholoma (OP781904) was selected as outgroup. Maximum Parsimony (MP) phylogenetic analyses were performed using the default settings and 500 bootstrap replicates with the Subtree-Pruning-Regrafting (SPR) algorithm for datasets determined using MEGA 7 (Kumar et al. 2016).

Result and discussion

Molecular characterization

nBLAST search using the nSSU sequence of the specimen, resulted in 98% identity with Inonotus hispidus (GenBank no.-ON063785, U59074). nBLAST result also showed 97% similarity with Inonotus lenteus (AB158296) and *Sanghuangporus baumii* (ON063798). The phylogenetic tree shows the placement of our collections among other related

taxa of order Hymenochaetaceae. In the phylogenetic tree, our specimen (PV731356) settled near to Inonotus hispidus with 99% bootstrap support.

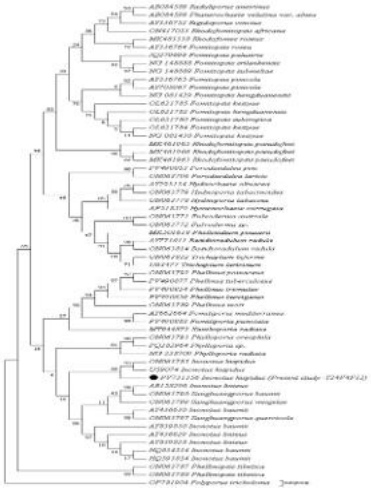

Fig.1. Maximum Parsimony tree generated using SSU sequence data. Values at the nodes indicate Maximum Parsimony bootstrap values. Inonotus hispidus is placed with bullets to highlight its phylogenetic position in the tree represent the GenBank accession numbers and voucher number given along with the taxa.

Inonotus hispidus (Bull.: Fr.) P. Karst., Meddn Soc. Fauna Flora fenn. 5:39, 1879. (Fig. 2)

Basidiocarp annual, solitary or imbricate, sessile with broad basal attachment. Pileus 4-15 x 6-25 x 2-10 cm, dimidiate to triquetrous, upper surface orange rust to fulvous, blackening on drying, strongly tomentose to pilose, changing to hispid and brittle with age; margin obtuse, straight. Pore surface at first fawn, then bright rust; pores irregular, polygonal, 2-4 per mm, dissepiments thick, lacerate; tubes 0.5-5 cm long, bright ochraceous rust. Context up to 7 cm thick, fulvous, paler towards upper surface and margin, blackening with KOH, zoned, radially fleshy fibrous. Hyphal system monomitic with setal hyphae. Generative Hyphae freely branching with a slightly thickened wall but broad lumen, simple septate, 4-12 µm diam. Setae typically present but often absent, ventricose to subulate, 15-28 x 6-11 µm. Basidia broadly clavate, 4 stergimate, 12-17 x 7-9 µm. Basidiospores subglobose to broadly ellipsoid, 8-12 x 7-9 µm. Anamorphic stage developing as a cushion shaped mass of brown tissue, soft and fleshy at first, exuding clear droplets of exudate from the entire surface, moist and velvety to the touch, up to 20 cm wide, 25 cm deep, and 15 cm thick, the entire structure resembling a sessile Inonotus basidiocarp but no tube layer ever develops, within a few weeks the fruiting body becomes drier and firmer and broken pieces show the crumbly and dusty texture resulting form chlamydospore. Chlamydospores abundant in context tissue, thick-walled, dark reddish brown in KOH, irregular in shape, smooth, globose to ellipsoid or often with an elongated cylindric appendage, 10-30 µm in diameter.

Fig.2. Inonotus hispidus (Bull.: Fr.) P. Karst.: a-d. Basidiomata, e. Generative hyphae, f. Setal hyphae with spore, g. Chlamydospores, h. Basidiospores. Scale Bars: a-d 2 cm, e-h 50 µm Speciemen examined: India, Maharahstra state, Kolhapur, on living *Delonix regia*, Shivaji University, Kolhapur; on living Delonix regia, (Y24V4P12), 09/08/2024, Rajaram College, Kolhapur, Yogesh Patil

Remark: Inonotus hispidus distinguished by its annual, sessile basidiocarp, orange rust to fulvous, hispid pileus, pore surface bright ochraceous rust, irregular, polygonal, 2-4 per mm, monomitic with setal hyphae, subglobose to broadly ellipsoid, 8-11 x 7-9 µm, abundant dark reddish brown, irregular, smooth, globose to ellipsoid Chlamydospores (Table 1.)

Table 1. Morphological comparison of Inonotus with its morphological similar species

Species	Basidiocarp	Hymenial surface	Setae	Basidia	Spores
Inonotus rickii (Pat.) D. Reid	Basidiocarps annual, sessile, tomentose, golden brown, becoming rough and dark rusty brown	Poroid, pores angular, 2-3 per mm, context becoming dark rusty brown	Hymenial setae frequent, subulate to ventricose, 15-20 x 4-6 µm.	-	Basidiospores dark reddish brown in KOH, 6-8.5 x 4.5-5.5µm. Chlamydospores dark reddish brown in KOH, irregular in shape, smooth, globose to ellipsoid or with an elongated cylindric appendage, 10-30 µm.
Inonotus cuticularis (Bull.) P. Karst.	Basidiocarps annual, sessile, yellowish brown, tomentose or radially fibrillose	pale brown, glancing, pores angular, 4-5 per mm	Abundant to rare, subulate to ventricose, frequentl y hooked, firm- to thick-walled, 16-30 x 6-11 µm.	Broadly clavate, 4-sterigmate, 16-21 x 7-8 µm, simple-septate at the base.	Basidiospores broadly ellipsoid to ovoid, pale to dark yellowish brown, 6-8 x 4.5-5.5 µm.
Inonotus tamaricis (Pat.) Bond. & Sing.	Basidiocarps pileate, annual, solitary, hispid to villose, rusty to cinnamon brown	deep rusty brown becoming black, pores angular to lacerate, 1-2 per mm	Absent	-	Basidiospores rusty brown to pale umber, thick-walled, subglobose to ellipsoid, 6.2-7.3 x 4.5-6 µm.
Inonotus quercustris M. Blackwell and Gilbn.	Basidiocarps annual, sessile, golden yellow at first, becoming rusty-brown	bright golden lustre when viewed obliquely, the pores angular, 3-5 per mm.	Absent	Basidia broadly ellipsoid, 18-23 x 9-11 µm.	Basidiospores pale golden yellow, ellipsoid, thick-walled, 9-10 x 6-8 µm.

Inonotus plorans (Pat.) Bondartsev & Singer	Basidiocarps annual,single, large, soft, semicircular, sessile, rusty brown slightly paler towards the margin	cinnamon to rusty brown, pores angular, 2-3 per mm	Absent	Basidia large, about 15–18 x 9–11 µm.	Basidiospores ellipsoid yellow to rusty brown, thick-walled, 8-11 x 6-8.5 µm.
Inonotus hispidus (Bull.: Fr.) P. Karst. (Present study)	Basidiocarp annual, solitary or imbricate, sessile	bright ochraceous rust, irregular, polygonal, 2-4 per mm.	Typically present but often absent, ventricose to subulate, 15-28 x 6-11 µm.	Broadly clavate, 4 stergimate, 12-17 x 7-9 µm.	Basidiospores subglobose to broadly ellipsoid, 8-12 x 7-9 µm. Chlamydospores dark reddish brown in KOH, irregular in shape, smooth, globose to ellipsoid or with an elongated cylindric appendage, 10-30 µm.

Hosts distribution: Mostly associated with Fraxinus and Malus, but also recorded on Acer, Aesculus, Alnus, Betula, Carya, Crataegus, Euonymus, Fagus, Ficus, Juglans, Liquidambar, Morus, Pistacia, Pyrus, Platanus, Populus, Prunus, Quercus, Robinia, Salix, Schinus, Sophora, Ulmus, Vitis and Zizyphus (Pegler and Waterston, 1968)

Geographical distribution: Africa (Morocco), Asia (China, Japan, India, Syria, U.S.S.R., West Pakistan), Europe (Belgium, Cyprus, Czechoslovakia, Finland, France, Germany, Great Britain, Greece, Hungary, Italy, Netherlands, Norway, Portugal, Spain, Sweden); North America (Canada, U.S.A.); South America (Brazil).

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