

Comparative Morphological Study of Species *Phellinus robustus* and *Fulvifomes squamosus* of Order Hymenochaetales from Nagpur District

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Abstract: The macrofungi of order Hymenochaetales belonging to class Agaricomycetes of phylum Basidiomycota are known for causing white wood rot by secreting ligninolytic enzymes, which degrades lignocellulosic substrates of cell walls of wood therefore, plays vital role in restoring nutrients back in the ecosystem. Despite their ecological significance, their species level identification often remains challenging due to overlapping of traits and underexplored morphotaxonomic studies in Central India region, especially Nagpur District.

This study presents a detailed comparative morphological characterization of one of the extremely important fungi of order Hymenochaetales. The total 18 specimens of the order were collected from Nagpur District of Maharashtra state among them two major distinct species, *Phellinus robustus* and *Fulvifomes squamosus* were considered for further studies of morphological characteristics. Comparative analysis revealed macroscopic features including shape, size, color, texture, attachment, etc. and microscopic features including spores, hyphal systems, sterile structures, etc. These observations were made with standard diagnostic reagents such as 5% KOH solution for Hymenochaetales differentiation, and Phloxin, Congo red and Melzer's reagent for proper staining.

The results showed that *P. robustus* is primarily characterized by its hard, brown, rough, horse hoof shape basidiocarps and three distinct color zones, whereas *F. squamosus* has comparatively large but applanate basidiocarp with centrally dark brown and peripherally light brown coloration. Furthermore, a significant variation observed in their pores geometry, pore density, basidiospores and hymenial setae. However, both of them share dimitic hyphal systems.

This study contributes to the broader understanding of *P. robustus* and *F. squamosus* fungi of order Hymenochaetales. The detailed morphological studies and documented taxa enrich the records of Nagpur District, providing basis of future taxonomic and ecological studies.

Keywords: Hymenochaetales, *Phellinus robustus*, *Fulvifomes squamosus*, dimitic hyphae, white rot, ligninolytic enzymes.

I. INTRODUCTION

The two species *Phellinus robustus* and *Fulvifomes squamosus* studied here are the most important fungi of the order Hymenochaetales for their ligninolytic enzyme activity of white wood rot and having some crucial medicinal properties. As this fungi are medicinally and ecologically important, each species of order need to be studied for their biotechnological and industrial potential. The comparative morphological study of the following two species revealed some distinct diagnostic key features like pore density, spore shapes, dimitic hyphal systems, absence of clamp connections and presence of hymenial setae. The *Fulvifomes* is mainly characterized by the absence of setae (Carlos A.



Salvador-Montoya *et al.*, 2018). However to the best of our knowledge, this study reported the first time observation of hymenial setae in the species *Fulvifomes squamosus* of the order Hymenochaetales.

II. RESEARCH METHODOLOGY

2.1 Survey of Selected Study Area: A detailed field survey and specimen collection was carried out through Nagpur district of Maharashtra state during the period of July 2025 to July 2026. The survey was conducted mostly in the pre-monsoon, monsoon and post-monsoon seasons.

2.2 Collection of Specimens and Documentation: The collection of fungal specimens was done from live tree barks, dead wooden logs and other infected plant parts. Particularly the hosts which are in contact with water bodies, near water bodied or those containing moisture, were considered for specimen collection. The specimens were GIS photographed before the removal, then including their host affinity, and field data such as location, date, time, temperature, weather, host and habitat was recorded and an unique specimen code was given to each specimen. Also the crucial positive Xanthochroic reaction (Potouillard, 1900) of 3-5% KOH solution test was used to confirm the specimens belongs to the order Hymenochaetales, which differentiates them from other Agaricomycetes. After this confirmation the collection was done and the specimens were kept in the breathable and moisture absorbing paper bags (Ryvarden and Melo, 2014) as to avoid any other microorganism growth by avoiding moisture and were taken to the lab for further proceedings.

2.3 Morphological characterization:

A. Macroscopic Characterization: The physical characteristics color of basidiocarps were compared and recorded based on color codes of Konerup and Wanscher(1967), and the characteristics such as shape of basidiocarp, size, texture, details of margins such as its angles which were acute or obtuse, its shapes- wavy, continuous or broken, and if it was sterile or fertile. Some more morphological features such as the color of its pilear surface, the context stipe length over the flat smooth surface membrane of fungus, the measurments of tube lengths and by taking a thin longitudinal section of basidicarp from pilear surface, the shape, size and the number of pores per mm were also noted down.

Lastly, a moist blotting paper was kept on the upper pilear surface while taking spore prints on both the black and white papers, which will be beneficial for the clear prints of darker color spores on white paper and lighter color spores on black paper. This process of spore print was repeated and on clean slides the prints were taken for its microscopic examinations (Ryvarden and Melo, 2014).

B. Microscopic Characterization: The microscopic characters of specimens such as spore size and shape were observed by staining a spore print on glass slide with 1% phloxin stain and mounting it in 5% KOH solution. After covering it with cover slip, the spores were observed under 10x, 45x and 100x (oil emersion) magnification in the microscope. This same method of staining and mounting was repeated with different stains like Cotton blue for staining of fungal cytoplasm, Congo red for staining fungal cell wall and Phloxine for the observation of nuclei and hyphal systems of basidiocarps. Through this, the structures of different types of hyphae, clamp connections of hyphae, structure of setae and cystidia were observed (Navpreet Kaur *et al.*, 2015).

2.4 Species Identification with Morphological Keys : After the observation of specimen at both macoscopic and microscopic level, the identification was done up to species level with the help of standard mycological keys and literatures such as,

Gilbertson and Ryvarden (1993-1994) fungal flora.

Poroid fungi of Europe by L. Ryvarden & I. Melo.

Aphylllophorales of Himalaya by J.R. Sharma (Botanical Survey of India).

2.5 Preservation of specimens in Fungarium:

The species were oven dried to preserved in the fungarium for future use. Then placed and held in the dehydrator at 43°C to 50°C for 12–48 hrs. These dried specimens then gone into archival-quality paper pockets with the chemical crystals of p-dichlorobenzene to avoid growth of other microorganisms (Ryvarden and Melo, 2014).



III. RESULTS AND DISCUSSION

A comparative evaluation of the selected species *Phellinus robustus* and *Fulvifomes squamosus* of order Hymenochaetales established distinct macro- and micromorphological features. The key comparative characters are given below in the following table;

Table 1: Comparative Morphological and Anatomical Profile

Character	<i>Phellinus robustus</i>	<i>Fulvifomes squamosus</i>
Basidiocarp	Pileate, sessile, perennial; horse-hoof shaped	Pileate, sessile, perennial; woody
Margin	Sterile, obtuse, continuous	Sterile, acute, cracked and with small fissures
Pileal surface	Woody, brown and rough	Centrally dark brown, peripherally light brown
Hymenial surface	Dark brown with numerous pores	Brown with numerous pores
No. of Pores, Pore size and shape	49–50 pores/mm ² , μm , Circular	22–24 pores/mm ² , 3-6 μm , Circular-Elongated
Spore shape	Ellipsoid	Sub-globose
Spore size	4.5-6 μm	3.5-4 μm
Hyphal system	Dimitic – generative + skeletal hyphae	Dimitic – generative + skeletal hyphae
Width of Generative Hyphae	3-4.5 μm	2.5-3.5 μm
Width of Skeletal Hyphae	3-5.5 μm	3.8-5.5 μm
Clamp connection	Absent	Absent
Setae	Present	Present
Context	0.1 mm	0.2 mm
Tube length	2-5 mm	1-4 mm
Key identifying character	Three distinct colour zones of context	Two different basidiocarp layers- dark peripheral and light central

3.1 Species Descriptions

1. *Phellinus robustus*

Macroscopic Features: The basidiocarp of *P. robustus* shows two different structural shapes, at the young age it shows tiny coin shape structure and at fully grown stage it shows prominent horse-hoop shape structure. The pileal surface is hard woody rough and brown in color. A sterile, obtuse and continuous margin is observed. The sectioning through the context reveals three distinct color zones. The hymenial surface is dark brown with very small size and high density of pores per mm.

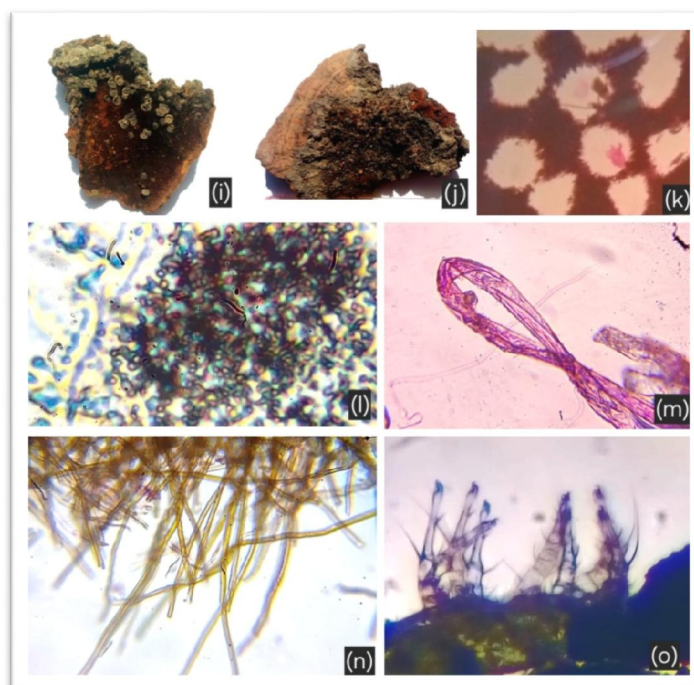
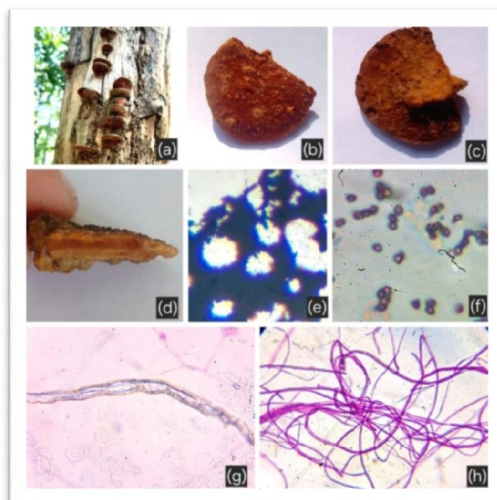
Microscopic Features: The dimitic hyphal system, consisting of generative and skeletal hyphae are observed with the absence of clamp connections. The sterile structure such as hymenial setae are present and the basidiospores are observed having distinct ellipsoid shape.

2. *Fulvifomes squamosus*

Macroscopic Features: The basidiocarp of *F. squamosus* is perennial, pileate, sessile and woody. The pileal surface is appanate with a dark brown central part and fading to a light greyish brown peripheral margin part. The hymenial surface is brown with a low pore density.

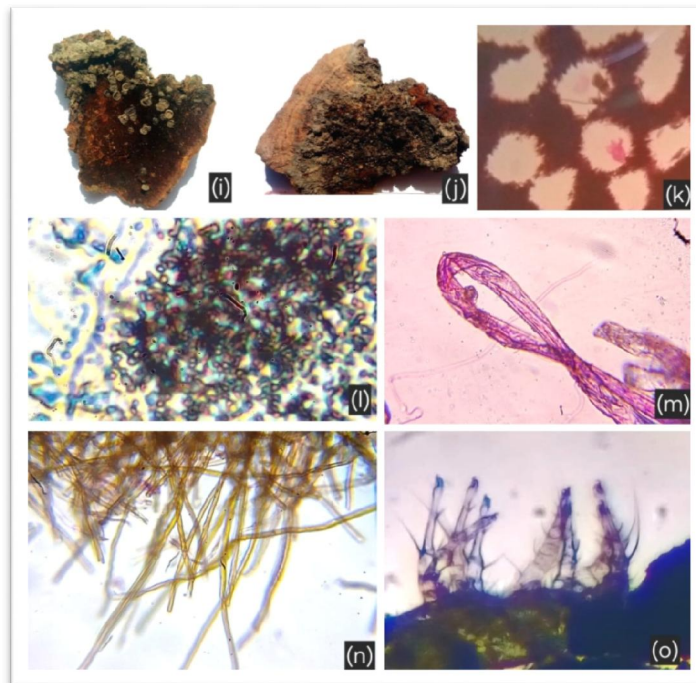
Microscopic Features: The hyphal system is dimitic with generative and skeletal hyphae. The clamp connection are absent and the sterile structure like hymenial setae are present.





Figs.: *Phellinus robustus* ; (a) – Habit, (b) – Pileal surface of young basidiocarp, (c) - Hymenial surface of young basidiocarp, (d) – Three different color zones, (e) – Pores, (f) – Spores, (g) – Skeletal Hyphae, (h) – Generative Hyphae





Figs.: *Fulvifomes squamosus* ; (i) – Hymenial surface, (j) - Pileal surface, (k) – Pores, (l) – Spores, (m) – Skeletal Hyphae, (n) – Generative Hyphae, (o) – Setae

IV. CONCLUSION

The species *Phellinus robustus* and *Fulvifomes squamosus* collected from Nagpur District region demonstrated clear key diagnostic morphological differences. The microscopic features such as dimitic hyphal systems, absence of clamp connections and presence of sterile structures like hymenial setae are common in both species. However, they can be easily differentiated by basidiocarp shape, pore density, context layering and spore morphology. These findings provide an important taxonomic reference for macrofungal and microfungal documentation in the Nagpur District of Central India region.

BIBLIOGRAPHY

1. Arumugam, E., Murugadoss, R., Gunaseelan, S., Karunarathna, S.C., Elgorban, A.M., Rampelotto, P.H., Kaliyaperumal, M. Characterization of New *Tropicoporus* Species (Basidiomycota, Hymenochaetales, Hymenochaetaceae) Discovered in Tamil Nadu, India. (2024). *Biology*, 13, 770. <https://doi.org/10.3390/biology13100770>.
2. Bakshi, B. K. (1958). New records of Hymenomycetes in India, *Indian Phytopathology*, 11: 88.
3. Bauhin, C. (1623). "Pinax Theatri Botanici", *Sumptibus & typis Ludovici Regis*. pp. 1-522.
4. Bourdot, H. & Galzin, A. (1928). Hymenomycetes de France, *Societe Mycologique de France, Sceaux.*, pp. 1-764.
5. Corner, E. J. H. (1947). Variation in the size and shape of spores, basidia and cystidia in Basidiomycetes, *New Phytologist*, 46: 195 -228.
6. Gilbertson, R. L. (1979). The genus *Phellinus* (Aphyllphorales: Hymenochaetaceae) in Western North America, *Mycotaxon*, 9: 51-89.



7. Kirk, P. M., Cannon, P. F., Minter, P. F. and Stalpers, J. A. (2008). Ainsworth and Bisby's Dictionary of the Fungi, *CAB International UK*, pp. 1-771.
8. Larsen, M.J. & L.A. Cobb-Poulsen. (1990). *Phellinus* (Hymenochaetaceae). A survey of the world taxa, *Fungi Flora*, Oslo, Norway. 1-206.
9. Kornerup, A. and Wanscher, J.H. (1967). *Methuen Handbook of Colour*, Methuen & Co. Ltd., London, pp. 1-243.
10. Ranadive, K. R., Jite, P. K., Ranade, V. D. & Vaidya, J. G. (2012). Host Distribution of *Phellinus* from India, *Indian Journal of Forestry*, 35(1): 67-72.
11. Ranadive, K. R. and Vaidya, J. G. (2012). Host diversity of genus *Phellinus* from world, *Elixir Appl. Botany*, 52: 11402-11408.
12. Ryvarden, L. & Gilbertson R. L. (1993-1994). European Polypores, *Vol. I-II, Fungiflora*, 743pp.
13. Salvador-Montoya, C. A., Popoff, O. F., Reck, M., & Drechsler-Santos, E. R. (2018). Taxonomic delimitation of *Fulvifomes robiniae* (Hymenochaetales, Basidiomycota) and related species in America: *F. squamosus* sp. nov. *Plant Systematics and Evolution*, 304(3), 445–459. <https://doi.org/10.1007/s00606-017-1487-7>
14. Sharma, J.R. (2012). Aphyllophorales of Himalaya. *Botanical Survey of India*, Calcutta. 590pp.

