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Cover: Himalayan Gray Langur *Semnopithecus ajax* (adult female) © Rupali Thakur.



First report of *Gymnopilus ochraceus* Høil. 1998 (Agaricomycetes: Agaricales: Hymenogastraceae) from India and determination of bioactive components

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Abstract: A rare *Gymnopilus* species *G. ochraceus* Høil. collected from live *Ficus platyphylla* Del. tree is described and illustrated for the first time from India. Morphological and microscopic characters with molecular and biochemical analysis has been discussed. To indicate that, 25 species of *Gymnopilus* have previously been recorded from India and the species *Gymnopilus ochraceus* is hereby reported for the first time, thus making a total of 26 species from India.

Keywords: Biochemical analysis, *Ficus platyphylla*, fungi, microscopic characters, mushroom, taxonomy.

The genus *Gymnopilus* (family Hymenogastraceae) is represented by 200 species in the world (Kirk et al. 2008). Members of the genus are characterized by yellow to brown, ferruginous or purple fruiting bodies, saprotrophic nature, cortinoid to membranous veil and a rusty brown spore print (Kaur et al. 2015). The genus was confirmed by roughened basidiospores that range from verrucose to rugulose; capitate to sub-capitate, ventricose cheilocystidia and clamp connections present on almost all kinds of hyphae (Khan et al. 2017). Shape and size of the spores and cystidia are considered important characters for distinction among the species (Rees et al. 2004). Based on the pigments and the non-mycorrhizal habit, Kühner (1984) classified *Gymnopilus* together with *Galerina* Earle in the Strophariaceae family. Later, Singer (1986) placed it in family Cortinariaceae

due to the ornamentation and lack of germinal pore of the basidiospores. Currently, according to the results of phylogenetic analysis, *Gymnopilus* forms an independent clade called "Gymnopilae" which is not related to any of the two families mentioned above by Matheny et al. (2006) and is currently placed in the family Hymenogastraceae (Kirk et al. 2008). 25 species of *Gymnopilus* have been reported from India (Kulkarni 1990; Thomas et al. 2003; Ministry of Environment and Forests 2011; Farook et al. 2013; Kumar et al. 2014; Kaur et al. 2015). Two species of *Gymnopilus* have been described from Maharashtra viz. *G. karnalensis* S.M.Kulk. and *G. chrysopellus* (Berk. & Crutis) Murril (Senthilarasu 2014; Patil 2019).

During the present study, mature fruiting body of *G. ochraceus* were collected on the living tree of *Ficus platyphylla* Del. from Kate Bhogaon, Panhala, Kolhapur district (M.S.), India. Morphological and microscopic analysis have been described in this paper. Analysis of ITS rDNA sequence was done to evaluate phylogenetic relationships and Gas Chromatography–Mass Spectrometry (GC-MS) was done for determination of bioactive components.

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MATERIAL AND METHODS

a) Morphological and microscopic analysis

Morphological and ecological characters were noted in the field. Microscopic observations of fresh fruiting body was done with the help of 1.5% phloxine B staining and Lawrence & Mayo N-300M research microscope.

b) Identification of fungal strains

The identification of isolates was carried out at the sequencing facility of National Centre for Microbial Resource (NCMR), National Centre for Cell Science, Pune. At the facility, genomic DNA was isolated by the standard phenol/chloroform extraction method (Sambrook et al. 1989), followed by PCR amplification of the ITS regions using universal primers ITS1 [5'-TCC GTA GGT GAA CCT GCG G-3'] and ITS4 [5'-TCC TCC GCT TAT TGA TAT GC -3']. The amplified ITS PCR product was purified by PEG-NaCl precipitation and directly sequenced on an ABI® 3730XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) as per manufacturer's instructions. Essentially, sequencing was carried out from both ends so that each position was read at least twice. Assembly was carried out using Lasergene package followed by NCBI BLAST against sequences from type material for tentative identification (Boratyn et al. 2013).

c) GC-MS analysis

10% methanolic extract of dried fruiting bodies was sonicated for 60 minutes at 35°C followed by centrifugation at 10,000 rpm for five minutes. Supernatant was used for GC-MS analysis using Shimadzu, Japan TQ 8050 plus HS 20. Helium was used as the carrier gas at a flow rate of 1ml/min and an injection volume of 1.0 μ L. Injector temperature was 250°C; ion source temperature 250°C. The oven temperature was 60°C isothermal for 2.0 min, with an increase of 10°C/min to 250°C, then 5°C/min to 275°C, ending with 10 min. isothermal at 275°C. Detector voltage was 0.7ev.

RESULTS AND DISCUSSION

Habitat: Growing on live *F. platyphylla* stem in a cluster (Image 1 A-G)

Pileus 2–8 cm wide, convex to plane, scaly, with appressed squamules at the centre, surface pale brown, honey brown, pale ochraceous to pale yellow brown, squamules pale to honey brown; lamellae adnexed to adnate, dark yellow brown; stipe 2–9 x 0.5–1.2 cm, cylindrical to somewhat clavate, fibrillose, concolourous with pileus, with an membranous pale yellow brown ring, context yellowish to pale buff, with bitter taste; Basidiospores (5.5) 5.6–7.1 (7.5) x (4) 4.2–5.3 (5.5) μ m,

ellipsoid to ovoid, verrucose to punctate, pale to yellow brown, dextrinoid; Basidia 16–26 x 5–6 μ m with four sterigmata; Cheilocystidia 15–18 x 4.5–7 μ m, ventricose-rostrate with a clavate or rounded apex with 3–3.5 μ m broad content; Pleurocystidia not seen; hyphae of pileipellis 6–18 μ m broad, smooth, yellow brown, hyaline, clamp connections present (Figure 1 A–D).

GC-MS analysis (Table 1 & Figure 2)

In GC-MS analysis three components are detected viz. 4,4'-Bipyridine, 9,12-Octadecadienoic acid, methyl ester and 9,12-Octadecadienoic acid (Z,Z). Application of 4,4'- 4,4'-bipyridine is a prototypical bridging ligand and an ideal connector between the transition metal atoms (Biradha et al. 2006). Heufler et. al. (1987) stated that 4,4'- bipyridine derivate orellanine causes acute renal failure in man. 9,12-Octadecadienoic acid (Z,Z)- methyl ester is used as fuel and fuel additive. It has potential cancer preventive, anti-inflammatory and anti-arthritis activities (Hagr et al. 2018).

CONCLUSION

The above described species *G. ochraceus* is reported for the first time from India. It clearly indicates that *G. ochraceus* is extremely rare species. Morphological and microscopic study along with ITS rDNA analysis confirms the species authentication. Many species of *Gymnopilus* are bitter or foul to taste, some are hallucinogenic and edibility of majority is unknown. There are many species and little consensus on identifying them. Microscopically they can be confused with *Pholiota* species and with the

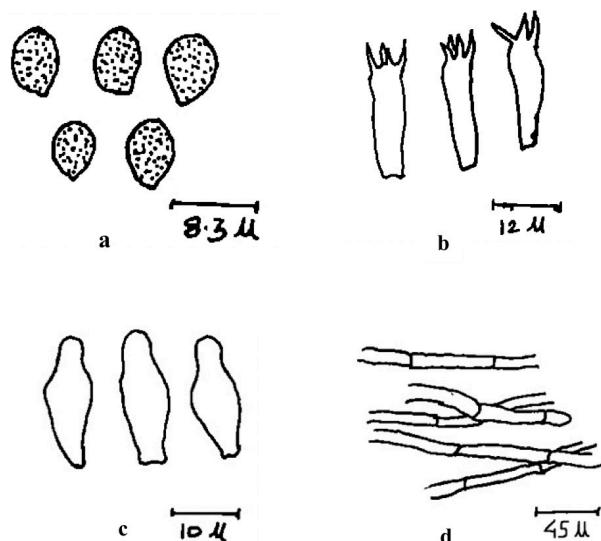


Figure 1. A—Basidiospores | B—Basidia | C—Cheilocystidia | D—Pileipellis hyphae.

Table 1. GC-MS analysis of *Gymnopilus ochraceus*.

Peak#	R.Time	I.Time	F.Time	Molecular formula	Area%	Name
1	16.395	16.070	16.875	C10H8N2	95.47	4,4'-Bipyridine
2	24.655	24.580	24.715	C19H34O2	3.41	9,12-Octadecadienoic acid, methyl ester
3	25.590	25.530	25.605	C19H34O2	1.12	9,12-Octadecadienoic acid (Z,Z)-
					100	



Image 1. *Gymnopilus ochraceus* Høil: A–C—Habit | D—Basidiospores | E—Basidia | F—Cheilocystidia | G—Pileipellis hyphae.
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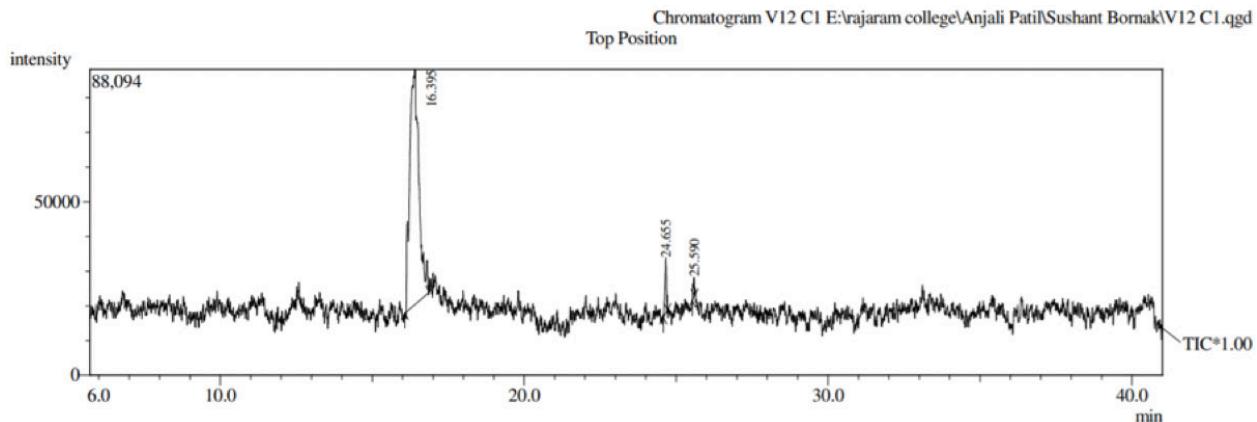


Figure 2. GC-MS chromatogram of *Gymnopilus ochraceus*

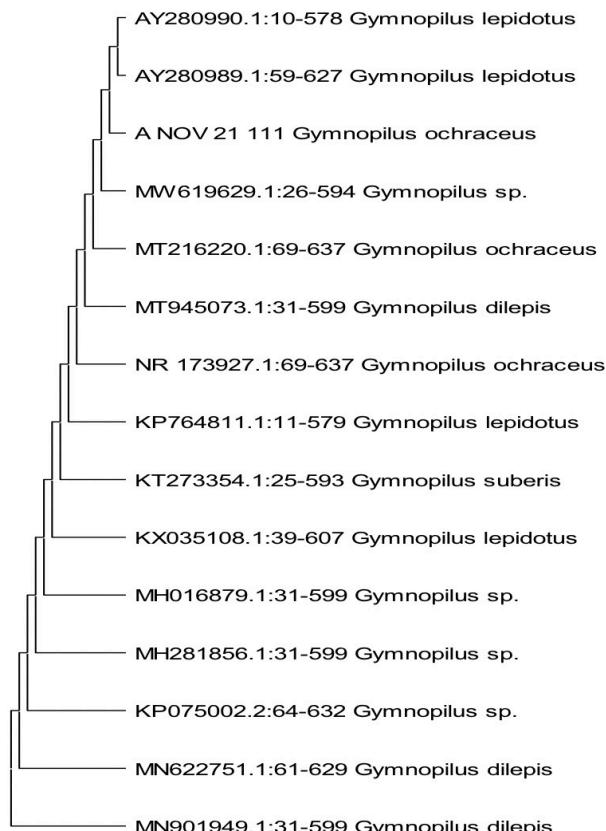


Figure 3. Phylogram of *G. ochraceus* represented by A NOV 21 111

deadly *Galerina marginata* complex. Microscopically, spores of *Gymnopilus* species are finely roughened (warty) and lack an apical pore.

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