

Distinctive Features of *Hypocrea microrufa*, Wood Decay Fungi, from Malaysia as Revealed Scanning Electron Microscopy

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전자현미경적 관찰에 의한 말레이시아산 목재부후균 *Hypocrea microrufa*의 특징

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ABSTRACT: The genus *Hypocrea*, a member of the Hypocreales, has yellow to pale yellow-ochre, perithecial stromata and angular to cuboid ascospores. The isolate of *Hypocrea microrufa* collected on decayed tropical wood at Malaysia is compared with *H. rufa* and *H. microsplendens*. This isolation of *H. microrufa* will be the first record from tropical region and the first description with scanning electron microscopy. The ornamentation of ascospores used one of main taxonomic keys for identification.

KEYWORDS: *Hypocrea*, *H. microrufa*, *H. rufa*, *H. microsplendens*, Taxonomical key, Identification

Since the generic name of *Hypocrea* was adopted for the first time by Fries in 1849, many taxonomists have tried to classify the genus *Hypocrea* (Saccardo, 1883, 1902, 1905; Theissen, 1911; Dingley, 1952; Doi, 1969). After Saccardo (1883) classified the genus under Hypocreaceae of the suborder Pyrenomycetes, Theissen (1911) represented six groups in the infrageneric classification of *Hypocrea* based on the species from Brazil. Later Doi (1969) submersed all taxonomical interests of Hypocreales in which morphological and ecology, even genetic and physiology, such as light response, tolerance of carbon disulphide, production of antibiotic. Furthermore, he described diagnostic characters in morphology of stromata and characteristics in culture. Even though, many researches have been focused on morphological identification, only light microscopy has been used. Septation of ascospores has been used as an important criterion for the taxonomy of the Hypocreales.

Hypocreales could be divided by fundamentally different developmental process into two group, *Hypocrea*-group and *Nectria*-group. However, the difficulties have been found to observe the process of furrowing and cell-plate formation of ascospores. Although roughness of the surface of the partspore seem to the most important characteristic for species delimitation, it is also difficult to distinguish partspore among the species belonging to the same

subsection in *Hypocrea* without higher magnification. Therefore, the development of electron microscopy provide possibilities that ascospore ornamentation will be an important taxonomical key for critical identification using scanning electron microscopy.

Doi (1972) described *H. microrufa*, isolated on decayed wood as new species in Japan. Since that nobody reported about this species. This report, therefore, would be that may endorse. Therefore, this report will be the first description of *H. microrufa* by a scanning electron microscope, which could reveal micro structural ornamentation of ascospores and asci.

Materials and Methods

Fungal isolates

Collections were carried out at University of Malay in Malaysia, on October 1998, from decayed broken branches and stems of an described tropical hardwood on the ground of the forest. Collected materials were placed in sterile plastic bags for transport to the laboratory. Samples were gently blow to remove any adhering surface contaminants such as soil particles.

Preparation for bright field microscopy

Fresh samples were used for observation of ascospores and asci. Reagents used with bright field microscopy were Melzer

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KOH (10%) solution. The color of the stroma was checked under a stereo microscope by using an acetone extract.

Preparation for scanning electron microscopy

For the analyses with the scanning electron microscopy (JEOL JSM-T330A), materials were stuck on tape affixed to an aluminum stub, vacuum coated with gold and then examined.

Taxonomy

Hypocrea microrufa Y. Doi

Stromata dispersed solitarily, often aggregated in 2-4, generally pulvinate to disciform or lenticular, with narrow bases and round margins, thin and round minutely pulvinate, yellow to pale yellow-ochre in young stages, becoming reddish yellow-ochre to blackish brown according to age or dryness, more or less irregular margins with ages, 1.5~5.0 mm diameter and 0.6~1.2 mm height, becoming uniformly dotted with umblicate perithecia. Textures soft and leathery in fresh material, becoming hard when dry. Perithecia obovate to ellipsoid with cylindrical necks of an ostiole. Ostiole open, mostly not projecting above the stromata. Asci narrow, cylindrical, 86~105 × 3.0~4.0 μm with short, 16-spored, apparatus not staining blue in Melzer's Iodine reagent. Ascospores mainly unicellular but one to three spores bicellular very few, with hyaline, warted verrucose, upper partspores obovate - subglobose, 3.2~4.2 × 3.0~4.8 μm, under partspores obovate - subcylindrical, 4.5~6.0 × 3.0~3.6 μm, semitransparent white to light brown.

Discussion

Although stromal form is very distinctive, ascospore ornamentation is the one of major species character, which can distinguish *Hypocrea* within the family. The surface of partspores is usually roughened, having discoidal cubical ornaments with margins, completely erupted above surface of partspore (Seaver, 1910; Doi, 1969, 1972).

In the genus *Hypocrea*, 8-ascospores are delimited by cell membrane, following which the binucleate ascospores become constricted, to from 16 partspores, after which chitinous cell wall of the partspores is formed (Doi, 1972) (Fig. 1). However, there is no enough information in ornamentation of ascospores, even though this study has mainly been focused on *H. microrufa*. Since Doi (1969) reported *H. microrufa*, isolated on unidentified decayed wood from Japan, as a new species, this isolation will be the second report. It means that his isolation and morphological identification could be fully supported by present report. Therefore, this report will be the first des-

cription of *H. microrufa* by a scanning electron microscope, which could reveal micro structural ornamentation of ascospores and asci. However, there is no comparison to other species of *Hypocrea* in ascospore ornamentation, because no observation was reported with a scanning electron microscopy observation. The result suggests that ornamentation of ascospores in the genus *Hypocrea* can be a main taxonomical key for identification. With present isolation *H. microrufa* can be extended geological distribution from Frigid Zone (Doi, 1972) to Tropical Zone (present collection). It means that *H. microrufa* can carefully be the cosmopolitan species and possibility of collection from Temperate Zone.

The classification of present study based on the system proposed by Doi (1972). However, no one has treated the ultra-structure classification of the genus *Hypocrea*. This species is very similar to *H. microsplendens* Doi and *H. rufa* (Pers.) Fri. in the appearance of stroma and the size, shape of ascospores and aci (Table 1). This species is also very close to a collection from Malaysia in 1996 by Jones and Lee (unpublished). The differences between *H. microrufa* and *H. microsplendens* in size of upper an under partspores and *H. rufa* in size of perithecia and under partspores is precisely described in Table 1. The color of stroma, having bright yellow-ochre in young stage to brackish brown with age, is the main characteristics. Even though its color is darker than the description by Doi (1972), this isolation could be identified as *H. microrufa*.

Septation of ascospores has been used an important criterion for the taxonomy of the Hypocreales. Hypocreales could be divided by fundamentally different developmental process into two group, *Hypocrea*-group and *Nectria*-group. However, the difficulties have been found to observe the process of furrowing and cell-plate formation of ascospores (Doi, 1969, 1972). Although roughness of the surface of the partspore seems to be the most important characteristics for specific identification, it is also difficult to distinguish partspore among the species belonging to the same subsection in *Hypocrea* without higher magnification. Therefore the development of electron microscopy give possibilities ascospore ornamentation to be important taxonomical key for critical identification using scanning electron microscopy. By the facts mentioned above and present observation (Table 1, Fig. 1), the author considers the ornamentation of ascospore one of the most important taxonomical keys. Therefore with more observation of ascospore ornamentation with higher magnification, the ornamentation of ascospores, which is generally consistent in each species, will be useful for identificaton of species in Hypocreales.

However, this study has focused on the description of a teleomorph stage, the consideration of anamorph stage

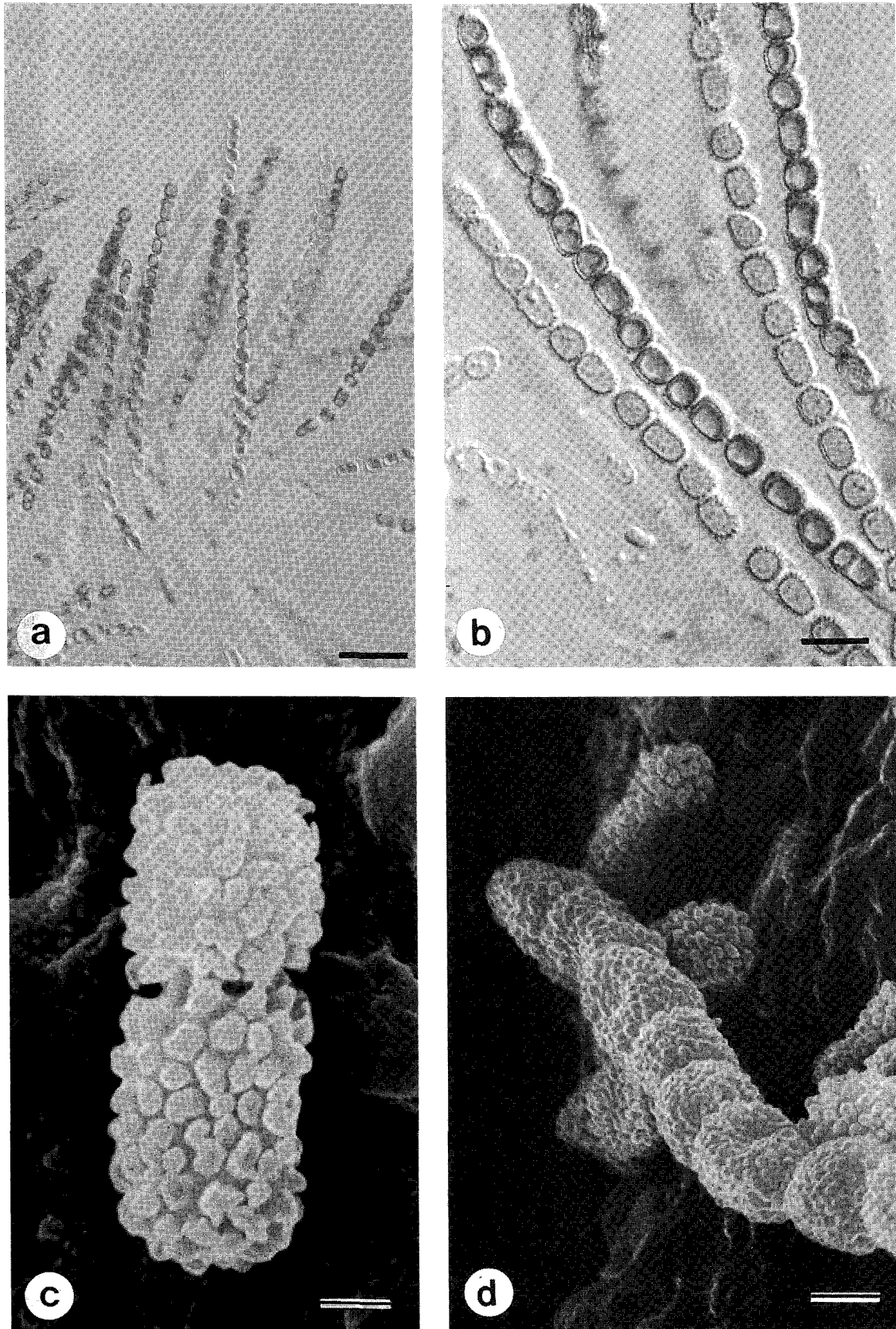


Fig. 1. a-b, Light micrographs of *Hypocrea microrufa*. a. Asci containing 16-spored ascospore (bar=20 μm). b. Higher magnification of asci containing obovate to subglobose upper partspores and obovate to subcylindrical under partspores and showing negative stained apical apparatus (arrowed) (bar=10 μm). c-d, Scanning electron micrographs of *Hypocrea microrufa* show warty verrucose ornamented ascospores with different shapes of partspores (c-bar=1 μm , d-bar=2 μm).

Table 1. Comparison of *Hypocrea microrufa* with allied taxa in morphological character

	<i>H. rufa</i> (Dingley, 1952)	<i>H. microsplendens</i> (Doi, 1969)	<i>H. microrufa</i> (Doi, 1969)	<i>H. microrufa</i> (present author)
Color of stroma	umber or chestnut brown	yellow orange to orange	bright orange	dark orange
Size of perithecia	220~250×500 μm	180~220 μm	180~210 μm	180~220 μm
Shape of perithecia	oval	obovate to ellipsoid	obovate to ellipsoid	obovate to ellipsoid
Size of upper partspores	3~3.5×3~3.5	1.8~2.4	3.8~4.5×3.1~4.0	3.2~4.2×3.0~4.8
Shape of upper partspores	globose to cuboid	obovate to subglobose	obovate to subglobose	obovate to subglobose
Size of under partspores	3~4×4~6 μm	1.9~2.9×1.7~2.0 μm	4.1~5.9×2.9~3.2 μm	4.5~6.0×3.0~3.6 μm
Shape of under partspores	ovate to pyriform	obovate to subcylindrical	obovate to subcylindrical	obovate to subcylindrical
Habitat	on rotten wood	Bamboo	on decayed wood	on decayed tropical hardwood

should be needed for conformation of identification of the genus of *Hypocrea* (Samuels and Rossman, 1992, Samuels *et al.*, 1994). The conclusion of the present study clearly requires further intensive investigation on the connection of teleomorph and anamorph stage.

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적 요

점버섯목의 점버섯속은 자낭각을 지닌 노란색이나 탁한 황토색의 자좌를 형성하며, 각진 입방형의 자낭포자를 지닌다. 열대지역인 말레이시아에서 발견된 *Hypocrea microrufa*를 형태적으로 유사한 *H. rufa*, *H. microsplendens*와 비교하여 동정하였다. 본 *H. microrufa*는 열대지방에서는 최초로 발견·기록되며, 또한 전자현미경을 이용한 최초의 보고이다. 전자현미경을 이용한 자낭포자의 표면무늬도 검색표의 주된 분류·동정기준으로 사용하였다.

References

- Dingley, J.M. 1952. The Hypocreales of New Zealand III. The genus *Hypocrea*. *Trans. Royal Soc. New Zealand* **79**: 323-337.
- Doi, Y. 1969. Revision of the Hypocreales with cultural observation. IV. The genus *Hypocrea* and its allies in Japan. (1) General part. *Bull. nat. Sci. Mus. Tokyo* **12**: 693-724.
- Doi, Y. 1972. Revision of the Hypocreales with cultural observation. IV. The genus *Hypocrea* and its allies in Japan. (2) Enumeration of the species. *Bull. nat. Sci. Mus. Tokyo* **15**: 649-751.
- Saccardo, R.A. *Sylloge fungorum hucusque cognitorum*. **2**: 520-536 (1883); **16**: 588 (1902); **17**: 799 (1905).
- Samuels, G.J. and Rossman, A.Y. 1992. *Thuemenella* and *Sarawakus*. *Mycologia* **84**: 26-40.
- Samuels, G.J., Petrini, O. and Manguin, S. 1994. Morphological and macromolecular characterization of *Hypocrea schweinitzii* and its *Trichoderma* anamorph. *Mycologia* **86**: 421-435.
- Seaver, F.J. 1910. The Hypocreales of North America III. *Mycologia* **2**: 58.
- Theissen, F.J. 1911. *Fragmenta Brasilica* III. *Ann. Mycol.* **9**: 55-61.