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Fungal Flora in Chiba Pref., Central Japan (IV) *Tuber indicum*, the First Record of Hypogenous Ascomycete Collected in Chiba

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Abstract *Tuber indicum* (Ascomycota, Pezizales), type specimen described in Himalaya, is reported from Chiba pref. central Japan with note of microscopic features of ascospores and asci. This is the first report of hypogenous macro-ascomycete from Chiba pref.

Key words: fungal flora, hypogenous ascomycete, *Tuber indicum*, southeast asiatic element.

The Natural History Museum & Institute, Chiba (CBM) has been conducting inventory work on macro fungi in Chiba pref. A total of 615 taxa of basidiomycota (Fukiharu *et al.*, 2002; Kasuya, 2004) and 63 taxa of ascomycota (Nagao and Fukiharu, 2002) have been recognized, but hypogenous macro-ascomycetes has not been reported. Genus *Tuber* F. H. Wigg is a hypogenous macro-ascomycota and famous as food in Europe and U.S. More than 60 species belonging to this genus have been reported in the world (Kirk *et al.*, 2001), but most of them occur in continental Europe. In contrast, the information about *Tuber* in Japan is lacking and only 4 species have been recognized; *T. californicum* Harkn. from Tottori pref. (Trappe, 1976), *T. hiromichi* (S. Imai) Trappe from Hokkaido pref. (Imai, 1940; Trappe, 1976, 1979; Otani, 1989a, b), *T. indicum* Cooke & Massee from Kyoto pref. (Yoshimi and Takayama, 1986; Yoshimi, 1988; Otani, 1989a, b) and *T. magnatum* Pico from Aichi pref. (Yoshimi and Takayama, 1986; Otani, 1989a, b). This time, *T. indicum* was collected in *Quercus* spp. coppice wood in Chiba pref., central Japan. This species is known only on lists (Otani, 1989a, b) or photographs (Yoshimi and Takayama, 1986; Yoshimi, 1988) and the microscopical characters of the Japanese specimens have not been fully described. In this report, the microscopic characters of the ascospore and ascus are described based on the specimens collected in Chiba.

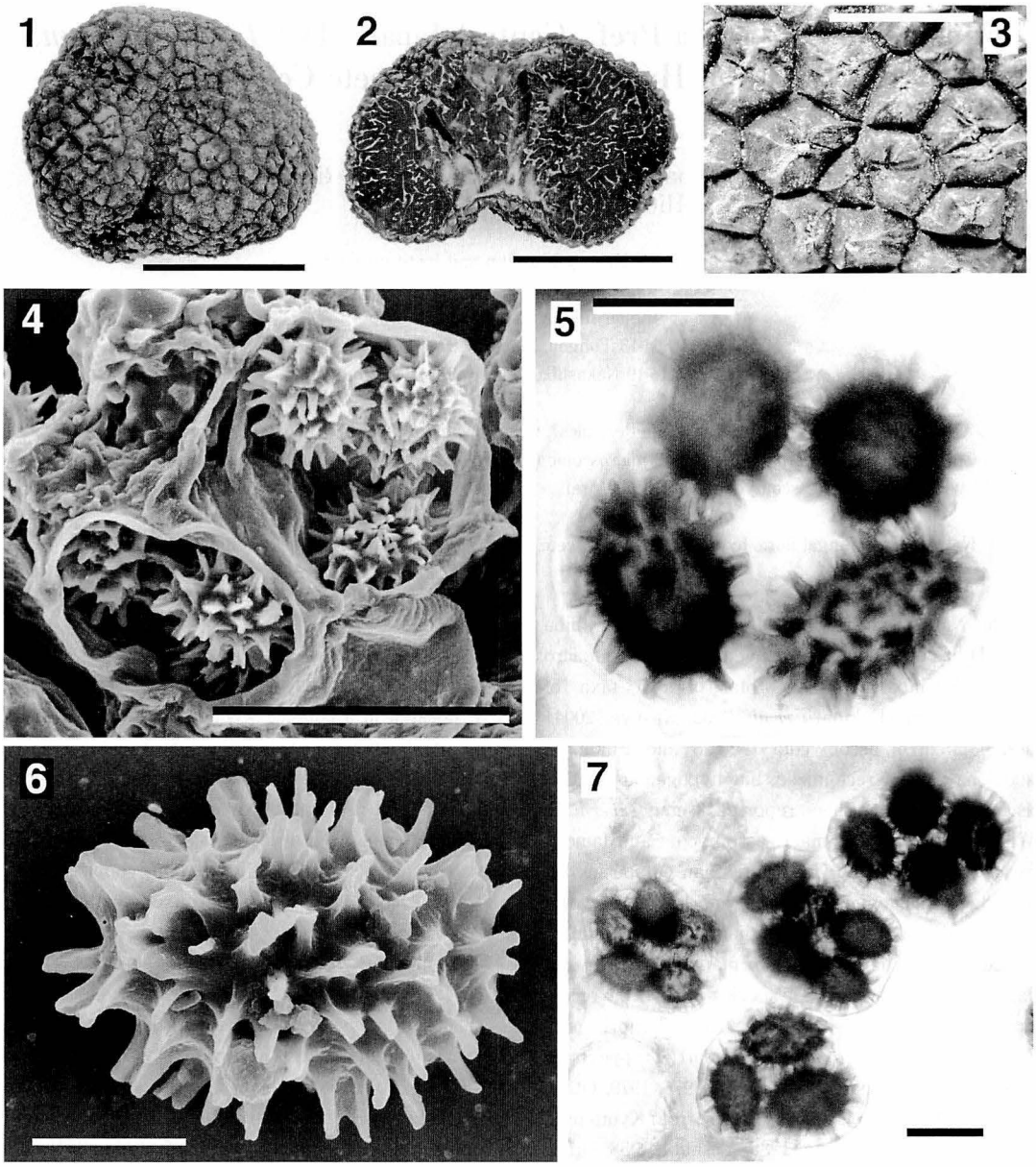
Materials and Methods

Field observations were conducted at two sites; Site 1: Located in a coppice wood dominated by *Quercus serrata*, boundaries of the school yard of Mishima elementary school, Masaki 149, Kimitsu-shi, Chiba pref., 35° 13' N, 140° 1' E, ca. 90 m above sea level. From Feb. 2004 to Aug. 2005, observations were conducted almost every week. Site 2: Located in a coppice wood of *Quercus myrsinaefolia* Blume. mixed with *Carpinus tschonoskii* Maxim. and *Cinnamomum camphora* (L.) Presl in a park of Ohtaki-machi, Isumi-gun, Chiba pref., 35° 17' N, 140° 14' E, ca. 80 m above sea level. Anatomical observations and measurements were made on materials mounted in 5% aqueous potassium hydroxide (KOH). For observation of ascospore ornamentation by scanning electron microscope (SEM), a small portion of a dried specimen was rehydrated in 25% aqueous ammonia, fixed in osmium acid (vapor), coated with platinum-palladium in an ion sputter-coater (Hitachi E-1030; Hitachi, Tokyo, Japan), and observed under a SEM (Hitachi S-800) operating at 15.0 kV. The specimens examined are deposited in the Natural History Museum and Institute, Chiba (CBM).

Taxonomy

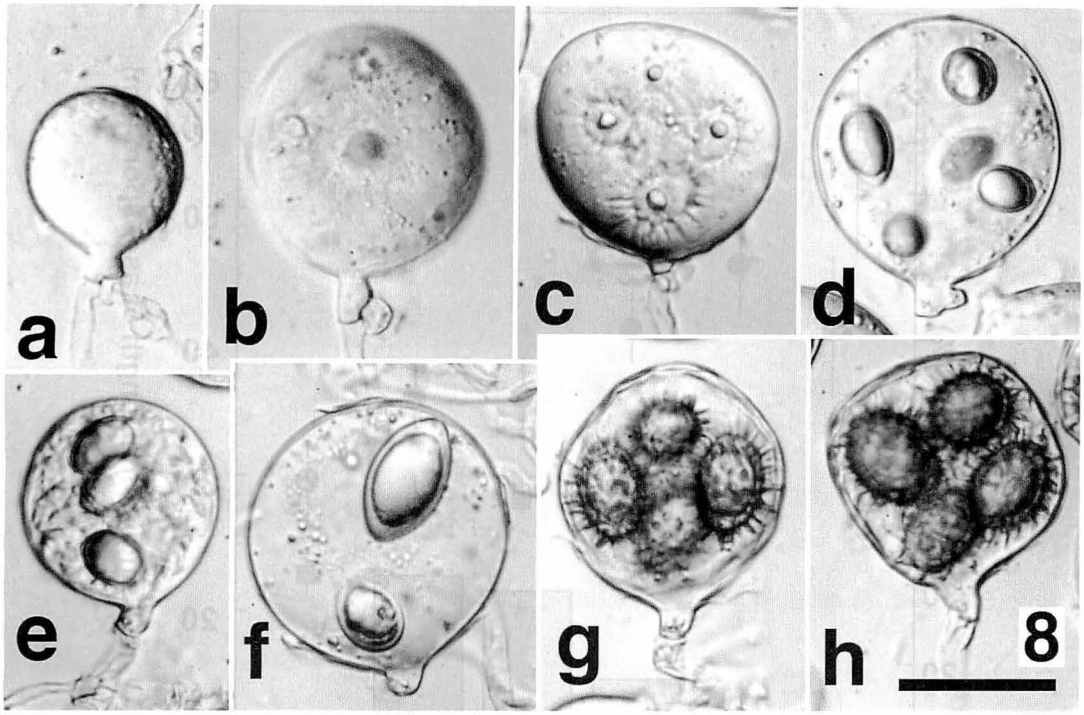
***Tuber indicum* Cooke & Massee**, Grevillea 20: 67 (1892) (Figs. 1-8)

External appearance of ascomata globose to ellipsoid, almost black, 20-45 mm diameter, occasionally



Figs. 1-7. *Tuber indicum* (CBM-FA-33922).
Fig. 1. Ascoma (bar = 2 cm). Fig. 2. Radial section of ascoma, showing the veins of gleba (bar = 2 cm). Fig. 3. Pyramidal warts on the ascomatal surface (bar = 5 mm). Fig. 4. SEM micrograph of ascus and ascospores. (bar = 50 μm). Fig. 5. Ascospores in a ascus, showing ornamentation of the spores (bar = 10 μm). Fig. 6. SEM micrograph of ascospores, showing ornamentation of the spores (bar = 10 μm). Fig. 7. Asci and ascospores, showing variable size and a number of spores in each ascus (bar = 10 μm).

lobed, with a coarsely warted surface (Figs. 1-2). Warts pyramidal, irregularly polygonal, some of them pentagonal or hexagonal, with splits along the side (Fig. 3). Peridium 500 - 800 μm thick (including warts), composed of two layers; outer layer composed of globose, round or elongated cells, inner layer composed of closely intricated hyphae. Outer layer, ca. 500 μm thick, outermost layer 50 - 100 μm, composed of globose or polygonal cell, 10 - 20 μm with dark brown cell wall about 3 - 4 μm thick, just below these dark outermost cells lie reddish brown, more elongated cells, about 10 x 20 - 30 μm in size, with 1.5-2

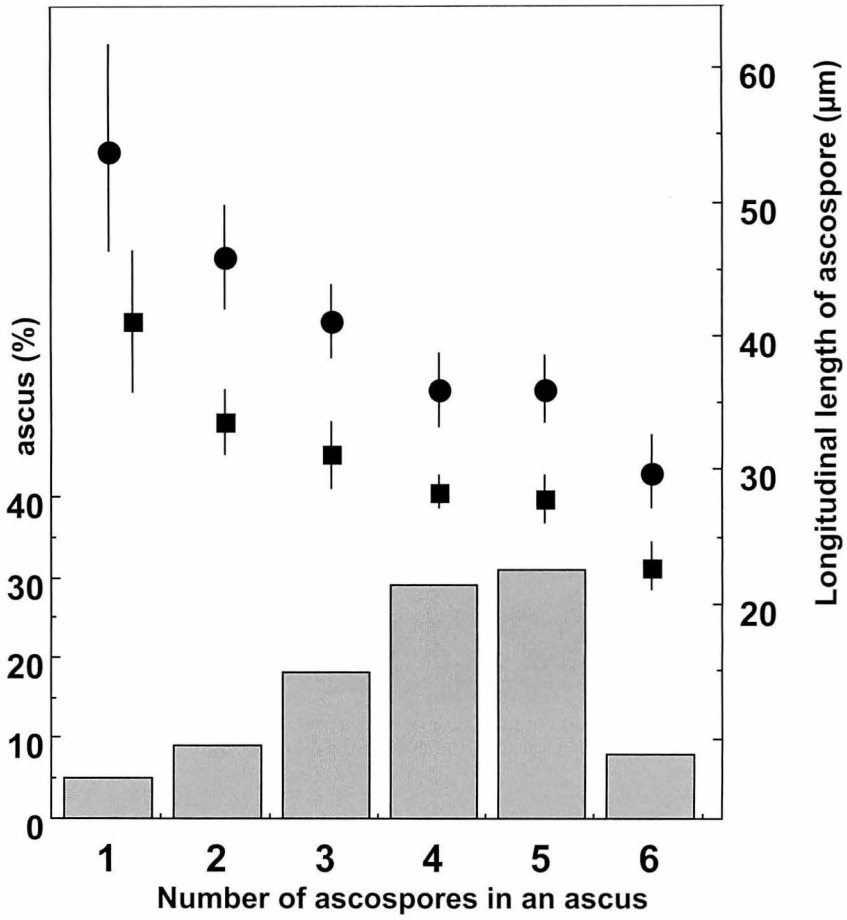


Figs. 8. a-h. *Tuber indicum*, asci and ascospores in each stage of development.
Each ascus has a short stalk connected to hyphae. CBM-FA 35773 (Scale bar = 20 μm).

μm cell wall. Inner layer, ca. 200 μm thick, divided into more than two layers; external area ca. 100 - 200 μm, composed of brown, thick-walled elongated hyphae, 4 - 5 diam., 1 - 1.5 μm thick cell wall, becoming paler and thinner-walled toward inside area. Gleba purplish black, marbled with numerous, very thin, whitish veins (Fig. 2). Asci 1 - 6-spored, with stalk connected to long hyphae; different stages in maturity present in one ascomata, young asci globose to ellipsoid (Fig. 8), nearly colorless; matured asci subglobose to irregular, depending on the number and arrangement of ascospores contained (Figs. 5, 7), 55 - 75 x 50 - 70 μm (65.8 ± 6.9 x 58.3 ± 4.9 μm, mean ± SD, n = 30) (CBM-FA-35589). Ascospores ellipsoid, spiny, pigmented, size very variable depending on the number of ascospores contained (Fig. 9). Ascospore size value showed including spines and [excluding spines]: ascospore in a single-spored ascus 53.8 ± 8.0 x 39.0 ± 6.1 μm [40.9 ± 5.8 x 23.9 ± 3.4 μm], spine 8.3 ± 1.5 μm long (n = 5); ascospore in a 2-spored ascus 45.9 ± 4.2 x 34.4 ± 2.4 [33.5 ± 2.5 x 22.7 ± 3.3], spine 7.1 ± 0.9 (n = 10); ascospore in a 3-spored ascus 41.2 ± 3.1 x 29.5 ± 2.5 [31.3 ± 2.5 x 19.9 ± 2.2], spine 6.0 ± 0.5 (n = 20); ascospore in a 4-spored ascus 36.0 ± 3.0 x 27.3 ± 2.5 [28.3 ± 1.3 x 18.3 ± 1.0], spine 5.6 ± 0.9 (n = 20); ascospore in a 5-spored ascus 36.0 ± 2.5 x 24.8 ± 2.0 [27.8 ± 1.9 x 16.2 ± 1.1], spine 4.8 ± 0.5 (n = 20); ascospore in a 6-spored ascus 29.8 ± 3.0 x 21.4 ± 1.9 [22.7 ± 1.9 x 14.7 ± 1.2], spine 3.7 ± 0.8 (n = 10). Pale brown when young, brown to dark brown when matureed. Spine blunt at the apex, not forming reticulation.

Habitat and Specimens Examined

Site 1: Ascomata were observed 6 times from Feb. 2004 to Aug. 2005 in the same place at this site: in 2004 Feb. 13 (CBM-FA-33922), Feb. 27 (CBM-FA-33927), Aug.13 (CBM-FA-35509); in 2005 May 6 (CBM-FA-35589, ascospore and ascus size were measured), Aug. 12 (CBM-FA-35764), Aug. 19 (CBM-FA-35773). Site 2: Ascomata were first observed in 1995, and almost every year ascomata appeared at the same site, on 29 Oct. 2005 (CBM-FA-36157), 29 Jan. 2006 (CBM-FA-36269). The ascospores of the specimens collected in January, February, May and October were almost mature, but the ascospores in August specimens collected were not mature. Genus *Tuber* has ectomycorrhizal association with vascular plants (Frank, 1885) and 19 host species have been reported, such as *Abies*, *Pinus*, *Fagus* and *Quercus* (Pacioni and Comandini, 1999).



Figs. 9. Ascospore size and ascospore numbers ratio of *Tuber indicum*.
Ascospore size showed in longitudinal length (mean value ± SD, n = 100) with a spine (●) and without any spine (■), CBM-FA-35589.

Type specimen of *T. indicum* observed under the tree of *Quercus incana* (Zhang and Minter, 1988). At site 1 and site 2, this fungus occurred in soil near *Quercus* spp., so *T. indicum* in Chiba also may have some relationship with *Quercus* spp.

Notes:

Taxonomically, genus *Tuber* is divided into two groups by its exoperidium characters; white-truffles with almost smooth exoperdium and black-truffles with pyramidal warts exoperidium. The black-truffles are divided into two groups by its ascospores ornamentation; spiny ornamentation and reticulate ornamentation (Montecchi and Sarasini, 2000). *Tuber indicum* belongs to black-truffles with spiny ascospores. In Europe, *T. blumale* Vittad. and *T. melanosporium* Vittad. are known as this type. *Tuber indicum* differs from the former in having darker colored ascospores and differs from the latter in having more robust and wide base spine of ascospore ornamentation (Montecchi and Sarasini, 2000). The specimens collected in the present study also have pyramidal warts in exoperidium and not reticulate-forming spiny ornamentation on ascospore. Recently species with reticulate-spiny ascospore species have been reported from China, such as *T. himalayense* B. C. Zhang & Minter (Zhang and Minter, 1988), *T. pseudoexcavatum* Wang, G. Moreno, L. J. Riousset, J. L. Manjn & G. Riousset (Riousset *et al.*, 2001) and *T. pseudohimalayense* G. Moreno, Manjn, Dez & Garca-Montero (Moreno *et al.*, 1997). Also in Japan, in ascospore morphologically different *Tuber* species were collected (Honda *et al.*, 1983; Yoshimi, 1994, 1997, 1998). Because hitherto reported *Tuber* species in Asia suggested to be as diverse as in

Europe, with more extensive taxonomic and mycofloristic studies are needed in this area. Type specimen of *T. indicum* was collected at north western Himalaya, Mussoorie, Uttar Pradesh, Garhwal Hills, about 2000 m alt. in *Quercus* vegetation. (Cooke and Massee, 1892; Zhang and Minter, 1988). From Himalaya, through South China, to the western part of Japan, the ectomycorrhizal basidiomycetes (Agaricales) called "Southeast Asiatic" element (Hongo, 1978; Hongo and Yokoyama, 1978) is known, such as *Amanita rubrovolvata* Imai, *Amanita pseudoporphyria* Hongo (Adhikari, 2000) and *Amanita sinensis* (Oda *et al.*, 2000). Such echtomycorrhizal Agaricales is closely related to *Quercus, Castanopsis* forest as symbiont and also distributed in close relationship with this vegetation (Oda *et al.*, 2000; Fukiharu and Oda, 2001). *T. indicum*, ectomycorrhizal ascomycetes, also may be the same element in this area. More extensive studies on *Tuber* spp. and also on ectomycorrhizal fungi in Southeast Asia are needed.

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千葉県菌類資料（IV） 千葉県産初の地下生子嚢菌イボセイヨウショウロ *Tuber indicum*

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