

SHORT COMMUNICATION

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Notes on Japanese Lycoperdaceae. 4. Validation of Japanese giant puffball, *Calvatia nipponica*

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Abstract *Calvatia nipponica*, an invalid name of the Japanese giant puffball, is formally validated as a new species by providing a necessary Latin description and designating the holotype specimen. Morphological features of *C. nipponica* including peridium, capillitia, basidia, and basidiospores are described and illustrated.

Key words *Langermannia* · *Lanopila* · *Lasiosphaera* · *nom. illegit.* · *nom. inval.*

The Japanese giant puffball was noticed by several naturalists in the Yedo Era of Japan because of its conspicuously large fruit body, and called “*Onifusube*” [“*Oni*” is a kind of devil, and “*fusube*” is a bump in Japanese] (Ito 1958). This Japanese name had been applied to some foreign species of Lycoperdaceae by Japanese mycologists before 1908: e.g., *Lycoperdon bovista* Pers. [= *Calvatia utrififormis* (Bull.) Jaap] (Shirai 1898), *Bovista maxima* Schaeff. [= *C. maxima* (Schaeff.) Morgan] (Shirai 1905), and *Lycoperdon giganteum* Batsch: Pers. [= *Langemannia gigantea* (Batsch) Rostk.] (Shirai 1905). Then this fungus was considered to be the same species as *Lasiosphaera* (*Las.*) *fenzlii* Reichardt [= *C. bicolor* (Lév.) Kreisel] by Lloyd (1908), being followed by some Japanese mycologists such as Yasuda (1908), Shirai and Miyake (1917), Kawamura (1914, 1929), and Kobayasi (1933a,b). Later, it was concluded by Kawamura (1937) that the Japanese giant puffball was a distinct species from *Las. fenzlii* after studying the holotype specimen of the latter, and a new species, *C. nipponica* Kawam., was established by him. Unfortunately, the origi-

nal description of *C. nipponica* (Kawamura 1937) was published in Japanese with English summary, and the Latin description was missing. Two combinations, *Las. nipponica* (Kawam.) Kobayasi (Kobayasi 1939) and *Lanopila* (*Lan.*) *nipponica* (Kawam.) Kobayasi (Kobayasi 1976) based on *C. nipponica*, were also published without any Latin descriptions or diagnoses. Therefore, *C. nipponica* has not been validly published since 1937, and the name remains invalid [Art. 36.1 of the International Code of Botanical Nomenclature (ICBN); McNeill et al. 2006]. Furthermore, its combinations, *Las. nipponica* and *Lan. nipponica*, are both *nomen illegitimum*. We agree with Kawamura (1937), who recognized the fungus as a good species in *Calvatia*; hence, the name is validated here, providing a Latin description and designating the type. A more comprehensive description is also given based on our observations of Japanese specimens.

The specimens examined in this study are deposited in the herbaria of the National Museum of Nature and Science (TNS) and the Kanagawa Prefectural Museum of Natural History (KPM). Macroscopic characters were described by observations of dried materials. For light microscopic observations, free-hand sections of gleba and peridium were mounted in water, 5% KOH (w/v), and 1% cotton-blue lactophenol on glass slides. Forty or 50 randomly selected basidiospores were measured for each specimen under a light microscope. For scanning electron microscope (SEM) observations of rhizomorphs, basidiospores, and capillitia, small portions of rhizomorphs and mature gleba were coated with platinum-palladium in an ion sputterer (Hitachi E-1030; Hitachi, Tokyo, Japan), and observed under a SEM (Hitachi S-4200) operating at 20 kV.

Calvatia nipponica Kawam. ex Kasuya & Katum., sp. nov.
Figs. 1–8

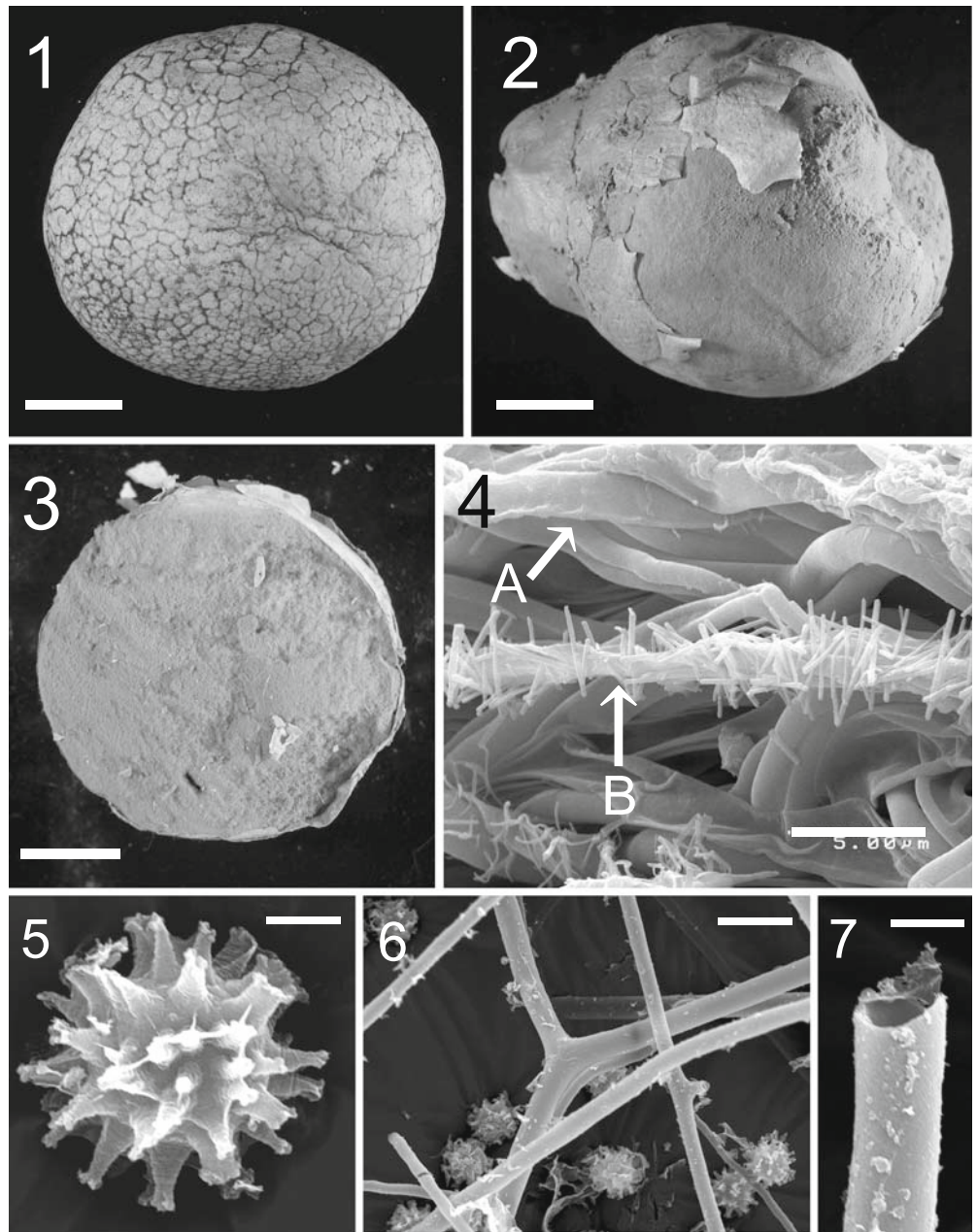
Pro. syn.: *Calvatia nipponica* Kawam., J. Jpn. Bot. 13:748, 1937 (*nom. inval.*); *Lasiosphaera nipponica* (Kawam.) Kobayasi, Nippon Inkwasyokubutu Dukan p. 581, 1939 (*nom. illegit.*); *Lanopila nipponica* (Kawam.) Kobayasi, Trans. Mycol. Soc. Jpn. 17:372, 1976 (*nom. illegit.*).

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Figs. 1–7. *Calvatia nipponica*.

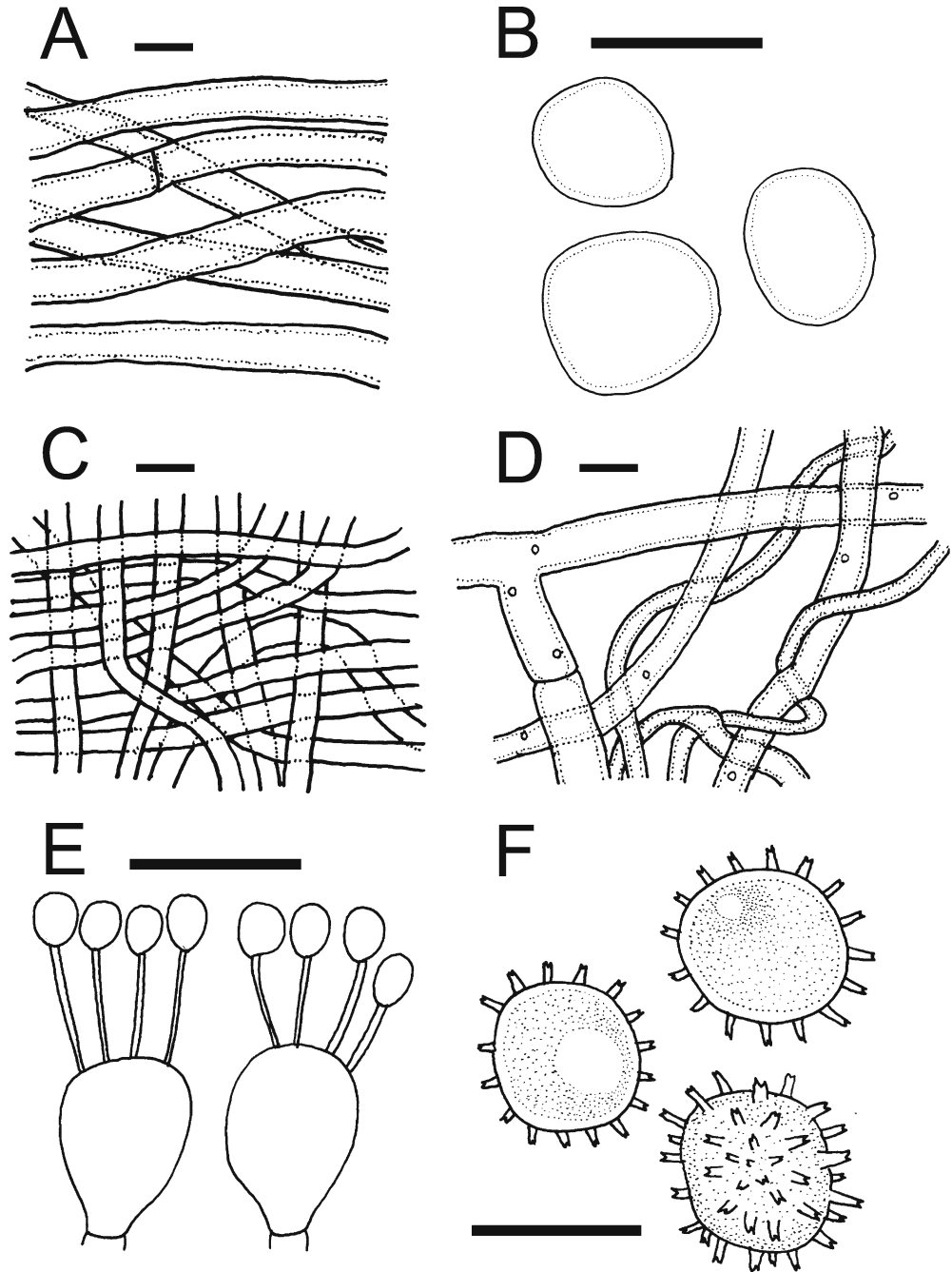
1 Mature basidioma (TNS-F-11860, holotype). **2** Old basidioma (TNS-F-11910). **3** Sectioned mature basidioma (TNS-F-243767). **4** Margin of rhizomorphs by SEM (TNS-F-11860). *A*, Thick-walled emanating hyphae; *B*, thin-walled cortex hyphae with needle-shaped crystals. **5** Basidiospore by SEM (TNS-F-11913). **6** Capillitia by SEM (TNS-F-11913). **7** Detailed structure of capillitium by SEM (TNS-F-11913). **Bars** **1**, 5 cm; **2** 4.5 cm; **3** 2 cm; **4** 5 μ m; **5** 1.5 μ m; **6** 6 μ m; **7** 3 μ m



Basidiomata globosa vel subglobosa, bene crescens, insigniter gigantea, 10–60 cm lata, 10–50 cm alta, rhizomorphis albis fragiles usque ad 1 cm latis 10 cm longis praedita. Exoperidium albidum vel lacteum, usque 800 μ m crassum, fragile, denique friabile, 2-stratosum, ex hyphis filamentosis 4–8 μ m crassis elongatis sub-parallelis raro septatus pachydermis albidis vel lacteis compositum; textura externa usque 500 μ m crassa, pseudoparenchymatica, ex cellulis 7–15 μ m diametro flavo-brunneis vel brunneis composita; textura interna usque 300 μ m crassa. Endoperidium cinereo-brunneum vel porphyro-brunneum, usque 300 μ m crassum, tenue, papyraceum, laevigatum, lucidum, denique friabile, ex hyphis filamentosis 3.5–7 μ m crassis implicatis leptoderis hyalinis compositum. Gleba juvenilis alba, lenis, incarnata, dein ochracea vel brunnea, pulverulenta vel gossypina,

sine pseudocolumella. Subgleba absens. Capillitium 2–6 μ m crassum, elongatum, raro dichotomico ramosum, flavo-brunneum vel brunneum, elasticum, septatum, poris exiguis irregulariter sparsis paucis praeditum; pariete 0.5–2 μ m crasso, raro foveolato. Paracapillitium absens. Basidia pyriforma vel sacciforma, 10–17 \times 15–20 μ m, 4-sporus, raro 2-sporus, sterigma usque ad 15 μ m longia praedita, dein evanescens. Basidiosporae subglobosae vel globosae, episporio insigniter spinuloso, 3.5–5.5 μ m diametro (5–6.5 μ m diametro spinulo incluso), flavo-brunneae vel brunneae, sine pedicello; spinulae usque ad 1.5 μ m alta, apice lobulis brevibus efferentes.

Holotypus: TNS-F-11860, ad terram in sylva, Meiji-jingu-gaien, Shinjuku-ku, Tokyo in Japonia, Sept. 27, 1994, F. Kudo leg., in Herbario fungorum in TNS conservatus.

Fig. 8. *Calvatia nipponica*.**A** Filamentous hyphae of outer layer of exoperidium (TNS-F-11860).**B** Pseudoparenchymatous cells of inner layer of exoperidium (TNS-F-11860).**C** Filamentous hyphae of endoperidium (TNS-F-11860).**D** Capillitia (TNS-F-11860).**E** Basidia (TNS-F-13393).**F** Basidiospores (TNS-F-11860). Bars **A, C,****D** 6 μm ; **B** 10 μm ; **E** 15 μm ;**F** 5 μm 

Basidiomata globose to subglobose, well developed, remarkably large, 10–60 cm broad, 10–50 cm high. White rhizomorphs attached to the base of basidiomata, up to 1 cm broad, 10 cm long, fragile when dried, consists of 3 layers; margin of rhizomorphs divided into thin-walled cortex hyphae up to 4 μm broad with numerous needle-shaped crystals, and thick-walled emanating hyphae up to 3.5 μm broad; the center of rhizomorphs comprise thick-walled vessel hyphae with septa, up to 15 μm broad. Exoperidium white to cream, up to 800 μm thick, fragile at maturity, finally breaking to pieces, divided into 2 layers; outer one up to 500 μm thick, composed of filamentous hyphae, 4–8 μm broad, elongate, subparallel, rarely septate,

thick-walled, white to cream; inner one up to 300 μm thick, composed of pseudoparenchymatous cells, 7–15 μm broad, yellowish-brown to brown. Endoperidium grayish-brown to purplish-brown, up to 300 μm thick, papery, smooth, shiny, finally breaking to pieces, composed of filamentous hyphae, 3.5–7 μm broad, interwoven, thin-walled, hyaline. Gleba white, soft, fleshy when young, later ochraceous to brown, pulverulent to cottony, lacking pseudocolumella. Subgleba absent. Capillitia *Lycoperdon* type, 2–6 μm broad, elongate, sometimes twining around other capillitial hyphae, rarely dichotomously branched, yellowish-brown to brown, elastic, septate, pores small, irregular, not abundant; wall of capillitia 0.5–2 μm thick, rarely pitted. Paracapillitium absent.

Basidia pyriform to sacciform, 10–17 × 15–20 µm, with long sterigmata up to 15 µm long, four-spored, rarely two-spored, collapsing at maturity. Basidiospores ovoid to subglobose, surface smooth when immature, later subglobose to globose, conspicuously spinulose at maturity, 3.5–5.5 µm in diameter excluding spines or 5–6.5 µm in diameter including spines, yellowish-brown to brown, lacking pedicel; spines up to 1.5 µm long with several short lobes at the apex.

Habitat: On rich soil or humus in grasses or woods, especially in bamboo groves.

Distribution: Known only from Japan.

Type specimens: Japan. Metropolitan Tokyo, Shinjuku-ku, Meiji-jingu-gaien, September 27, 1994, F. Kudo, TNS-F-11860 (holotype); TNS-F-11871, TNS-F-11917 (isotypes).

Other specimens examined: Japan. Ibaraki Pref.: Naka-gun, Tokai-mura, Toyooka, May 28, 2005, T. Kasuya, TNS-F-11914; Mito-shi, San-nomaru, September 29, 2007, T. Kasuya and M. Kakishima, TNS-F-16983. Saitama Pref.: Kitaadachi-gun, Ina-machi, Kobariuchijuku, May 20, 1997, T. Isoda, TNS-F-11913; Toda-shi, October 1984, Y. Doi, TNS-F-11912. Metropolitan Tokyo: Kita-ku, Rikugien, September 30, 1991, S. Yoshimi, TNS-F-11910; Shinjuku-ku, Shinjuku-gyoen, May 15, 1969, K. Morita, TNS-F-182042; Minato-ku, Shirogane-dai, Shizen-kyoikuen, October 17, 1985, Y. Doi, TNS-F-199157, October 16, 1986, Y. Doi, TNS-F-169636, September 23, 1991, Y. Doi, TNS-F-11911; Oume-shi, Kurosawa, October 23, 1999, Y. Doi, TNS-F-101053. Kanagawa Pref.: Yokohama-shi, Hodogaya-ku, August 23, 2005, Y. Ohno, KPM-NC-0012971; Sagamihara-shi, Sagamihara Chuo-Ryokuchi, September 15, 2001, F. Sawada, KPM-NC-0009550; Isehara-shi, September 27, 2005, Y. Inoue, KPM-NC-0013044; Hadano-shi, Tochikubo, October 1, 1978, S. Kigawa, KPM-NC-0001708; Aikou-gun, Kiyokawa-mura, September 4, 2004, S. Kadokura, KPM-NC-0012564. Nagano Pref.: Inashi, July 15, 2005, A. Yamada, TNS-F-13393. Kyoto Pref.: Kyoto-shi, Sakyo-ku, Ginkakuji Temple, October 11, 1987, S. Yoshimi, TNS-F-243767. Kochi Pref.: Kami-gun, Yoshikawa-mura, Yoshihara, December 25, 2005, R. Yamamoto, TNS-F-11915.

Japanese name: Onifusube.

The name of Japanese giant puffball was applied by Lloyd (1908) to *Las. fenzlii* based on the material sent to him from Japan. Later, the authentic material of *Las. fenzlii* was examined by Kawamura (1937), comparing with materials of the Japanese giant puffball, and it was concluded that these two fungi were morphologically distinguishable from each other (Kawamura 1937). The former fungus had capillitia 1.5–2(–3) µm broad and basidiospores 4–4.5 µm in diameter, the latter fungus, in contrast, had capillitia 2.5–4(–5) µm broad and basidiospores 4–5(–5.5) µm in diameter (Kawamura 1937). Kawamura (1937, 1954) also examined specimens of *C. maxima* (= *L. gigantea*) and recognized the differences among morphological features of these three fungi. Because at that time *Lasiosphaera* Reichardt was a monotypic genus and generally treated as synonymous with *Calvatia* Fr., the Japanese fungus was considered by Kawamura (1937) to be a new species in

Calvatia, *C. nipponica*. Dissing and Lange (1962) noted the distribution of *Las. fenzlii* in Japan based on the Lloyd (1908) misapplied name for the Japanese fungus. Afterward, the absence of subgleba in *Lasiosphaera* was emphasized, and this genus was again distinguished from *Calvatia*, having distinct subgleba, and *C. nipponica* was transferred to *Lasiosphaera* (Kobayasi 1939; Kawamura 1954). However, now *Lasiosphaera* is put into synonymy of *Calvatia* (Kreisel 1992).

According to Kobayasi (1976), although there is very little morphological distinction between *Lanopila* Fr. and *Calvatia*, *C. nipponica* should be transferred to *Lanopila*. In the case of *Lanopila*, the subgleba is absent and the gleba remains a compact, coherent mass at maturity, whereas in *Calvatia*, a subgleba is present and the gleba disintegrates (Ahmad 1950; Homrich and Wright 1973; Kobayasi 1976). However, compact glebas have also been observed in several species of *Calvatia*, such as *C. candida* (Rostk.) Hollós (Coetsee and van Wyk 2007). Furthermore, the gleba in some specimens of *Lanopila* may also disintegrate to some degree (Coetsee and van Wyk 2007). Therefore, we approve Kreisel (1992, 1994) and Coetsee and van Wyk (2007), who put *Lanopila* into synonymy of *Calvatia*.

Langermannia Rostk., morphologically a related genus of *Calvatia*, has the same characters of basidiomata, basidiospores, and capillitia, especially of basidiospores and capillitia, as *Calvatia* (Kreisel 1992, 1994). Recent work on the molecular phylogeny of Lycoperdaceae supports the monophyly of *Calvatia* and the distinct lineage including *L. gigantea* [≡ *C. gigantea* (Batsch) Lloyd], *C. pachydermica* (Speg.) Kreisel, and *C. bicolor* (Bates 2004). These species also share several important morphological characteristics such as basidiospores and capillitia (Kreisel 1992, 1994). However, the gleba development of *L. gigantea* is distinctively different from that of *C. bicolor* (Swoboda 1937) and the other members of the genera *Bovista* Pers., *Calvatia*, *Lycoperdon* Pers., and *Vascellum* F. Šmarda (Gube 2007). Therefore, Gube (2007) suggests keeping the genus *Langermannia* separate from *Calvatia*. Although we have not been able to examine gleba development of the Japanese giant puffball, Kobayasi (1933a) described numerous cavities in developing gleba of this fungus, which are not the same type as *L. gigantea* but similar to the “lacunar type,” those of other members of Lycoperdaceae (Gube 2007). Gube (2007) pointed out that two-layered rhizomorphs without cortex hyphae are another generic characteristic of *Langermannia*. However, rhizomorphs of the Japanese giant puffball consist of three-layered hyphae with cortex. This rhizomorph structure is typical of Lycoperdaceae and is morphologically similar to those of *C. excipuliformis* (Scop.) Perdeck and *Lycoperdon pyriforme* Schaeff. (Agerer 2002). Therefore, the Japanese giant puffball should be excluded from *Langermannia*. For the foregoing reasons, the effective name of the Japanese giant puffball is designated under the accepted genus *Calvatia*. However, further studies are needed for determination of the phylogenetic placement of the Japanese giant puffball, because this fungus has not yet been analyzed by DNA sequences.

Calvatia nipponica is well defined by the following four characteristics: (1) remarkably large, globose to subglobose basidiomata; (2) absence of subgleba; (3) capillitia that sometimes wind around other capillitial hyphae; and (4) subglobose to globose, conspicuously spinulose at maturity, non-pedicellate basidiospores. There are three other known species of *Calvatia* having large, globose to subglobose basidiomata: *C. bicolor*, *C. booniana* A.H. Sm., and *C. polygonia* Lloyd ex A.H. Sm. *Calvatia bicolor*, a pantropical species, is also microscopically similar to *C. nipponica* in its verruculose to spinulose basidiospores (Kreisel 1994). However, *C. bicolor* differs from *C. nipponica* by its stiff, blackish-brown to blackish endoperidium and its capillitia, which do not twine around other capillitial hyphae. Further, *C. booniana* and *C. polygonia* are clearly distinguished from *C. nipponica* by their polygonal, scaly exoperidium and rudimentary, compact subgleba (Zeller and Smith 1964; Kreisel 1994; Calonge et al. 2004). *Langermannia gigantea* is also morphologically similar to *C. nipponica* in its large basidiomata and absence of subgleba. However, *L. gigantea* is well distinguishable from *C. nipponica* by its finely warty to punctate basidiospores and its capillitia that do not twine around other capillitial hyphae.

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References

- Agerer R (2002) Rhizomorph structures confirm the relationship between Lycoperdales and Agaricaceae (Hymenomycetes, Basidiomycota). *Nova Hedwigia* 75:367–385
- Ahmad S (1950) Studies in the Gasteromycetes IV. The morphology of *Lanopila wahlbergii* Fr. *Sydowia* 4:39–43
- Bates ST (2004) Arizona members of the Geastraceae and Lycoperdaceae (Basidiomycota, Fungi). Master's thesis, Arizona State University, Tempe
- Calonge FD, Guzmán G, Ramirez-Guillén F (2004) Observaciones sobre los Gasteromycetes de Mexico depositados en los herbarios XAL y XALU. *Bol Soc Micol Madrid* 28:337–371
- Coetzee JC, van Wyk AE (2007) Proposal to conserve *Calvatia* nom. cons. (Basidiomycota, Lycoperdaceae) against an additional name, *Lanopila*. *Taxon* 56:598–599
- Dissing H, Lange M (1962) Gasteromycetes of Congo. *Bull Jard Bot État Brux* 32:325–416
- Gube M (2007) The gleba development of *Langermannia gigantea* compared to other Lycoperdaceae, and some systematic implications. *Mycologia* 99:396–405
- Homrigh MH, Wright JE (1973) South American Gasteromycetes. The genera *Gastropila*, *Lanopila* and *Mycenastrum*. *Mycologia* 65:779–794
- Ito S (1958) Miscellaneous notes on mushrooms (2) (in Japanese). *Trans Mycol Soc Jpn* 1(9):7–15
- Kawamura S (1914) Illustrations of Japanese fungi no. 3 (in Japanese). The Bureau of Forestry of the Department of Agriculture and Commerce, Tokyo
- Kawamura S (1929) The Japanese fungi (in Japanese). Daichi-shoin, Tokyo
- Kawamura S (1937) The Japanese giant puffball differs from the giant puffball in America and Europe or *Lasiosphaera fenzlii* from Nicobar Islands (in Japanese with English summary). *J Jpn Bot* 13:748–757
- Kawamura S (1954) *Icones of Japanese fungi*, vol VI (in Japanese). Kazama-shobo, Tokyo
- Kobayasi Y (1933a) Notes on *Lasiosphaera* Reich. (I) (in Japanese). *J Jpn Bot* 9:468–480
- Kobayasi Y (1933b) Notes on *Lasiosphaera* Reich. (II) (in Japanese). *J Jpn Bot* 9:546–556
- Kobayasi Y (1939) Gasteromycetes (in Japanese). In: Asahina Y (ed) *Nippon Inkwasoyokubutu Dukan*. Sanseido, Tokyo, pp 570–605
- Kobayasi Y (1976) On the systematic position of the Japanese giant puffball (in Japanese with English summary). *Trans Mycol Soc Jpn* 17:366–373
- Kreisel H (1992) An emendation and preliminary survey of the genus *Calvatia* (Gasteromycetidae). *Persoonia* 14:431–439
- Kreisel H (1994) Studies in the *Calvatia* complex (Basidiomycetes) 2. *Feddes Repert* 105:369–376
- Lloyd CG (1908) A possible mistake in Japan. *Mycol Writ* 2(31):411
- McNeill J, Barrie FR, Burdet HM, Demoulin V, Hawksworth DL, Marhold K, Nicolson DH, Prado J, Silva PC, Skog JE, Wiersma JH, Turland NJ (2006) International code of botanical nomenclature (Vienna code). ARG Gantner Verlag, Koenigstein
- Shirai M (1898) On a giant Gasteromyces (in Japanese). *Bot Mag Tokyo* 12:323–327
- Shirai M (1905) A list of Japanese fungi hitherto known (in Japanese). Nippon Engei Kenkyukai, Tokyo
- Shirai M, Miyake I (1917) A list of Japanese fungi hitherto known, 2nd revised edn (in Japanese). Tokyo-shuppansha, Tokyo
- Swoboda F (1937) Über den fruchtkörperbau und die systematische stellung von *Lanopila* Fries. *Ann Mycol* 35:1–14
- Yasuda A (1908) Babotsu no gakumei (in Japanese). *Bot Mag Tokyo* 22:166
- Zeller SM, Smith AH (1964) The genus *Calvatia* in North America. *Lloydia* 27:147–186