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Distribution Data of Bird's Nest Fungi in Japan: *Nidula niveotomentosa* and *Crucibulum laeve*

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Abstract *Nidula niveotomentosa* and *Crucibulum laeve* are distributed widely in temperate countries, but the true distribution in Japan has not been investigated. We hereby update the distribution record of these two species in Japan based on observation of morphological characters from recently collected samples from some areas in Japan, e.g., Chichibu, Nikko, Mt. Fuji and Mt. Daisen, as well as the distribution data from GBIF and other herbarium records. Detailed description of macro- and microscopic characters with photographs and distribution records in each prefecture in Japan are provided.

Key words: GBIF, herbarium, mushrooms, specimens, taxonomy.

Introduction

The small mushrooms *Nidula* V.S.White and *Crucibulum* Tul. & C.Tul (Agaricales, Basidiomycota), are two genera in the family Agaricaceae. They are morphologically characterized by having peculiar cup-shaped basidiomata filled with peridioles, and are commonly known as the "Bird's Nest Fungi". Both genera are well represented in temperate countries around the world, though predominately in the Americas and Europe, and have been known for centuries (Brodie, 1975).

Nidula is characterized by a white or light colored peridium covered by a prominent tomentum, mostly white, and contains small and numerous brown peridioles, without funiculus, that are immersed in a gelatinous substance, which enables fixation onto nearby vegetation during dispersion (White, 1902; Brodie, 1975). *Crucibulum* has a yellow-orange to grey peridium covered with fine tomentum, and peridioles that are covered by a thick tunic layer with a simple funiculus in a button-like purse at one side (Brodie, 1975).

Nidula niveotomentosa (Henn.) Lloyd and *Crucibulum laeve* (Huds.) Kambly are the most frequently encountered species in either genus among 5 and 3 recognized species, respectively (Das and Zhao, 2013; Kirk *et al.*, 2008). However, the old literature indicates no specific localities in Japan and records are often without voucher specimens (Brodie, 1975). For example, Kawamura (1954) referred to only one specimen of *Crucibulum laeve* (as *C. vulgare*) from Chiba Prefecture. Ito (1959) and Imazeki and Hongo (1989) indicated *C. laeve* is widespread in Japan, but no voucher specimens were deposited. The distribution of *N. niveotomentosa* was shown to be in Hokkaido and Honshu by Ito (1959), and widespread in Japan by Imazeki and Hongo (1989), but no information of voucher specimens was given.

Distribution data from the Global Biodiversity

Information Facility (GBIF, 2016a; 2016b) show from Japan only six records of *Nidula niveotomentosa*—from Gunma, Ibaraki and Kanagawa Prefectures; and eight of *Crucibulum laeve* —from Ibaraki, Kanagawa and Tochigi Prefectures (Table 1). These facts clearly indicate that the distribution data for *Crucibulum* and *Nidula* in Japan are insufficient.

The information of distribution and abundance of common species, like *C. laeve* and *N. niveotomentosa*, is important to understand the local ecosystem composition and function, but such information is often unavailable online or from literature (Braga-Neto *et al.*, 2013). Knowing the distribution of organisms is also an important factor to determine their conservation status and management strategies (Mota-Vargas and Rojas-Soto, 2012). Furthermore, both genera produce bioactive compounds such as salfredins and various phenolic metabolites (Ayer and Singer, 1980; Matsumoto *et al.*, 1995; Böker *et al.*, 2001). Understanding the current distribution of *Crucibulum* and *Nidula* in Japan may lead us to finding new bioactive compounds.

In this study, we update the distribution records of *Nidula niveotomentosa* and *Crucibulum laeve* in Japan, including a detailed morphology, photos and distribution data.

Materials and Methods

Field expeditions were performed during July to October of 2015 in four areas of Japan: Chichibu (Saitama Prefecture), Nikko Botanical Garden (Nikko, Tochigi Prefecture), Mt. Fuji (Yamanashi Prefecture) and Mt. Daisen (Tottori Prefecture). The collection methodology was adapted from Fidalgo and Bononi (1984) and each specimen was photographed and dried at 45°C for 24 hours.

Observation of macro- and micro-morphology was conducted in the Mycology laboratory at the Department of Botany, National Museum of Nature and Science, Tsukuba, Japan. Species identification followed Brodie (1975, 1984) as well as more recent literature and descriptions (Das and Zhao, 2012; Das and Zhao, 2013).

Macro-morphology was observed using a stereomicroscope OLYMPUS SZX12 with capture camera. Micro-morphology was observed from sections of peridioles mounted in 5% KOH and examined using a light microscope OLYMPUS BX50, under 400X magnification.

Abbreviations used in the basidiospore descriptions were based on Zhao *et al.* (2008): "Qm" as the mean of the quotient of length ("L") and width ("W"); and "n" is the number of basidiospores measured per specimen. The descriptions of basidiospore shape were in accordance with Bas (1969), based in the "Qm" value. Color codes and names were defined using Kornerup and Wanscher (1978), and the specimens were deposited in the fungal herbarium, Department of Botany, National Museum of Nature and Science, Tsukuba, Japan (TNS).

In addition to newly collected specimens, the occurrence records of these two species in Japan were retrieved from the GBIF (http://www.gbif.org/) database. Furthermore, there are numerous specimen data of these two species at the TNS herbarium that have not yet been integrated into the GBIF database. We retrieved such data and kept only the records with complete information of collection date and locality (at least at the Prefecture level).

Description

Nidula niveotomentosa (Henn.) Lloyd, Mycological Writings 3 (34): 455 (1910) [Fig. 1A–E]

Basidiomata (Fig. 1A, B) campanulate, 5.3–7 mm in height, 5–7 mm in width at the upper part; slightly expanded at the mouth but not tapering abruptly at the base; emplacement inconspicuous. Exterior (Fig. 1A, B) hirsute to cottonous at the touch, white to greyish-orange in color (5A2–5B3). Tomentum 0.54–0.78 mm, arranged in regular and flexible tufts; external wall without plication and mouth smooth, recurved; interior greyish-orange to brownish-orange (5B4–5C5), smooth, not shiny, not contrasting with the exterior. Epiphragm operculate, concolorous to exte-

Table 1. Specimen records of *Crucibulum laeve* and *Nidula niveotomentosa* from each Region/Prefecture of Japan

Region	Prefecture	*Crucibulum laeve*	*Nidula niveotomentosa*
Hokkaido	Hokkaido	TNS-F-43566	TNS-F-245061[*3]
Tohoku	Aomori	—	—
	Iwate	TNS-F-189368	TNS-F-25275
	Akita	—	—
	Miyagi	TNS-F-12299	TNS-F-44057[*3]
	Yamagata	—	—
	Fukushima	—	—
Kanto	Tochigi	GMNHJ-FF-1000405[*1]	TNS-F-25100, KH-JPN15-563 (TNS)[*2]
	Ibaraki	INM-Fu-41058[*1, 3]	INM-Fu-42857[*1]
	Gunma	—	GMNHJ-FF-1001328[*1, 3]
	Saitama	—	TNS-F-24962, KH-JPN15-920 (TNS)[*2], KH-JPN15-921 (TNS)[*2]
	Chiba	TNS-F-24414	—
	Tokyo	TNS-F-5379[*3]	TNS-F-5387[*3]
	Kanagawa	KPM-NC0015851[*1, 3]	KPM-NC0020068[*1, 3]
Chubu	Niigata	—	—
	Toyama	—	—
	Ishikawa	TNS-F-22394[*3]	TNS-F-38275[*3]
	Fukui	—	—
	Nagano	TNS-F-18602[*3]	TNS-F-48852[*3]
	Gifu	—	—
	Yamanashi	—	TNS-F-47702[*3] KH-JPN15-582 (TNS)[*2] KH-JPN-15-583 (TNS)[*2] KH-JPN15-584 (TNS)[*2]
	Shizuoka	—	TNS-F-24957
	Aichi	—	—
Kinki	Shiga	TNS-F-37910	—
	Mie	TNS-F-44154	—
	Kyoto	TNS-F-43577[*3]	TNS-F-42897[*3]
	Hyogo	TNS-F-203899	—
	Nara	TNS-F-41774[*3]	—
	Osaka	TNS-F-43001[*3]	—
	Wakayama	—	—
Chugoku	Tottori	TNS-F-44734[*3] RH-20151011-7 (TNS)[*2]	TNS-F-42631[*3]
	Okayama	—	—
	Shimane	—	—
	Hiroshima	TNS-F-57715[*3]	—
	Yamaguchi	—	—
Shikoku	Kagawa	—	—
	Tokushima	—	—
	Ehime	TNS-F-205132	—
	Kochi	TNS-F-200481	—
Kyushu	Fukuoka	—	—
	Oita	—	TNS-F-17867[*3]
	Saga	—	—
	Nagasaki	—	—
	Miyazaki	TNS-F-206783	—
	Kumamoto	—	—
	Kagoshima	—	TNS-F-13340[*3]
Okinawa	Okinawa	—	—

All specimens housed at the National Museum of Nature and Science are indicated with the voucher numbers of "TNS".

*1 Specimen data retrieved from the GBIF database.

*2 Specimens examined in this study.

*3 More than one specimen was retrieved from the database, but only one is shown here.

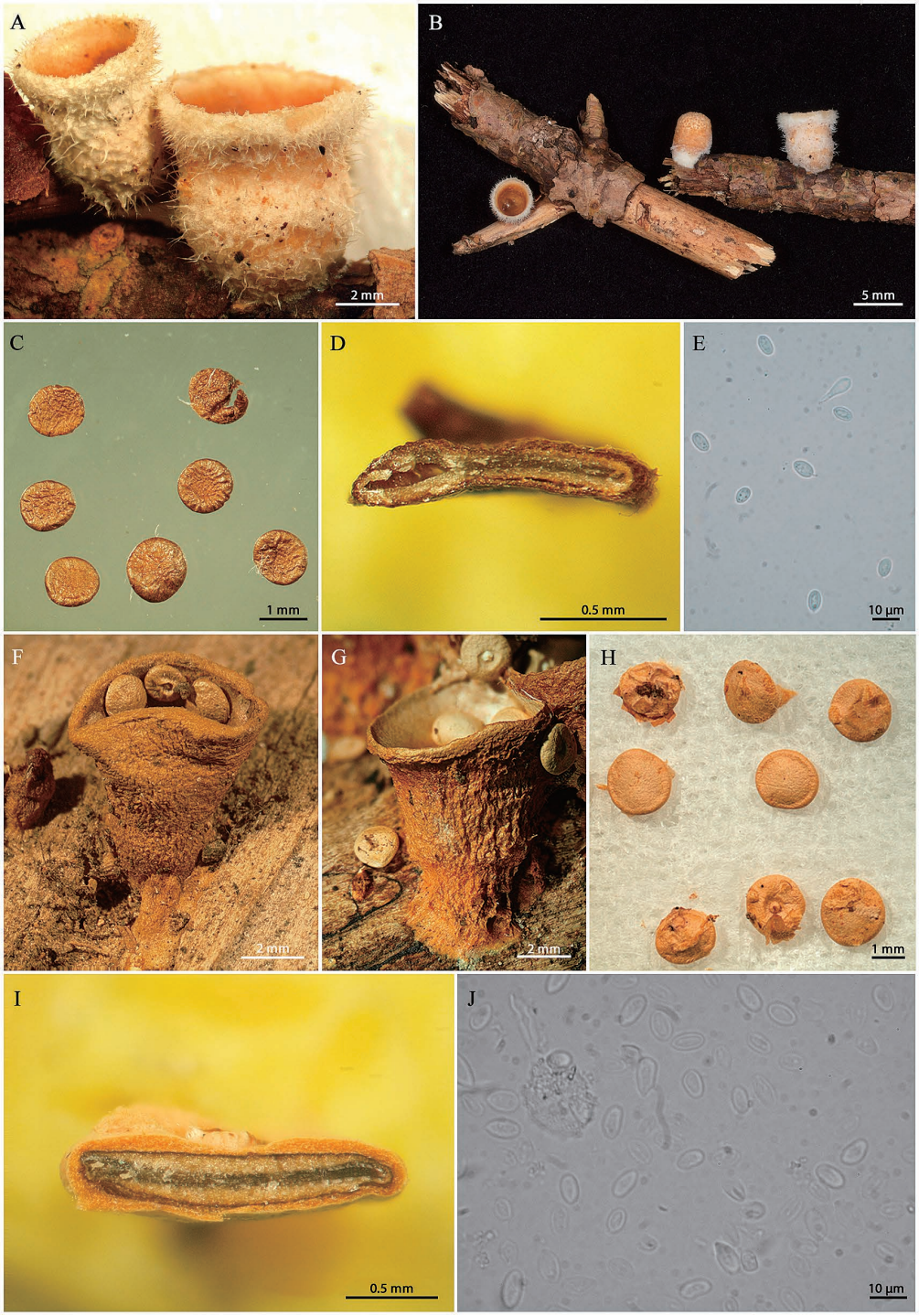

Fig. 1. *Nidula niveotomentosa* (A–E; KH-JPN15-563, TNS) and *Crucibulum laeve* (F–J; RH-20151011-7, TNS). A. Mature fruit bodies. B. Fruit bodies at different stages of development, on dead branches. C. Peridioles. D. Peridiole in section showing the double-layered cortex (subhomogeneous single-layer). E. Basidiospores. F, G. Mature fruit bodies. H. Peridioles. I. Peridiole in section showing the single-layered cortex with thick tunic. J. Basidiospores.

rior, flexible. Peridioles (Fig. 1C) brown to reddish-brown (6E4–6F5), 1.2–1.4 × 1.1–1.3 mm, circular to slightly elliptical in shape, embedded in mucilaginous liquid when covered by the epiphragm; tunic absent, double-layered cortex (Fig. 1D), subhomogeneous single-layered. Basidiospores (Fig. 1E) smooth, hyaline, 5–8 × 4–5 μm (L = 7.58 μm; W = 4.25 μm; n = 30); slightly elliptical to elongated (Q = 1.2–2), elongated in mean (Qm = 1.65), apicule present in some, prominent, and spore wall 0.5–1 μm.

Specimens examined: Nikko Botanical Garden, Nikko, Tochigi Prefecture, Japan, 18 July 2015, col. Kentaro Hosaka, solitary on fallen branch (KH-JPN15-563, TNS); Mount Fuji, Yamanashi Prefecture, Japan, 12 October 2015, col. Rhudson H. S. F. Cruz, solitary to gregarious on fallen branches (KH-JPN15-582, TNS); col. Megumi Otsuka, solitary to gregarious on fallen branches (KH-JPN15-583, TNS); col. Rhudson H. S. F. Cruz (KH-JPN15-584, TNS); Chichibu, Saitama Prefecture, 28 September 2015, col. Rhudson H. S. F. Cruz, solitary to gregarious on fallen branches (KH-JPN15-920, TNS); col. Rhudson H. S. F. Cruz, solitary to gregarious on fallen branches (KH-JPN15-921, TNS).

Crucibulum laeve (Huds.) Kambly, University of Iowa Studies in Natural History 17 (4): 167 (1936) [Fig. 1F–J]

Basidiomata (Fig. 1F, G) campanulate, rigid (rarely funnel-shape), 6–8 mm in height, 6–9 mm in width at the upper part; slightly expanded at the mouth but not tapering abruptly at the base; emplacement inconspicuous to conspicuous, 4 mm in diameter, with the same color of the exterior. Exterior (Fig. 1F, G) wooly, fine texture, greyish-orange in color (5B5). Tomentum not arranged in tufts; external wall without plication and mouth smooth, recurved; interior alabaster (5B2), smooth, perceptible bright, contrasting with the exterior. Epiphragm operculate, outside golden yellow (5B6), inside white, rigid. Peridioles (Fig. 1H) pale orange (6A3), 1.81–2 × 1.55–1.87 mm, circular to elliptical in shape; tunic present, thick, bronze, single-layered cortex (Fig. 1I). Basidiospores (Fig. 1J) smooth, hyaline, 6–12.5 × 3.80–5.50 μm (L = 8.78 μm; W = 4.58 μm; n = 30); elliptical to cylindrical (Q = 1.50–2.98), elongated in mean (Qm = 1.92), or ovoid in few spores, apicule present in some, and spore wall 0.5–1 μm.

Specimens examined: Mount Daisen, Saihaku, Tottori Prefecture, Japan, 11 October 2015, col. Shuji Ushijima, cespitose to gregarious on decaying wood (RH-20151011-7, TNS).

Results and Discussion

Nidula niveotomentosa and *Crucibulum laeve* are common species in temperate areas, both in Northern and Southern Hemispheres (Brodie, 1975), but in Japan all the records found in the GBIF dataset are restricted to the east part of the country (Table 1). Further investigation of the in-house database at the TNS fungal herbarium, however, indicated that both species are distributed widely across Japan (Table 1, Fig. 2). Both species are recorded in all major "regions" (e.g., Tohoku and Kanto) except for the Okinawa region. This finding is consistent with Imazeki and Hongo (1989) who claimed that both species are distributed widely in Japan. To our knowledge, this is the first study showing the distribution pattern of both species based on voucher specimens.

The absence of records from many prefectures in Japan based on GBIF, TNS herbarium or literature is not because the two taxa are truly absent from those areas. Many specimens in herbaria are identified only to genus, and they can be identified as *C. laeve* or *N. niveotomentosa* in the future. The other problem is a potential misidentification of three genera of bird's nest fungi (*Nidula*, *Crucibulum* and *Cyathus*), which can change the distribution records of those species. Furthermore it is often difficult to find fruit bodies of bird's nest fungi in nature, due to their small size and similar color to the substrate. More sampling effort is necessary to cover the whole distribution area of the two species in Japan.

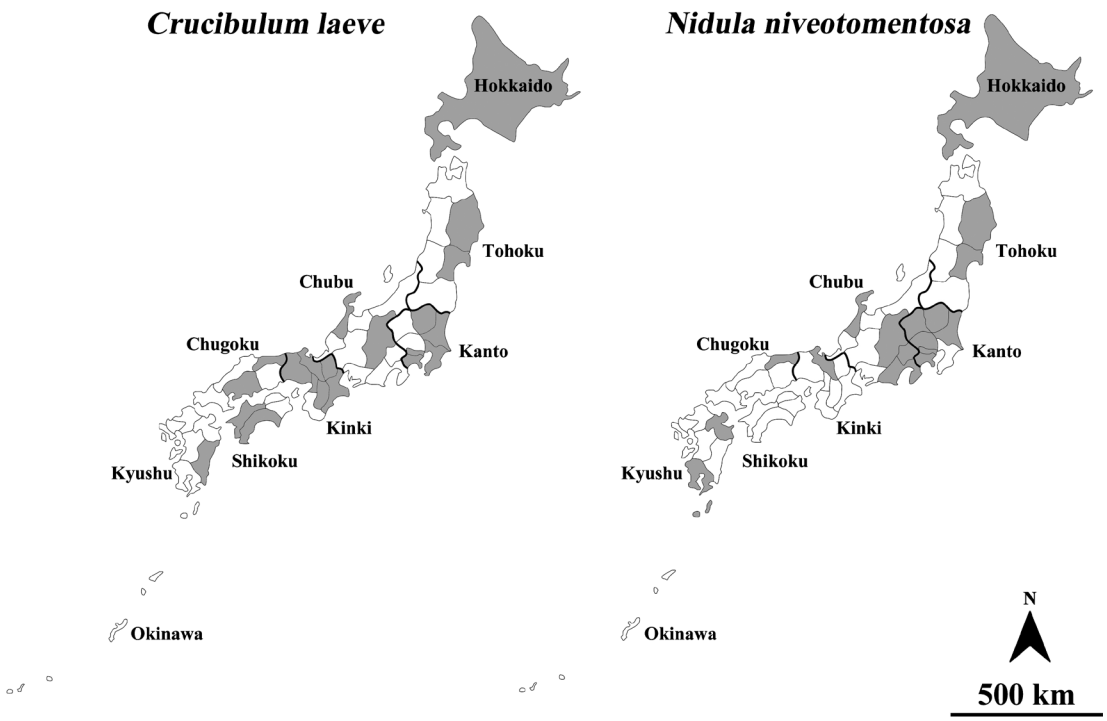

Fig. 2. Distribution map of *Crucibulum laeve* and *Nidula niveotomentosa* based on each Region/Prefecture of Japan. Prefectures with representative voucher specimens are indicated by gray color.

The absence of the two species from the Okinawa region is worth mentioning. Although it can be simply because of a lack of sampling effort in Okinawa, as indicated above, Okinawa is the only area in Japan with "subtropical" climate. These two species are known from the rest of the world in areas with a similar climate to Okinawa (Baseia and Milanez, 2001), but the presence of both species in Okinawa cannot be proven until new collections are made.

The materials of both species examined in this study present consistent characteristics with the descriptions in the literature and no differences were found between the Japanese materials and specimens reported from the rest of the world (Baseia and Milanez, 2001; Bottomley, 1948; Brodie, 1975; Calonge and Demoulin, 1975; Gomez and Pérez-Silva, 1988; Liu, 1984; White, 1902). However, future molecular works may reveal the presence of cryptic species. The distribution data of these two species in Japan need to be updated with more specimens and molecular data.

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