A new species of *Gastrosporium* (Phallales) from coastal sand dunes of Ibaraki Prefecture, central Japan

茨城県の海岸砂丘において採集された*Gastrosporium*属（スッポンタケ目）の一新種

Taiga Kasuya¹,²*, Shota Hanawa², Kentaro Hosaka³

糟谷大河¹,²*, 塙祥太², 保坂健太郎³

¹ Department of Biology, Keio University, 4-1-1 Hiyoshi, Kohoku-ku, Yokohama-shi, Kanagawa 223-8521, Japan
２Faculty of Risk and Crisis Management, Chiba Institute of Science, 3 Shiomi-cho, Choshi-shi Chiba 288-0025, Japan
３Department of Botany, National Museum of Nature and Science, 4-1-1 Amakubo, Tsukuba-shi, Ibaraki 305-0005, Japan

* Corresponding author
E-mail: tkasuya@keio.jp

Abstract

An hypogeous basidiomycete fungus was collected in coastal sand dunes of Ibaraki Prefecture, central Japan. Based on morphological observations and phylogenetic analyses using nuclear ribosomal DNA sequences, the present fungus was recognized as a member of *Gastrosporium*, belonging to Gastrosporiaceae (Phallales). The genus *Gastrosporium* produces hypogeous to subhypogeous, small, globose, subglobose to ovoid basidiomata, and the genus includes only two known species, *G. simplex* and *G. asiaticum*. Previously, this genus has not been recorded from Japan. Japanese specimens are distinguishable from other known species of the genus by the exoperidium forming a cottony mycelial mass and they constitute a phylogenetically distinct monophyletic group. Therefore, Japanese specimens are described as a new species, *G. gossypinum*.

要旨

茨城県の海岸砂丘において、担子菌門に属する地下生菌の一種が採集された。本菌について、形態的特徴の観察および子実体より得られた核リボソームDNAの塩基配列を用いた系統解析を行った。その結果、本菌はスッポンタケ目のGastrosporiaceaeに属するGastrosporium属の一新種と判断された。Gastrosporium属は地中生～半地中生の小型で球形、類球形あるいは卵形の子実体を形成する菌群で、G. simplexとG. asiaticumの2種のみを含むが、日本からはこれまでに報告されていなかった。日本産標本は本属の既知の2種とは外皮が締毛状の菌糸塊からなる点で異なり、系統的にも独立した単系統群を形成したため、これを新種と判断し、G. gossypinumの学名を与え記載した。外皮が締毛状の菌糸塊からなり、砂地生であることから本菌の和名をワタゲスナツブタケ、また*Gastrosporium*属の和名をスナツブタケ属とする。
Introduction

During the course of our study of gasteroid fungi from sand dunes of the Japanese coast (Kasuya et al., 2009, 2011, 2015), several specimens of a hypogeous basidiomycete fungus were found in sand of coastal dunes in Ibaraki Prefecture, central Honshu, Japan. Based on morphological observations and phylogenetic analyses using nuclear ribosomal DNA sequences, the present fungus was recognized as a member of Gastrosporium Mattir, belonging to Gastrosporiaceae (Phallales). Although the classification of Gastrosporiaceae has not been clear for a long time, a recent molecular phylogenetic study (Trierveiler-Pereira et al., 2014) revealed that Gastrosporiaceae is sister to Phallaceae in the order Phallales. The genus Gastrosporium produces hypogeous to subhypogeous, small, globose, subglobose to ovoid basidiomata with conspicuous white mycelial cords at the base (Montecchi & Sarasini, 2000; Rimóczki et al., 2011), and they are characterized by a chalk-white exoperidium, gelatinized endoperidium, clamped paracapillitium and globose to subglobose, somewhat angular, slightly warted basidiospores (Miller & Askew, 1982; Montecchi & Sarasini, 2000; Rimóczki et al., 2011). Only two known species, G. simplex Mattir. and G. asiaticum Dörfelt & Bumžaa, have been described as members of the genus in the world (Dörfelt & Bumžaa, 1986; Trierveiler-Pereira et al., 2014). The genus was originally described from Italy based on G. simplex, and thereafter it has been recorded in Europe to Siberia (Montecchi & Sarasini, 2000; Kreisel 2001; Rimóczki et al., 2011), Mauritius (Kreisel & Hausknecht, 2002), the Middle East (Kreisel 2001; Kreisel & Al-Fatimi, 2008), Asia (Ahmad, 1950, 1952; Dörfelt & Bumžaa, 1986), North America (Miller & Askew, 1982) and Argentina (Dominguez de Toledo & Castellano, 1997). Although Gastrosporium presumably has a worldwide distribution, this genus has not been found in Japan. In Asia, G. simplex has been known from India and Pakistan (Ahmad, 1950, 1952), and the second species G. asiaticum was only recorded from its type locality, Mongolia (Dörfelt & Bumžaa, 1986).

Our morphological observations and phylogenetic analyses of the Japanese specimens revealed that it is distinguishable from known species of the genus. Therefore, in this paper we describe Japanese specimens as a new species of Gastrosporium.

Materials and methods

Sample collecting and morphological observations

Fresh hypogeous basidiomata were collected from sandy soil of coastal dunes covered with Imperata cylindrica (L.) P. Beauv., Calystegia soldanella (L.) Roem. & Schult., Lathyrus japonicus Willd. and Pittosporum tobira (Thunb.) W.T. Aiton at the seashore in Hasaki, Kamisu, Ibaraki Prefecture in 2015 and 2017 (Table 1). Specimens were photographed and observed macroscopically. Fresh basidiomata of specimens were dried using a food dehydrator (Snackmaster Express FD-60; Nesco/American Harvest, Milwaukee, WI, USA) under 46 ºC. For light microscopy, hand-cut sections of both fresh and dried specimens were mounted in water, 3% KOH or Melzer’s reagent. Dimensions of basidiospores were measured from water-mounted sections. More than 50 randomly selected basidiospores were measured under a light microscope at 1000× magnification. All measurements were performed with Photometer 1.1.3 (http://inocybe.info). Five specimens examined in this study were deposited at mycological herbaria of the National Museum of Nature and Science (TNS) or Ibaraki Nature Museum (INM) in Japan.

DNA preparation, PCR and sequencing

DNA extraction, PCR and DNA sequencing were carried out according to the methods introduced by Kasuya et al. (2012). First, small fragments of global tissue from freshly collected samples were soaked in DMSO buffer (Seutin et al., 1991) with the addition of 100 mM Tris-HCl (pH 8.0) and 0.1 M sodium sulfite (Na2SO3) at 4 ºC, following the procedures of Hosaka (2009), Hosaka & Castellano (2008), and Hosaka et al. (2010). DNA of the above specimens was extracted from the tissue fragments stored in DMSO buffer. DNA extractions used the modified CTAB extraction followed by glass milk purification methods as summarized by Hosaka (2009) and Hosaka & Castellano (2008). DNA sequence data were obtained from the nuclear ribosomal ITS region and large subunit (LSU). For amplifying the ITS region, the primer combination of ITS5 and ITS4 (White et al., 1990) was used. For amplifying the LSU, the combination of LR0R and LR5 (Vilgalys & Hester, 1990) was used. Polymerase chain reactions (PCR) were carried out using 20 µl reaction volume, each containing 1 µl genomic DNA, 1 µl dNTP (4 mM), 1 µl each primer (8 µM), 0.5 units Taq polymerase (Takara), 2 µl MgCl2 (25 mM), and 2 µl bovine serum albumin (BSA). Cycling parameters for ITS region and LSU followed Kasuya et al. (2012). PCR products were electrophoresed in 1% agarose gels stained with ethidium bromide and visualized under UV light. When amplification bands were confirmed, PCR products were then purified using the ExoSap-IT (Millipore, Molsheim, France) and directly sequenced using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Norwalk, CT, USA), following the manufacturer’s instructions. A total of 9 newly generated sequences from this study were deposited in GenBank (Table 1).
Phylogenetic analyses

Five ITS and four LSU sequences newly generated from Japanese specimens were used for the phylogenetic analyses (Table 1). Additionally, 15 ITS and 17 LSU sequences of Phallales fungi were retrieved from the NCBI GenBank databases (https://www.ncbi.nlm.nih.gov/) and included in the analyses. DNA sequences were initially aligned using Muscle v.3.6 (Edgar, 2004a, b), followed by manual alignment in the data editor of BioEdit ver. 7.0.1 (Hall, 1999). The total 18 ITS and 30 LSU nucleotides were excluded from the analyses because they were recognized as ambiguously aligned regions and introns. Phylogenetic analyses were performed for ITS and LSU sequences using MEGA X (Kumar et al., 2018) with the help of the maximum likelihood (ML) method after testing the best models. According to the lowest BIC (Bayesian Information Criterion) scores, Kimura 2-parameter (Kimura, 1980) with proportion of invariant sites (K2+I) and Tamura 3-parameter (Tamura, 1992) with gamma distributed rate heterogenetic and a proportion of invariant sites (T92+G+I) were chosen as the optimal substitution models for the analyses of the ITS and LSU datasets, respectively. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. For the ML analyses, clad robustness was assessed using a bootstrap analysis with 1000 replicates (Felsenstein, 1985). Sequences of Clathrus archeri (Berk.) Dring and C. ruber P. Micheli ex Pers. were selected for outgroups, which were strongly supported as the sister of the major clade containing Gastrosporiaceae, Lysuraceae, Phallaceae and Protophallaceae (Trierveiler-Pereira et al., 2014).

Results

Morphological observations

Japanese specimens were morphologically identical to the described species of *Gastrosporium* in their globose, subglobose to ovoid basidiomata with white mycelial cords at the base, gelatinized endoperidium, clamped paracapillitium and globose to subglobose, somewhat angular, slightly warted basidiospores (Miller & Askew, 1982; Montecchi & Sarasini, 2000; Rimóczi et al., 2011). However, the specimens clearly differ from *G. simplex* in their exoperidium that is formed by a cottony, white mycelial mass. The specimens were also different from *G. asiaticum* in the size of basidiospores.

A detailed description and illustrations of the salient features of the Japanese specimens are given below.

Phylogenetic analyses

The ITS dataset includes 745 sites consisting of 18 ingroup taxa and 2 outgroup taxa. The resulting ML topology with the highest log likelihood (-4758.52) is shown in Fig. 1. The rate variation model allowed for some sites to be evolutionarily invariable [+I, 34.50% sites]. The LSU dataset includes 987 sites consisting of 19 ingroup taxa and 2 outgroup taxa. The resulting ML topology with the highest log likelihood (-3131.58) is shown in Fig. 2. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.5982)]. The rate variation model allowed for some sites to be evolutionarily invariable [+I, 59.63% sites]. Tree topology by ML analysis of LSU is almost identical to those of ITS. Sequences of *Gastrosporium* specimens from the coastal sand dunes in Ibaraki Prefecture, Japan constitute a distinct monophyletic group in Gastrosporiaceae with strong bootstrap support (99%) in both loci (Figs. 1–2). *Gastrosporium simplex* was resolved as the sister group of Japanese specimens in the ML trees with strong support (Figs. 1–2).

Taxonomy

*Gastrosporium gossypinum* T. Kasuya, S. Hanawa & K. Hosaka, sp. nov.

[MycoBank ID: MB 833998]

Diagnosis: Similar to *G. simplex*, hypogeous basidiomata with long, white mycelial cords at the base, gelatinized endoperidium, clamped paracapillitium and globose to subglobose, somewhat angular, slightly warted basidiospores, but differing by the exoperidium that is formed by a cottony, white mycelial mass.

Etymology: From Latin (“gossypinum” = cottony), refers to cottony...
Fig. 1. A phylogenetic tree of the nuclear ribosomal ITS region of selected Phallales species based on ML method, inferred by using K2+I model. ML bootstrap values greater than 60% are shown for each node. Scale bar indicates the number of substitutions per site.

Fig. 2. A phylogenetic tree of the nuclear ribosomal LSU of selected Phallales species based on ML method, inferred by using T92+G+I model. ML bootstrap values greater than 60% are shown for each node. Scale bar indicates the number of substitutions per site.
Kasuya et al.: A new species of Gastrosporium from Japan

© The Japanese Association for Truffle Science (JATS), 2020

surface of exoperidium.

Description: Basidiomata (Fig. 3C–F) hypogeous, globose, subglobose to ovoid, 3–15 mm high, 3–10 mm wide, arising from a conspicuous, thickened mycelial cord, which connects the basidiomata with each other; surface covered with adherent sand, apex not eroded at maturity. Peridium 1–2 mm thick, two-layered (Fig. 4A). Exoperidium (Fig. 4B–C) up to 2 mm thick, forming a cottony, white mycelial mass with adherent numerous sand, and not disintegrating when rubbed or mature. Endoperidium (Fig. 4D) thin, up to 0.5 mm thick, gelatinized, plant when moist but brittle when dry, light yellowish brown to light olivaceous. Gleba (Fig. 3F and Fig. 4A) occupies the entire cavity, apparently homogenous, white when young, becoming light yellowish brown to light olivaceous, powdery at maturity. Basidiospores (Fig. 4E) 3.5–6.5 × 3–5 µm (mean = 4.8 × 3.7 µm; n = 60), subglobose, ovoid to ellipsoid, sometimes somewhat angular, irregular in profile,
varying from almost smooth with scattered warts to densely warty, with or without a short pedicel, light green to light greyish blue in 3% KOH, pale yellow in Melzer’s reagent. Basidia not observed. Capillitium absent. Paracapillitium (Fig. 4F) of branched elements with numerous clamp connections, up to 4 µm diameter, thin-walled, with occasional irregular swellings, hyaline in 3% KOH, pale yellow in Melzer’s reagent. Exoperidium (Fig. 4G) composed of loosely interwoven, thin-walled, branched hyphae up to 4 µm diameter, hyaline in 3% KOH and Melzer’s reagent, with numerous clamp connections, interspersed with crystals. Endoperidium distinctly separated from the exoperidium, a thick gelatinized, refractive layer of interwoven, frequently branched hyphae up to 4 µm diameter with numerous clamp connections. Cortex of mycelial cords proliferating from the base of basidiomata homologous to exoperidial hyphae.

Habitat: Hypogeous, about 3–10 cm deep in sand, usually small groups or scattered in sandy soil of coastal dunes covered with Imperata cylindrica, Calystegia soldanella, Lathyrus japonicus and...
Pittosporum tobiara along seashore (Fig. 3A–B). Fruitings in the type locality occurs in winter to late spring (January to May) or early autumn (September).


Known distribution: Known only from the type locality.

Japanese name: Watage-sunatsubu-take (“watage” = cottony; “sunatsubu-take” = the Japanese name of Gastrosporium; “sunatsubu” refers to the arenicolous nature).

Remarks: Gastrosporium gossypinum is morphologically similar to G. simplex, the type species of the genus, in its structure of endoperidium and gleba, the shape and the size of basidiospores and paracapillitium. However, G. gossypinum is clearly distinguishable from G. simplex by morphology of the exoperidium that is formed from a cottony, white mycelial mass. Exoperidium of G. simplex is opaque, chalky, finely powdery and scaly (Dominguez de Toledo & Castellano, 1997; Montecchi & Sarasini, 2000; Rimóczi et al., 2011). Moreover, when basidiomata are gently rubbed or reach maturity, exoperidium of G. simplex is disintegrating (Montecchi & Sarasini, 2000), flaking away in pulverulent patches (Rimóczi et al., 2011) or splitting irregularly at the apex (Miller & Askew, 1982). However, exoperidium of G. gossypinum is not collapsing or splitting, even in mature basidiomata, because of numerous sand persistently adhering to its exoperidial hyphae. Phylogenetically, G. gossypinum is distinct from G. simplex, which supports our morphological observations. Another species of the genus, G. astiaticum is also distinguished from G. gossypinum by its much smaller basidiospores (2.2–4.5 µm; Dörfelt & Bumžaa, 1986). Although Kreisel & Al.-Fatimi (2008) recorded an unidentified Gastrosporium specimen which is apparently a new species from Yemen, it is distinct from G. gossypinum because it has smooth basidiospores. Results of our phylogenetic analyses are almost consistent with Trierveiler-Pereira et al. (2014), showing the monophyly of the genus. More comprehensive phylogenetic studies including sequences of G. astiaticum and the Yemeni specimen are required to discuss monophyly and phylogeography of Gastrosporium.

Monthoux & Röllin (1976) reported that the hyphae of G. simplex create a network among poaceous plant roots and they invade roots in a parasitic manner. Montecchi & Sarasini (2000) regarded G. simplex as a symbiont of Poaceae. Also, Rimóczi et al. (2011) reported that G. simplex is a typical species of sandy Stipa steppes in Hungary. In Japan, basidiomata of G. gossypinum were collected around coastal plant communities dominated by a poaceous plant Imperata cylindrica. While it is unclear whether they are truly symbions, parasites or saprobionts, Gastrosporium species presumably prefer poaceous plants. However, in Mauritius, rhizomorphs of G. simplex were connected with roots of a tropical tree or shrub (Kreisel & Hausknecht, 2002); G. simplex was also found under an arecaceous plant Trithrinax campestris in Argentina (Dominguez de Toledo & Castellano, 1997). In Mongolia, G. astiaticum were collected among Allium, Artemisia and Achnatherum plants (Dörfelt & Bumžaa, 1986). Further studies on the ecological nature of Gastrosporium species are needed to clarify the relationship between them and associated plants. Although G. gossypinum is known only from the type locality, there are several similar habitats in Japanese coasts. Thus, G. gossypinum presumably has several further localities in Japan.

Acknowledgements

We are very much obliged to Kyung-Ok Nam, Kazuo Nishibori and Megumi Otsuka of the National Museum of Nature and Science for help with assisting molecular experiments. We also thank Ikuo Asai, the late Yoshiko Asai, Kazuki Kobayashi, Takashi Maruyama, Maya Omori and Shohei Wada for facilitating the fieldwork. This work was supported by JSPS KAKENHI Grant Number JP15K16279 and the commissioned research grant from the Division of Environmental Affairs, Kamisu City Office, Ibaraki Prefecture.

References

Kasuya et al.: A new species of Gastrosporium from Japan

Truffology - Volume 3, Issue 1, 2020


