Taxonomic study of polymorphic basidiomycetous fungi Sirobasidium and Sirotrema: Sirobasidium apiculatum sp. nov., Phaeotremella translucens comb. nov. and rediscovery of Sirobasidium japonicum in Japan

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Research Article

Keywords: Basidiomycetous yeasts, Phylogeny, Tremellales, Tremellomycetes, Taxonomy

Posted Date: March 23rd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1461453/v1

Abstract

Species in the genera *Sirobasidium* and *Sirotrema* (Tremellales, Tremellomycetes, Agaricomycotina, Basidiomycota) were described based on the morphology of teleomorph, and many of them lack both isolates of anamorphic yeast state and nucleotide sequence data. Strains of *Sirotrema translucens* and *Sirobasidium japonicum* were established for the first time from basidiocarps collected in Japan. Also, an undescribed species in the genus *Sirobasidium* was isolated. Molecular phylogenetic analysis based on the sequences of D1/D2 region of large subunit ribosomal RNA gene (LSU D1/D2), ITS-5.8S ribosomal RNA gene (ITS), and small subunit ribosomal RNA gene (SSU) showed that *St. translucens*, which has been considered to be related to the genus *Sirobasidium* based on its basidial ontogeny, was placed in a phylogenetically separated clade including the genus *Phaeotremella.* The analysis also revealed that *Sirobasidium* sp. and *Sb. japonicum,* previously assumed to have a close affinity to the order Auriculariales, were members in the Tremellales. *Sirobasidium* sp. was characterised by its apiculate epibasidia and 2-celled basidia, which is a unique combination of characteristics in the genus. In conclusion, we keep *Sirobasidium japonicum* in the Tremellales and propose *Sirobasidium apiculatum* sp. nov. and *Phaeotremella translucens* comb. nov.

Introduction

The genera *Sirobasidium* and *Sirotrema* are basidiocarp-forming fungal taxa belonging to the order Tremellales (Tremellomycetes, Agaricomycotina). The genus *Sirobasidium* is characterised by the morphological characteristics: basidia formed in chain and deciduous primary spores defined as epibasidia (Bandoni 1957). Basidiocarps of *Sirobasidium* spp. usually closely associate with pyrenomycetes, especially Xylariales, suggesting that they are mycoparasites (Chen 1998; Roberts and Meijer 1997). A parasitic structure called tremelloid haustorium (= haustorial branch) is common in the Tremellales (Grube and de los Ríos 2001; Bandoni 1987). However, as the structure was reported only one time in the genus *Sirobasidium* (Bandoni et al. 2011; Bandoni 1987), the parasitic nature of *Sirobasidium* species remains unclear. There are around 10 described species in the genus, but only two species have been cultured and have nucleotide sequence data available. Based on the morphological features, there are many discussions about the species delimitation or their taxonomic position in higher rank for the species without strains (Dämon and Hausknecht 2002; Roberts and Spooner 1998). For instance, transversally septate basidia of *Sirobasidium japonicum* Kobayasi caused the suspicion that the species is closely related to another order, Auriculariales (Agaricomycetes; Dämon and Hausknecht 2002).

The other genus, *Sirotrema* is characterised by an occasional formation of basidia in chain and parasitizing rhytismataceous fungi via tremelloid haustoria (Bandoni 1986). Based on its similarity in basidial ontogeny, Bandoni (1986) speculated that *Sirotrema* is a close relative of the genus *Sirobasidium.* Because the genus *Sirotrema* lacks both strains and nucleotide sequence data, the phylogenetic relationship between these two genera remains unknown.

In this study, we established strains and observed morphological features of *Sirobasidium japonicum,* one undescribed species of *Sirobasidium*, and *Sirotrema translucens* collected in Japan. Based on the phylogenetic analysis, morphological observations, and physiological characteristics, we discuss phylogenetic relationships between these two genera and the taxonomic status of the species.

Materials And Methods

Morphological observations

Basidiocarps growing on fallen branches or pine needles were collected in the middle and the southern part of Japan (Table 1). To study microscopic characteristics, handmade sections were mounted in sterile water or 3% (w/v) potassium hydroxide stained with or without phloxine. The sections were observed under a Zeiss Axioskop Microscope (Carl Zeiss, Oberkochen, Germany) or a BX53 upright microscope (Olympus, Tokyo, Japan). For the comparison, a herbarium specimen of *Sirobasidium japonicum* (TNS-F-196751) was observed in the same way. Single-basidiospore isolates of each taxon were established following Wong et al. (1985) using Malt Agar (MA; Nissui, Tokyo, Japan). Morphological features of the anamorphic yeast states were observed according to Kurtzman et al. (2011). Yeast cells for these observations were cultivated on 5% (w/v) malt extract agar (MEA; Kurtzman et al. 2011) at 25 °C for 3 days. Dried specimens of basidiocarps were deposited in the National Museum of Nature and

Science, Tokyo (TNS), and strains were in the Japan Collection of Microorganisms (JCM), the CBS collection (housed at Westerdijk Fungal Biodiversity Institute), and the Portuguese Yeast Culture Collection (PYCC) as shown in Table 1.

Physiological tests

Physiological tests of the strains were performed according to Kurtzman et al. (2011). Assimilation of carbon and nitrogen compounds were tested using liquid and solid media, respectively. Growth at various temperatures were tested in YM broth (Difco).

DNA sequencing and phylogenetic analysis

Genomic DNAs were extracted according to Ishida et al. (1999). The D1/D2 region of large subunit ribosomal RNA gene (LSU D1/D2), ITS-5.8S ribosomal RNA gene (ITS), and small subunit ribosomal RNA gene (SSU) were amplified by PCR with Ex Taq (Takara Bio, Otsu, Japan). The LSU D1/D2 was amplified using a primer pair of LR0R (Rehner and Samuels 1994) and LR5 (Vilgalys and Hester 1990), and a pair of ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) was used for the ITS region. For the SSU, a primer pair NS1 (White et al. 1990) and NS8 (White et al. 1990) was used. PCR amplifications were carried out as follows: for the primer pairs LR0R/LR5 and ITS1F/ITS4, initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 1 min, 51 °C for 30 s, 72 °C for 1 min, and then final extension at 72 °C for 15 min. For the primer pair NS1/NS8, initial denaturation at 94 °C for 2 min followed by 10 cycles of 98 °C for 10 s, 55 °C for 30 s dropping by 0.5 °C per cycle, and extension at 72 °C for 2 min. Those 10 cycles were followed by 25 cycles of 98 °C for 10 s, 50 °C for 30 s, 72 °C for 2 min. The PCR products were purified by polyethylene glycol precipitation. Sequence reactions containing BigDye terminator v3.1 (Applied Biosystems) and primers, namely LR0R and LR5 for the LSU D1/D2, ITS1F, and ITS4 for the ITS region, and NS1, NS3, NS5, and NS8 for the SSU, were carried out following manufacturer's instructions. DNA sequences were analysed with ABI PRISM 3130 Genetic Analyzer (Applied Biosystems).

The dataset used for molecular phylogenetic analysis is shown in Table S1. Sequences were aligned using the online version of MAFFT v.7.490 (Katoh and Standley 2013; Katoh et al. 2019) and ambiguous sites were trimmed in SeaView v.5.0.4 (Gouy et al. 2010) using Gblocks v.0.91b (Castresana 2000), allowing less strict flanking positions. The alignments of three genes were concatenated in SeaView, and maximum likelihood (ML) analysis was performed using RAxML-NG v1.0.3 (Kozlov et al. 2019). The concatenated dataset was partitioned by each gene and appropriate substitution models for partitioned ML analysis were selected by Modeltest-NG v.0.2.0 (Darriba et al. 2020) based on the corrected Akaike information criterion (AICc). The applied substitution models for ML analysis were as follows: TIM3+I+G4 for SSU, SYM+I+G4 for ITS, and TIM2+I+G4 for LSU D1/D2. Branch supports for ML analysis were estimated by 1,000 bootstrap replicates.

Mating experiment of yeast cells

Intraspecific mating experiments were performed to confirm the mating type. In each species, single-basidiospore strains were established from the same basidiocarp. Pairs of the strains were inoculated on conjugation medium (CJM; Flegel 1981) and kept at 25 °C. Method for the inoculation was according to Flegel (1976), and inoculated cells were covered with flame-sterilized coverslips to reduce yeast cell growth and promote conjugation of yeast cells (Flegel 1981). One month after incubation, mycelia were cut off and inoculated onto weakly nutrient medium (wMY; Spiegel 1990) and kept at 25 °C to promote basidia and basidiocarp production. Those cultivations were directly observed under a light microscope by covering the surface of the media with coverslips, or mounted in phloxine with 3% potassium hydroxide or lacto-cotton blue.

Results & Discussion

Isolation and Morphological observation

We collected 4 and 2 specimens of *Sirobasidium* and *Sirotrema,* respectively. Their collecting sites, herbarium numbers, and single-basidiospore derived strains are listed in Table 1. According to the morphological observation, 3 specimens of *Sirobasidium* were identified as *Sb. japonicum* Kobayasi. The other specimen of the genus, however, could not be designated to any previously described species.

In newly collected specimens of *Sirobasidium japonicum,* microscopic features (morphology of basidia, epibasidia, and basidiospores) were fitted with the original description by Kobayasi (1962). Basidiocarps were closely associated with ascomata of *Biscogniauxia* spp. (Table 1; Fig. 1a), which has not been reported before.

Sirobasidium sp. was characterised by the combination of the following features: pulvinate to cerebriform and white to grayish-white basidiocarps associated with *Eutypella scoparia* (Schwein.) Ellis & Everh. (Fig. 2a, b), broadly ellipsoid basidia divided to 2-cells (Fig. 2c), fusiform to cylindrical epibasidia apiculate at the tip (Fig. 2d), subglobose basidiospores, which are (8.0–)8.5–10.5 × 7.0–8.5(–9.0) µm (Fig. 2e, f), and ellipsoid to oblong yeast cells, which are 3.0–5.5(–5.8) × (1.6–)1.7–3.6 µm (Fig. 2g). The species was similar to *Sb. albidum* Lagerh. & Pat., *Sb. brefeldianum* Möller, *Sb. intermedium* Kundhalkar & M.S. Patil and *Sb. sandwicense* Gilb. & Adask (Lagerheim and Patouillard 1892; Möller 1895; Kundalkar and Patil 1986; Gilbertson and Adaskaveg 1993). It can be distinguished from *Sb. albidum* by 2-celled basidia. From the other species, it can be distinguished by apiculate epibasidia.

The specimens of the genus *Sirotrema* were identified as *Sirotrema translucens* (H.D. Gordon) Bandoni 1986. Their basidiocarps parasitized *Lophodermium conigenum* (Brunaud) Hilitzer growing on fallen needles of Japanese red pine (*Pinus densiflora* Siebold et Zucc.; Fig. 3a). Their microscopic features agreed well with the descriptions by Gordon (1938), Reid and Minter (1979), and Bandoni (1986). Formation of basidia in chain was not detected, which also agreed with observations in the previous studies (Gordon 1938; Reid and Minter 1979).

The descriptions of each species are given in the taxonomy section.

Phylogeny

For the strains of *Sirobasidium japonicum, Sirobasidium* sp., and *Sirotrema translucens*, LSU D1/D2, ITS, and SSU were sequenced (Table. 1). In the ML analysis using these regions, all the species were located in the order Tremellales with a high support value (Fig. 4). In this order, *Sirobasidium* sp. (as "*Sb. apiculatum*") formed a well-supported clade with *Fibulobasidium* spp. (Fig. 4), which is characterised by basidia produced by the expansion of clamp connection and forming cluster (Bandoni 1979). This clade also formed a moderately supported clade with *Sb. magnum* (Fig. 4). Among the *Sirobasidium* species, *Sb. intermedium* CBS 7805 and *Sb. japonicum* were phylogenetically distant from other *Sirobasidium* spp., respectively; the former forming a well-supported clade with *Tremella exigua* and the latter a unique lineage (Fig. 4). Two strains of *St. translucens* (as *"Phaeotremella translucens"*) were shown to be not closely related to the above *Sirobasidium* spp. but included in the genus *Phaeotremella* (Fig. 4).

Physiological tests

The results of assimilation, fermentation, and other physiological tests were shown in Table 2. All the tested species did not ferment glucose. Two *Sirobasidium* species differed in the assimilation abilities of maltose, raffinose, melezitose, L-arabinose, D-ribose, L-rhamnose, ethanol, methyl-α-D-glucoside, D-glucuronic acid, and *N*-acetyl-D-glucosamine as a sole carbon source.

Mating experiments

As a result of the mating experiments on CJM, 9 tested strains of *Sirobasidium japonicum* formed true hyphae with clamp connections in all the pairs and single inoculations, but neither basidia nor basidiocarps (Table 3). The hyphae, which germinated from single yeast cells, also had clamp connections (Fig. 1g). Hyphal formation after conjugation of two yeast cells was detected only in some pairs (Table 3; Fig. 1h). After transferring the mycelia to wMY agar plates, basidia (Fig. 1i), epibasidia, and

basidiospores (Fig. 1j) were produced from the inoculations in which conjugation of yeast cells was detected on CJM (Table 3). These compatible pairs could be categorised into 4 groups (Table 3), suggesting that the species has a tetrapolar mating system as in *Sb. magnum* and other basidiomycetes (Flegel 1976; Raudaskoski and Kothe 2010).

In *Sirobasidium* sp., we performed a mating experiment using 8 single-basidiospore strains. Conjugation of yeast cells was not detected in all the pairs or single inoculations during one month on CJM or wMY. All the inoculations, however, produced true hyphae with false clamp and tremelloid haustoria (Fig. 2h, i). The results suggest that the true hyphae with false clamps derived from single-cell growth, and the strains would require other conditions to differentiate sexual reproduction.

In *Sirotrema translucens,* 8 single-basidiospore strains were crossed. In all the pairs or single inoculations, neither conjugations of yeast cells, formation of basidia nor hyphal growth were detected on both CJM and wMY during one month. As in *Sirobasidium* sp., cultural conditions would not be suitable for the species to induce sexual reproduction.

Taxonomy

The ML analysis demonstrated that *Sirobasidium* sp. is a novel lineage in the order Tremellales. Furthermore, as its morphology is different from any species in the genus *Sirobasidium*, a new taxon should be established. According to the ML tree, however, *Sb. intermedium* and *Sb. japonicum* were separated from the other *Sirobasidium* species (Fig. 4). Therefore, the genus is polyphyletic, which agrees with the previous studies (Boekhout et al. 2011; Millanes et al. 2011; Liu et al. 2015). In a phylogenetic tree in Liu et al. (2015), *Sb. intermedium* CBS 7805 formed a clade with another *Sirobasidium* species named *Sb. brefeldianum* AM71. However, since their sequences have the same origin represented by a single isolate (see Millanes et al. 2011), its phylogenetically isolated position needs to be confirmed by new isolates, as pointed out by Boekhout et al. (2011). Additionally, nucleotide sequence data of the type species (*Sb. sanguineum* Lagerh. & Pat.) has not yet been obtained. For these reasons, we keep the generic name *Sirobasidium* at present.

Sirobasidium japonicum was not closely related to the Auriculariales, but a member of Tremellales, although it did not form a supported clade with other *Sirobasidium* spp. (Fig. 4). Phylogenetic analyses of previous studies using LSU D1/D2 sequences yielded different results for monophyly between *Fibulobasidium* spp. and the three *Sirobasidium* spp. (*Sb. japonicum*, *Sb. magnum*, and *Sirobasidium* sp.). For example, Liu et al. (2015) and Kachalkin et al. (2019) showed that they formed a moderately supported monophyletic group (Sirobasidiaceae), whereas Li et al. (2020) showed that the monophyly of *Fibulobasidium* spp. and *Sirobasidium* sp. was well supported, but that of *Sb. magnum* and *Sb. japonicum* was not supported. Our phylogenetic analysis of LSU D1/D2 concatenated with SSU and ITS showed the monophyly of *Fibulobasidium* spp. and *Sirobasidium* sp. consistent with the above studies, but *Sb. magnum* was only weakly supported with the monophyletic group (*Fibulobasidium* spp. + *Sirobasidium* sp.) (bootstrap value = 64), and *Sb. japonicum* did not show monophyly with any of these species (Fig. 4). Therefore, the phylogenetic relationship between *Fibulobasidium* spp. and the three *Sirobasidium* species cannot be resolved by LSU D1/D2 alone, and the addition of SSU and ITS to the LSU D1/D2 dataset is still insufficient to resolve the phylogenetic relationships with the other members in Tremellales.

Sirotrema translucens and the genus *Sirobasidium* was not closely related from a phylogenetic point of view, but *St. translucens* formed a clade with the species of the genus *Phaeotremella* (Fig. 4), which was recently emended from the 'foliacea clade' recognized in the genus *Tremella* (Liu et al. 2015). In *St. translucens,* both morphological features of teleomorph and physiological traits of anamorph agree with those of the genus *Phaeotremella* (Liu et al. 2015). Therefore, it is reasonable that the species is transferred to the genus *Phaeotremella.*

Description of the species

Sirobasidium apiculatum M. Yamada, Endoh et Degawa *sp. nov.*

MycoBank No.: MB 821218 (Fig. 2)

Etymology: referring to its apiculate epibasidia.

Basidiocarp gelatinous, pulvinate to cerebriform, 2–4.5 mm in diam., white to grayish-white (Fig. 2a). Basidia basipetally formed in chain, broadly ellipsoid, 13.0–18.0(–21.0) × 8.5–11.5 µm, divided 2-cells by a longitudinal septum (Fig. 2c). Epibasidia fusiform to cylindrical, apiculate at the tip, 17.5–26.0 × 6.0–7.0 µm (Fig. 2d), passively detached from the top of basidia, producing a sterigma from lateral side (Fig. 2e). Basidiospores actively discharged from the tip of sterigmata, subglobose, (8.0–) 8.5–10.5 × 7.0–8.5(–9.0) µm (Fig. 2e, f), germinating by budding or repetition. Hyphae with clamp connection, 1.5–2.5 µm in diam. in hymenium layer, 3.0 µm in diam. under hymenium layer, anastomoses. After 3 days incubation of the yeast cells on 5% MEA at 25 °C, yeast cells ellipsoid to oblong, 3.0–5.5 (–5.8) × (1.6–) 1.7–3.6 µm (Fig. 2g), colony surface shiny, white to cream. Physiological and biochemical characters are shown in Table 2.

Type materials: **holotype** TNS-F-66691 (deposited to the National Museum of Nature and Science, Ibaraki, Japan), JAPAN, Nishiagina, Amagi-cho Oshima-gun, Kagoshima Pref., 27°46' N 128°57' E, 24 Jun 2014, col. M. Yamada. Ex-holotype culture JCM 32018 (= CBS 14977) was established from a basidiospore obtained from a basidiocarp (TNS-F-66691) on a fallen branch of broadleaf tree, associated with *Eutypella scoparia* (Schwein.) Ellis & Everh. A single-basidiospore isolate JCM 32019 (= CBS 14978) was also established from the same basidiocarp.

Notes: Although *Sb. apiculatum* was associated with *Eutypella scoparia,* we could not observe tremelloid haustoria in a basidiocarp. Nevertheless, there are many reports of co-occurrence of sirobasidiaceous fungus with Xylariales (e.g., Chen 1998; Gilbertson and Adaskaveg 1993), no reports of tremelloid haustoria were there until Bandoni et al. (2011) mentioned the rare formation of them at the interface with associated ascomycetous stroma in *Sb. magnum.* Although tremelloid haustoria were not detected during the observation of the basidiocarp in *Sb. apiculatum*, their formation under the cultural conditions suggests the mycoparasitic nature of the species.

Sirobasidium japonicum Kobayasi, Trans. Mycol. Soc. Japan 4: 29 (1962)

MycoBank No.: MB 339295 (Fig. 1)

Basidiocarps pulvinate to applanate-cerebriform, white to pale yellow (Fig. 1a). Basidia basipetally formed in chain, cylindrical to ellipsoid, (15.0–)17.5–32.5 × 5.0–8.0 µm, divided to 4-cells by transverse to oblique septa (Fig. 1b, c). Epibasidia fusiformis, (7.5–)9.0–15.0(–20.5) × 4.0–7.0 µm, producing a sterigma from lateral side (Fig. 1c, d). Basidiospores globose to subglobose, 4.0–7.0(–7.5) × 3.5–6.5 µm, actively discharged from the tip of sterigmata (Fig. 1d, e), germinating by budding or repetition. After 3 days incubation of the yeast cells on 5% MEA at 25 °C, yeast cells globose to subglobose, (2.5–)3.0–5.0 × (2.1–)2.5–4.2 µm (Fig. 1f), colony surface shiny, white to cream colour. Physiological and biochemical characters are shown in Table 2.

Materials examined: JAPAN, Nagata, Kumage-gun, Yakushima-cho, Kagoshima Pref., 24 Oct 1961, col. H. Indoh, TNS-F-196751 (holotype); JAPAN, Mt. Yonaha, Hiji, Kunigami-gun, Kunigami-son, Okinawa Pref., 26°43'19.3"N 128°12'54"E, alt. 360 m, on a fallen branch of *Alnus japonica* (Thunb.) Steud. var. *formosana* (Burkill) Callier, associated with *Biscogniauxia capnodes* (Berk.) Y.M. Ju & J.D. Rogers, 13 Oct 2013, TNS-F-66692, its compatible single-basidiospore isolates deposited as JCM 32020 (= CBS 14979 = PYCC 6704), JCM 32021 (= CBS 14980 = PYCC 6706), PYCC 6703, and PYCC 6705; JAPAN, Mt. Nishime, Uka, Kunigami-gun, Kunigami-son, Okinawa Pref., 26°48'32.364" N 128°16'19.085" E, alt. 350 m, on a fallen branch of *Styrax japonica* Siebold et Zucc., associated with *Biscogniauxia capnodes* (Berk.) Y.M. Ju & J.D. Rogers, 13 Oct 2013, col. M. Yamada, TNS-F-66693; JAPAN, Susami, Susami-cho, Nishimuro-gun, Wakayama Pref., 33°33'36.26" N 135°32'27.99" E, alt. 85 m, on a fallen branch of broadleaf tree, associated with *Biscogniauxia* sp., 19 Jul 2015, col. K. Yamamoto, TNS-F-66694.

Notes: *Sirobasidium japonicum* had been reported only from Yakushima, Kagoshima, Japan and Jianfengling, Hainan, China (Kobayasi 1962; Peng and Liu 1992), and this is the first report of the isolates and their nucleotide sequence data. The type material was deposited in the TNS herbarium, but its number was not mentioned in the original description (Kobayasi 1962). We found one specimen of *Sb. japonicum* numbered TNS-F-196751. Although the collection date (24. Oct. 1961) was one day earlier than that of the description (Kobayasi 1962), the other data (locality and collector) were identical. In the specimen, any basidiospores were not found, however, basidia (22–32 × (3.0–)5.0–7.0 µm) and epibasidia (10.5–13.0 × 4.0–6.0 µm) were observed and corresponded to those in the original description (Kobayasi 1962). As a result, it is reasonable to regard the specimen (TNS-F-196751) as the type material.

Phaeotremella translucens (H.D. Gordon) M. Yamada, Endoh et Degawa *comb. nov.*

Mycobank No.: MB 821219 (Fig. 3)

Basionym: *Tremella translucens* H.D. Gordon, Transactions of the British Mycological Society 22 (1-2): 111 (1938); MB 280499.

≡*Pseudostypella translucens* (H.D. Gordon) D.A. Reid & Minter, Transactions of the British Mycological Society 72 (2): 345 (1979); MB 321880.

≡*Sirotrema translucens* (H.D. Gordon) Bandoni, Canadian Journal of Botany 64 (3): 674 (1986); MB 103818.

Basidiocarps pulvinate, translucent, 0.5–1.0 mm in diam., 0.5 mm high (Fig. 3a). Basidia spherical, 4-celled with longitudinal septa (tremelloid), 9.0–11.0(–13.0) × 8.0–10.0 µm, formed singly or in cluster at the tip of hyphae (Fig. 3b). Basidiospores ovoid, (3.5–)4.0–5.5 × (7.0–)8.0–11.0 µm (Fig. 3c), germinating by budding. Hyphae 1.0 µm in diam., lack vesicles and swollen cells (*sensu* Chen 1998), with clamp connections, bearing tremelloid haustoria (Fig. 3e). In some cases, hyphae swollen up to 4.5 µm beside clamp connections (Fig. 3d). After 3 days on 5% MEA at 25 °C, yeast cells ellipsoid to oblong, 4.9–8.3(–9.2) × (2.8–)3.0–5.0(–5.3) µm (Fig, 3f), colony surface dull, pale orange. Physiological and biochemical characters are shown in Table 2.

Materials examined: JAPAN, Sugadaira Montane Research Center (now as Sugadaira Research Station), University of Tsukuba, Sugadaira-Kogen, Ueda, Nagano Pref., 36°31'20.7"N 138°21'2.2"E, alt. 1327 m, on fallen leaves of *Pinus densiflora* Siebold et Zucc., associated with *Lophodermium conigenum* (Brunaud) Hilitzer, 24 Aug 2014, col. M. Yamada, TNS-F-66695; JAPAN, Sugadaira Montane Research Center, University of Tsukuba, Sugadaira-Kogen, Ueda, Nagano Pref., 36°31'20.7"N 138°21'2.2"E, alt. 1327 m, on fallen leaves of *Pinus densiflora* Siebold et Zucc., associated with *Lophodermium conigenum* (Brunaud) Hilitzer, 20 Oct 2014, col. M. Yamada, TNS-F-66696, its single-basidiospore isolates deposited as JCM 32022 (= CBS 14981) and JCM 32023 (= CBS 14982).

Notes: Formation of basidia in chain was not detected in the specimen examined, which is consistent with some previous reports (Gordon 1938; Reid and Minter 1979). Bandoni (1986) indicated the different frequencies of basidia in chain among collections. These inconsistent observations suggest that the formation of basidia in chain is an unstable characteristic.

In the genus *Phaeotremella*, *P. mycetophiloides* (Kobayasi) Millanes & Wedin, *P. mycophaga* (G.W. Martin) Millanes & Wedin, and *P. simplex* (H.S. Jacks. & G.W. Martin) Millanes & Wedin are also known as mycoparasites other than *P. translucens*. They parasitize basidiomycetous hosts, *Aleurodiscus* spp. (Kobayasi 1939; Martin 1940; Bandoni and Ginns 1993), and they have hyphal swellings near clamp connections similar to *P. translucens* (Bandoni and Ginns 1993). While phylogenetic relationships of the mycoparasitic species in the genus are still not clear, this character appears to be common to the mycoparasitic taxa in the genus.

Declarations

Acknowledgments

This manuscript was almost drafted by Muneki Yamada, a young talented personable postgraduate student who had an enthusiasm for the study of fungi, especially Tremellomycetes, although sadly passed away at the age of 25 on 20 May 2017, by an unexpected accident. We are very grateful to the member of the Laboratory of Mycology, Sugadaira, Dr. Takamichi Orihara (KPM), Dr. Kyohei Watanabe (KPM), Dr. Mitsuru Moriguchi of Okinawa University, Dr. Yusuke Takashima and Dr. Kohei Yamamoto for providing specimens and supports in sampling. We would like to thank Dr. Tsuyoshi Hosoya (TNS) for his kind supports for loaning a specimen.

Funding

This study was partially funded by JSPS KAKENHI Grant Number 15K18720 to RE, and JSPS KAKENHI Grant Number 19H03281 to YD and HM.

Comepeting of interest

The authors declare that they have no conflict of interest.

Authors' contribution

Muneki Yamada and Yousuke degawa designed the study; Muneki Yamada performed field sampling, isolation of the fungi, morphological observation, molecular characterization, and wrote the original draft; Hiroshi Masumoto and Yuma Yoshihashi performed phylogenetic analysis; Muneki Yamada and Rikiya Endoh performed phenotypic characterization; Yousuke degawa, Rikiya Endoh, Moriya Ohkuma, and Hiroshi Masumoto edited the original draft; Yousuke degawa and Moriya Ohkuma supervised the study. All authors approved the final manuscript.

Data availability

Ethical approval

This article does not contain any studies with human and animals.

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Tables

Table 1 List of specimens, strains and their sequence depositions

Specimen	Collected site	Associated fungus	Strain*	DDBJ accession numbers		
				ITS	LSU D1/D2	SSU
Sirobasidium apiculatum						
TNS-F-66691^{T}	Sirobasidium apiculatum Nishiagina, Amagi-cho Oshima-gun, Kagoshima Pref., Japan, 27°46'N 128°57'E	*Eutypella scoparia*	JCM 32018^{T} (= CBS 14977^{T})	LC203425	LC203426	LC203427
			JCM 32019 (= CBS 14978)	LC203428	LC203429	LC203430
Sirobasidium japonicum						
TNS-F-66692	Mt. Yonaha, Hiji, Kunigami-gun, Kunigami-son, Okinawa Pref., Japan, 27°46'N 128°57'E	*Biscogniauxia capnodes*	JCM 32020 (= CBS 14979 =PYCC 6704)	LC203420	LC016573	LC203421
			JCM 32021 (= CBS 14980 = PYCC 6706)	LC203422	LC203423	LC203424
			PYCC 6703	nd†	nd	nd
			PYCC 6705	nd	nd	nd
TNS-F-66693	Mt. Nishime, Uka, Kunigami-gun, Kunigami-son, Okinawa Pref., Japan, 26°48'32.364"N 128°16'19.085"E	*Biscogniauxia capnodes*		nd	nd	nd
TNS-F-66694	Susami, Susami-cho, Nishimuro-gun, Wakayama Pref., Japan, 33°33'36.26"N 135°32'27.99"E	*Biscogniauxia* sp.		nd	nd	nd
***Phaeotremella translucens* (= *Sirotrema translucens*)**						
TNS-F-66696	Sugadaira Montane Research Center, University of Tsukuba, Sugadaira-Kogen, Ueda, Nagano Pref., Japan, 36°31'20.7"N 138°21'2.2"E	*Lophodermium conigenum*	JCM 32022 (= CBS 14981)	LC203431	LC203432	LC203433
TNS-F-66695	Sugadaira Montane Research Center, University of Tsukuba, Sugadaira-Kogen, Ueda, Nagano Pref., Japan, 36°31'20.7"N 138°21'2.2"E	*Lophodermium conigenum*	JCM 32023 (= CBS 14982)	LC203434	LC203435	LC203436

*T, holotype or ex-holotype strain; JCM, Japan Collection of Microorganisms, Tsukuba, Japan, CBS, Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands, PYCC, Portuguese Yeast Culture Collection, Caparica, Portugal

†nd, not determined

Table 2 Physiological traits of *Sirobasidium japonicum, Sirobasidium apiculatum,* and *Phaeotremella translucens*

Physiological test	*Sb. japonicum*	*Sb. apiculatum*	*P. translucens*
Assimilation of carbon source			
Glucose	+	+	+
Galactose	−	w	+
L-Sorbose	w	−	−
Sucrose	+	v	−
Maltose	+	−	s/−
Cellobiose	+	w	−
Trehalose	+	+	+
Lactose	−	−	−
Melibiose	−	−	−
Raffinose	+	−	−
Melezitose	+	−	w/−
Inulin	−	−	−
Soluble starch	−	−	v
D-Xylose	+	+/−	+
L-Arabinose	+	−	−
D-Arabinose	+/s	−/w	+
D-Ribose	+	−	w
L-Rhamnose	+	−	−
Ethanol	+	−	−
Methanol	−	−	−
Glycerol	−	−	−
meso-Erythritol	+/s	−	−
Ribitol	w	s/w	+
Galactitol	w	s	−
D-Mannitol	+	+	+
D-Glucitol	+/w	w	w/−
Methyl-α-D-glucoside	+	−	−
Salicin	w	−	−
Arbutin	+	+	+
Glucono-δ-lactone	+	−/w	+

2-Keto-gluconic acid	+/s	w	w/−
5-Keto-gluconic acid	w/−	w	w/−
DL-Lactic acid	−	−	−
Succinic acid	−	−/w	−
Citric acid	w	−	−
myo-Inositol	−	−	−
D-Glucuronic acid	+	−	+
D-Galacturonic acid	+	−/w	+
D-Gluconic acid	+	w	s/w
D-Glucosamine	w	−	−
D-Glucurono lactone	+	+	+/w
L-Arabinitol	w	−	−
Quinic acid	−	−	−
Saccharic acid	+	+	−
Propyleneglycol	−	−	−
Xylitol	w/−	−	+
N-Acetyl-D-glucosamine	+	−	−
Butane-2,3-diol	−	−	−
Xylo-oligosaccharide	−	−	−
n-Decane	−	−	−
n-Hexadecane	−	−	−
Assimilation of nitrogen source			
Ammonium sulfate	+	+	+
Potassium nitrate	−	−	−
Sodium nitrite	−	−	+
Etylamine hydrochloride	+	+	−

Lysine hydrochloride	+	+	−
Cadaverine dihydrochloride	+	+	−
Creatine	−	−	−
Creatinine	−	−	−
D-glucosamine	+	−	−
Imidazole	−	−	−
Growth at			
25 °C	+	+	+
30 °C	−	+	−
35 °C	−	−	−
Other tests			
Cycloheximide 0.01%	+	−	−
Cycloheximide 0.1%	−	−	−
10% NaCl	−	−	−
Vitamin free	−	−	−
Gelatin liquefaction	−	−	−
Acid production	−	−	−
1% Acetic acid	−	−	−
50% Glucose	−	−	−
Urease	+	+	+
Starch formation	−	−	−
Fermentation (D-Glucose)	−	−	−
DBB test	+	+	+

+, Positive; s, slowly positive; w, weakly

positive; −, negative; v, variable

Table 3 Reactions in the paired or single inoculations of *Sirobasidium japonicum* on CJM, and those after transferring the mycelium from CJM onto wMY

			Strains								
			type 1				type 2		type 3	type 4	
			MY111-02	MY111-03	MY111-04	JCM 32020	PYCC 6703	MY111-06	JCM 32021	MY111-07	PYCC 6705
Strains	type 1	MY111-02		+/+	+/+	+/+	+/+	+*/B	+*/B	+/+	+/+
		MY111-03			+/+	+/+	+/+	+*/B	+*/B	+/+	+/+
		MY111-04				+/+	+/+	+*/B	+*/B	+/+	+/+
		JCM 32020					+/+	+*/B	+*/B	+/+	+/+
	type 2	PYCC 6703						+/+	+/+	+*/B	+*/B
	type 3	MY111-06							+/+	+/+	+/+
		JCM 32021								+/+	+/+
	type 4	MY111-07									+/+
		PYCC 6705									
Single inoculations			+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+

Left, reactions of inoculated yeast cells on CJM; right, reactions of the mycelium transferred from CJM onto wMY. +, true hyphae with clamp connections; +*, true hyphae from conjugated yeast cells; B, formation of basidia and basidiospores. All the strains were derived from a single basidiocarp (TNS-F-66692)

Figures

Figure 1

Sirobasidium japonicum (TNS-F-66693; JCM 32020; JCM 32021). **a** Basidiocarp on a fallen branch (arrowhead) associated with *Biscogniauxia capnodes* (arrow). **b** Transversally to obliquely septate, 4-celled basidia in chain. **c** Basidia producing epibasidia. **d** Epibasidia forming a sterigma and a basidiospore. **e** Basidiospores. **f** Yeast cells on 5% MEA, 3 days at 25°C. **g** Yeast cells of JCM 32020 forming true hyphae with a clamp connection (double-arrowhead) on CJM, 3 days at 25°C. **h** True hyphae with a clamp connection (double-arrowhead) formed after mating between JCM 32020 and JCM 32021 on CJM, 3 days at 25°C. **i** Basidia on wMY, after transferring the hyphae formed between the mating on CJM. **j** An epibasidium and a basidiospre formed after the basidial formation on wMY. Bars, **a** 1 cm, **b-j**, 10 µm

Figure 2

Sirobasidium apiculatum (TNS-F-66691; JCM 32018). **a** Basidiocarp on a fallen branch. **b** Associated fungus, *Eutypella scoparia* (arrows). **c** Two-celled basidia (left) and collapsed basidia in chain (right). **d** Matured epibasidia with apiculate tips. **e** Epibasidia forming sterigmata and basidiospores. **f** Basidiospore. **g** Yeast cells on 5% MEA, 3 days at 25°C. **h, i** True hyphae with a tremelloid haustorium and basal false clamp (arrowhead) from 3 days cultures on CJM agar. Bars, **a** 5 mm, **b** 1 mm, **c-i** 10 µm

Figure 3

Phaeotremella translucens (TNS-F-66693; JCM 32023). **a** Basidiocarps (arrowheads) growing on *Lophodermium conigenum* (arrows). **b** Basidia. **c** Basidiospores. **d** Swollen nodes near clamp connections (double arrowheads). **e** Tremelloid haustoria. **f** Yeast cells on 5% MEA, 3 days at 25 °C. Bars, **a** 1 mm, **b-f** 10 µm

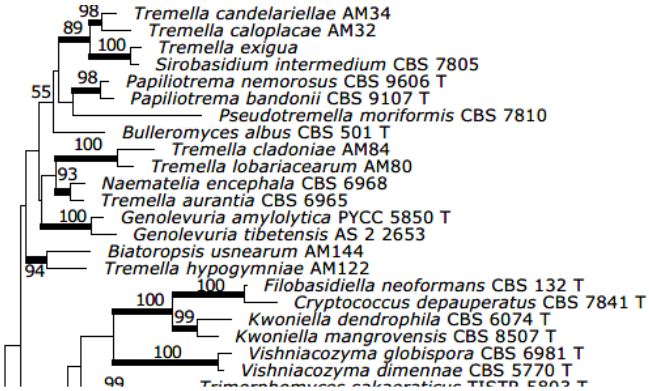

Figure 4

ML tree of the Tremellales and related basidiomycetes, based on the LSU D1/D2, ITS and SSU sequences. Bootstrap values over 50% were shown on each branch. Branches with bootstrap values over 70% were indicated by thick lines. The scale bar represents

nucleotide substitution per site

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