



Full paper

Taxonomic and ecological significance of synnema-like structures/acanthophyses produced by *Physisporinus* (*Meripilaceae*, *Polyporales*) species

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ABSTRACT

Physisporinus, a genus in *Polyporales*, *Basidiomycota*, is a versatile fungus that lives as a wood decomposer, a potential pathogen of standing trees, and an orchid mycobiont. We previously reported that some *Physisporinus* species inhabiting wet wood in aquatic environments such as streams and waterfalls form synnema-like structures (SSs) bearing acanthophyses at their apices, and that they produce acanthophyses on vegetative hyphae when cultured on agar media. In this study, we investigated the acanthophysis-forming ability in *Physisporinus* and allied genera, and experimentally demonstrated the function of SSs. Phylogenetic analyses and observations of *Meripilus*, *Physisporinus* and *Rigidoporus* cultures showed that all of the strains forming acanthophyses belonged to *Physisporinus*, whereas strains of *Meripilus* and *Rigidoporus* did not produce acanthophyses. These findings suggest that SS/acanthophysis formation is a useful taxonomic character for members of *Physisporinus*. When *Physisporinus* strains were cultured under oxygen (O₂) concentrations of 5, 10, 20 and 40%, most of those cultured under 20% O₂ formed the most acanthophyses. According to these experimental data, the SSs/acanthophyses in *Physisporinus* were considered to have a respiratory function. *Physisporinus* probably acquired the SS/acanthophysis-forming ability to adapt to moist and/or aquatic habitats and to decay wet wood in which the O₂ concentration is often low.

Keywords: freshwater, *Meripilus*, oxygen concentration, phylogenetic analysis, *Rigidoporus*

Article history: Received 5 July 2023, Revised 14 September 2023, Accepted 18 September 2023, Available online 20 November 2023.

1. Introduction

Physisporinus P. Karst. is a poroid fungus in *Meripilaceae*, *Polyporales*, *Agaricomycetes*, *Basidiomycota* (Justo et al., 2017). This cosmopolitan genus mainly decays dead broad-leaved and coniferous trees (Breitenbach & Kränzlin, 1986; Dai, 2012; Gilbertson & Ryvardeen, 1987; Núñez & Ryvardeen, 2001; Ryvardeen et al., 2022; Ryvardeen & Gilbertson, 1994; Ryvardeen & Melo, 2017). Some species may cause butt rot in living Japanese cedars [Noguchi et al., 2007 (as *Basidiomycete*-B)], or establish mycorrhizal relationships with mycoheterotrophic orchids (Yamashita et al., 2020). Based on morphological and phylogenetic studies of aquatic fungi inhabiting wet wood in streams, we previously reported that five clades of fungi in the genus *Physisporinus*, i.e., two new species (*P. microacanthophysis* Shino, Sotome & Nakagiri and *P. rhizomorphae* Shino, Sotome & Nakagiri) and three unidentified groups (*P. cf. 1 eminens*, *P. cf. 2 eminens*, and *P. cf. furcatus*) form synnema-like structures (SSs) and produce numerous acanthophyses at their apices (Shino et al., 2022). Since cultures isolated from SSs or basidiocarps pro-

duced several types of acanthophyses on agar media, we considered that these characters might be useful as a taxonomic trait of *Physisporinus*. However, our previous study did not examine whether genera that are closely related to *Physisporinus* also produce SSs/acanthophyses. Phylogenetically, *Physisporinus* is closely related to both *Meripilus* P. Karst. (Chen & Dai, 2021; Shino et al., 2022; Tomšovský et al., 2010), which is the type genus of the *Meripilaceae* (Binder et al., 2013; Jülich, 1981; Justo et al., 2017), and *Spongipellis* Pat. (Kotiranta et al., 2017; Spirin et al., 2022; Wang & Dai, 2022). *Spongipellis* forms basidiocarps composed of a duplex context and generative hyphae with clamp connections (Ryvardeen, 1991; Spirin et al., 2022) and can be distinguished from *Physisporinus* having a simplex context and generative hyphae without clamp connections in the basidiocarps (Gilbertson & Ryvardeen, 1987). *Meripilus* species produce pileate basidiocarps with single to numerous brownish pilei arising from a short stipe or a base. This type of basidiocarp differs from the whitish resupinate basidiocarps found in *Physisporinus*, but the micromorphological features in basidiocarps of *Meripilus* (monomitic hyphal system, generative hyphae without clamp connections, and smooth and broadly ellipsoid to subglobose basidiospores with inamyloid reaction in Melzer's reagent) are similar to those found in *Physisporinus* (Gilbertson & Ryvardeen, 1987). These characteristics are also observed in

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Leucophellinus Bondartsev & Singer, *Oxyporus* (Bourdot & Galzin) Donk, and *Rigidoporus* Murrill, which have been phylogenetically assigned to *Hymenochaetales* (Wu et al., 2017). *Leucophellinus*, which produces clavate and occasionally septate cystidia and has distinctly thick-walled basidiospores (Núñez & Ryvardeen, 2001), is distinguishable from *Physisporinus* and related genera. Many species of *Oxyporus* and *Rigidoporus* resemble each other in that they form cystidia in their basidiocarps, but these taxa differ from *Physisporinus*, which has no cystidia (Ryvardeen & Gilbertson, 1994). Regarding the former two genera, Pouzar (1966) treated *Oxyporus* as a subgenus of *Rigidoporus* because of their morphological similarities including the above features. However, several mycologists (Corner, 1987; Donk, 1967; Ryvardeen & Johansen, 1980) proposed that these genera should remain separate because *Rigidoporus* typically produces basidiocarps with bright colors and forms cystidia in tramae while *Oxyporus* has pale-colored basidiocarps with cystidia in hymenia (Ryvardeen, 1991). Recently, Wu et al. (2017) integrated *Oxyporus* into *Rigidoporus* since their phylogenetic analysis showed that the type species of the two genera grouped in the same clade in *Hymenochaetales*. Moreover, they transferred part of the remaining species of *Rigidoporus*, which were found belonging to *Polyporales*, to *Physisporinus*. As a result of this and other studies, *Physisporinus* currently accommodates several species that form apically encrusted cystidia [e.g., *P. eminens* (Y.C. Dai) F. Wu, Jia J. Chen & Y.C. Dai, formerly treated as *R. eminens* Y.C. Dai (Dai, 1998); *P. furcatus* (Núñez & Ryvardeen) F. Wu, Jia J. Chen & Y.C. Dai, formerly *R. furcatus* Núñez & Ryvardeen (Núñez et al., 2001); *P. lineatus* (Pers.) F. Wu, Jia J. Chen & Y.C. Dai, formerly *R. lineatus* (Pers.) Ryvardeen (Ryvardeen, 1972; Ryvardeen & Johansen, 1980); *P. pouzarii* (Vampola & Vlasák) F. Wu, Jia J. Chen & Y.C. Dai, formerly *R. pouzarii* Vampola & Vlasák (Vampola & Vlasák, 2012)] and have pore surfaces with vivid colors when fresh [e.g., *P. lavendulus* F. Wu, Jia J. Chen & Y.C. Dai (Wu et al., 2017); *P. roseus* Jia J. Chen & Y.C. Dai (Chen & Dai, 2021); *P. sulphureus* Y.C. Dai (Dai & Dai, 2018)]. Thus, *Physisporinus* and *Rigidoporus* have become difficult to clearly distinguish by the morphology of their basidiocarps.

Hence, the first objective of this study is to evaluate the taxonomic significance of SS/acanthophysis formation among *Physisporinus*, phylogenetically related genus *Meripilus* and morphologically similar genus *Rigidoporus*, and to identify other taxonomically important characters found in their cultures, such as formation of clamp connections at hyphal septa, as well as the presence of conidia and plectenchymata in mycelia.

We previously reported that acanthophyses on the apices of SSs are not conidia because they neither easily detach from SSs nor germinate hyphae (Shino et al., 2022). Since SSs have been often found at the water-boundary part of wet wood in aquatic environments such as streams and waterfalls, we hypothesized that the SSs of *Physisporinus* may be associated with the respiration for mycelia creeping in the water-saturated wood tissue where oxygen (O_2) levels tend to be lower than in the atmosphere. Therefore, as the second objective of this study, we aim to verify this hypothesis by experiments using cultures of *Physisporinus* and to discuss the ecological significance of SSs/acanthophyses.

2. Materials and methods

2.1. Samples

24 specimens and 41 strains were tested in this study (Figs. 1, 6; Table 1). Procedures for the establishment of dried specimens and living isolates followed Shino et al. (2022). We also used strains preserved in the Fungus/Mushroom Resource and Research Center (FMRC), Faculty of Agriculture, Tottori University, and strains obtained from the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands (Table 1).

2.2. Molecular phylogeny

2.2.1. DNA extraction, amplification and sequencing

DNA extraction from mycelia cultured on agar media was performed using a modified cetyltrimethylammonium bromide

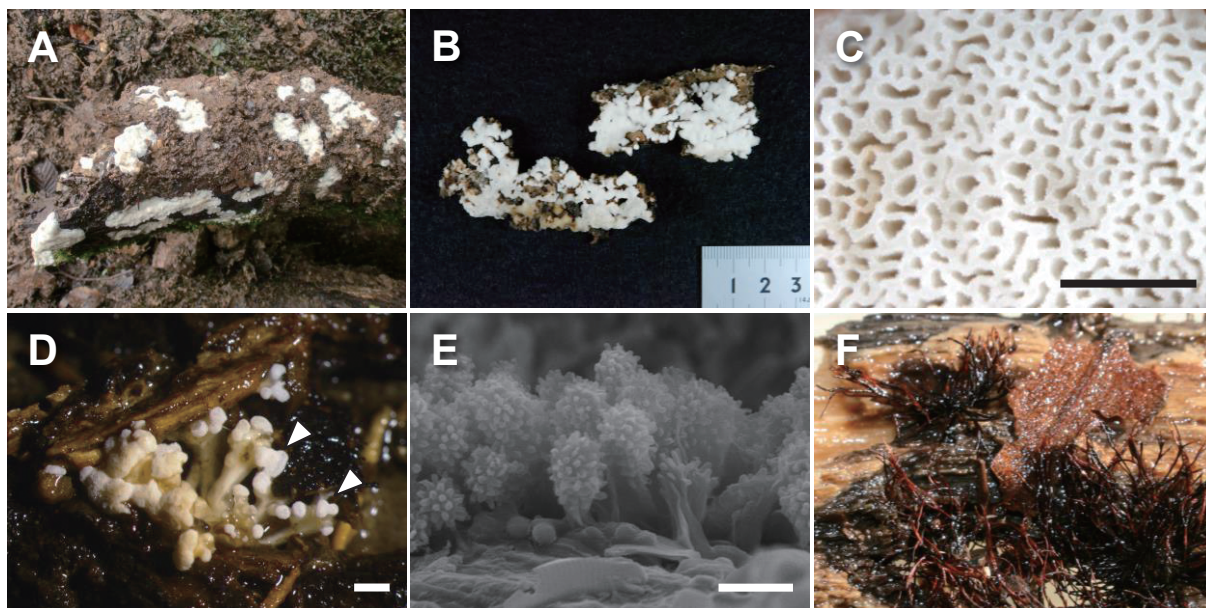


Fig. 1 – Basidiocarps (A–C), SSs (D) bearing acanthophyses (E), and rhizomorphs (F) of *Physisporinus* in nature. A: Whitish basidiocarps produced on wet wood nearby streams (*P. cf. 2 eminens* TUMH 65445). B: Basidiocarps (*P. cf. 1 eminens* TUMH 65440). C: Pore surface of basidiocarps (*P. cf. 1 eminens* TUMH 65442). D: SSs on the water-boundary part of wood in streams (the source for *P. pouzarii* TUF 101965). Arrowheads show parts forming acanthophyses. E: Acanthophyses on the apex of SS (*P. microacanthophysis* TUMH 64311). F: Rhizomorphs on the submerged part of wood in streams (*P. rhizomorphae* TUMH 64298). Bars: C, D 1 mm; E 30 μ m.

Table 1. Data of samples used in this study.

Species name	Strain No. ^a	Herbarium Specimen No.	Locality	Collection date	Isolation date	Source ^b	Habitat ^c
<i>Meripilus giganteus</i>	CBS 421.48	–	Germany	–	–	–	–
<i>M. giganteus</i>	TUFC 100564	(TUMH 60367) ^d	Tottori Pref., Japan	17 Aug 2012	17 Aug 2012	T	F
<i>Physisporinus</i> cf. 1 <i>eminens</i>	TUFC 101880	TUMH 64307	Miyagi Pref., Japan	22 Sep 2014	22 Sep 2014	S	A
<i>P. cf. 1 eminens</i>	TUFC 101957	TUMH 65440	Tottori Pref., Japan	05 Nov 2019	05 Nov 2019	B	A
<i>P. cf. 1 eminens</i>	TUFC 101958	TUMH 65441	Tottori Pref., Japan	05 Nov 2019	05 Nov 2019	B	A
<i>P. cf. 1 eminens</i>	TUFC 101959	TUMH 65442	Tottori Pref., Japan	05 Nov 2019	05 Nov 2019	B	A
<i>P. cf. 2 eminens</i>	TUFC 101881	TUMH 64308	Tottori Pref., Japan	25 Jun 2014	25 Jun 2014	S	A
<i>P. cf. 2 eminens</i>	TUFC 101960	TUMH 65443	Hokkaido Pref., Japan	26 Sep 2017	26 Sep 2017	B	F
<i>P. cf. 2 eminens</i>	TUFC 101961	TUMH 65444	Tottori Pref., Japan	14 Oct 2017	14 Oct 2017	B	A
<i>P. cf. 2 eminens</i>	TUFC 101962	TUMH 65445	Tottori Pref., Japan	30 Oct 2019	30 Oct 2019	B	A
<i>P. cf. furcatus</i>	TUFC 101883	TUMH 64310	Gifu Pref., Japan	29 May 2012	29 May 2012	S	A
<i>P. cf. furcatus</i>	TUFC 101884	TUMH 64310	Gifu Pref., Japan	29 May 2012	29 May 2012	S	A
<i>P. crocatus</i>	CBS 107806	–	Canada	–	1982	–	–
<i>P. lineatus</i>	CBS 167.65	–	USA	–	24 Aug 1960	–	–
<i>P. lineatus</i>	CBS 700.94	–	Germany	–	–	–	–
<i>P. lineatus</i>	CBS 109425	–	Taiwan	27 Aug 1996	–	–	–
<i>P. lineatus</i>	TUFC 13809	TUMH 60931	Tokyo Metropolis, Japan	19 Nov 2010	–	T	F
<i>P. lineatus</i>	TUFC 13812	TUMH 60932	Tokyo Metropolis, Japan	19 Nov 2010	–	T	F
<i>P. microacanthophysis</i>	TUFC 101885	TUMH 64311	Tottori Pref., Japan	07 Jul 2011	07 Jul 2011	S	A
<i>P. microacanthophysis</i>	TUFC 101888	TUMH 64312	Miyazaki Pref., Japan	24 Nov 2013	24 Nov 2013	S	A
<i>P. microacanthophysis</i>	TUFC 101889	TUMH 64313 ^{Tc}	Tottori Pref., Japan	23 Oct 2019	23 Oct 2019	B	A
<i>P. microacanthophysis</i>	TUFC 101963	TUMH 65446	Tottori Pref., Japan	04 Apr 2020	16 Apr 2020	S	A
<i>P. microacanthophysis</i>	TUFC 101964	TUMH 65447	Tottori Pref., Japan	19 May 2020	19 May 2020	B	A
<i>P. pouzarii</i>	TUFC 101965	No specimen	Osaka Pref., Japan	01 Apr 2013	01 Apr 2013	S	A
<i>P. pouzarii</i>	TUFC 101966	TUMH 65448	Tottori Pref., Japan	05 Nov 2019	05 Nov 2019	B	A
<i>P. rhizomorphae</i>	TUFC 101870	TUMH 64297	Tottori Pref., Japan	24 Sep 2013	24 Sep 2013	S	A
<i>P. rhizomorphae</i>	TUFC 101871	TUMH 64298	Tottori Pref., Japan	19 Oct 2014	19 Oct 2014	R	A
<i>P. rhizomorphae</i>	TUFC 101876	TUMH 64303 ^T	Tottori Pref., Japan	14 Oct 2017	14 Oct 2017	B	A
<i>P. rhizomorphae</i>	TUFC 101967	TUMH 65449	Tottori Pref., Japan	04 Apr 2020	08 May 2020	S	A
“ <i>P. sanguinolentus</i> ”	CBS 139.76	–	Belgium	Sep 1975	–	–	–
“ <i>P. sanguinolentus</i> ”	CBS 193.76	–	Netherlands	–	–	–	–
“ <i>P. sanguinolentus</i> ”	CBS 679.70	–	USA	–	–	–	–
“ <i>P. sanguinolentus</i> ”	CBS 107146	–	Denmark	–	1980	–	–
<i>Physisporinus</i> sp.	TUFC 101892	TUMH 64316	Kagoshima Pref., Japan	04 Sep 2018	04 Sep 2018	B	A
<i>Physisporinus</i> sp.	TUFC 101968	TUMH 65450	Tottori Pref., Japan	05 Sep 2020	05 Sep 2020	T	F
<i>Rigidoporus ulmarius</i>	CBS 186.60	–	USA	05 Nov 1952	–	T ^f	–
“ <i>R. vinctus</i> ”	CBS 153.84	–	New Zealand	–	16 Oct 1973	–	–
“ <i>R. vinctus</i> ”	CBS 174.71	–	Costa Rica	20 Jun 1963	–	–	–
“ <i>R. vinctus</i> ”	TUFC 11175	(TUMH 60851)	Kagoshima Pref., Japan	19 Sep 2007	19 Sep 2007	B	F
“ <i>R. vinctus</i> ”	TUFC 13815	(TUMH 63562)	Tokyo Metropolis, Japan	20 Nov 2010	–	B	F
“ <i>R. vinctus</i> ”	TUFC 35082	(TUMH 60896)	Okinawa Pref., Japan	13 Jul 2003	13 Jul 2003	B	A

^a Strains in bold were used for the experiments incubating cultures under different O₂ concentrations.

^b “B”, “R”, “S”, and “T” mean basidiospores, a rhizomorph, a SS, and tissue of the basidiocarp as the source of isolation, respectively.

^c “A” means that the sample was collected in or nearby an aquatic area. “F” means the sample from a forest area, not aquatic.

^d Parenthesis means the specimen that we did not examine in the present study.

^e T means the type specimen.

^f FP 103737, other strain number of CBS 186.60, was isolated from tissue of the basidiocarp (Lombard et al., 1960).

(CTAB) method (Shino et al., 2022). From the obtained genomic DNA, the internal transcribed spacer (ITS) region and D1/D2 domains of the large subunit (LSU) of nuclear ribosomal DNA (nrDNA) were amplified by the polymerase chain reaction (PCR) using a thermal cycler (PC-812 or PC-818; ASTEC Co., Ltd., Fukuoka, Japan). As primers, we used ITS5 and ITS4 for the ITS region (White et al., 1990), and LR0R and LR5 for the LSU region (Rehner & Samuels, 1994; Vilgalys & Hester, 1990). PCRs were conducted using the protocol described in Shino et al. (2022). Amplicons were purified using NucleoSpin Gel and PCR Clean-up (Takara Bio Inc., Shiga, Japan), and Fasmac Co., Ltd. (Kanagawa, Japan) was commissioned to perform the DNA sequencing. All of the sequences except the ITS regions of CBS 186.60 and TUFC 101965 were readable by direct sequencing. Of the above two samples showing partial heterogeneity between the gene copies, we performed a cloning for TUFC 101965 using pGEM-T Easy Vector Systems (Promega K.K., Tokyo, Japan) and competent bacterial cells (*Escherichia coli*

(Migula) Castellani & Chalmers JM109). Sequence data were deposited at the DNA Data Bank of Japan (DDBJ; <https://www.ddbj.nig.ac.jp/index-e.html>).

2.2.2. Sequence alignment and phylogenetic analyses

Alignment of the data sets and creation of phylogenetic trees were performed online using MAFFT v. 7 (Katoh & Standley, 2013; <https://mafft.cbrc.jp/alignment/server/>; Jun 2023) and MEGA7 (Kumar et al., 2016). DNA sequences of the ITS and/or LSU regions of nrDNA retrieved from the GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>) were included in phylogenetic analyses which were performed using the maximum likelihood (ML) method. Based on the results of the best-fitting model test in MEGA7, the GTR+G+I model was adopted as a model of molecular evolution in the ML analyses using a combined data set of nrDNA ITS and LSU sequences for the *Meripilaceae* group (*Meripilus*, *Physisporinus*, and *Spongipellis*) and only nrDNA ITS for the *Cer-*

renaceae group [*Cerrena* Gray, *Irpiciporus* Murrill, *Pseudolagarobasidium* J.C. Jang & T. Chen, *Pseudospongipellis* Y.C. Dai & Chao G. Wang, *Radulodon* Ryvardeen, “*Rigidoporus hypobrunneus*” (Petch) Corner, and “*R. vincus*” (Berk.) Ryvardeen] in *Polyporales*, whereas the TN93+G model was applied to only nrDNA LSU for *Rigidoporus* in *Hymenochaetales*. The confidence coefficient of each node in the phylogenetic trees was confirmed by bootstrap (BS) analysis with 1,000 replicates (Felsenstein, 1985). Outgroups for the phylogenetic analyses of each data set for the above three clusters were selected as follows; *Abortiporus biennis* (Bull.) Singer (FD-319), *Hypoderma setigerum* (Fr.) Donk (FD-312), and *Hypochnicium* sp. (FP-110227-sp) (Floudas & Hibbett, 2015; Justo et al., 2017) for the data set consisting of the nrDNA ITS and LSU sequences of the *Meripilaceae*, *Polyporales* group, following Yamashita et al. (2020); *Cymatoderma* sp. (OMC-1427), *Panus conchatus* (Bull.) Fr. (Miettinen 13966), and *P. fragilis* O.K. Mill. (HHB-11042-Sp) (Floudas & Hibbett, 2015; Justo et al., 2017; Miettinen et al., 2012) for the data set consisting of nrDNA ITS sequences of the *Cerrenaceae*, *Polyporales* group, referring to Justo et al. (2017); *Bridgeoporus sinensis* (X.L. Zeng) F. Wu, Jia J. Chen & Y.C. Dai (Cui 10013), *Leucophellinus hobsonii* (Berk. ex Cooke) Ryvardeen (Cui 6468), and *L. irpicoides* (Bondartsev ex Pilát) Bondartsev & Singer (Yuan 2690) (Wu et al., 2017) for the data set consisting of nrDNA LSU sequences of *Rigidoporus*, *Hymenochaetales*, referring to Wu et al. (2017). The DNA sequences analyzed in this study and retrieved from GenBank to infer phylogenetic relationships among the *Meripilaceae*, *Polyporales* group are listed in Table 2. The lists of DNA sequences for the *Cerrenaceae*, *Polyporales* group and *Rigidoporus*, *Hymenochaetales* are shown in Supplementary Table S1 and S2. Sequence alignment data are added as Supplementary alignment S1 for the *Meripilaceae*, *Polyporales* group, S2 for the *Cerrenaceae*, *Polyporales* group, and S3 for *Rigidoporus*, *Hymenochaetales*.

2.3. Observation of cultures and specimens

Strains including new isolates were precultured on an antibiotic-added corn meal agar medium (Shino et al., 2022) at room temperature (20–25 °C) for 1–2 mo. After agar discs containing mycelia were cut out or stamped out from the precultured plates using a flame sterilized scalpel or autoclaved sterilized plastic straws (6 mm diam), they were inoculated on the following four media; a corn meal agar medium [CMA; Corn Meal Agar “Nissui” (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan; containing 2 g/L cornmeal extract and 15 g/L agar)], a malt extract agar medium [MA; 15 g/L malt extract (Oriental Yeast Co., Ltd., Tokyo, Japan) and 15 g/L agar (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan)], a potato dextrose agar medium [PDA; Potato Dextrose Agar “Nissui” (Nissui Pharmaceutical Co., Ltd.; containing 3.9 g/L potato extract, 21 g/L glucose and 14.1 g/L agar)], and a starch agar medium [SA; 10 g/L starch, soluble (FUJIFILM Wako Pure Chemical Corporation), 5 g/L malt extract, and 20 g/L agar]. These plates were incubated at 25 °C for 7–31 d and the following cultural properties were examined using a Nikon ECLIPSE 80i differential interference contrast microscope (DICM) (Nikon Corporation, Tokyo, Japan); presence/absence and morphology of acanthophyses, clamp connections, conidia, and plectenchymata. Samples were mounted in 3% potassium hydroxide (KOH) on a slide glass, and the size was measured by PhotoRuler ver. 1.1.3 software (<http://inocybe.info>). Specimens of SSs and basidiocarps from which strains were isolated were observed by the above method to confirm the consistency to the results of phylogenetic analyses. Acanthophyses were also observed using a scanning electron microscope (SEM) (SU1510; Hitachi High-Tech Corporation, Tokyo,

Japan). Preparation and observation of SEM samples followed Shino et al. (2022).

2.4. Investigation of acanthophysis production on agar media during incubation under different O₂ concentrations

A schematic illustration of the following experiment is shown in Fig. 2. Selected strains (*Physisporinus* cf. 1 *eminens* TUFC 101880, *P.* cf. 2 *eminens* TUFC 101881, *P.* cf. *furcatus* TUFC 101883, *P. lineatus* TUFC 13809, *P. microacanthophysis* TUFC 101885, and *P. pouzarii* TUFC 101965; see Table 1) were preincubated at room temperature (20–25 °C). After mycelia covered the entire surface of the medium, they were stamped out as discs with agars using autoclaved sterilized plastic straws (6 mm diam) and the agar discs were used to inoculate CMA plates: six discs were inoculated per a plate (three discs were placed facing up and the other three discs were placed facing down). After checking the number of acanthophyses on the surface of the discs under the DICM, as they were already formed during preincubation before experiment, the CMA plates with their inoculated agar discs were placed in four desiccators [Vacuum Polycarbonate Desiccator 240G (or 240GA) or 300G (or 300GA) (AS ONE Corporation, Osaka, Japan)]. Then, the air in each desiccator was exhausted using a diaphragm type dry vacuum pump (DA-20D; ULVAC KIKO, Inc., Miyazaki, Japan) and each desiccator was filled with one of the following standard gas mixtures or air to prepare four different O₂ conditions: 5% O₂ (O₂ 4.95%, CO₂ 402 ppm, and N₂ as a base gas), 10% O₂ (O₂ 10.0%, CO₂ 405 ppm, and N₂), 20% O₂ (the atmospheric air: O₂ 21%, CO₂ 400 ppm, and N₂ 78%; all the values of concentrations are approximate), and 40% O₂ (O₂ 39.5%, CO₂ 395 ppm, and N₂). To prepare the 5, 10 and 40% O₂ conditions, we used calibration gas cylinders that were prepared by Taiyo Nippon Sanso JFP Corporation (Kanagawa, Japan) and a gas regulator (GHN-3; CHIYODA SEIKI Co., Ltd., Hyogo, Japan). The gas in each desiccator was exchanged daily: the process of exhausting and filling of each gas was repeated twice per exchange. Uncovered plates in desiccators were incubated for 2–5 d at room temperature. After finishing the incubation, the number of acanthophyses that formed on the upper surface and side of each disc was counted under the DICM. The number of acanthophyses produced during preincubation was excluded from the data.

3. Results

3.1. Relationships between molecular phylogeny and cultural characteristics with emphasis on acanthophysis formation

The phylogenetic analysis of *Meripilaceae* in *Polyporales* that was based on the combined sequences of the ITS and LSU regions of nrDNA showed that *Meripilus*, *Physisporinus*, and *Spongipellis* form a clade, and *Meripilus* was included within the *Physisporinus* clade (BS = 99%; Fig. 3). The topology of this phylogenetic tree was almost correspondent with trees estimated in several recent studies (Chen & Dai, 2021; Shino et al., 2022; Spirin et al., 2022; Wang & Dai, 2022). Acanthophysis-forming strains on agar media in the present study belonged only to the *Physisporinus* clade (Figs. 3–5). In this clade, a monophyletic cluster including *P. castanopsisidis* Jia J. Chen & Y.C. Dai, *P. crocatus* (Pat.) F. Wu, Jia J. Chen & Y.C. Dai, *P. microacanthophysis*, *P. pouzarii*, “*P. sanguinolentus*” (Alb. & Schwein.) Pilát, *P. subcrocatus* F. Wu, Jia J. Chen & Y.C. Dai, *P. tibeticus* F. Wu, Jia J. Chen & Y.C. Dai, and *P. vitreus* (Pers.) P. Karst. (BS = 100%) formed shorter acanthophyses (10–30 µm long: the ornamented part with warts or spines, but not including spines) than

Table 2. DNA sequence data newly obtained in this study (bold-face type) and employed from GenBank for the phylogenetic analysis of *Meripilaceae* in *Polyporales*.

Species name	Sample No.	Locality	GenBank accession No.	
			nrDNA ITS	nrDNA LSU
<i>Abortiporus biennis</i>	FD-319	USA	KP135300	KP135195
<i>Hypoderma setigerum</i>	FD-312	USA	KP135297	KP135222
<i>Hypochnicium</i> sp.	FP-110227-sp	USA	KY948804	KY948862
<i>Meripilus giganteus</i>	CBS 421.48	Germany	MH856418 ^a	LC770099
<i>M. giganteus</i>	FP-135344-Sp	UK	KP135307	KP135228
<i>M. giganteus</i>	TUFC 100564	Japan	LC643683	LC643708
<i>Physisporinus castanopsidis</i>	Dai 20396 ^{Tb}	China	MT309485	MT309470
<i>P. castanopsidis</i>	Dai 20397	China	MT309486	MT309472
<i>P. castanopsidis</i>	Dai 20398	China	MT840113	MT840131
<i>P. cf. 1 eminens</i>	TUFC 101880	Japan	LC643670	LC643695
<i>P. cf. 1 eminens</i>	TUFC 101957	Japan	LC770057	LC770082
<i>P. cf. 1 eminens</i>	TUFC 101958	Japan	LC770058	LC770083
<i>P. cf. 1 eminens</i>	TUFC 101959	Japan	LC770059	LC770084
<i>P. cf. 2 eminens</i>	TUFC 101881	Japan	LC643671	LC643696
<i>P. cf. 2 eminens</i>	TUFC 101960	Japan	LC770060	LC770085
<i>P. cf. 2 eminens</i>	TUFC 101961	Japan	LC770061	LC770086
<i>P. cf. 2 eminens</i>	TUFC 101962	Japan	LC770062	LC770087
<i>P. cf. furcatus</i>	TUFC 101883	Japan	LC643673	LC643698
<i>P. cf. furcatus</i>	TUFC 101884	Japan	LC643674	LC643699
<i>P. cinereus</i>	Cui 3266	China	KY131844	KY131903
<i>P. crataegi</i>	Dai 15497 ^T	China	KY131845	KY131904
<i>P. crataegi</i>	Dai 15499	China	KY131846	KY131905
<i>P. crocatus</i>	CBS 107806	Canada	LC770074	LC770100
<i>P. crocatus</i>	Dirks 374051	USA	ON364084	ON369536
<i>P. crocatus</i>	MJ 19/09	Slovakia	JQ409466	OM669978
<i>P. eminens</i>	Cui 9520	China	KY131847	KY131906
<i>P. eminens</i>	Cui 10341	China	KY131849	KY131907
<i>P. eminens</i>	Cui 10344	China	KY131850	KY131908
<i>P. eminens</i>	Cui 10475	China	MT840114	MT840132
<i>P. eminens</i>	Dai 11400	China	KY131852	KY131909
<i>P. eminens</i>	Dai 12685	Czechia	MT840115	MT840133
<i>P. eminens</i>	Dai 17200	Unknown	MT279690	MT279911
<i>P. eminens</i>	Dai 19861	China	MT840116	MT840134
<i>P. eminens</i>	Dai 20832	China	MT279689	MT279910
<i>P. eminens</i>	Dai 20868	China	MT840117	MT840135
<i>P. eminens</i>	Dai 22472	China	OM669900	OM669983
<i>P. furcatus</i>	Dai 2105	China	KY131854	KY131911
<i>P. furcatus</i>	Dai 2544	China	KY131855	KY131912
<i>P. furcatus</i>	Dai 11313	China	KY131856	KY131913
<i>P. furcatus</i>	Dai 12938	China	KY131857	KY131914
<i>P. furcatus</i>	Dai 20976	Belarus	MT840118	MT840136
<i>P. furcatus</i>	Dai 20977	Belarus	MT840119	MT840137
<i>P. furcatus</i>	TAA 15097 ^T	Russia	KY131853	KY131910
<i>P. lavendulus</i>	Dai 9925	China	KY131858	KY131915
<i>P. lavendulus</i>	Dai 13587A ^T	China	KY131859	KY131916
<i>P. lineatus</i>	CBS 167.65	USA	LC770075	LC770101
<i>P. lineatus</i>	CBS 700.94	Germany	LC770076	LC770102
<i>P. lineatus</i>	CBS 109425	Taiwan	LC770077	LC770103
<i>P. lineatus</i>	Dai 17986	China	MT840121	MT840139
<i>P. lineatus</i>	Dai 18280	Vietnam	MT840122	MT840140
<i>P. lineatus</i>	JV 1407/37	Costa Rica	OM669903	OM669986
<i>P. lineatus</i>	TUFC 13809	Japan	LC770063	LC770088
<i>P. lineatus</i>	TUFC 13812	Japan	LC770064	LC770089
<i>P. longicystidius</i>	Cui 16630	Australia	ON417177	ON417227
<i>P. longicystidius</i>	Cui 16725	Australia	ON417178	ON417228
<i>P. microacanthophysis</i>	TUFC 101885	Japan	LC643675	LC643700
<i>P. microacanthophysis</i>	TUFC 101888	Japan	LC643678	LC643703
<i>P. microacanthophysis</i>	TUFC 101889 ^T	Japan	LC643679	LC643704
<i>P. microacanthophysis</i>	TUFC 101963	Japan	LC770065	LC770090
<i>P. microacanthophysis</i>	TUFC 101964	Japan	LC770066	LC770091
<i>P. pouzarii</i>	Dai 15005	China	KP420014	KP420017
<i>P. pouzarii</i>	Dai 21043	Belarus	MT840124	MT840142
<i>P. pouzarii</i>	JV 0511/23	Czechia	JQ409465	KY131921
<i>P. pouzarii</i>	TUFC 101965	Japan	LC770067	LC770092
<i>P. pouzarii</i>	TUFC 101966	Japan	LC770068	LC770093
<i>P. rhizomorphae</i>	TUFC 101870	Japan	LC643660	LC643685
<i>P. rhizomorphae</i>	TUFC 101871	Japan	LC643661	LC643686
<i>P. rhizomorphae</i>	TUFC 101876 ^T	Japan	LC643666	LC643691

Species name	Sample No.	Locality	GenBank accession No.	
			nrDNA ITS	nrDNA LSU
<i>P. rhizomorphae</i>	TUFC 101967	Japan	LC770069	LC770094
<i>P. roseus</i>	Dai 19877 ^T	China	MT840126	MT840144
" <i>P. sanguinolentus</i> "	CBS 139.76	Belgium	MH860969 ^c	MH872738 ^c
"<i>P. sanguinolentus</i>"	CBS 193.76	Netherlands	LC770078	LC770104
" <i>P. sanguinolentus</i> "	CBS 679.70	USA	MH859899 ^c	MH871689 ^c
"<i>P. sanguinolentus</i>"	CBS 107146	Denmark	LC770079	LC770105
" <i>P. sanguinolentus</i> "	Dai 20995	Belarus	MT309483	MT309480
" <i>P. sanguinolentus</i> "	DM1068	Denmark	MT644902	MT644902
" <i>P. sanguinolentus</i> "	JV 1610/2	Czechia	OM669921	OM669999
" <i>P. sanguinolentus</i> "	KHL 11913	Sweden	JX109843	JX109843
<i>Physisporinus</i> sp. 1	Dai 11693	China	KY131865	KY131922
<i>Physisporinus</i> sp. 2	Dai 6720	China	KY131867	KY131923
<i>Physisporinus</i> sp. 4	Dai 15184	Unknown	KY131868	KY131924
<i>Physisporinus</i> sp.	Cui 16852	Puerto Rico	ON417179	ON417229
<i>Physisporinus</i> sp.	CWU 3874	Ukraine	OM971903	OM971889
<i>Physisporinus</i> sp.	JV 0308/58	USA	OM669909	OM669991
<i>Physisporinus</i> sp.	JV 0509/47	USA	OM669906	OM669988
<i>Physisporinus</i> sp.	JV 0709/188	USA	OM971904	OM971890
<i>Physisporinus</i> sp.	JV 0909/3	Czechia	OM669939	OM670011
<i>Physisporinus</i> sp.	JV 1407/36	Costa Rica	OM669933	OM670008
<i>Physisporinus</i> sp.	Miettinen 15239	Indonesia	KY948732	KY948867
<i>Physisporinus</i> sp.	Miettinen 16699	USA	KY948733	KY948863
<i>Physisporinus</i> sp.	TUFC 101892	Japan	LC643682	LC643707
<i>Physisporinus</i> sp.	TUFC 101968	Japan	LC770070	LC770095
<i>P. subcrocatus</i>	Dai 12800	USA	KY131869	KY131925
<i>P. subcrocatus</i>	Dai 15917 ^T	China	KY131870	KY131926
<i>P. sulphureus</i>	Dai 17839 ^T	Singapore	MG132179	MG132181
<i>P. sulphureus</i>	Dai 17841	Singapore	MG132180	MG132182
<i>P. tibeticus</i>	Cui 9381 ^T	China	KY131871	KY131927
<i>P. tibeticus</i>	Cui 9588	China	KY131873	KY131929
<i>P. tibeticus</i>	Cui 10478	China	MT840128	MT840146
<i>P. undatus</i>	JV 0110/48	Czechia	OM669931	OM670005
<i>P. undatus</i>	Miettinen 13591	Finland	KY948731	KY948870
<i>P. undatus</i>	MJ 129/04	Czechia	OM669932	OM670006
" <i>P. vinctus</i> "	Cui 16903	China	MT840129	MT840147
<i>P. vitreus</i>	Dai 21060	Belarus	MT840130	MT840148
<i>P. vitreus</i>	KHL 11959	Norway	JQ031129	JQ031129
<i>P. yunnanensis</i>	CLZhao 21583	China	OP852341	OP852343
<i>P. yunnanensis</i>	CLZhao 21647 ^T	China	OP852340	OP852342
<i>Polyporales</i> sp.	422b	Japan	AB470242	AB470242
<i>Polyporales</i> sp.	TK-10	Japan	AB716748	AB762089
Uncultured mycorrhizal fungus	Polyporales218	Taiwan	KP238183	KP238182
Uncultured mycorrhizal fungus	Polyporales859	Taiwan	KP238186	KP238184
Uncultured mycorrhizal fungus	Physisporinus222	Taiwan	KP238185	KP238181
<i>Spongipellis ambiens</i>	Niemelä 6407	China	ON979313	ON979313
<i>S. ambiens</i>	Spirin 5389	Russia	ON979322	ON979322
<i>S. profissilis</i>	Dai 3934	China	ON979321	ON979321
<i>S. profissilis</i>	Kotiranta 26990	Russia	OP104014	OP104014
<i>S. spumea</i>	JV 1511/6	Czechia	ON979318	ON979318
<i>S. spumea</i>	Kotiranta 26889	Finland	ON979311	ON979311
<i>S. spumea</i>	Spirin 6741	Russia	ON979326	ON979326
<i>S. variispora</i>	Niemelä 6423	China	ON979320	ON979320
<i>S. variispora</i>	Spirin 3737 ^T	Russia	ON979312	ON979312

^a The nrDNA ITS sequence of *M. giganteus* CBS 421.48 had been already registered with this accession number in GenBank before our study. We used it for the analysis of the *Meripilaceae* group because the sequence of this strain obtained in the present study corresponded to the above sequence with high homology (99%) by the Standard Nucleotide BLAST (Basic Local Alignment Search Tool) of the GenBank database.

^b ^T means the type specimen or ex-type culture.

^c The nrDNA ITS and LSU sequences of "*P. sanguinolentus*" CBS 139.76 and CBS 679.70 had been already registered with these accession numbers in GenBank before our study. We used them for the analysis of the *Meripilaceae* group because the sequences of the two strains obtained in the present study corresponded to the above sequences with high homology (Both were 100% in the ITS and 99% in the LSU region) by the BLAST.

the rest of species in this genus which are known to produce SSS/ acanthophyses (20–70 µm long), except for *P. cf. 1 eminens* (12–29 µm long). Moreover, the strains in this cluster had sparse clamp

connections at the septa of vegetative hyphae (Figs. 3, 4, 6E), whereas strains in other clades of *Physisporinus* lacked clamp connections on the hyphae. *Meripilus* strains did not produce acantho-

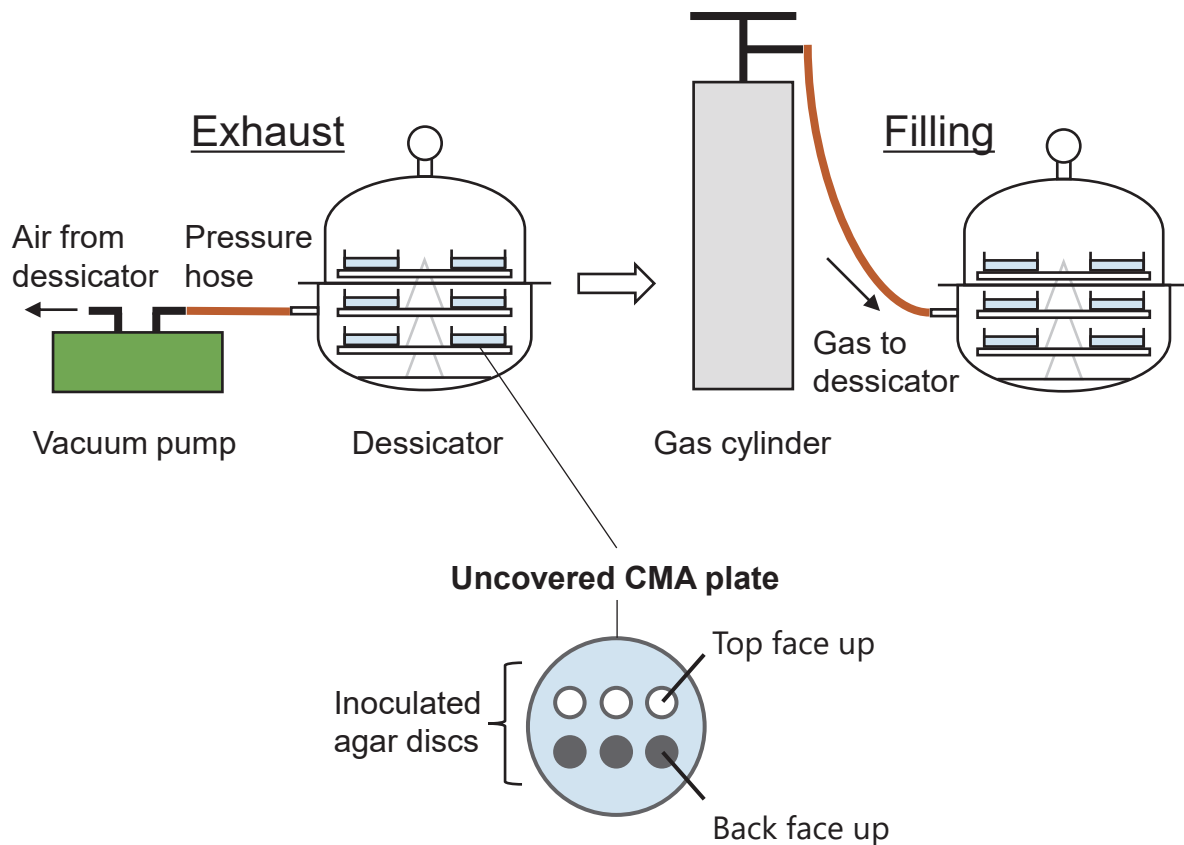


Fig. 2 – The outline of experiment for acanthophysis production under different O_2 concentrations (For details, see section 2.4. in materials and methods).

physes on the agar media employed in this study. Both *Meripilus* and *Physisporinus* formed plectenchymata in cultures (Fig. 6B, F). Formation of the plectenchymata in both genera have been described previously, e.g., Larsen and Lombard (1988) and Lombard and Chamuris (1990).

The phylogenetic analysis based on the nrDNA ITS or LSU region showed that “*Rigidoporus*” species in the traditional usage separated in two different lineages, *Polyporales* and *Hymenochaetales* (Supplementary Figs. S1, S2) as reported by Wu et al. (2017). The phylogenetic tree of the *Cerrenaceae* group in *Polyporales* (Supplementary Fig. S1) showed that this family clusters with *Cerrena*, *Irpiciporus*, *Pseudolagarobasidium*, *Pseudospongipellis*, *Radulodon*, and “*Rigidoporus*” (BS = 100%), as reported in previous studies (Justo et al., 2017; Wang & Dai, 2022; Westphalen & Motato-Vásquez, 2022), and a highly supported clade (BS = 99%) of “*R. hypobrunneus*”/“*R. vincetus*” accommodates five strains of “*R. vincetus*” examined in this study. Arthroconidia production in “*R. vincetus*” strains was observed (Supplementary Fig. S1) as reported previously [Setliff, 1972 (as oidia); Stalpers, 1978], but they did not produce any acanthophyses. The topology of the tree estimated for *Rigidoporus* in *Hymenochaetales* was similar to that in Wu et al. (2017) and Yuan et al. (2020). *Rigidoporus ulmarius* (Sowerby) Imazeki CBS 186.60 formed a clade together with nine sequences, including four sequences of *R. microporus* (Sw.) Overeem (BS = 98%; Supplementary Fig. S2) in *Hymenochaetales*. This strain did not form acanthophyses, but it did produce vesicular cells on vegetative hyphae laterally and terminally in culture (Supplementary Fig. S2), as described previously in Lombard et al. (1960) and Stalpers (1978; as terminal vesicles).

3.2. Acanthophysis production on agar media under different O_2 concentrations

The results of the experiments using six strains (*Physisporinus* cf. 1 *eminens* TUF 101880, *P. cf. 2 eminens* TUF 101881, *P. cf. furcatus* TUF 101883, *P. lineatus* TUF 13809, *P. microacanthophysis* TUF 101885, and *P. pouzarii* TUF 101965) cultured under four different O_2 conditions (5, 10, 20, and 40% O_2) are shown in Fig. 7. In the case of the agar discs placed facing up, the three strains (TUF 101880, TUF 101881, and TUF 101885) formed acanthophyses most abundantly under the atmospheric condition, i.e., 20% O_2 , whereas the two strains (TUF 101883 and TUF 101965) formed most acanthophyses under 40% O_2 . The four strains other than TUF 13809 and TUF 101880 tended to form more acanthophyses on the upper surface than on the sides of the agar discs in the situation placed facing up, probably because aerial vegetative hyphae on the discs are easy to contact to the air.

In the case of the agar discs placed facing down, the four strains (TUF 101880, TUF 101881, TUF 101885, and TUF 101965) produced more acanthophyses under the 20% O_2 condition than under the 40% O_2 condition. TUF 101883 produced acanthophyses only under the 40% O_2 condition. When the agar discs were set on the plates with facing down, the aerial vegetative hyphae at the upper surface were facing the CMA plates, which resulted in most of the hyphae existing inside the discs. Hence, this latter situation in which the hyphae spreading inside the agar disc is somewhat analogous to that of hyphae growing within water-saturated wood tissue in the natural wet habitats of SS-forming *Physisporinus* species.

The number of acanthophyses formed by the five strains (TUF 101880, TUF 101881, TUF 101883, TUF 101885, and TUF

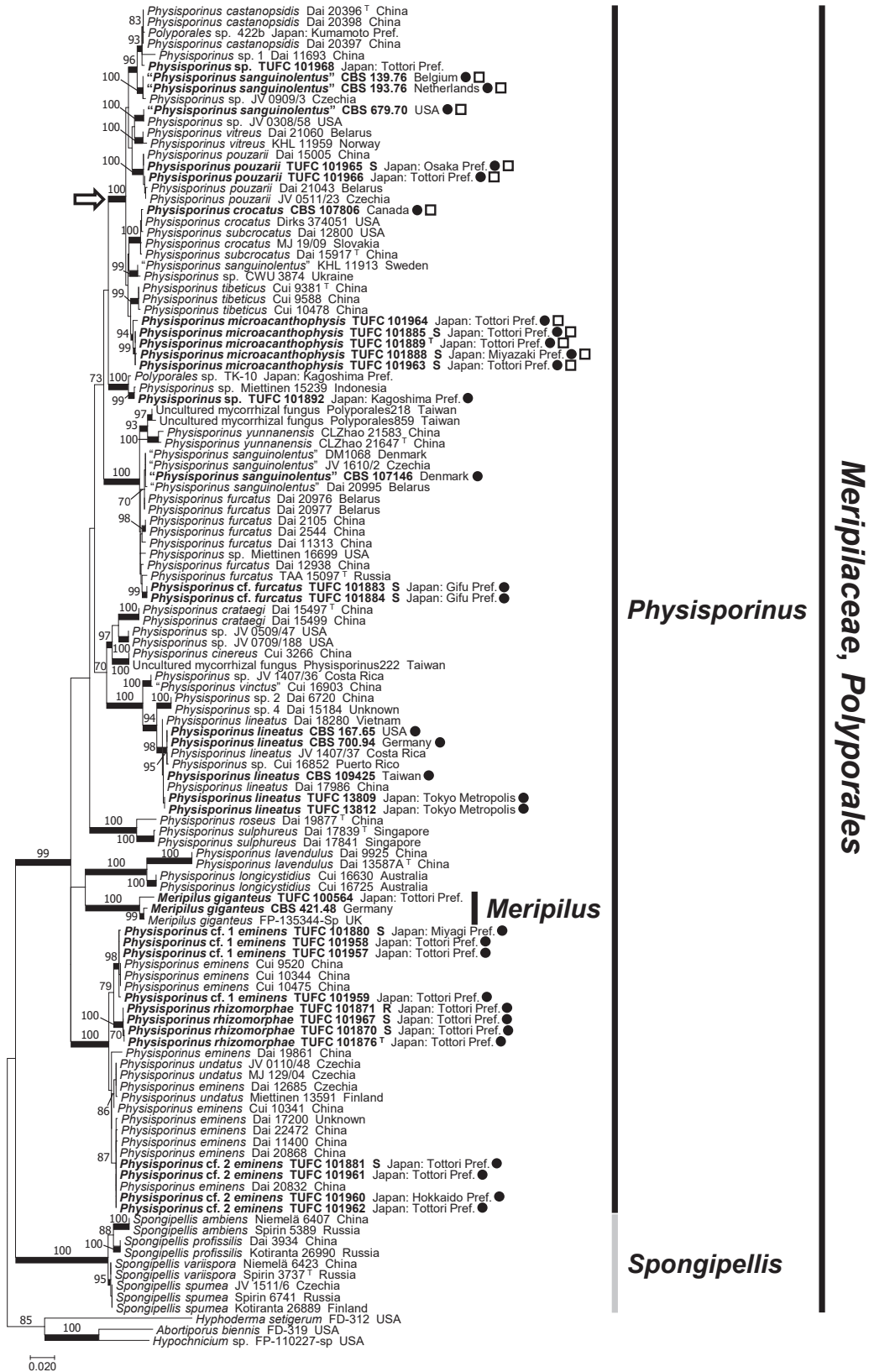


Fig. 3 – Phylogenetic tree of Meripilaceae in Polyporales inferred from connected sequences of the ITS and LSU regions of nrDNA by ML method. A total of 1,171 sites in the final data set were used for this analysis. The values at nodes indicate BS in ML method ($\geq 70\%$), and bold branches mean BS $\geq 90\%$ in the above method. The species names and numbers of strains used in this study are shown in bold, and the strain number followed by “S” or “R” indicates the isolate from a SS or rhizomorph. [†] on the sample number means the sequence obtained from the type specimen or ex-type culture. Filled circles show the strains forming acanthophyses in culture. Open squares show the strains having clamp connections at the septa of vegetative hyphae on agar media (white arrow indicates a monophyletic clade characterized by this feature). The strains without sufficient cultural investigations in the present study are unmarked.

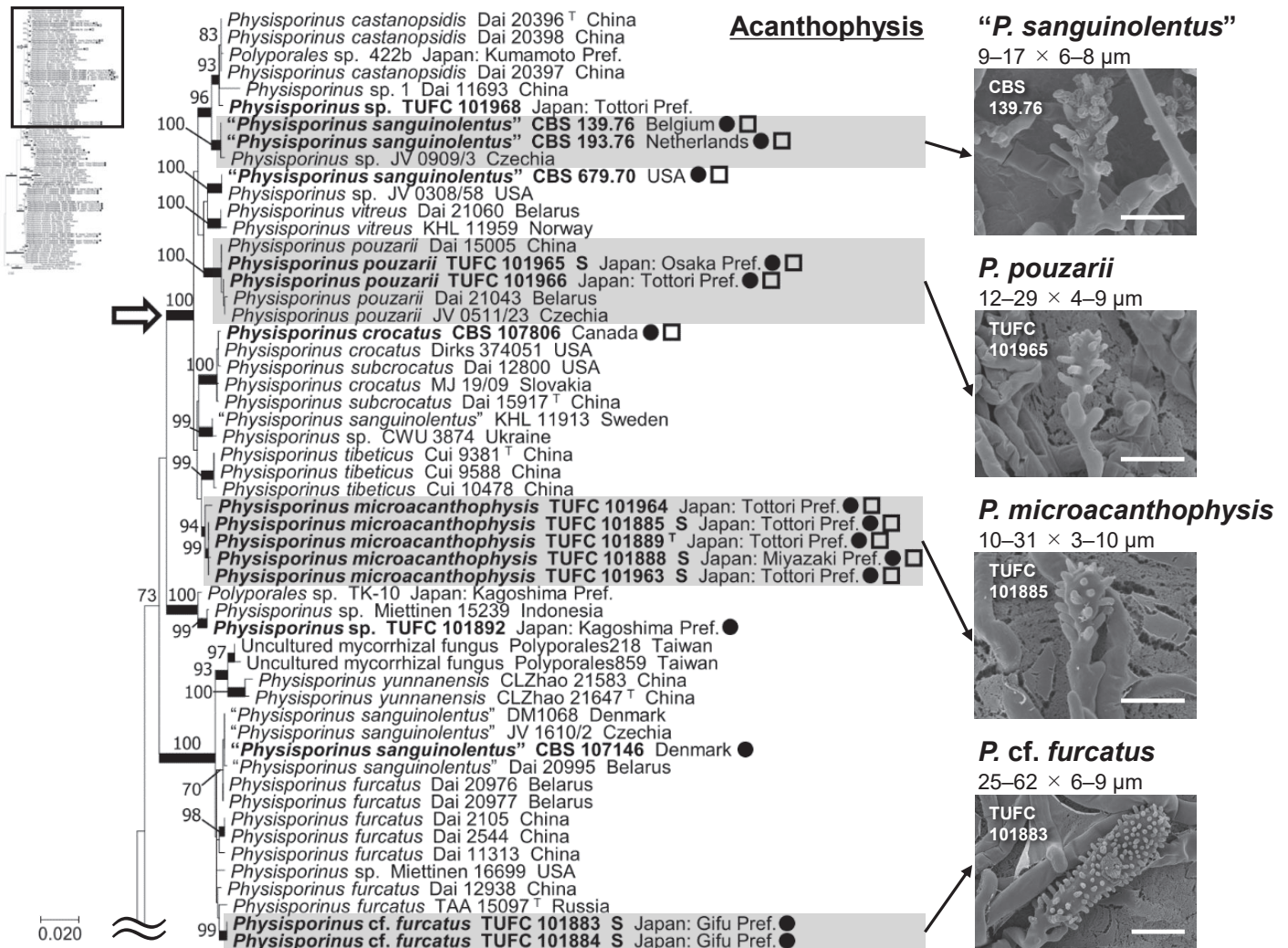


Fig. 4 – The relationship between molecular phylogeny and acanthophysis formation in *Physisporinus*. A figure at the upper left is the reduced Fig. 3, and a box in it shows a magnified part for this figure. As with Fig. 3, filled circles show the strains forming acanthophyses in culture, and open squares show the strains having clamp connections at the septa of vegetative hyphae on agar media (white arrow indicates a monophyletic clade characterized by this feature). The strains without sufficient cultural investigations in the present study are unmarked. The sizes of acanthophyses of *Physisporinus* species investigated in this study are described under the species name. The appearance of a typical acanthophysis of each species is exhibited by photographs using SEM at the right of this figure. Bars: 10 μm.

101965) on the discs placed both facing up and down under the 5% and 10% O₂ conditions was lower than 20% or 40% O₂ conditions, but TUFC 13809 produced acanthophyses most abundantly under 10% O₂ in the case of discs being placed facing up and under 5% O₂ in the case of discs being placed facing down. The above five strains tended to mainly produce acanthophyses from the upper surface and/or side face of the agar discs placed facing up or down on CMA plates, while TUFC 13809 formed acanthophyses abundantly on the aerial vegetative hyphae that spread on the plates as well as on the entire surface of the agar discs.

4. Discussion

Some basidiomycetes have been known to produce acanthophyses on vegetative hyphae in culture, and most of these taxa are now placed in *Physisporinus*; for example, *P. crocatus*, which was formerly treated as *Poria nigrescens* Bres. (Nobles, 1958); *P. lineatus*, formerly treated as *Polyporus zonalis* Berk. [Bakshi et al., 1963; Davidson et al., 1942 (acanthophyses were described as hyphal ends covered with short knobs or definite spines); Nobles, 1958], as

Rigidoporus zonalis (Berk.) Imazeki (Kobayashi, 1972), and as *R. lineatus* [Hood et al., 1997 (as acanthohyphidia); Motato-Vásquez et al., 2016 (as the spiny and clavate cystidia); Stalpers, 1978 (as acanthohyphidia)]; *P. undatus* (Pers.) Pilát, formerly treated as *R. undatus* (Pers.) Donk (Motato-Vásquez et al., 2016); *P. vitreus*, formerly treated as *R. vitreus* (Pers.) Donk (Lombard & Chamuris, 1990; Schmidt et al., 1996, 1997). In these previous studies, species identification was based mainly on the morphological characteristics of basidiocarps. Our phylogenetic studies showed that the above acanthophysis-forming species are accommodated in *Physisporinus*. Except for *Physisporinus*, some species of *Xylobolus* P. Karst. have also been reported to form acanthophyses on agar media; for example, *X. frustulatus* (Pers.) Boidin [Lombard & Chamuris, 1990; Nakasone, 1990 (termed as acanthohyphidia); Stalpers, 1978 (as acanthohyphidia)]; *X. subpileatus* (Berk. & M.A. Curtis) Boidin (Stalpers, 1978), although the two species scarcely produce acanthophyses (Stalpers, 1978). In addition to *Xylobolus*, *Aleurodiscus* Rabenh. ex J. Schröt. sensu lato (Wu et al., 2001), *Megalocystidium* Jülich [only *M. diffusum* (Sacc.) K.H. Larss. & Spirin (Spirin et al., 2021)], and *Stereum* Hill ex Pers. are also known to

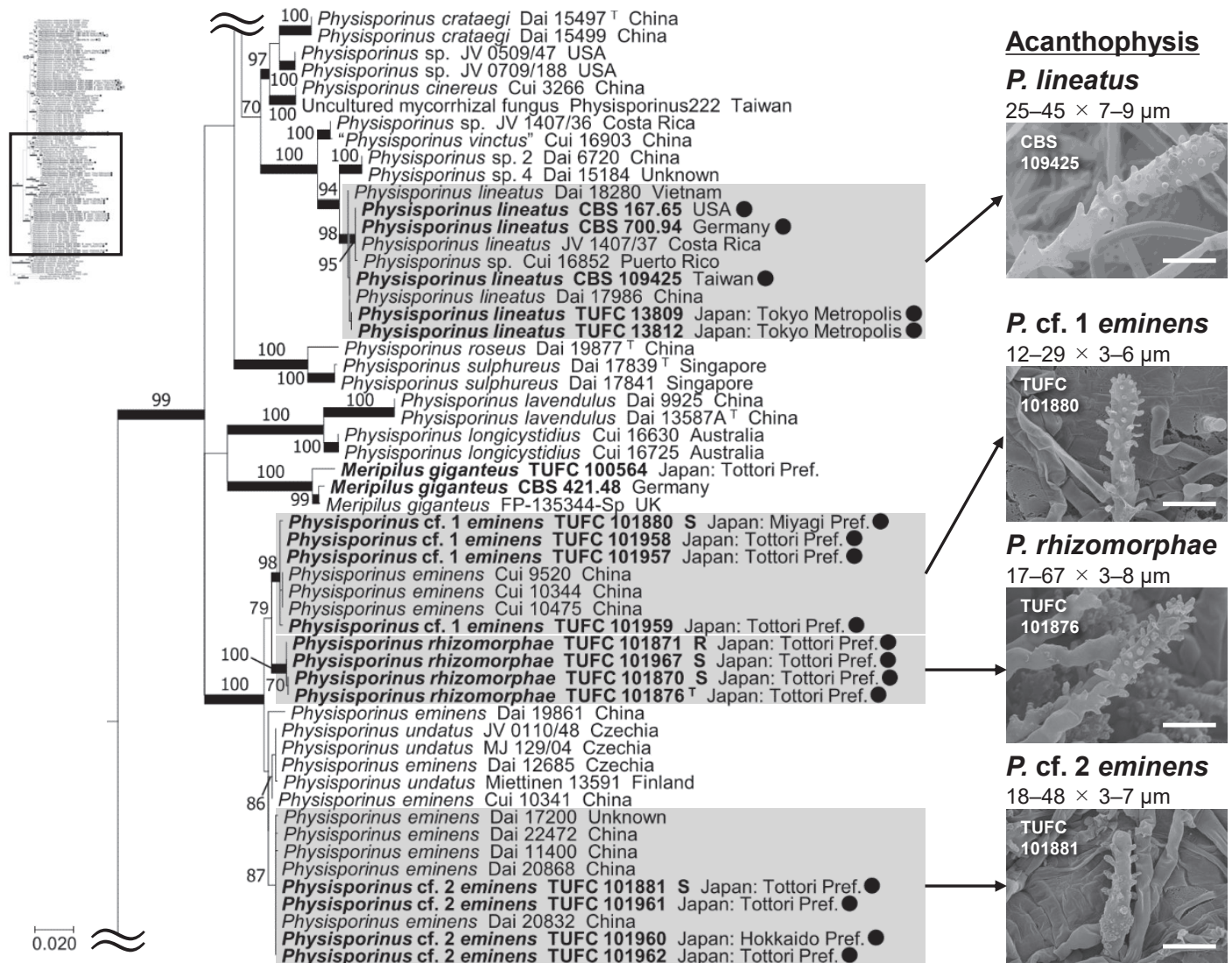


Fig. 5 – The relationship between molecular phylogeny and acanthophysis formation in *Physisporinus*. A figure at the upper left is the reduced Fig. 3, and a box in it shows a magnified part for this figure. As with Fig. 3, filled circles show the strains forming acanthophyses in culture. The strains without sufficient cultural investigations in the present study are unmarked. The sizes of acanthophyses of *Physisporinus* species investigated in this study are described under the species name. The appearance of a typical acanthophysis of each species is exhibited by photographs using SEM at the right of this figure. Bars: 10 µm.

produce acanthophyses (or termed as acanthocystidia or acanthohyphidia) in their basidiocarps (e.g., Bernicchia & Gorjón, 2010; Larsson & Ryvarden, 2021). These genera belong to *Stereaceae*, *Russulales* (Miller et al., 2006; Wu et al., 2022), and acanthophysis formation on vegetative hyphae in culture has been known only in the above *Xylobolus* species. On the other hand, *Physisporinus* seldom or never produce acanthophyses in their basidiocarps. Among *Physisporinus*, the closely related *Meripilus* and morphologically similar *Rigidoporus*, only *Physisporinus* species produce SSs in nature and/or acanthophyses in culture. Previous studies on the culture of *Meripilus* and *Rigidoporus* species, except for the species currently transferred to *Physisporinus*, did not observe SS/acanthophysis formation (Campbell, 1937; Davidson et al., 1942; Go et al., 2021; Kaewchai et al., 2010; Larsen & Lombard, 1988; Lombard et al., 1960; Nobles, 1948, 1965; Setliff, 1972; Stalpers, 1978). These results support our previous suggestion that SS/acanthophysis formation could be a taxonomic character for defining the genus *Physisporinus*, which is currently difficult to distinguish from *Rigi-*

doporus based on the morphology of the basidiocarp (Shino et al., 2022). All the *Physisporinus* samples used in our previous study were collected from aquatic environments (Shino et al., 2022). However, in addition to samples from streams, this study includes new samples from terrestrial environments (Table 1). Therefore, the *Physisporinus* species that inhabit forest areas may also form acanthophyses on their vegetative hyphae. *Physisporinus* species that have not been proven to produce SSs or acanthophyses, especially those that form perennial and/or brightly colored basidiocarps, should be investigated for their SS/acanthophysis-forming ability on wet wood or on media. Moreover, we found that the species group producing small acanthophyses (10–30 µm long) and rare clamp connections on septa of vegetative hyphae formed a highly supported clade that harbored at least eight *Physisporinus* species (*P. castanopsidis*, *P. crocatus*, *P. microacanthophysis*, *P. pouzarii*, “*P. sanguinolentus*”, *P. subcrocatus*, *P. tibeticus*, and *P. vitreus*), whereas other acanthophysis-forming clades in this genus produce larger acanthophyses (20–70 µm long, except for *P. cf. 1 eminens*

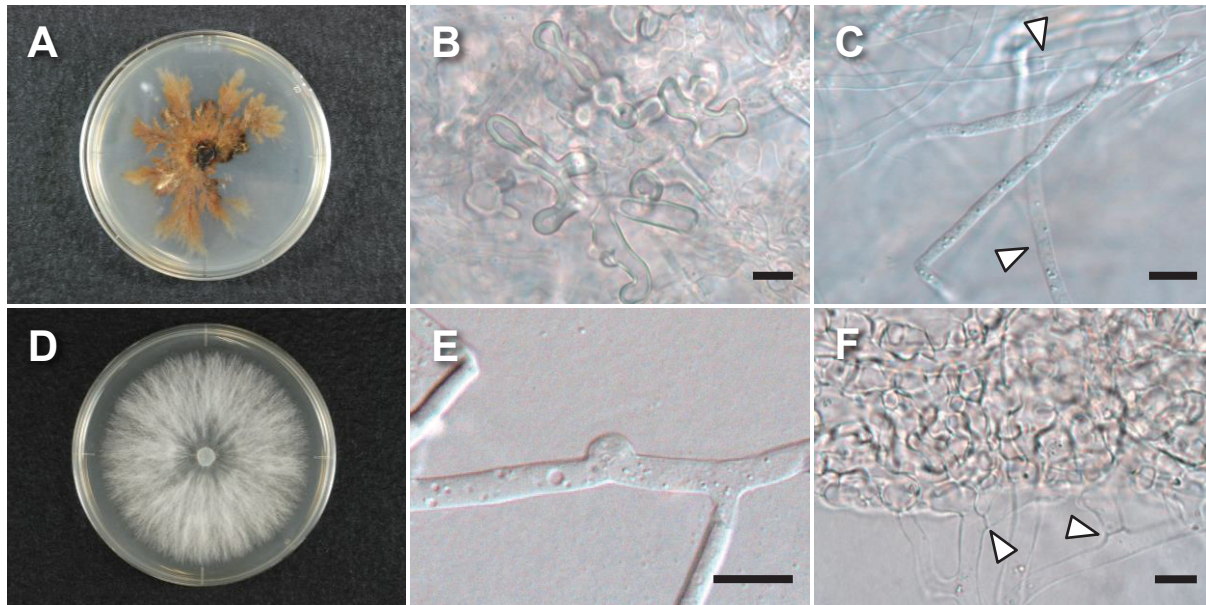


Fig. 6 – Cultural characteristics of the strains of *Meripilus giganteus* (A–C) and *Physisporinus* species (D–F) in *Meripilaceae*. A: Colony on 1.5% MA (TUFC 100564). B: Plectenychmata in mycelia (CBS 421.48). C: Vegetative hyphae (CBS 421.48). Arrow heads show clampless septa. D: Colony on 1.5% MA (*P. pouzarii* TUFC 101965). E: Clamp connection on a septum of vegetative hyphae (*P. pouzarii* TUFC 101965). F: Plectenychmata and vegetative hyphae (*P. lineatus* CBS 167.65). Arrow heads show clampless septa. Bars: B, C, E, F 10 μ m.

which forms acanthophyses of 12–29 μ m long) and no clamp connections (Figs. 3–5). These characteristics suggest that the size of acanthophyses is related to the phylogeny of *Physisporinus*. In this study, we were unable to find any clearly distinctive characteristics in basidiocarps of the species group having short acanthophyses and clamp connections in culture in comparison with other *Physisporinus* species contained in different clades. Further studies focusing on both the basidiocarps and isolates are therefore needed.

We currently face a raft of challenges related to the taxonomy of *Physisporinus* and the allied genera, *Meripilus* and *Rigidoporus*. *Physisporinus* still contains taxonomically confused species probably composed of plural species [e.g., *P. sanguinolentus* (Runnel et al., 2021, refer to Additional file 5; Wu et al., 2017); *P. furcatus* group and *P. undatus* group (Chen & Dai, 2021); *P. vitreus* (Runnel et al., 2021)]. The question remains about the validity of *P. subcrocatus* from the perspective of the very close similarity to *P. crocatus* in terms of morphology of basidiocarps and phylogeny. The phylogenetic position of *Meripilus* (i.e., whether this genus is truly nested in the *Physisporinus* clade or not) has not been confirmed by multi-gene phylogenetic analyses with sufficient sequences yet, although this study showed that, unlike *Physisporinus*, *Meripilus* species do not produce acanthophyses. In the *Cerrenaceae*, *Polyporales* group, a taxonomic problem regarding the “*R. hypobrunneus*”/“*R. vinctus*” clade, which was also pointed out by Nakasone and Ortiz-Santana (2022), was more clearly highlighted by our phylogenetic analysis (Supplementary Fig. S1). This clade should be treated as a new or another genus, but we do not treat it as such in this study because we could not investigate the type specimens of these species. In addition, it is important to reexamine specimens that are currently treated as *P. vinctus* (Berk.) Murrill, a synonym of *R. vinctus*, in the phylogenetic trees by Chen and Dai (2021), Shino et al. (2022), Wu et al. (2017), and this study (Fig. 3). To solve these taxonomic issues, investigations of currently overlooked or underestimated characters, such as the cultural properties of asexual states and vegetative hyphae, as well as the ecological characteristics of these species should be conducted in addition to the currently dominant studies focusing on the morphology of sexual states (basidiocarps)

and molecular phylogeny.

When we find SSs of *Physisporinus* species in freshwater areas, they are often formed on the water-boundary part of dead and wet wood of broad-leaved or coniferous trees. The wood substrate is carried by water flow as drift and caught between rocks, then exposed to the flow and splash for extended periods, mostly resulting in barkless and sometimes partly getting mossy. The insides of wet wood at the water-boundary and submerged parts are saturated with water and the dissolved O_2 concentrations within the wood tissue most likely decrease due to the low level of gas exchange. The results of the present experiments that exposed cultures of *Physisporinus* to gas mixtures with different O_2 concentrations suggested that acanthophyses were produced in response to higher O_2 concentrations and that they probably play a role in obtaining O_2 . Our experiments clearly showed that the number of acanthophyses produced on agar discs was markedly increased when incubated under O_2 concentrations of 20–40% compared to when incubated under O_2 concentrations of 5–10% O_2 (Fig. 7). The numerous spines of the acanthophyses probably function to increase the surface area of acanthophysis cells for gas exchange. This thought is supported by the fact that acanthophyses are formed on aerial vegetative hyphae, not on submerged hyphae in agar medium. Thus, it is possible that SSs furnished with numerous acanthophyses are formed at the water-boundary part that is exposed to the air and that they play a role in respiration at the closest site to the submerged part. The synnematos morphology of the SSs possibly serves to maintain the distance from the water surface and the water-saturated part of the wood substrate, so that the acanthophyses are exposed to the atmosphere, and also to withstand the force of the water flow. When the agar discs containing mycelia were placed on media facing down, four strains (*P. cf. 1. eminens* TUFC 101880, *P. cf. 2. eminens* TUFC 101881, *P. microacanthophysis* TUFC 101885 and *P. pouzarii* TUFC 101965) formed acanthophyses more abundantly under 20% O_2 condition than under 40% O_2 condition (Fig. 7). This finding might be explained as follows; under the 40% O_2 condition, high levels of O_2 permeate the agar media, so only a fewer number of acanthophyses need to be produced in order to obtain sufficient

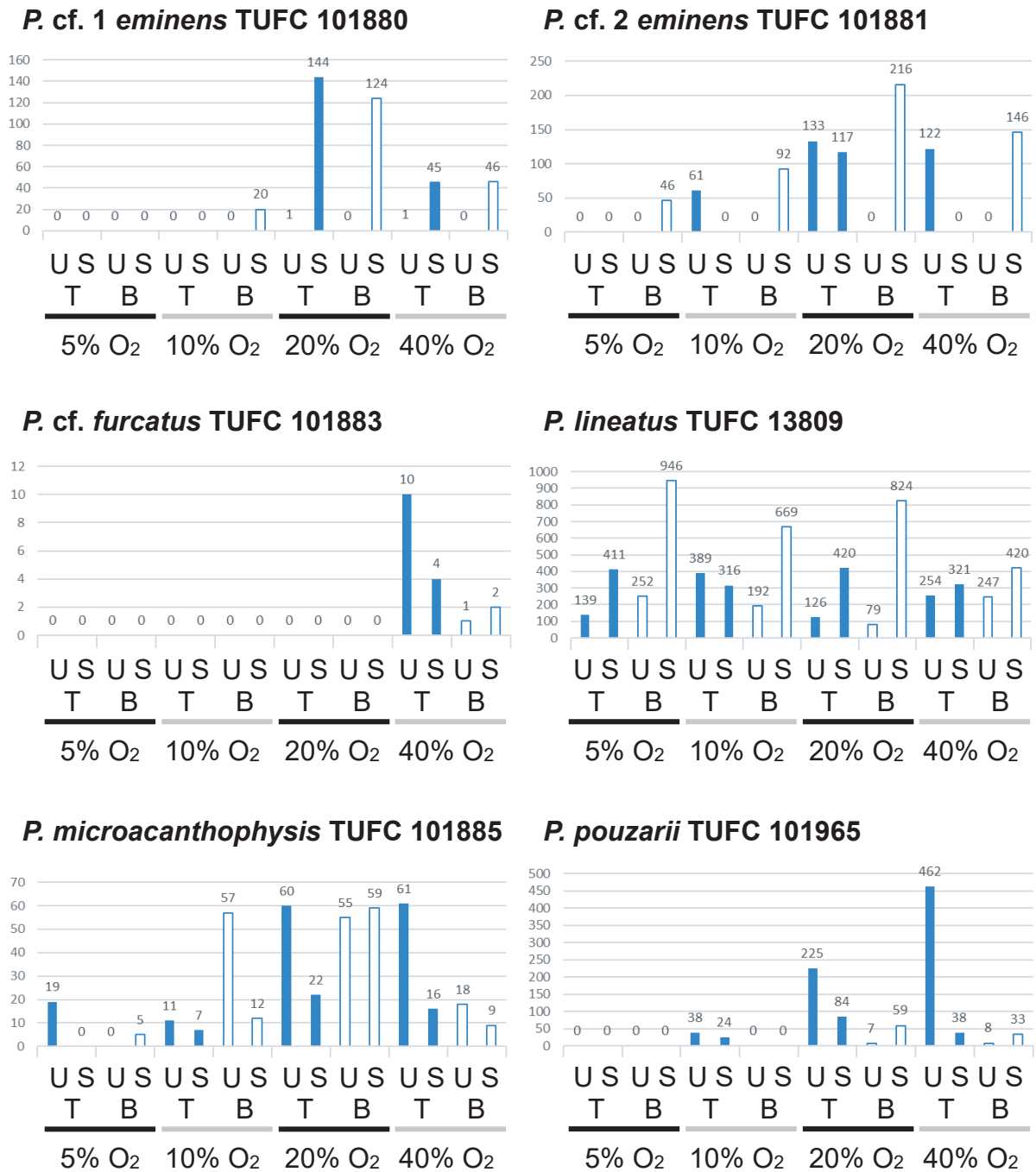


Fig. 7 – Differences in the number of acanthophyses produced on agar discs under four different O₂ concentrations (5, 10, 20, and 40%) by six *Physisporium* strains. The vertical axis of the bar graph shows the number of acanthophyses. “T” and “B” under the horizontal axis mean the agar discs inoculated on a CMA plate as top face up and back face up, respectively. “T” is shown by a blue bar and “B” by a white bar. “U” and “S” indicate the upper surface and side face of the agar discs.

O₂ for extending hyphae into the media. However, *P. cf. furcatus* TUF 101883 responded differently, as producing more acanthophyses under the 40% O₂ condition than under the 20% O₂ condition (Fig. 7). Therefore, the sensitivity to O₂ may differ among species and/or strains. In the present experiments, most strains formed less acanthophyses under the low O₂ conditions, 5% or 10%. This is assumed that there was insufficient difference in oxygen concentration between inside and outside the culture medium to induce acanthophysis formation. Further detailed physiological study is required to verify this hypothesis. Interestingly, *P. lineatus* TUF 13809 exceptionally formed numerous acanthophyses, even

under 5% O₂ condition (Fig. 7). This species is known to show a high level of mycelial growth rate even in low O₂ concentrations (Hood et al., 1997) and to cause the decay in heartwood, especially root and butt rot of living trees in Asia and North and South America [Dai et al., 2007 (as *Rigidoporus lineatus*); Kobayashi, 1972 (as *R. zonalis*); Overholts, 1953 (as *Polyporus zonalis*); Rajchenberg & Robledo, 2013 (as *R. lineatus*)]. The internal part of trees is not normally exposed to the atmosphere, so such heart rot fungi must be adapted to the low oxygen condition. Though SS formation by *P. lineatus* has not been reported yet, the ability to produce large numbers of acanthophyses even under low O₂ concentrations is likely to

contribute to the high rate of hyphal growth and the heart rot in trees. Additional investigations on the ecology of this species are needed. Hyde and Goh (1998) reported an unidentified fungus that formed tufts of acanthophyses on the apices of root-like hyphal strands on wet wood collected in several tropical streams. Based on the habits and observations of the characteristics of the fungus, they guessed that the function of acanthophyses was to take up O₂ in water. However, their discussion was speculative at the time. Because the fungus was not identified or observed in its sexual state, the taxonomic assignment of their fungus should be clarified by further study.

Basidiomycetous fungi have been considered to prefer terrestrial environments to aquatic environments; this is suggested by the fewer number of aquatic species compared to terrestrial species (Jones et al., 2014; Shearer et al., 2007). However, our findings showed that *Physisporinus* species have adapted to humid environments such as streams and waterfalls by acquiring the ability to form SSS/acanthophyses, which appear to function as respiratory organs. This may be a strategy for terrestrial fungi in origin to adapt to aquatic habitats and decay water-saturated wood with low O₂ concentrations. Further research of the basidiomycetous fungi inhabiting wet habitats should be undertaken to better clarify their biodiversity and ecology.

Disclosure

The authors declare no conflicts of interest. All the experiments undertaken in this study comply with the current laws of the country where they were performed.

Acknowledgments

This study was supported, in part, by a Grant-in-Aid from the Institute for Fermentation, Osaka (IFO). We appreciate staff of Westerdijk Fungal Biodiversity Institute for providing living cultures of *Meripilus*, *Physisporinus* and *Rigidoporus* species. We also great thank Ms. Sachiko Ueta and Kaori Shimizu, FMRC, for taking on the cryopreservation and maintenance of strains investigated in the present study. Deposition and utilization of Tottori University Fungal Culture Collection (TUFC) strains were supported by FMRC through the National BioResource Project (NBRP) of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan (<http://nbrp.jp>).

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