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Article

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Abstract: Typhulaceae Jülich is one of the cold-adapted fungal families in basidiomycetes. The representative genera, *Typhula* (Pers.) Fr. and *Pistillaria* Fr. are distinguished by the discontinuity between stems and hymenia in the former and the continuity in the latter (Fries 1821). This taxonomic criterion is ambiguous, and consequently the view of Karsten (1882) has been widely accepted; *Typhula* develops basidiomata from sclerotia, while basidiomata develop directly from substrata in *Pistillaris*. But, Corner (1970) observed basidiomata of *Pistillaria petasitis* S. Imai developed from sclerotia in Hokkaido, Japan. We later recognized *P. petasitis* basidiomata emerged directly from substrates on the ground also in Hokkaido. An aberrant form of *Typhula hyperborea* H. Ekstr. was found in Upernavik, West Greenland. This specimen had a stem-like structure on a Poaceae plant, and sclerotia developed on its tip. Similar phenomena were found in other *Typhula* sp. in Japan. These findings imply that both *Typhula* and *Pistillaria* have the potential to produce sclerotia as well as the capacity of mycelial growth at ambient air temperatures in each locality when samples were collected. These findings suggest that *Typhula* spp. developed basidiomata not only from the sclerotia dispersed by the basidiospores but also mycelia generated by the spore germination formed basidiomata multiple times depending on their growth environments.

Keywords: cryophilic; ecophysiology; growth temperature; life history; local climate

1. Introduction

Approximately 80% of the biosphere are constantly and seasonally cold and have a temperature below 5°C, and polar regions occupies 14% of the biosphere (e.g. [1]). The biosphere, with an exception of deep sea and stratosphere, is almost identical to the cryosphere. The term cryosphere was proposed by the Polish geophysicist and meteorologist, A.B. Dobrowolski [2,3] and collectively describes the portions of the Earth's surface where water exists as the frozen state – snow cover, glaciers, ice sheets and shelves, freshwater ice, sea ice, icebergs, permafrost, and ground ice [4].

Several kinds of microorganisms especially fungi were reported from the cryosphere [5–9]. Fungi are eukaryotic organisms without plastids, nutrition absorptive, unicellular or filamentous and consisting of multicellular coenocytic hyphae [10]. The true fungi form kingdom Fungi and the organisms studied by mycologist, fungi are mostly placed here, but others belong to the kingdoms Protozoa and Chromista [10,11]. Fungal species were less frequently recorded from the cryosphere than those of temperate zone despite that all fungal taxa have already been found in the cryosphere. These records suggest a possibility that various fungi are active under various cold environments.

We proposed the term 'cryophilic fungi' to denote fungi adapted to the cryosphere [12]. The concept of cryophilic fungi is defined as fungi that are present in the cryosphere, spend a certain life stage or whole life cycle (sexual and/or asexual reproductive stages), and grow under subzero temperature where water remains in the solid state such as snow and ice. The concept of cryophilic fungi also applies to uncultured fungi such as mycorrgizal fungi.

Their life cycles are affected by environmental factors [13]. This group of fungi includes psychrophile and psychrotolerant. The psychrophily is defined by physiological characteristics at each stage of their lifecycle [13]. Snow molds, representing the cryophilic fungi, develop mycelia to attack dormant plants such as forage crops, winter cereals, and conifer seedlings under snow cover [9]. Some of them pass the dormancy from spring to autumn in the form of sclerotia in temperate and frigid zones, as well as the Arctic [14–27] and Antarctica [8,29,30], e.g., *Typhula incarnata* Lasch, *Typhula ishikariensis* complex (consisting to *T. ishikariensis* S. Imai, *T. canadensis* (J.D. Sm. & Årsvoll) Tam. Hoshino, T. Kasuya & N. Matsumoto and *T. hyperborea* H. Ekstr.), *Sclerotinia boreslis* Bubák & Vleugel., and *Sclerotinia antarctica*.

In 2007, the first author collected aberrant sclerotia of *Typhula* sp. (Figure 1D–F) from Upernavik (72.7869, -56.1472), West Greenland. They resembled those of *T. hyperborea* (Figure 1C), but were often formed on the tip of a stem-like structure on the Poaceae host. The stem-like structure was indistinguishable from the stems of *Typhula* sp. (Figure 1D–F) and seemed identical to secondary sclerotia of hybrids of *T. ishikariensis* var. *ishikariensis* S. Imai × *T. ishikariensis* var. *idahoensis* (Remsberg) Årsvoll & J.D. Sm. produced under artificial conditions [31,32].

Pistillaria Fr. is close to the genus of *Typhula* Fr. in Typhulaceae Jülich [33] and saprophytic. The hymenium of *Typhula* is distinguishable from the stem, but indistinguishable to the stem in the genus *Pistillaria* [34]. However, this criterion proved unclear to separate both genera. Consequently, Karsten's view has been widely accepted; *Typhula* spp. develop basidiomata from sclerotia, and *Pistillaris* spp., lacking sclerotia, develop basidiomata directly from substrata [35]. But, Corner [36] observed basidiomata of *Pistillaria petasitis* S. Imai developed from sclerotia in Hokkaido, Japan.

In this study, we aimed to elucidate the plasticity in the genera *Typhula* and *Pistillaria* though the interactions between their ecophysiological potential and environmental conditions in their localities. The results here revealed that repeated basidioma production was dependent on both ambient temperatures and the length of time without snow cover after basidioma formation.

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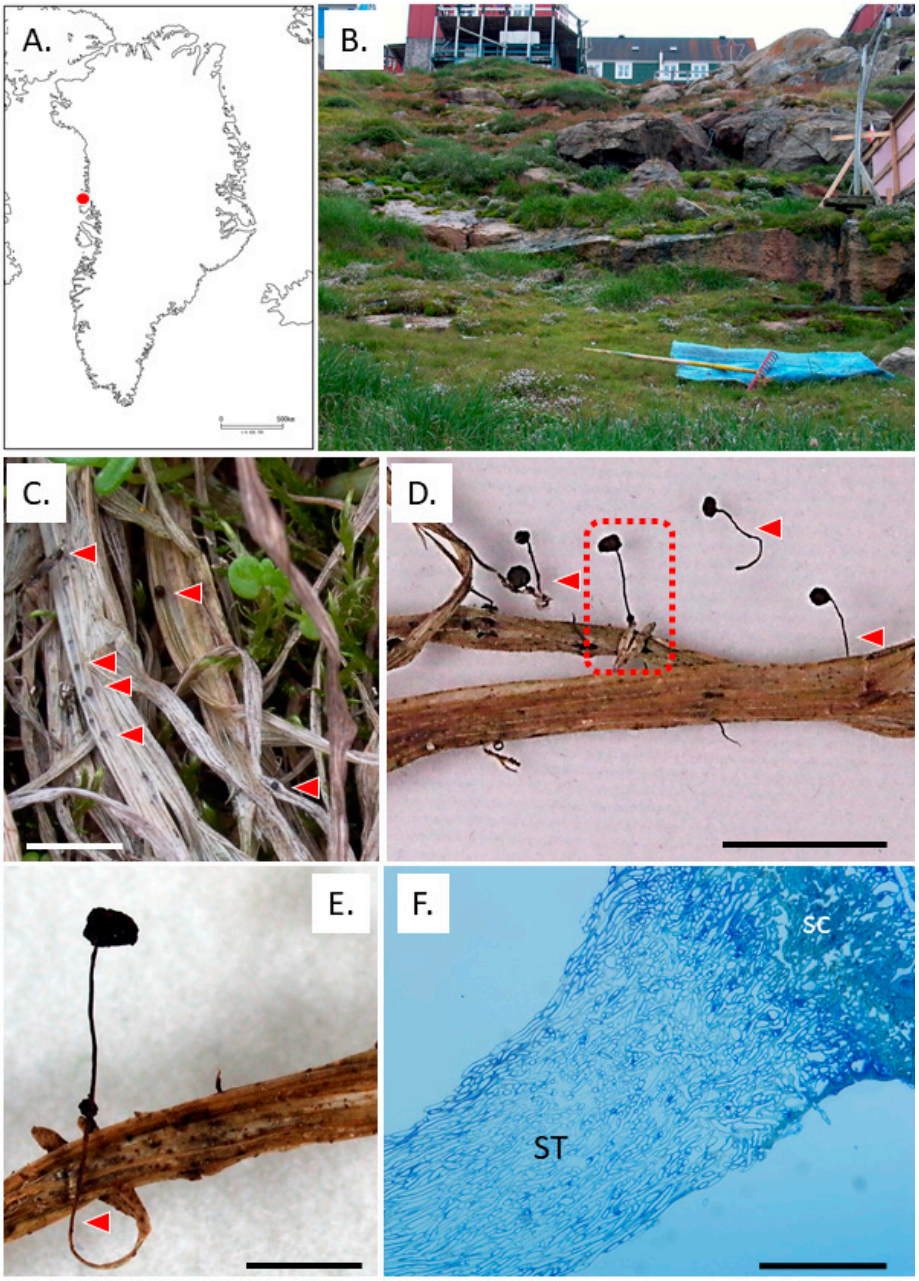

Figure 1. Aberrant sclerotia from Upernavik, West Greenland. Collected locality (**A**). Collected site (**B**). Normal sclerotia of *T. hyperborea* (**C**). Collected aberrant sclerotia (**D**). Red triangles: stem-like structures. Close-up view of the rectangular in D (**E**). Vertical section of aberrant sclerotia. SC: sclerotia, ST: stem-like structure. Bars 1 cm (**C**–**E**), and 100 μm (**F**).

2. Materials and Methods

2.1. Fungal materials

Fungal sclerotia were collected from decayed leaves and stems of decayed plants during the surveys. Sclerotium samples were packed in paper envelopes and dried at room temperature during transportation. Sclerotia were surface-sterilized in 70% (v/v) ethanol for 10 sec, in 0.5% (as active chlorine) sodium hypochlorite solution for 30 sec, and thoroughly rinsed in sterilized distilled water. They were then cut with sterilized steel blades, placed on potato dextrose agar (PDA: Difco, Sparks, MD, USA) and incubated at 4°C. Mycelia from growing colony margins were transferred to PDA slants and maintained at 0°C.

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Collected basidiomata were put in plastic cases with wet cotton balls and kept in a refrigerator. Basidiomata were attached to the inside of Petri dish lids with double-sided adhesive tape, and spores were collected on PDA plates to incubate at 4°C for 1 or 2 days. Basidiospores subsequently germinated and mated to produce heterokaryons and sclerotia. These sclerotia were transferred to fresh PDA plates and incubated at 4°C for 2 weeks. Isolates were maintained on PDA slants at 0°C.

These specimens were kept in the mycological herbaria of National Museum of Nature and Science, Tokyo (TNS).

2.2. Mating experiments

Monokaryons of *T. ishikariensis* var. *ishikariensis* (strains PR7-6-7 and PR9-4-3 from Japan) and *T. canadensis* (strains 35-8 and 8-2 from Japan) were designated as testers and paired with dikaryons of collected strains (di-mon mating; [37]) on PDA plates and incubated at 4°C for one month. A small agar block was cut from monokaryon colonies near the colony junction and transplanted to another PDA plate. Growth from the block was then examined for the presence of clamp connections on hyphae after incubation for 5 to 7 days at 4°C. The presence of clamp connections on hyphae was the criterion of mating compatibility

2.3. Phylogenic analyses

Fungal strains were cultured for 1 month at 10°C on PDA or Wort agar (HiMedia Laboratories, Pvt. Ltd., Mumbai, India). Sclerotia of *T. hyperborea* from Upernavik, West Greenland and *S. nivale* were harvested and DNA was extracted by the protocol of DNeasy Plant MiniPrep (QIAGEN GmbH, Germany). ITS regions including the 5.8S gene of genomic rDNA were amplified using primer pair ITS1 (5'TCCGTAGGTGAACCTGCGG) and ITS4 (5'-TCCTCCGCTTATTGATATGC), as described by White *et al.* [38]. PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN GmbH, Germany) and sequenced in one direction on ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA) using the ITS1 primer. Multiple alignment of the ITS sequences was performed, and the nucleotide substitution rate (Knuc value) was calculated in CLUSTAL W [39]. A phylogenetic tree was constructed by neighbor-joining method [40,41] using the program CLUSTAL W.

2.4. Morphological observations

Colors of basidiocarps and sclerotia were described according to the color identification chart of the Royal Botanic Garden Edinburgh (Flora of British Fungi) [42].

For light microscope section, aberrant sclerotia of *Typhula* sp. were fixed with 2% glutaraldehyde (Nisshin EM, Co., Ltd., Tokyo, Japan) in 50 mM phosphate buffer, washed the same buffer. The samples then post-fixed with 1% osmium tetroxide (Nisshin EM, Co., Ltd., Tokyo, Japan), dehydrated with an ethanol series, and embedded in Quetol 651 (Nisshin EM, Co., Ltd., Tokyo, Japan). The sections (0.8 μm thick) were stained with toluidine blue (Wako Ltd., Osaka, Japan) and observed under a light microscope.

2.5. Mycelial growth temperature

Five mm diam discs with mycelia were cut from the margins of actively growing colonies, inoculated to the centers of PDA plates, and incubated at six different temperatures from 0° to 25°C in duplicate. After 1, 2, and 3 weeks of inoculation, colony diameters were determined. Linear mycelial growth rate per week was calculated after the initial lag period.

3. Results

3.1. Typhula hyperborea in Greenland

Fungal cultures from aberrant sclerotia from Upernavik West Greenland had feather-like mycelia when grown on PDA at 4°C (Figure 2A), which were typical physiological reaction of *T. hyperborea* [28], and our strain did not react when paired with both tester monokaryons of *T.*

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ishikariensis var. *ishikariensis* and *T. canadensis*. Phylogenic analysis of ITS regions also supported this assumption (data not shown).

When the cultures were kept at 4°C for 2 years, sclerotia were found to have stme-like structures with intercalary, small secondary sclerotia as described by Christen [31,32] (Figure 2B). Cultures of *T. ishikariensis* var. *ishikariensis* from Hokkaido, Japan also produced similar aberrant sclerotia. Stem-like structures and aberrant sclerotia of *T. hyperborea* and *T. ishikariensis* var. *ishikariensis* on PDA were dark brown (19 bay) as same color with field samples (Figure 1D,E). However, normal stems of both fungi were white (2 B) to pale yellow (6 E) or pale brown (30 clay pink) [28]. Collected stem-like structures of *T. hyperborea* from Upernavik West Greenland were gradually connected with aberrant sclerotia (Figure 1F), and similar findings were reported the base of the stem of *Typhula sclerotioides* (Pers.) Fr. at its origin from the sclerotium [43]. These results suggested that the germination of sclerotia and the secondary sclerotium formation were reversible for *T. hyperborea* and *T. ishikariensis* var. *ishikariensis*.

The strain OUP1811 from Nuuk (64.1666, -57.7500), also in West Greenland was different in culture morphology on PDA plates (Figure 2C). This strain had weak pathogenicity against host [28], and they showed normal growth at 4–10°C and had abundant aerial mycelia without sclerotia on PDA. Sclerotium formation was, however observed on oatmeal agar plates (Figure 2D).

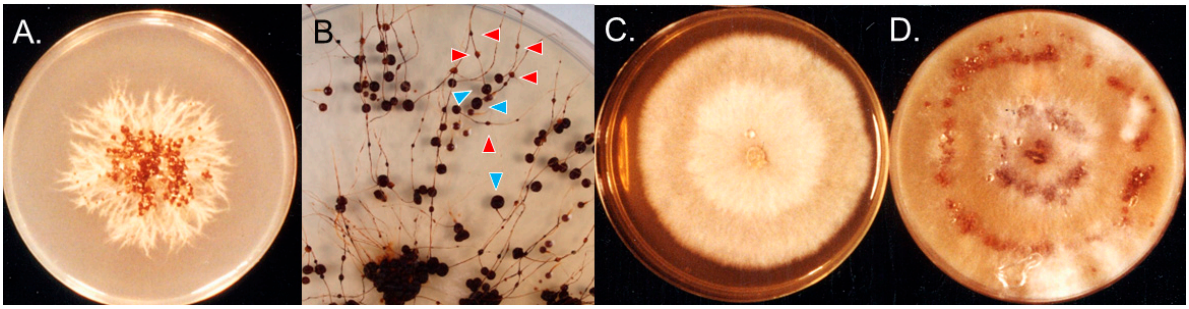

Figure 2. Mycelial growth of *Typhula hyperborea* from Greenland. Isolate from irregular sclerotia from Upernavik on PDA at 4°C for 1 month (**A**) and 2 years (**B**). Blue triangles: original sclerotia. Red triangles: secondary triangles. OUP1811 from Nuuk on PDA at 4°C for 1 month (**C**) and oatmeal agar plates at 4°C for 1 month (**D**).

3.2. Typhula sp. *and Pistillaria petasitis in Hokkaido, Japan*

Basidiomata of *Typhula* sp. whose hymenium was distinguishable from the stem were found on the dead petioles of *Kalopananax septemlobus* in the Ashoro Research Forest, Kyushu University, Hokkaido, Japan (43.2507, 143.5498, altitude 114–471m) in October 2010 (Figure 3A,B). Basidioma: ca. 1.5–3.0 cm high, white (2 B). Head: ca. 2.5–8.5 × 0.5– 1.8 mm, clavate to cylindric, obtuse, straight or curved. Stem: ca. 3.5–21.5 × 0.5–1.0 mm, opaque, white (2 B). Although the specimens lacked, resultant cultures produced sclerotia on PDA (Figure 3C). Sclerotium: ca. 1–2.5 mm diam, globose to subglobose, almost black (36 fuscous black).

Basidiomata of *P. petasitis* without sclerotia were observed on the hillside of Mt. Asahidake (43.6511, 142.7990, altitude 1,100m), Higashikawa, Hokkaido (Figures 3A and 4A,B), in August 2010. Basidioma: ca. 1–3.8 cm high, white (2 B). Head: ca. 2.5–26.5 × 1.5–6.5 mm, clavate to cylindric, obtuse, straight or curved. Stem: ca. 5–12.5 × 0.8–3.5 mm, opaque, white (2 B). The fungus also observed in the forest neighboring agricultural field in Hokkaido Agricultural Research Center, NARO, Sapporo, Hokkaido, Japan in September 2010.

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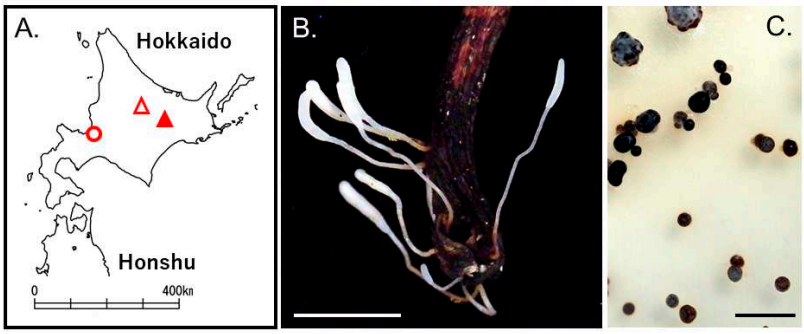

Figure 3. *Typhula* sp. on *Kalopananax septemlobus* in Ashoro, Hokkaido, Japan. Collected locality (**A**). Closed red triangle: Ashura Research Forest, Kyushu University, open red triangle: Mt. Asahidake, Higashikawa, and red circle: Sapporo. Basitiomata of *Typhula* sp. (**B**). Sclerotia of *Typhula* sp on PDA at 4°C for 1 month (**C**). Bars 1 cm (**B**) and 5 mm (**C**).

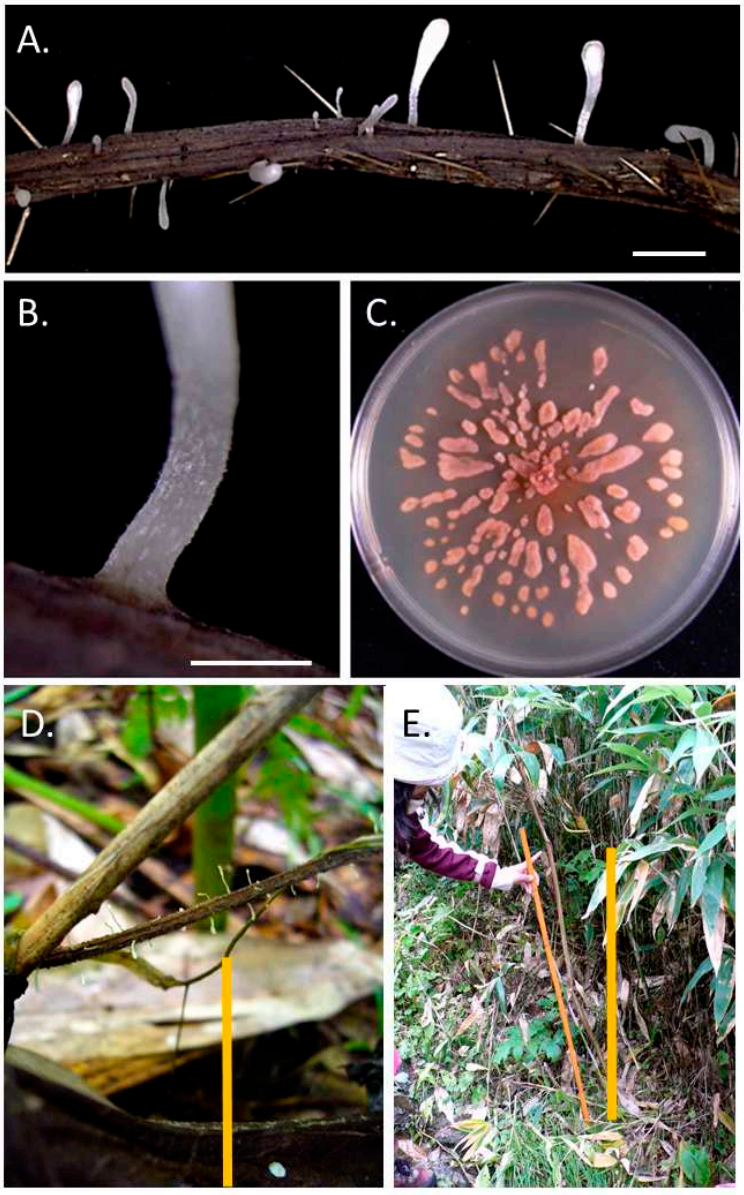

Figure 4. *Pistillaria petasitis* on Mt. Asahidake, Higashikawa, Hokkaido, Japan. Basitiomata of *P. petasitis* (**A**, **B**). Sclerotia of *P. petasitis* on PDA at 4°C for 1 month (**C**). Field observations on 3rd August (**D**) and 23rd August in 2010 (**E**). Bars 2 cm (**A**), 1 cm (**B**), 5 mm (**C**), 10 cm (**D**), and 80 cm (**E**).

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Pistillaria petasitis basidiomata were found on various substrates such as *Petasites japonicus*, *Conioselinum filicinum*, and *Cirsium kamtschaticum*. Basidiomata of *P. petasitis* were found on substrates on the ground surface to 10cm on 3rd August 2010 (Figure 4D). Up to 80cm on 23rd August 2010 (Figure 4E). All *P. petasitis* isolates produced sclerotia under culture conditions (Figure 4C). Sclerotia of our isolates were similar as described Corner [36], and ca. 2.5–22.5 × 2.5–5.5 mm, fusiform, somewhat flattened, light brown (15 brick).

3.3. Effect of temperature on mycelial growth

Optimal mycelial growth temperatures of *T. hyperborea* from Upernavik, West Greenland, *Typhula* sp. from Ashoro, and *P. petasitis* from Higashikawa occurred at 5, 15, and 20°C, respectively (Figure 5). Maximal growth temperatures were 15, 25 and 30°C, respectively. The mycelial growth range of *T. hyperborea* Upernavik, West Greenland, was psyhrophilic and typical in this fungus [28]. According to ranges of their mycelial growth temperature, *Typhula* sp. from Ashoro and *P. petasitis* from Higashikawa were psychrotolerant. This is the first record of the mycelial growth range of *P. petasitis*.

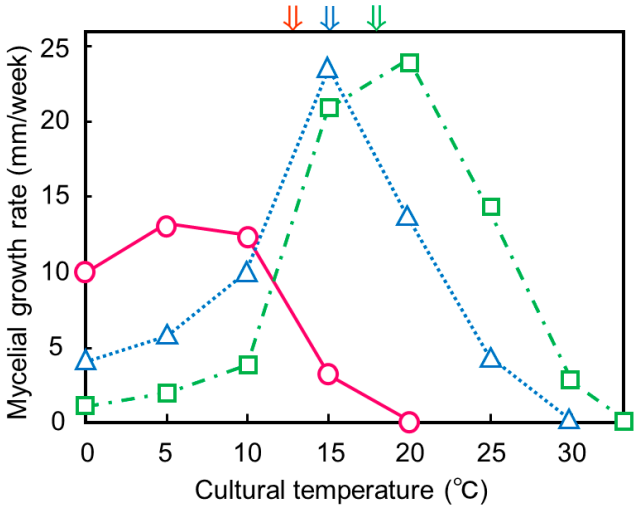

Figure 5. *Effect of cultural temperature on mycelial growth.* Red circles: *Typhula hyperborea* in Upernavik, West Greenland. Blue triangles: *Typhula* sp. on Ashoro, Hokkaido, Japan. Green squares: *Pistillaria petasitis* on Mt. Asahidake, Higashikawa, Hokkaido, Japan. Magenta arrow: 12°C, maximal air temperate in Upernavik. Blue arrow: 15.5°C, average air temperature in September in Ashoro. Green arrow: 18°C, average air temperature in September in Sapporo.

These growth temperature relations agreed with the ambient temperatures when basidiomata samples were collected in each locality. These results suggested that the mycelia of these three fungi could survive and be active without snow in their localities.

4. Discussion

Snow molds representing cryophiles resume growth typically by sporogenic germination of sclerotia (sexual cycle) in autumn. Mycelia prevails on dormant plants under the snow to produce sclerotia in late winter before dormancy (asexual cycle). The lifecycle of the genus *Typhula* is illustrated in Figure 6. Basidiospores germinate to develop into monokaryons which subsequently mate with their counterparts differing in mating incompatibility alleles to produce dikaryon. Dikaryons are capable of sexual recombination through carpogenic germination of sclerotia, which is critical to generate diversity to cope with fluctuating environments and flexibility, as we found in this study.

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Kawakami *et al.* [44] elucidate conditions required for stem elongation from the sclerotium and fertile head development in *T. ishikariensis* from Hokkaido, Japan. Stem elongation occurred at low temperatures and high humidity, but the light was not essential. In contrast, light and moderate day length (8h/day) were essential for fertile head development. Several strains of *T. hyperborea* also produced basidiomata under Kawakami's condition [28]. *T. hyperborea* in the Arctic also act to produce the asexual formation of basidiomata dispersing basidiospores under light condition (red dashed line in Figure 6). Mycelia of *T. hyperborea* did not produce basidiomata under snow and dark condition. They formed sclerotium on the top of the remaining stems (Figure 1D,E). Our observation of sclerotia with stems was similar to secondary sclerotia described by Christen [31,32]. In addition, Tkachenko [45] reported another type of secondary sclerotia in the original sclerotia of *T. ishikariensis* on tulip bulbs in Russia. One to seven secondary sclerotia were found as original sclerotia. We also observed this type of secondary sclerotia from bloated original sclerotia from *T. hyperborea* from Upernavik (Figure 2B) and other strains of *T. ishikariensis* complex (Hoshino *et al.* unpublished results).

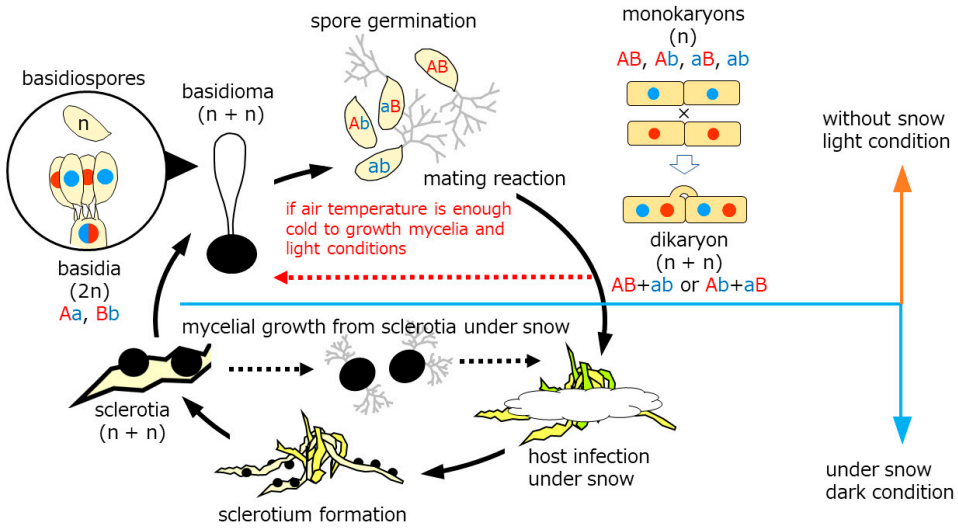

Figure 6. *Lifecycle of Typhula spp. in the cryosphere.* Solid lines: sexual reproduction stages. Black dashed lines: known asexual reproduction stages. Red dashed line: our finding stage.

Corner [43] suggested that the aggregation of hyphae of *Typhula gyrans* (Batsch) Fr. developed into the tissue of the stem and sclerotium. Those of *Macrotyphula phacorrhiza* (Reichard) Olariaga, Huhtinen, Læssøe, J.H. Petersen & K. Hansen also extended throughout the head. However, the texture of the hyphal walls in the head and central part of the stem was never as tough as in the sclerotium or on the surface of the stem. In addition, the mycelia of *M. phacorrhiza* and other *Macrotyphula* spp. on PDA plates were harder than those of *Typhula* spp. (Hoshino *et al.* unpublished results). *M. phacorrhiza* was past type species and *T. incarnata* is present type species of *Typhula* [46]. These points suggested that steam and sclerotium had common properties in *Typhula* and related genera. Lind [47] noted that comparatively, many rust, smuts, and species of Dothideales Lindau in the Arctic have a perennial mycelium in the host, enabling them to grow as soon as the season starts. Previously we reported that several strains of *T. hyperborea* in West Greenland were weak pathogenic activity [28] and had abundant aerial mycelium and less productivity of sclerotium (Figure 2C,D). These physiological characteristics supported the adaptation to the Arctic summer climate (blight and cold conditions).

Similar phenomena were also recorded from other *Typhula* sp. in the Arctic (loss of sclerotium-forming ability under cultural conditions) [27] and *T.* cf. *subvariavilis* in Antarctica (no-sclerotia at the field survey) [30]. Basidiomata of *T.* cf. *subvariavilis* in Antarctica emerged directly from substrates, and this fungus had high homology of ITS region with *Typhula* sp. Wh-1 in Iran [48] and *Typhula variabilis* Riess. Snow mold symptoms of *T.* cf. *subvariavilis* in Antarctica and *Typhula* sp. Wh-1 in Iran

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were not observed sclerotia just after the snow melts, and *T. variabilis* was widely reported in the Northern Hemisphere, including rarely snow areas such as the Azores [49]. Sclerotia of *Typhula* sp. in Iran were formed at ca. 2 wk to 1 month after the snow melt (Figure 7). *Typhula* sp. in Iran formed immature sclerotia that were mainly mycelial aggregations after the snow melt, and sclerotia matured without snow. *T. canadensis* in Norway also had sclerotia with aerial mycelia in the field, and these sclerotia with aerial mycelia could act as airborne propagules [50,51]. However, these sclerotia matured under the snow cover. Therefore it was different than the ecophysiological characteristics of sclerotia from *T.* cf. *subvariavilis* in Antarctica and *T. canadensis* in Norway. These results suggested that *T.* cf. *subvariavilis* in Antarctica and other related species could still act after the snow melts in their localities.

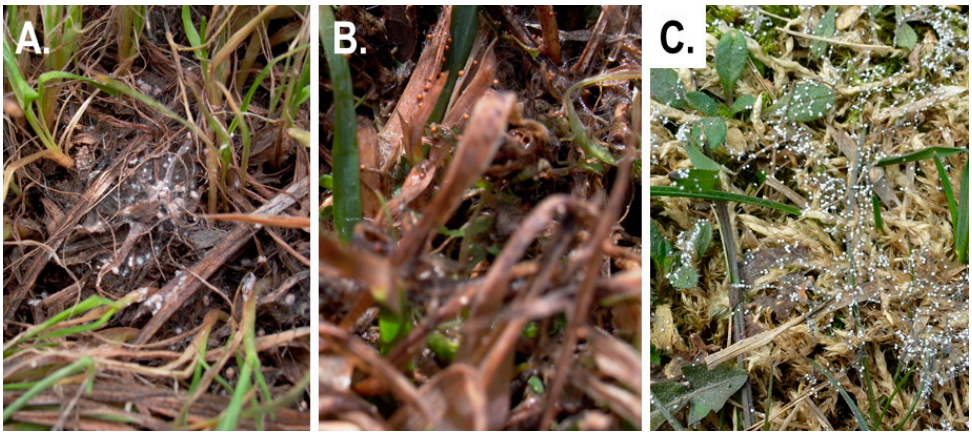

Figure 7. *Sclerotia of Typhula sp. in northern Iran and Sclerotium nivale in Moscow, Russia.* Immature sclerotia, mainly mycelial aggregations, ca. 2wks after snow melt in Nir (38.0360, 48.0105), Ardabil Province at 27thFebruary 2004 (**A**). Mature sclerotia, ca. 1 month after snow melt in Qazvin (36.2311, 49.9982), Qazvin Province at 7th March 2004 (**B**). Sclerotia of *S. nivale* on lawn in Russian State Agricultural University – Moscow Agricultural Academy at 24th April 2013 (**C**).

Tkachenko [52] described other white sclerotia of snow mold, *Sclerotium nivale* Elenev Nom. Inval. Art. 39.1 (ICN Shenzhen Code) [53]. Their Russian name is "Снеговая крупка" in English as "Snow tiny pellets" or "Snow tiny grain." This fungus was recorded in Russia, Ukraine, and Estonia [54]. Mycelia were web-like without clamp connections, and sclerotia were white (2 B), globose 0.5–1 mm diam. on leaves and stems of host plants. The sexual stage of this fungus was not reported (Sokirko *et al.* [55] described the apothecia of this fungus from matured black sclerotia. This description is not correct for *S. nivale*). ITS sequence (KY703612) of our strain of *S. nivale* was high homology (>99%) with ascomycetes, *Karstenula* Speg. and *Paraphaeosphaeria* O.E. Erikss. (Tkachenko *et al.* unpublished results), and both genera did not record producing the sclerotium. However, *Paraphaeosphaeria* included sclerotium parasites such as *Paraphaeosphaeria minitans* (W.A. Campb.) Verkley, Göker & Stielow [56]. These findings suggested that *S. nivale* was the possibility of the mycoparasite under the snow, and their white sclerotia were not related to the ecophysiological functions of the above *Typhula* spp.

Dominated strains of *T. hyperborea* showed irregular growth on PDA more than 10°C (Figure 2A). However, these strains showed normal growth at the same temperature on corn meal agar or PDA with free radical scavengers such as ascorbic acid or β-carotene [28]. When *T. ishikariensis* complex, *T. incarnata*, and *Typhula trifolii* Rostr. from Canada were first exposed to the maximum growth temperature (20 or 25°C) and then incubated their optimal growth temperatures, the *T. ishikariensis* complex formed a "fan-like" irregular colony that was similar to the colony morphology of typical *T. hyperborea* [57]. Oxygen uptake of the *T. ishikariensis* complex was optimal at 20°C (maximum growth temperature), about 15°C higher than its optimal growth temperature (5 to 10°C). Typical strains of *T. hyperborea* had strong pathogenicity against host plants. Therefore, they obtained free radical scavengers from hosts.

On the other hand, several strains of *T. hyperborea* from West Greenland obtained saprophytic activity (we did not collect such strains from East Greenland). Probably, they lost pathogenicity and acquired resistance to oxidative stress near maximum growth temperature. There is a positive correlation between virulence and psychrophily. However, this significance is unclear [58,59]. Most species belonging to Typhulaceae are psychrotolerant and saprophyte. Pathogenic species of *Typhula* spp. found the new resource of overwintering plants and evolved in a cold environment under snow cover.

We dived into general and specific ecophysiological strategies that fungi adapt to cold climates [59]. General strategies include "the increase of cell or spore size" [60–62], "the usage of natural cryoprotectants" [7,63], and "the production of ice-binding proteins (IBPs)" [64,65]. *T. hyperborea also* has large spores among the *T. ishikariensis* complex [28,66], and they have 7 isoforms of IBPs [67]. Specific strategies are "the shortcut of the life cycle" [68], "the changes of host plants" [69], and "the adaptation of osmotic stress" [70–73]. Many rusts in the Arctic have a simplified life cycle, only producing one kind of spore (micropuccinia) instead of a life cycle with three spore forms (eupuccinia) more commonly seen in warmer areas [47,68]. It is the first finding that the new lifecycle stage of the *T. ishikariensis* complex and similar phenomena were observed in *Typhula* sp. in Ashoro, Hokkaido (Figure 3) and *P. petasitis* in Higashikawa and Sapporo, Hokkaido (Figure 4). Many types of research of the *T. ishikariensis* complex were carried out in temperate or frigid zones where were higher air temperature than those of their psychrophily. Therefore, dikaryons of the *T. ishikariensis* complex grew only under the snow cover and formed sclerotia for the passing spring to summer seasons.

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