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Article

What Is "*Sclerotium nivale*"? Old and New Snow Mold in Russia

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Abstract: It has been reported that *Sclerotium nivale* Elenev causes winter damage (Sclerotium disease) to winter rye and turfgrass in the European part of Russia, Ukraine and Estonia. This fungal name is invalid, and their biological characteristics are limited. Since all reports on this fungus and their disease are written in Russian and Estonian without our recent review (Tkachenko 2013), few phytopathologists outside former Union of Soviet Socialist Republics know about this fungus. We found symptoms of Sclerotium disease in Moscow, Russia, and we obtained asexual spore-forming ascomycetous (absent clamp connections) and basidiomycetous (had clamp connections) isolates from their white sclerotia. "*S. nivale*" was composed of several fungal species of ascomycetes and basidiomycetes. The ascomycetous isolate had the ability to grow under the snow cover, and phylogenic analysis was suggested that ascomycetous isolate had high homology (98–99%) with genera of *Karstenula* and *Paraphaeosphaeria*. We previously reported sclerotia of *Typhula* sp. were formed ca. 2 weeks to 1 month after the snow melt, remained immature as mycelial aggregations after snow melt, and matured without snow. *In addition, Paraphaeosphaeria* included sclerotium parasites such as *P. minitans*. Several cold-adapted fungi infected basidiomycetous sclerotia of *Typhula* spp. and others. These findings suggested that ascomycetous *S. nivale* was the possibility of the mycoparasite under the snow.

Keywords: cold-adapted; cryophilic fungi; mycoparasite; psychrophile; psychrotolerant

1. Introduction

Snow molds are incited by many fungi that attack dormant plants, such as forage crops, winter cereals, and conifer seedlings under snow cover [1]. Although some of their taxonomic and ecological features have only recently been elucidated in basidiomycetous snow mold, *Typhula ishikariensis* S. Imai complex [2]. Several fungi, including unidentified species, were found under snow [3–5]. «Снеговая крупка» (Snegovaya krupka; Sclerotium disease) [6], *Sclerotium nivale* Elenev in European part of Russia and Ukraine is typical among unidentified fungi [7,8]. Kask [9,10] also recorded this fungus in Estonia. P.F. Elenev (Eleneff in some English literature), who was one of the pioneers of snow mold researchers in Russia, described this fungus in 1926 [11], but his diagnosis was invalid (no Latin diagnosis or description; ICN Shenzhen Code Art. 39.1). Therefore, this fungal name was not registered in any fungal database, such as Fungal Names, Faces of Fungi, Index Fungorum, or MycoBank.

Morphological and physiological characteristics of "*S. nivale*" were limited. Elenev mentioned that this fungus formed web-like mycelia and white sclerotia after snow melt, was widespread on wild grasses, and had weak pathogenicity against winter rye [11]. Kask [9] also described that the sclerotia of "*S. nivale*" were covered with their white-fluffy mycelia, and the diameters of their sclerotia were 0.5–1 mm under the natural condition. Dark gray masses of mycelia were formed in

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fluffy mycelia. Gutner et al. [12] illustrated the white sclerotia of "*S. nivale*" on cereal seedings. Kask [9] and Tkachenko [7] showed photos of the symptom with their sclerotia. Peresypkin [12] briefly described their distribution in the former Soviet Union and their life cycles. "Sclerotium disease was common in southern regions. Their sclerotia remained until autumn, germinating and infecting host plants under moist conditions." Sokirko et al. [13] also described, "After the snow melts from the fields, a gray coating with cotton wool-like clusters of mycelium appears on the leaves and stems of plants," and "Large quantities of first whitish, then blackening, dense, indefinitely shaped sclerotia are formed on the mycelium. They are well preserved in the soil and germinate, forming apothecia on the stalks — the apothecia with mature asci containing ascospores. Ascospores carry out primary infection of winter cereal seedlings". Unfortunately, there were no results or references to their sexual stages and reproduction.

Before O.B.T. summarized Russian snow molds in his review [7], "*S. nivale*" was recorded only as a "Phytopathological dictionary Russian–English–German–French" [6] in English literature. Since all reports on "*S. nivale*" were written in Russian or Estonian, few mycologists and plant pathologists outside the former Soviet Union know about this fungus. Several times, O.B.T. found and collected their white sclerotia, like "*S. nivale,*" in Moscow, Russia (Figure 1). In this article, we tried to elucidate the biological (morphological, physiological, and phylogenetic) characteristics of "*S. nivale*".

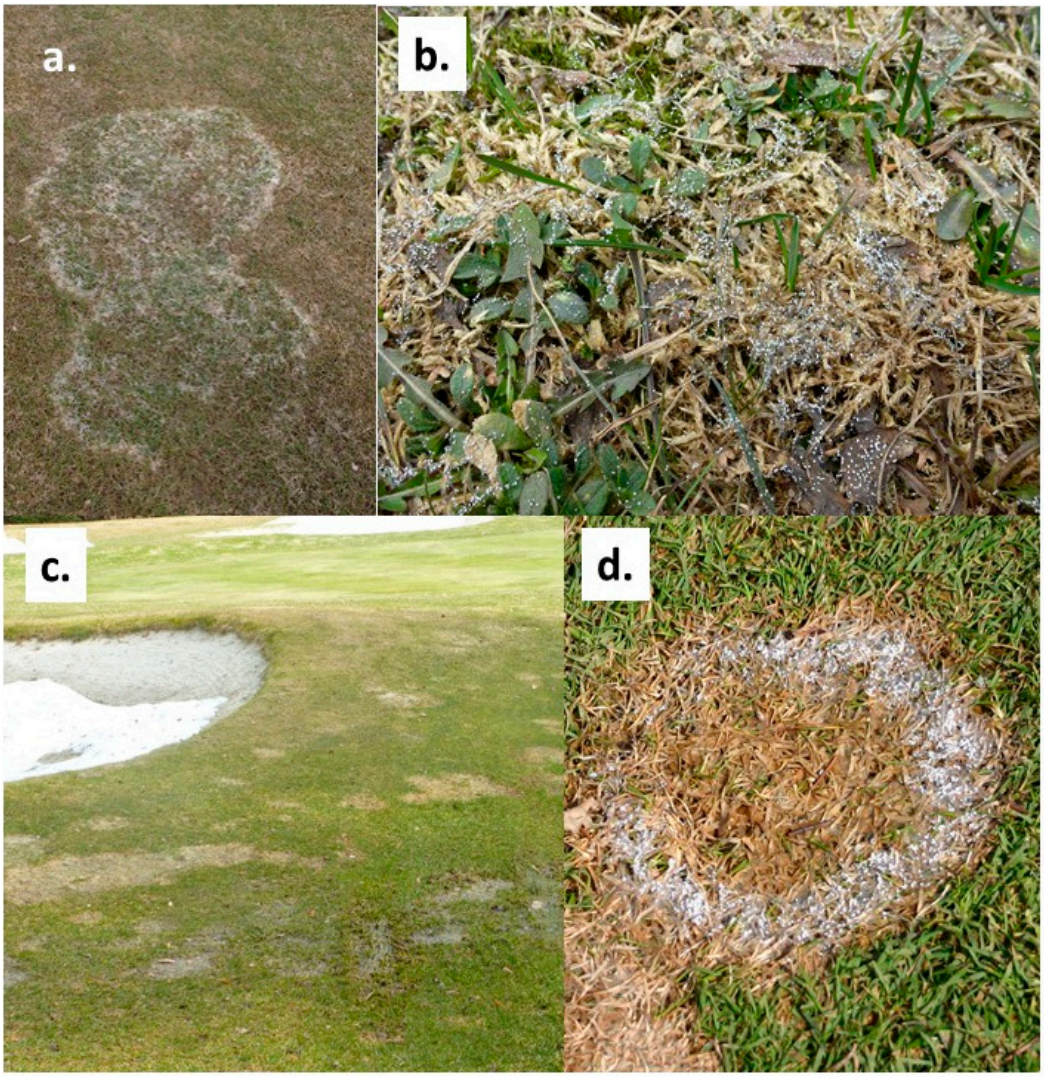

Figure 1. Symptoms and sclerotia of "*Sclerotium nivale*" in Moscow, Russia. (**a**) Symptoms; (**b**) Close-up of symptoms and white sclerotia on the lawn of Russian State Agrarian University – Moscow Agricultural Academy (RSAU-MAA) in 2013. (**c**) Symptoms; (**d**) Close-up of symptoms and white sclerotia on International Golf Course in Nakhabino (IGCN), Moscow district, Russia in 2020. Other

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photos of their symptoms were shown in supplemental figures (RSAU-MAA in Figure S1 and IGCN in Figure S2).

2. Materials and Methods

2.1. Fungal Materials

Fungal sclerotia were collected from decayed lawns at Russian State Agrarian University – Moscow Timiryazev Agricultural Academy (RSAU-MAA) in 2013 and International Golf Course in Nakhabino (IGCN), Moscow district, Russia, in 2020–2023. Collected sclerotium samples were packed in paper envelopes and dried at room temperature during transportation. Sclerotia were surface-sterilized in 70% (v/v) ethanol for 5 sec and thoroughly rinsed in sterilized distilled water. They were then cut with sterilized steel blades, placed on potato dextrose agar (PDA; HiMedia Laboratories, Mumbai, India), and incubated at 10°C. Mycelia from growing colony margins were transferred to PDA slants and maintained at 2°C.

2.2. Morphological Observations

Colors of sclerotia and mycelia were described according to the color identification chart of the Royal Botanic Garden Edinburgh (Flora of British Fungi) [15].

Confocal laser microscopy (CLM). Unfixed samples of mycelium and sections of sclerotia, made by hand with a razor blade, were stained on a glass slide for 1–2 h in a humid chamber with 100 µg/ml Berberine (BRB, Sigma-Aldrich, Merck Life Science LLC, Moscow, Russia), 5 mg/ml Calcofluor (CF, Fluorescent Brighter 28, Sigma-Aldrich, Merck Life Science LLC, Moscow, Russia), 50 µg/ml propidium iodide (PI, Sigma-Aldrich, Merck Life Science LLC, Moscow, Russia) in distilled water or 10-12 mg/ml dipyridamole (DPM, 2,2',2'',2'''-{(4,8-dipiperidinylpyrimido[5,4-d]pyrimidine-2,6-diyl)dinitrilo}tetraethanol, Sigma-Aldrich, Merck Life Science LLC, Moscow, Russia) in 5% acetic acid. The specimens were washed with water or 50% glycerol (DPM) and embedded in 50% glycerol. Samples were viewed on an Olympus FV1000-D confocal microscope (Olympus Corporation, Tokyo, Japan) immediately after staining under excitation with a combination of 405 nm, 473 nm, and 560 nm lasers. The stained samples were examined using 20–50% laser power. The signal was recorded in blue (425–460 nm), green (485–530 nm), red (560–660 nm) and differential interference contrast (DIC) channels. Channel parameters followed standard settings for DAPI (4,6-diamidino-2-phenylindole), Alexa Fluor 488, and rhodamine, respectively. All the dyes we use in confocal microscopy give a polychromatic image, nonspecifically interacting with chemical compounds of fungal cellular structures.

To examine frozen samples by scanning electron microscopy (cryoSEM), native sclerotia were adhered with thermal paste, AlSil-3 (OOO "ANT" Alsil-novye teknologii, Moscow, Russia) or KPT-8 (OOO Paika i Montaje, Moscow, Russia) to a 2 × 4 cm copper plate, which was then fixed with the same paste on a Deben CoolStage cooling stage in the chamber of a LEO-1430 VP scanning electron microscope (Carl Zeiss AG, Jena, Germany). Computer thermal paste improves sample cooling and image quality [16]. The samples were examined in the high vacuum mode at a temperature of -25°C and 15 kV acceleration voltage using a QBSD backscattered electron detector.

2.3. Mycelial Growth Temperature.

Five mm diam discs with mycelia were cut from the margins of actively growing colonies, inoculated to the centers of Czapek-Dox agar plates (Merck Life Science Sp. z.o.o., Warszawa, Poland, an affiliate of Merck KGaA, Darmstadt, Germany), and incubated at five different temperatures from 2°C to 14°C (pharmaceutical refrigerator MPR-311D(H), SANYO Electric Co., Ltd., Gunma, Japan) 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.

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2.4. 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 lawns at RSAU-MAA were harvested, and DNA was extracted using the protocol of DNeasy Plant MiniPrep (QIAGEN GmbH, Hilden, Germany). ITS1-5.8S-ITS2 (ITS) region of genomic rDNA were amplified using primer pair ITS1 (5'TCCGTAGGTGAACCTGCGG) and ITS4 (5'-TCCTCCGCTTATTGATATGC), as described by White *et al.* [17]. PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN GmbH, Hilden, Germany) and sequenced in one direction on ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using each primer set. Multiple alignments of the ITS sequences were performed, and the nucleotide substitution rate (Knuc value) was calculated in CLUSTAL W [18] and then manually edited in MEGA X [19]. Ambiguously aligned regions were excluded from the analyses. The final alignments were deposited in TreeBASE (https://treebase.org). Briefly, the analyses were performed using MEGA X [19] after testing the best models. According to the lowest BIC (Bayesian Information Criterion) scores, K2 +G were chosen as the optimal substitution models for the analyses of the ITS datasets. BI analyses were conducted using the same method reported by Kasuya and Ono [20], using MrBayes v.3.0b4 [21]. In BI analyses, the best-263 fit substitution models for the ITS dataset were estimated using MrModeltest v.3.7 [22] based on the hierarchical likelihood-ratio test (hLRT). The GTR+G+I model was selected as the best evolutionary model for the dataset.

3. Results

3.1. Morphological Characteristics of Sclerotium Nivale

Collected white (2B [15]) sclerotia were ca. 0.5–1mm in diameter and were covered with their mycelia and soil particles (Figures 1 and 2a, Figures S1 and S2). Sclerotia from IGCN, Moscow district had a layer of rind cells and their thickness ca. 4–7µm (Figure 2e), and their hypha had clamp connections (Figure 2f). We did not observe the rind cell surface structure of these sclerotia — the inside of the sclerotia (medulla) was composed of closely packed iso-diametric or oval-shaped cells (Figure 2b–e).

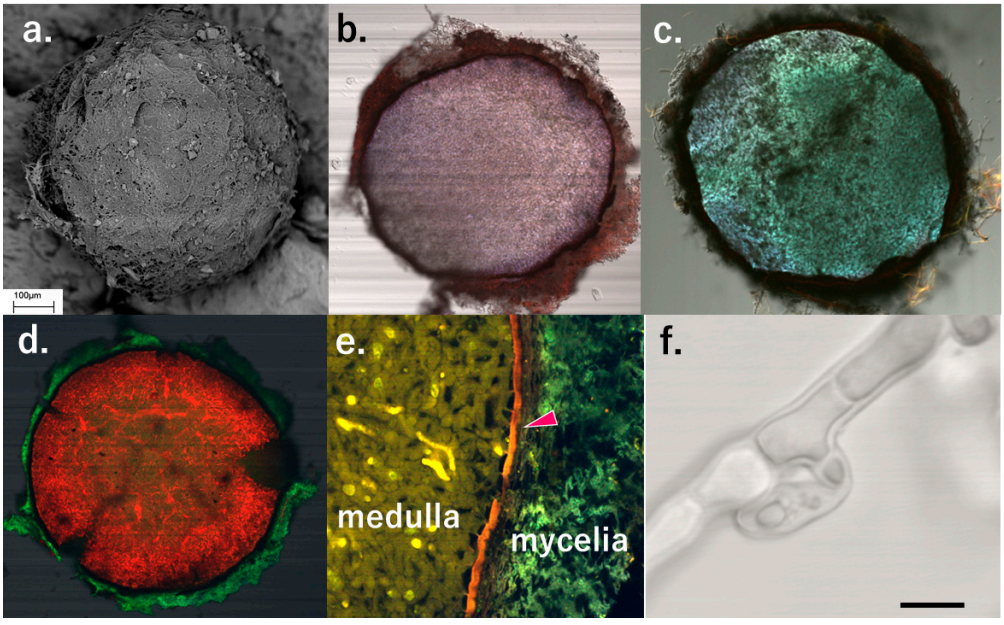

Figure 2. Sclerotia of "*Sclerotium nivale*" in IGCN, Moscow district, Russia, in 2020. (**a**) cryoSEM; (**b**) The cross section stained with DPM, (CLM); (**c**) with CF ;(**d**) with PI; (**e**) Close-up of sclerotium stained BRB. Magenta triangle: rind structure; (**f**) Hypha (DIC image), bar = 10µm.

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Our observations were similar to *those of S. nivale* by Elenev [10] and Kask [8]. Therefore, we identified that "*S. nivale*" caused our observed symptoms. O.B.T. observed these sclerotia and their symptoms several times (2013, 2020–2023) in Moscow, Russia. However, O.B.T. did not find any sexual stage (describe by Sokirko et al. [13]) of this fungus in nature.

The isolate IS-2013a was obtained from fungal sclerotia in the lawns of RSAU-MAA (Figure 3). Their mycelia were white (2B [15]) the early cultivation (Figure 3a) and gradually turned olivaceous buff (63 [15]) on wort agar plates (Figure 3b). This result was similar to the field observation by Kask [8]. White (78 [15]) and the mass of mycelia were also formed on wort agar plates (Figure 3a), but we did not observe sclerotium formation under cultural conditions.

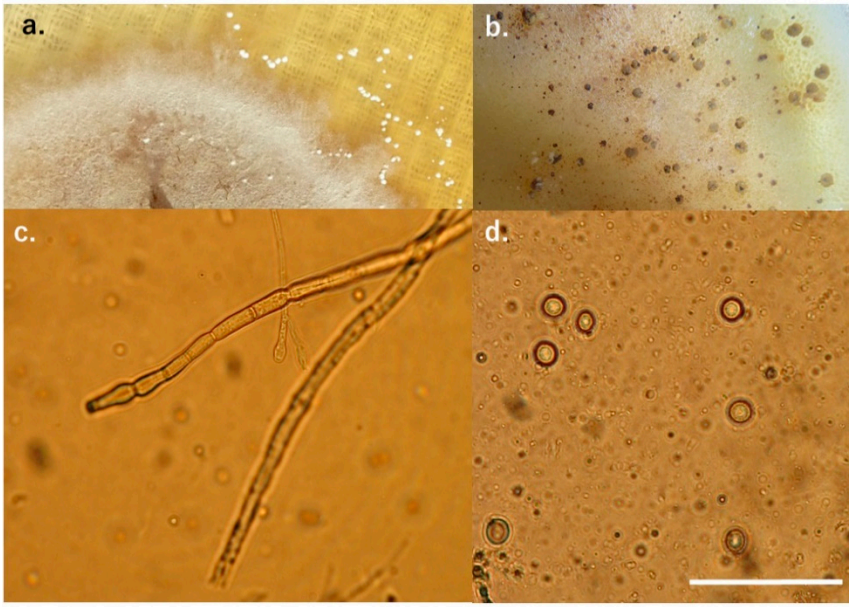

Figure 3. Cultural morphologies of "*Sclerotium nivale*" from RSAU-MAA (**a**) Fungal colony (IS-2013a) and white mass of mycelia on wort agar plates; (**b**) Old fungal colony (IS-2013a) formed asexual spores on wort agar plates; (**c**) Their hyphae without clamp connections; (**d**) Asexual spores (IS-2013a), bar = 50μm.

Hypha had septum but did not have clamp connections (Figure 3c), and citrine (64 [15]) masses of mycelia formed asexual spores (Figure 3d and the illustration of their conidia in Figure S3), which were round to ellipse (ca. 3—5 μm in diameters) and had smooth surfaces. This is the first report on the spore production of "*S. nivale.*" We identified that the isolate of IS-2013 was the ascomycete.

We also obtained another isolate, IS-2020, mycelia with clamp connections from sclerotia in IGCN, Moscow district, Russia (Figure 4). Their mycelia were white (78 [15], Figure 4a) and accumulated in intracellular (irregular growth "knot like" hyphae) and extracellular substances stained via rhodamine (Figure 4e and f). Mycelium formed snuff brown (17 [15]) sclerotia on PDA and wort agar (Figure 4b and c). Their size of sclerotium was as same as that in our field observation (Figure 1c and d). These results suggested that the isolate IS-2020) belonged to basidiomycetes, and symptoms of "*S. nivale*" were composed of several fungal species of ascomycetes and basidiomycetes.

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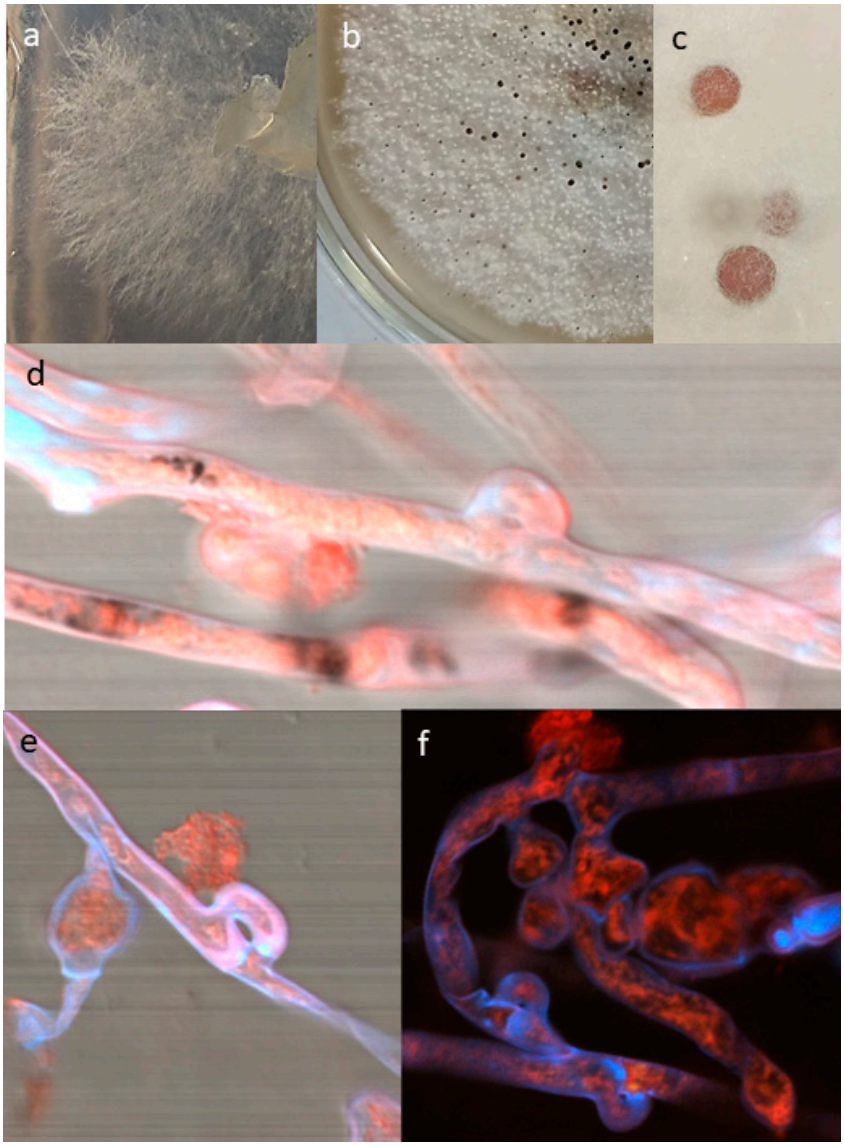

Figure 4. Cultural morphologies of "*Sclerotium nivale*" from IGCN (**a–c**) Fungal colony (IS-2020) with sclerotia; (**d–f**) Their hyphae with clamp connections, (**d** and **e**) fluorescence merged with DIC (CF+PI); (**f**) fluorescence (CF+PI).

3.2. Physiological Characteristics of "Sclerotium nivale"

Mycelia of *S. nivale* IS-2013a (without clamp connections) could grow between 2–14°C, and their optimal growth temperature was ca. 10°C (Figure 4). Mycelial growth of IS-2013a on Czapek-Dox agar was 4mm/week at 2°C and 7 mm/week at 5°C.

3.3. Phylogenic Analysis of "Sclerotium nivale"

We obtained ITS sequence (364 bp, DDBJ/EMBL/GenBank accession no. KY703612) from the isolate IS-2013a. The ITS dataset consisted of 13 ingroup taxa, including the isolate IS-2013a, and sequences had high homology. It had an aligned length of 2027 characters, including gaps, of which 326 were phylogenetically informative.

The ML tree with the highest log likelihood (-509.37) is shown in Figure 5. The isolate IS-2013a was the same clade with uncultured fungus clone CMH239 [23], and both sequences had high homology with the ascomycetous species of *Karstenula* Speg. and *Paraphaeosphaeria* O.E. Erikss.

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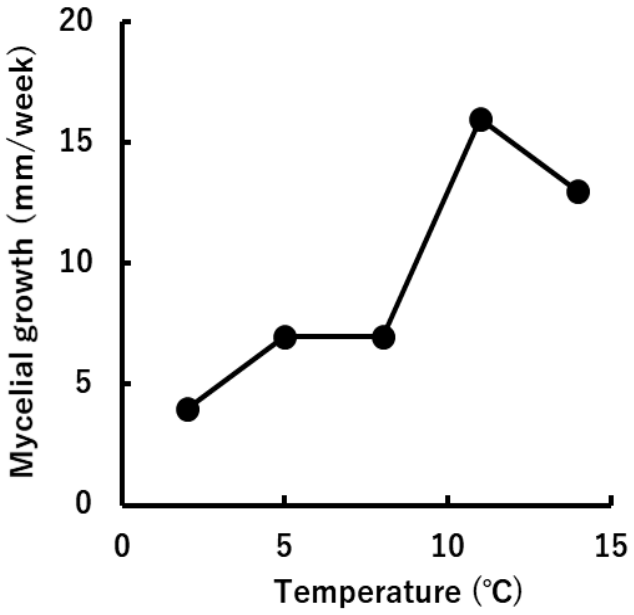

Figure 5. Effects of cultural temperatures on mycelial growth of "*Sclerotium nivale*" isolate IS-2013a.

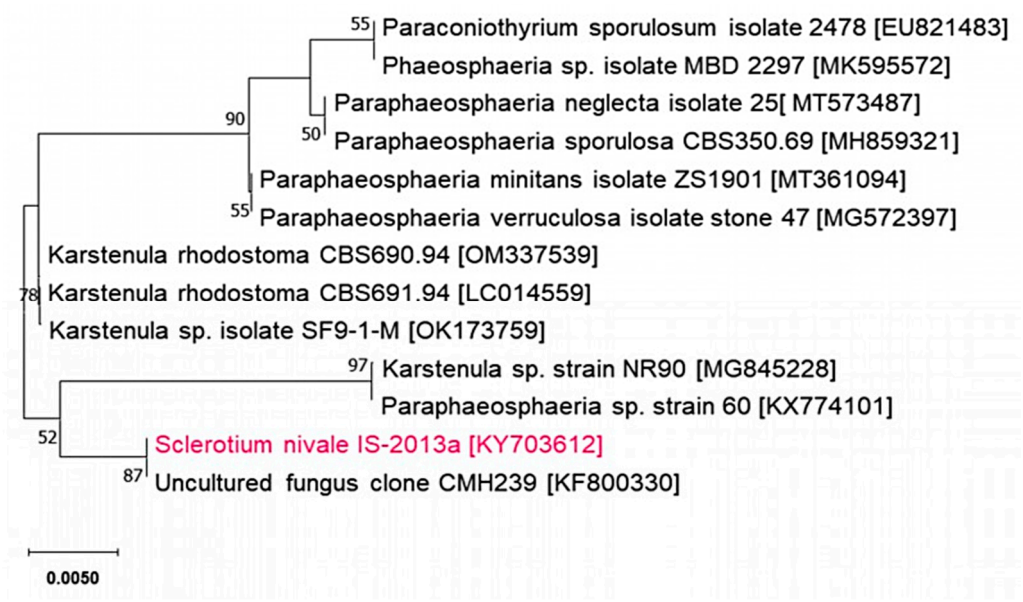

Figure 6. Maximum likelihood tree based combined ITS region sequences for "*Sclerotium nivale*" and other related species. The numbers given on branches given on branch are frequencies (>50%) with which a given branch appeared in 1,000 bootstrap replications. The scale indicates the number of nucleotide substitutions.

4. Discussion

Sclerotia are hardened masses of hyphae that serve as survival structures for ascomycetes and basidiomycetes [24]. White sclerotia are obvious morphological characteristics of "*S. nivale*"; white sclerotia are generally considered immature, and mature sclerotia are mainly brown to black [25]. However, several basidiomycetous mature sclerotia were pale yellow to light brown, such as termite balls of the species *Athelia* Pers. [26] or saprophytic species of *Typhula* (Per.) Fr. [27,28] (ex. *Typhula ochraceosclerotiata* Olariaga & Salcedo [29] Figure 7a and b). *Typhula canadensis* (J.D. Sm. & Årsvoll) Tam Hoshino, T. Kasuya & N. Matsumoto in Norway also had sclerotia with

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aerial mycelia in the field, which were considered to facilitate dispersal by the wind [1,30,31]; however, they matured under the snow cover (Figure 7c).

Figure 7. Morphology of basidiomycetous mature sclerotia (**a**) Pale yellow (5F [15] sclerotia of cold adapted and saprophytic *Athelia* sp. in Tanzawa, Kanagawa Prefecture, Japan (collected 9th April 2005 by T.H. and Y.D.) [32], bar = 1 cm; (**b**) cinnamon (19 [15]) sclerotia of *Typhula* sp. in Nakatsugawa, Saitama Prefecture, Japan (collected 21st March 2005 by T.H.), bar = 1 cm; (**c**) sclerotia with mycelium of *T. canadensis* on PDA (Courtesy, Dr. N. Matsumoto), bar = 5 mm.

We previously reported that basidiomycetes, *Typhula*, and *Pistillaria* Fr. can produce sclerotia and the capacity for mycelial growth at ambient air temperatures in each locality [33]. Sclerotia of *T.* cf. *subvariabilis* Berthier in northern Iran were formed ca. 2 weeks to 1 month after the snow melt, were not observed or remained immature as mycelial aggregations after snow melt, and matured without snow [33,34]. Several strains of *Typhula hyperborea* H. Ekstr. in West Greenland had weak pathogenic activity [2], abundant aerial mycelium, and less productivity of sclerotium [33]. These physiological characteristics supported the adaptation to the Arctic summer climate (blight and cold conditions). Similar phenomena were recorded other *Typhula* sp. in the Arctic (loss of sclerotium forming ability under cultural conditions) [35] and *T.* cf. *subvariabilis* in Antarctica (no-sclerotia at the field survey) [36]. *T. subvariabilis* was widely distributed in snowy low latitude such as Spain [37], France [28], Germany [38], Poland [39], Lithuania [40], Estonia [41], and Bashkorstan Rep. in Russia [42]. *T.* cf. *subvariabilis* was also recorded in the mainland of Japan [32] and Uzbekistan [43].

Therefore, it differed from the ecophysiological characteristics of sclerotia from *T.* cf. *subvariabilis* in northern Iran and *T. canadensis* in Norway. Previous findings suggested that *T.* cf. *subvariabilis* and similar ecophysiological species could still act in their localities after the snow melts. Our basidiomycetous isolates (IS-2020 having clamp connections) from sclerotia of Sclerotium disease, Moscow, Russia (Figures 1c, 1d and 2) had similar ecophysiological characteristics of *T.* cf. *subvariabilis* in northern Iran. O.B.T. observed brown sclerotia under cultural conditions. Sokirko et al. [13] also described that "*S. nivale*" was first whitish, then blackening, and dense, indefinitely shaped sclerotia were formed on the mycelium. T.H. thought that their observations supported our hypothesis. Mycelia of IS-2020 were cultured on PDA at 2°C, and several hyphae showed "knot like" irregular shapes (Figure 4). We found similar phenomena from Arctic *Pythium ultimum* var. *ultimum* under the chilling stress [44]. Chilling stress probably caused an imbalance in hyphal growth and intercellular substance transport, resulting in "knot like" irregular hyphal morphologies. These findings suggested that the isolate IS-2020 was cold adapted fungus but not psychrophile. They could grow relatively higher environmental temperature than psychrophilic snow molds such as *T. ishikariensis* complex and *Sclerotinia borealis* Bubák & Vleugel.

Sclerotia of basidiomycetous "*S. nivale*" from IGCN composed the rind and medulla (Figures 2b–e). Townsend and Willetts [24] divided into 3 types of fungal sclerotia, and sclerotia of "*S. nivale*" from IGCN were loose type in their typification as same as *Rhizoctonia solani* J.G. Kühn and *Typhula* spp. [27,28]. *Typhula* spp. included typical basidiomycetous snow molds such as *Typhula incarnata* Lasch or *T. ishikariensis* complex. *Ceratobasidium gramineum* (Ikata & T. Matsuura) Ogoshi & T. Araki (one of the teleomorphs of the pathogenic binucleate *Rhizoctonia* DC fungi [45]) was snow mold of winter wheat and barley in Japan [46]. Other *Rhizoctonia* spp. also infected pine seeds [47] or acorns [1 and personal communication from Dr. H. Masuya, Forestry and Forest Products Research Institute,

Japan] under snow cover in Japan. However, snow molds of *Rhizoctonia* were not recorded for sclerotium production.

We also isolated ascomycetous isolates (ex. IS-2013a) from RSAU-MAA from sclerotia of Sclerotium disease, Moscow, Russia (Figure 3c). The mycelial growth of IS-2013a (Figure 5) was faster than psychrophilic ascomycete, Sclerotinia snow blight, *S. borealis* (its optimal mycelial growth temperature at ca. 5°C, 16.5 mm/month) [48] and psychrotolerant ascomycete, pink snow mold, *Microdochium nivale* (Thümen) M. Hern-Restr. & Crous and *Microdochium majus* (Wollenw.) Glynn & S.G. Edwards (its optimal mycelial growth temperature at 20–25°C, 3.5–14 mm/week at 2°C [49] and 5.8 mm/week [50]). This result suggested that the isolate of IS-2013a had enough ability to grow and infect host plants under the snow cover.

Phylogenetic analysis showed that the isolate of IS-2013a had high homology (100% with aligned data set) with uncultured fungus clone CMH239 [23] (Figure 6). This clone was detected in indoor environments of Kansas City, KS, USA, where there was less yield loss of winter wheat by snow mold diseases [51]. However, *M. nivale* and *Typhula ishikariensis* var. *idahoensis* (Remsberg) Årsvoll & J.D. Sm. were recorded there [52]. These situations were similar to the southern former Soviet Union, where Sclerotium disease of winter rye was recorded [12]. Fungi in genus *Sclerotium* Tode form sclerotia and sterile mycelia but no spores [53]. The isolate of IS-2013a produced unsexual spores under cultural conditions (Figures 3b, d, and S3). These findings supported the fact that a similar fungus was detected in indoor environments [23], and the isolate of IS-2013a was not *Sclerotium*.

We obtained DNA sequence of ITS region of IS-2013a isolate that had high homology (98–99% with aligned data set) with *Karstenula* and *Paraphaeosphaeria* spp. in Didymosphaeriaceae Munk. Both genera were not listed in known snow molds [1,7,8,54,55] and did not record producing the sclerotium [56]. *Karstenula rhodostoma* (Alb. & Schwein.) Speg. and *Paraphaeosphaeria michotii* (Westend.) O.E. Erikss are a type species of both genera, respectively. The holotype of *K. rhodostoma* was collected in southern Sweden [57], and the first record of *P. michotii* was in the Himalayas [58]. Therefore, species in both genera could survive under the snow. In addition, *Paraphaeosphaeria* was one of the genera in Coelomycetes based on past morphological taxonomy and included sclerotium parasites such as *Paraphaeosphaeria minitans* (W.A. Campb.) Verkley, Göker & Stielow [59]. Various ascomycetes, including *Cladosporium* Link spp., Dematiaceae Fr., and Coelomycetes Grove (including *Paraphaeosphaeria*) from sclerotia of *T. ishikariensis* from collected from an alfalfa field in late autumn, Sapporo, Hokkaido, Japan [1,60]. Matsumoto also isolated 47 fungal species from sclerotia of *T. incarnata* and *T. ishikariensis* [61]. Seven species were mycoparasites, including *P. minitans* (*Coniothyrium minitans* W.A. Campb. in original text). Our IST region of IS-2013a isolate was not enough from phylogenetical identification, therefore we should need to obtain additional DNA sequences from other loci.

Various cold-adapted fungi could infect basidiomycetous sclerotia of *Typhula* spp. and *Macrotyphula phacorrhiza* (Reichard) Olariaga, Huhtinen, Læssøe, J.H. Petersen & K. Hansen [50]. Basidiomycete, *Cylindrobasidium parasiticum* D.A. Reid is a mycoparasite of sclerotia of *T. incarnata* in Scotland [62], and ascomycetes, *Episclerotium sclerotiorum* (Rostr.) L.M. Kohn and *Episclerotium sclerotipus* (Boud.) L.M. Kohn are also probably obligate parasites of sclerotia of *M. phacorrhiza* and *Sclerotinia* Fuckel spp. [63,64]. Cyst-like bodies were also observed in nivicolous myxomycete fruiting bodies [65]. Partial sequences of 18S rDNA gene from infected *Lamproderma* Rostaf. sp. contained a novel sequence, suggesting cyst-like bodies belonged to the Cryptomycota M.D.M. Jones & T.A. Richards. Several bacteria also isolated from immature sclerotia under the snow cover and mature sclerotia of snow molds from late autumn [66]. These bacteria inhibited the mycelial growth of *T. incarnata* and *T. ishikariensis*.

Physical stress tolerances, especially against high temperatures, of immature (white) sclerotia of *R. solani* and *S. sclerotiorum* were weaker than those of mature sclerotia [25]. *P. minitans* infected *S. sclerotiorum* showed irregular growth and formed immature sclerotia [67]. These findings suggested that ascomycetous "*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. T.H. thought that this hypothesis supported our filed observation that basidiomycetous sclerotia including inactive

states (Figure 2b). PI was a fluorescent intercalating agent that could be used to stain cells and nucleic acids. PI was not membrane-permeable, making it useful to differentiate necrotic, apoptotic and healthy cells based on membrane integrity [68]. They were alive that sclerotia of *Typhula* spp. cut with steel blades [2 and in this study] or lightly crushed with tweezer [60]. According to these biochemical features of PI, T.H. thought that some sclerotia collected in 2020, were probably dead but covered with active mycelia (Figure 2d).

Typhula corallina Quél. & Pat. (syn. *Typhula bulbosa* (Pat.) Corner) had sclerotia with basidiocarps and discoid conidiophores [27,28,69]. Other known *Typhula* spp. did not record these structures. T.H. thought that sclerotia with discoid conidiophores could be reproductive organs of ascomycetous mycoparasites. Sokirko et al. [13] described the apothecia of "*S. nivale*" on the host stalks. Their description did not match sclerotia with discoid conidiophores.

Sclerotium-forming snow molds, *Sclerotinia* spp., and *Typhula* spp. are important plant pathogens that cause economic loss in cereal crops [2]. Some chemical fungicides have been developed for snow mold diseases. However, snow molds can grow and cause pathogenic activities under the snow cover. Therefore, a scattering of fungicides before the stable snow cover is the most critical factor for the management of snow mold diseases, and keepers of some golf courses scatter fungicides several times in winter. A surplus of fungicides in soil caused to pollute underground water. This is a severe environmental problem in northern Japan [70]. Recently, many types of researches on biological control of snow molds have been carried out: non-pathogenic psychrophilic fungi (ex. *M. phacorrhiza*) and psychrotrophic bacteria (ex. *Pseudomonas* spp.) [71–76]. However, there were no reports to apply mycoparasites for biological control agents under the snow cover. Our ascomycetous isolate was one of the candidates for these applications. We should identify their taxonomical position of ascomycetous "*S. nivale*" for the safety usage of industrial applications.

5. Conclusions

The genus *Sclerotium* Tode includes more than 40 plant-pathogenic species including ascomycetes and basidiomycetes [77]. Many *Sclerotium* species do not or rarely reproduce sexually and are known only from their asexual stage. We rediscovered symptoms of "*Sclerotium nivale*" Elenev from Moscow, Russia, in 2013 and 2020. The isolate from sclerotia collected in 2013 was the asexual spore-forming ascomycete without sclerotium, and that collected in 2020 was the basidiomycete producing sclerotia under the cultural conditions. According to previous studies, we concluded that "*S. nivale*" was mainly basidiomycetes (ex. *Typhula subvariabilis*) whose sclerotia matured after the snow melts. T.H. thought that some of these sclerotia were probably weakly parasitized by or co-existed with ascomycetes of (ex. *Paraphaeosphaeria minitans*).

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1: symptoms of Sclerotium disease in RSAU-MAA, Moscow, Russia. Figure S2: symptoms of Sclerotium disease in IGCN, Moscow, Russia. Figure S3: illustration of conidia of IS-2013a.

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