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Taxonomic revision of the *Typhula ishikariensis* complex.

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ABSTRACT

Typhula ishikariensis and the related fungi were separated into three biological species by morphological and physiological characteristics, as well as DNA sequences and mating reactions. We propose that the *T. ishikariensis* complex should be divided into three species (*T. ishikariensis*, *T. canadensis* and *T. hyperborea*) and two varieties (*T. ishikariensis* var. *ishikariensis* and var. *idahoensis*). *Typhula hyperborea* was reappraised to be recognized also as a separate species of the *T. ishikariensis* complex.

Keywords: ecophysiology, *Pleurotineae* (*Typhulaceae*), snow mold, speciation, species complex

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1. Introduction

Snow mold is incited by many fungi that attack dormant plants such as forage crops, winter cereals and conifer seedlings under snow cover (Matsumoto & Hsiang, 2016). Although some of their taxonomic and ecological features have only recently been elucidated (e.g., Saito, 1998; Hoshino, Tkachenko, Kiriaki, Yumoto, & Matsumoto 2004b; Ikeda, Hoshino, Matsumoto, & Kondo, 2015), the taxonomic confusion of the most important fungus, *Typhula ishikariensis* S. Imai still remains unsolved, and requires thorough comparison. McDonald (1961), after literature review, concluded that *T. ishikariensis* had priority among related fungi. Since then, different fungi related to *T. ishikariensis* were reported from different regions by different authors such as Bruehl and Cunfer (1975), Årsvoll and Smith (1978), Matsumoto, Sato and Araki (1982), and Matsumoto, Tronsmo and Shimanuki (1996).

Imai (1930) first described this fungus on dead leaves and stems of winter wheat and red clover in Hokkaido, Japan. A similar fungus, *T. idahoensis* Remsberg was described as a snow mold pathogen on winter wheat in the USA (Remsberg, 1940a, 1940b). In Scandinavia, Ekstrand (1937, 1939, 1955) also found similar fungi and named *T. borealis* H. Ekstr. 1955 and *T. hyperborea* H. Ekstr. 1955. His first diagnoses of *T. borealis* at 1937, 1939 and *T. hyperborea* at 1937 were invalid (no Latin diagnosis or description; ICN Shenzhen Code Art. 39.1). Legitimate nomenclatures accompanied with Latin diagnoses of both species were published 1955 (Ekstrand).

Tomiyama (1955) recognized the significance of *T. ishikariensis* as an important snow mold pathogen on winter wheat in Hokkaido, Japan. Gulaev (1948) described *T. graminearum* Gulaev Nom. Inval. Art. 39.1 (ICN Shenzhen Code) on grasses and conifer seedlings, and Kuznetzova (1953) also described *T. humulina* Kuznezowa on hop roots in Russia. Potatosova (1960a, 1960b) on the basis morphological features considered these species to be

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synonyms of *T. idahoensis*, and Hoshino et al. (2004b) suggested that *T. graminearum* and *T. humulina* should be regarded as *T. ishikariensis* by mating experiments.

Later in the USA, Bruehl and Cunfer (1975) claimed that *T. ishikariensis* and *T. idahoensis* should be regarded as separate species based on morphology, host range, and distribution. They also revealed genetic isolation of both species by mating experiments (Bruehl, Machtmes, & Kiyomoto, 1975). However, *T. ishikariensis* and *T. idahoensis* mated rarely (Bruehl et al., 1975), but Christen and Bruehl (1979) confirmed that their hybrids were virulent and capable of survival in nature. Bruehl and Machtmes (1980) ultimately concluded that these two species were indistinguishable based on culture morphology.

Årsvoll and Smith (1978), on genetic evidence, regarded isolates from Europe and North America as a single species and established three varieties in the *T. ishikariensis* complex, i.e., var. *ishikariensis* S. Imai, var. *idahoensis* (Remsberg) Årsvoll & J.D. Sm., and var. *canadensis* J.D. Sm. & Årsvoll based on sclerotial rind cell pattern.

Matsumoto et al. (1982) found two biological species (biotypes A and B) in Japan that were sexually incompatible with each other. They also differed in morphology, distribution, host range, and pathogenicity (Matsumoto, 1989) – different enough to be regarded as separate species. However, both biotypes were genetically related through the medium of North American fungi (Matsumoto, Sato, Araki, & Tajimi, 1983). Matsumoto et at. (1996) divided *T. ishikariensis* strains from Norway into three groups based on mating reactions of tester monokaryons of biotypes A and B. Isolates of group I, sharing common culture morphology (Matsumoto & Tronsmo, 1995), were basically compatible with biotypes A but showed various mating reactions. Group II was compatible with biotype B, and group III was not compatible with either biotype.

These results imply that populations of *T. ishikariensis* in each locality are genetically separate to various extent but that they have potential to exchange genetic traits especially under laboratory conditions. Hsiang and Wu (2000) examined phylogenic relationships of *T. ishikariensis* in North America to detect differences at an infraspecific level, using internal transcribed spacer-restriction fragment length polymorphism (ITS-RFLP). Thus, the situation of the *T. ishikariensis* complex is comparable to that of *Armillaria mellea* (Vahl) Kumm. (Cha, Sung, & Igarashi, 1994) and *Heterobasidion annosum* (Fr.) Bref. (Garbelotto & Gonthier, 2013).

In this study, we aimed to elucidate the extent of variation within the *T. ishikariensis* complex, using 480 strains from various localities in Northern Hemisphere based on di-mon mating reactions, phylogenic analyses, morphological characteristics, host range and their distribution. The results obtained here indicate biological species differentiation sufficient to propose a new universal nomenclature system of the *T. ishikariensis* complex.

2. Material and methods

2.1. Fungal strains from overwintered plants

Fungal sclerotia were collected from decayed leaves and stems of various plants during our surveys (Table 1). Collected sclerotium samples were packed in paper envelopes and dried at room temperature during transportation. Fungal sclerotia were surface-sterilized first in 70% (v/v) ethanol and in 0.5% (as active chlorine) sodium hypochlorite solution and, then, 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 and maintained on PDA slants at 0°C.

Table 1 – Biological species separation within the strains belonging to the *Typhula ishikariensis* complex based on mating reaction of testers.

Localities [a]	Number of strains designated as [b]			
	Biological species I		II	III
	A+ B+	A+ B-	A-B+	A-B-
West Greenland (7)	0	0	0	48
East Greenland (3)	1	0	0	13
Iceland (3)	0	4	2	0
Svalbard (4)	0	0	0	3
Tromsø og Finmark, Norway (3)	0	0	2	58
Switzerland (5)	0	72	0	1
Liechtenstein (2)	0	5	0	0
Austria (2)	0	5	0	0
Russia				
European (8)	0	31	0	7
Siberia (7)	0	41	21	8
Far East (12)	0	25	8	18
Hokkaido, Japan (3)	0	38	45	0
Alaska, USA (3)	0	0	2	0
Quebec, Canada (2)	3	13	2	4

[a] Figures in parentheses indicate the number of sampling site.
[b] Figures indicate the number of strains. Isolates belonging to different vegetative compatible groups (VCGs) were regarded as separate strains. VCGs between localities were not determined. A: biotype A; B: biotype B; +: compatible; -: incompatible.

2.2. Production of basidiomata

Sclerotia formed on oatmeal agar (Difco) plates were placed on the surface of a mixture of humid unsterile commercial artificial soil (Hokusan Co. Ltd., Kita-Hiroshima, Japan) and sea sand (14—20 mesh, Wako Pure Chemical Industries, Ltd., Osaka, Japan) in glass dishes (55 mm diam, 45 mm in height) and incubated under the fructification regime of 12 h light at 10°C and 12 h dark at 4°C up to two mo (Kawakami, Matsumoto, & Naito, 2004).

2.3. Morphological observations

Colors of basidiomata and sclerotia were described according to the color identification chart of the Royal Botanic Garden Edinburgh (*Flora of British Fungi*) (Anonymous, 1969). Basidiospores from fresh specimens were mounted in water for light microscopic examination. About 30 basidiospores were randomly chosen for determination of length and width excluding the apiculus. Surface features of basidiomata and sclerotia were observed by phase-contrast microscopy and scanning electron microscopy (SEM). For SEM, basidiomata and sclerotia were cut on a piece of double-sided adhesive tape attached to a specimen holder and then coated with platinum-palladium using a JFC-1100 Ion Sputter (JEOL, Tokyo, Japan). They were examined using a JSM-T330A SEM (JEOL) operating at 10 kV.

2.4. Mating experiments

Basidiomata were soaked separately in 500 μL autoclaved water in test tubes and kept overnight in a refrigerator (4°C). Test tubes were shaken to remove basidiospores from basidiomata. Basidiospore suspension was appropriately thinned, spread on PDA plates containing lactic acid, and incubated at 4°C for two weeks. Colonies with smooth hyphae (monokaryons) were subcultured on PDA slants at 4°C. Monokaryons of biotypes A (strains PR7-6-7 and PR9-4-3 from Japan) and B (strains 35-8 and 8-2 from Japan) were designated as testers and paired with dikaryons of collected strains (di-mon mating; Bruehl et al., 1975) 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.

2.5. Phylogenic analyses

Sclerotia were harvested from one-month-old PDA cultures grown at 4°C, and DNA was extracted by the protocol of DNeasy Plant MiniPrep (QIAGEN GmbH, Hilden, Germany). ITS1-5.8S-ITS2 (ITS) region of genomic rDNA were amplified using the primer pairs ITS1 and ITS4 (White, Bruns, Lee, & Taylor, 1990), and mitochondrial large ribosomal RNA (the partial mitochondrial LrRNA) with ML5 and ML6 (Bruns et al., 1998). PCR products were purified using QIAquick PCR Purification Kit (QIAGEN GmbH, Germany) and sequenced in one direction on ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using each primer set. A total of 186 sequences newly generated from the present study were deposited in DDBJ/EMBL/GenBank (Table 2).

Representative sequences of *Typhula* spp. and related fungi were included in the dataset of the present phylogenetic analyses based on the recent phylogenetic study on typhuloid fungi reported by Olariaga, Huhtinen, Læssøe, Petersen, & Hansen (2020). Each ITS and the partial mitochondrial LrRNA dataset consisted of 129 or 109 taxa including two outgroups, *Pleurotus ostreatus* (Jacq.) P. Kumm. and *P. eryngii* (DC.) Quél., following Olariaga et al. (2020). Namely, each dataset was aligned with Muscle v.3.6 (Edgar, 2004a, b) and then, manually edited in BioEdit v.7.0.1 (Hall, 1999). Ambiguously aligned regions were excluded from the analyses. The final alignments were deposited in TreeBASE (https://treebase.org) under the accession number S27664. Phylogenetic analyses were performed for ITS and the partial mitochondrial LrRNA datasets under maximum likelihood (ML) and Bayesian inference (BI). Briefly, the analyses were performed using MEGA X (Kumar et al., 2018) after testing the best models. According to the lowest BIC (Bayesian Information Criterion) scores, K2 and T92+G were chosen as the optimal substitution models for the analyses of the ITS and the partial mitochondrial LrRNA datasets, respectively. BI analyses were conducted by the same method reported by Kasuya and Ono (2018), using MrBayes v.3.0b4 (Huelsenbeck & Ronquist, 2001). In BI analyses, the best-263 fit substitution models for the ITS and the partial mitochondrial LrRNA datasets were estimated using MrModeltest v.3.7 (Nylander, 2004) based on the hierarchical likelihood-ratio test (hLRT). The GTR+G+I model was selected as the best evolutionary model for each dataset.

2.6. Characterization of Typhula hyperborea

2.6.1. Growth temperature relations

Mycelial discs of 5 mm diam were cut from the margins of actively growing colonies, transferred to the center of PDA plates, and incubated at 5 different temperatures from 0 to 25°C, in triplicates. After 1, 2 and 3 wk of incubation, colony diameters were measured. The linear mycelial growth rate per week was calculated after initial lag period.

2.6.2. Pathogenicity

Five each of Engmo timothy seedings were transplanted to 9 cm plastic pots filled with steamed, fertilized peat/soil mixture, and grown for 6 wk in a greenhouse at 12–18°C, supplemented with 8000 lux form cool white and warm fluorescent light for 12 h, and hardened at 1°C for 2 wk under the same light conditions, according to Tronsmo (1984, 1985).

Table 2 – Sequenced specimens used this study.

Fungal name	Voucher no.	Strain no.	Localities	Accession numbers	
				ITS [a]	ML [b]
Pistillaria petasitis		PP1, HIT	Hokkaido, Japan	LC005088	LC192706
Typhula incarnata		IS11in, HIT	Akureyri, Iceland		LC192600
		IS12in, HIT	Glaumbæ, Iceland		LC192599
		IS13in, HIT	Reykholt, Iceland	LC192841	LC192598
		S1in, HIT	Skælingur, Faroe Is.		LC192602
		V1in, HIT	Vestmanna, Faroe Is.		LC192601
		Rin, HIT	Błonie, Poland	LC192842	LC192603
		Komi1in, HIT	Komi, Russia		LC192606
		Y1in, HIT	Mari El, Russia	LC192843	LC192605
	IRAN 11961F	H-I2, HIT	East Azerbaijian, Iran	AB267391	LC192604
		IK1in, HIT	Irkutsk, Russia	LC192844	LC192607
		SD1in, HIT	Sakhalin, Russia	LC192845	
		SD11in, HIT	Sakhalin, Russia		LC192609
		YS1in, HIT	Sakhalin, Russia	LC192608	LC192608
		QBC1in, HIT	Quebec, Canada		LC192610
T. intermedia		TI1, HIT	Hokkaido, Japan		LC192704
T. ishikariensis var. *ishikaiensis*	TNS-F-24965	IS-1, HIT	Akureyri, Iceland	LC192584	LC192611
	TNS-F-24696	IS-3, HIT	Akureyri, Iceland	LC192585	LC192612
	TNS-F-24697	IS-4, HIT	Akureyri, Iceland	LC192846	LC192613
		OPU1809	Buskerud, Norway	LC192577	LC192615
		2-2-5, NIBIO [d]	Oppland, Norway	LC192578	LC192616
		5-5-11, NIBIO	Troms og Finnmark, Norway		LC192618
		KP-10, MBG [c]	Murmansk, Russia	LC192569	LC192628
	TNS-F-24681	KP-13, MBG	Murmansk, Russia	LC192851	LC192629
	TNS-F-24682	KP-16, MBG	Murmansk, Russia	LC192570	LC192630
	TNS-F-24683	SPb-2, HIT	St Petersburg, Russia	LC192571	LC192625
		SPb-4, HIT	St Petersburg, Russia		LC192626
	TNS-F-24684	SPb-6, HIT	St Petersburg, Russia	LC192572	LC192627
	TNS-F-24679	92-Tr-13, MBG	Moscow, Russia	LC192567	LC192631
	TNS-F-24680	MSC-1, HIT	Moscow, Russia	LC192568	LC192632
	TNS-F-24676	C-2, HIT	Chuvashia, Russia	LC192565	
	TNS-F-24677	C-4, HIT	Chuvashia, Russia	LC192566	LC192633
	TNS-F-24678	C-6, HIT	Chuvashia, Russia	LC192852	LC192634
	TNS-F-24675	*T. humulina*, HIT	Mari El, Russia	LC192564	LC192638
	TNS-F-2673	Y-2, HIT	Mari El, Russia,	LC192562	LC192635
		Y-6, HIT	Mari El, Russia,		LC192637
		E-2, HIT	Sverdlovsk, Russia	LC192558	LC192641
	TNS-F-24670	E-5, HIT	Sverdlovsk, Russia	LC192854	LC192643
	TNS-F-24671	*T. graminearum*, HIT	Sverdlovsk, Russia	LC192560	LC192644
	TNS-F-24663	N3A3, HIT	Novosibirsk, Russia	LC1928554	LC192645
	TNS-F-24664	N4A1, HIT	Novosibirsk, Russia	LC192555	LC192646
		N4A3, HIT	Novosibirsk, Russia	LC192855	LC192647
		N4B2, HIT	Novosibirsk, Russia	LC192856	LC192648
		N5-1, HIT	Novosibirsk, Russia		LC192649
		IK1-1, HIT	Irkutsk, Russia		LC192651
		IK2-1, HIT	Irkutsk, Russia		LC192652
		IK3-1, HIT	Irkutsk, Russia		LC192653
	TNS-F-24661	YS1-1, HIT	Sakhalin, Russia	AB194769	
		YS3-3, HIT	Sakhalin, Russia	AB127951	AB187583
		YS5-3, HIT	Sakhalin, Russia	AB194770	
		YS8-2, HIT	Sakhalin, Russia	AB194771	
		Y10-1, HIT	Sakhalin, Russia	AB194772	
		A1-222, HIT	Hokkaido, Japan	LC192859	LC192673
	TNS-F-24716	MAFF306136	Hokkaido, Japan	AB127949	LC192671
		MF1-5, HIT	Hokkaido, Japan	LC192858	LC192672
		Rebun, HIT	Hokkaido, Japan	AB127950	
		PR7-6, HIT	Hokkaido, Japan	AB127952	LC192670
		Noheji, HIT	Aomori, Japan	LC192860	
	TNS-F-24704	QBC4-1, HIT	Quebec, Canada	LC192591	LC192677
		QBC7-1, HIT	Quebec, Canada		LC192680
T. ishikariensis var. *idahoensis*	CUP25153		Montana, USA	LC613246	
	CUP27221		Idaho, USA	LC192550	
	TNS-F-24709	ida1-72, HIT	North America	LC192595	LC192675
	TNS-F-24710	ida2-101, HIT	North America	LC192596	LC192676
		idal-3, HIT	North America		LC192674
T. canadensis		KUS2-1, HIT	Kulusk, Greenland		LC192701
		IS5, HIT	Akureyri, Iceland		LC192614
	TNS-F-24692	5-2-21, NIBIO	Troms og Finnmark, Norway	LC192581	LC192622
	TNS-F-24693	5-4-13, NIBIO	Troms og Finnmark, Norway	LC192582	LC192623
		IK4-3, HIT	Irkutsk, Russia		LC192654
		KPK2-1	Kamchatka, Russia		LC192661
		KPK3-1, HIT	Kamchatka, Russia		LC192662
		KPK4-1, HIT	Kamchatka, Russia		LC192663
		KPK5-1, HIT	Kamchatka, Russia		LC192664
		KPK11-2, HIT	Kamchatka, Russia		LC192666
		KS1-1, HIT	Kamchatka, Russia		LC192659
	TNS-F-24659	KS1-2, HIT	Kamchatka, Russia	LC192574	LC192660
	TNS-F-24662	SD-1, HIT	Sakhalin, Russia	LC192553	LC192658
		YS2-1, HIT	Sakhalin, Russia		LC192655
		YS3-1, HIT	Sakhalin, Russia		LC192656
	DAOM 160550		Saskatchewan, Canada	LC192551	
	TNS-F-24707	QBC7-4, HIT	Quebec, Canada	LC192594	LC192681
		QBC10-1, HIT	Quebec, Canada		LC192682
		QBC10-2, HIT	Quebec, Canada		LC192683
		Cam14, HIT	North America		LC192687
		Can II, HIT	North America		LC192686
	TNS-F-2411	DE7228, HIT	North America	LC192597	LC192685
T. hyperborea	TNS-F-24694	FERM P-18741	Barentsburg, Svalbard	LC192583	LC192624
		SSM1-1, HIT	Sisimiut, Greenland		LC192688
	TNS-F-24703	SSM2-1, HIT	Sisimiut, Greenland	LC192588	LC192689
		SSM3-1, HIT	Sisimiut, Greenland		LC192690
	TNS-F-24698	OPU1811	Nuuk, Greenland	LC192586	LC192691
	TNS-F-24699	OPU1812	Nuuk, Greenland	LC192587	LC192692
	TNS-F-24700	OPU1814	Nuuk, Greenland	LC192862	LC192693
		OPU1816	Nuuk, Greenland		LC192694
		AGM1-1, HIT	Tasiilaq, Greenland		LC192695
		AGM2-1, HIT	Tasiilaq, Greenland	LC613245	LC192696
		AGM4-1, HIT	Tasiilaq, Greenland		LC192697
	TNS-F-24701	AGM5-1, HIT	Tasiilaq, Greenland	LC192589	LC192698
		KUS1-1, HIT	Kulusk, Greenland		LC192699
	TNS-F-24703	KUS1-2, HIT	Kulusk, Greenland	LC192590	LC192700
	TNS-F-24687	OPU1810	Oppland, Norway	LC192579	LC663960
	TNS-F-24688	5-4-9, NIBIO	Troms og Finnmark, Norway	LC192847	
	TNS-F-24689	6-1-1, NIBIO	Troms og Finnmark, Norway	LC192580	LC192619
	TNS-F-24690	6-1-9, NIBIO	Troms og Finnmark, Norway	LC192848	LC192620
	TNS-F-24691	Tana, NIBIO	Troms og Finnmark, Norway	LC192849	LC192621
	TNS-F-24674	Y4-2, HIT	Mari El, Russia	LC192563	LC192563
	TNS-F-24672	K-1, HIT	Tatarstan, Russia	LC192561	LC192639
	TNS-F-24666	E-1, HIT	Sverdlovsk, Russia	LC192557	LC192640
	TNS-F-24668	E-3, HIT	Sverdlovsk, Russia	LC192853	LC192642
	TNS-F-24669	E-4, HIT	Sverdlovsk, Russia	LC192559	
	TNS-F-24665	N6A-1, HIT	Novosibirsk, Russia	LC192556	LC192650
	TNS-F-24658	KPK8-2, HIT	Kamchatka, Russia	LC192573	LC192665
		KK2-1, HIT	Kamchatka, Russia		LC192668
	TNS-F-24660	KK8-1, HIT	Kamchatka, Russia	LC192575	LC192669
		KE1-1, HIT	Kamchatka, Russia		LC192667
	TNS-F-24657	AND1-1, HIT	Chukotka, Russia	LC192576	
	TNS-F-24705	QBC5, HIT	Quebec, Canada	LC192592	LC192678
	TNS-F-24706	QBC6, HIT	Quebec, Canada	LC192593	LC192679
	TNS-F-24708	QBC13, HIT	Quebec, Canada	LC192861	LC192684
T. intermedia	SAPA 100038	MAFF 244400	Hokkaido, Japan	AB267394	LC192704
T. maritima	TNS-F-17093	NBRC 104266	Hokkaido, Japan		LC192703

[a] 18S rRNA, ITS1, 5.8S rRNA, ITS2, LSU rRNA, partial and complete sequence.
[b] mitochondrial gene for large subunit ribosomal RNA, partial sequence.
[c] Hachinohe Institute of Technology, Aomori, Japan.
[d] Norwegian Institute of Bioeconomy Research, Ås, Norway.
[e] N.V. Tsitsin Main Botanical Garden, Russian Academy of Sciences

A total of 4 pots were used for inoculation experiments for each strain. Inoculated plants were incubated under the simulated snow cover conditions at 1°C for 8 wk, as described by Årsvoll (1977). Plant damage was evaluated three weeks after recovery in the greenhouse. Inoculation experiments were all made in Ås, Norway.

3. Results and discussion

3.1. Biological species designation

Strains of the *T. ishikariensis* complex collected from snowy regions of Northern Hemisphere were divided into three biological species based on mating reactions of testers of biotypes A and B: biological species I strains were mostly compatible only with biotype A and included those compatible with both biotypes; biological species II strains were all compatible exclusively with biotype B; and both biotypes did not react when paired with biological species III (Table 1).

3.2. Phylogenic analyses

The ITS dataset consisted of 127 ingroups and two outgroup taxa. It had an aligned in length of 763 characters including gaps, of which 37 characters were constant, 402 variable and phylogenically uninformative, and 324 phylogenically informative. The partial mitochondrial LrRNA dataset consisted of 107 ingroup and two outgroup taxa. It had an aligned length of 1088 characters including gaps, of which 19 characters were constant, 700 variable and phylogenetically uninformative, and 318 phylogenetically informative.

The ML and BI analyses resulted in trees that were almost identical in topology for both of ITS and the partial mitochondrial LrRNA sequence datasets. Hence, only the ML trees with the highest log likelihood (-1006.12 and -832.25) were shown in Figs. 1 and 2 for ITS and the partial mitochondrial LrRNA, respectively. Phylogenetic analyses revealed the monophyly of *Typhula* from outgroups (*Pleurotus* spp.) and also that of the *T. ishikariensis* complex for *Typhula* of ITS (Fig. 1: 82/100, 82/0.61) and the partial mitochondrial LrRNA (Fig. 2: 100/1.00, 89/0.74).

We here referred to biological species I, II, and III as *T. ishikariensis*, *T. canadensis* stat. nov. (syn. *T. ishikariensis* var. *canadensis*), and *T. hyperborea*, respectively. The partial mitochondrial LrRNA supported biological species separation (*T. ishikariensis* including both varieties: 97/0.97, *T. canadensis*: 97/0.92, *T. hyperboea*: 99/0.97). However, mating reactions and phylogenic clades were discrepant in some strains due to their anomalities in mating reactions as follows: i) *T. ishikariensis* var. *idahoensis* strains, belonging to biological species II, constituted a distinct subclade within the clade of *T. ishikariensis* var. *ishikariensis* (Figs. 1, 2). The phylogenic evidence as well as morphological similarities of both fungi (Bruehl & Machtems, 1980) led us regard *T. ishikariensis* var. *idahoensis* as a valid taxon within the *T. ishikariensis* complex. ii) A group of *T. ishikariensis* strains from North America was compatible with both testers of biotypes A and B and considered as hybrids between var. *ishikariensis* and var. *idahoensis* (Årsvoll & Smith, 1979; Christen & Bruehl, 1979), and some strains in Norway showed the same feature (Matsumoto et al., 1996). We failed to determine sequences of both ITS and the partial mitochondrial LrRNA and were, consequently, unable to conduct their phylogenic analyses. These strains were identified as *T. ishikariensis* var. *ishikariensis* on morphological evidence; iii) there were three strains of *T. canadensis* from North America that mated with biotype A testers (Table 1; Fig. 1); and iv) the *T. hyperborea* clade included a strain from Wisconsin, USA that mated with biotype B and biological species III (Fig. 1) and showed normal growth on PDA at 10°C (Millett & Maxwell, 1997; Millet, 1999). *T. hyperborea* usually failed to show normal growth on PDA at 10°C (Matsumoto et al., 1996; Hoshino, Tronsmo, Matsumoto, Ohgiya, & Ishizaki, 1997).

3.3. Morphological characteristics

Cultural morphology and physiological characteristics of the *T. ishikariensis* complex were well described by many authors (e.g., Remsberg, 1940a; Ekstrand, 1955; Potatosova, 1960a; Årsvoll & Smith, 1978; Bruehl & Machtmes, 1980, Smith, 1987; Smith, Jackson, & Woolhouse, 1989; Matsumoto, 1989; Matsumoto & Tajimi, 1991; Matsumoto et al., 1996; Hoshino, Kiriaki, Yumoto, & Kawakami, 2004a; Hoshino et al., 2004b). We summarized taxonomically important characteristics of the *T. ishikariensis* complex including our findings in Table 3.

Aerial mycelia of *T. ishikariensis* are more or less appressed to PDA plates and less fluffy as compared to *T. canadensis* and *T. hyperborea*. Remsberg (1940a) and Årsvoll and Smith (1978) described the abundant production of aerial mycelia by *T. ishikariensis* var. *idahoensis*. However, Bruehl and Machtmes (1980), after examination of ca. 400 isolates of *T. ishikariensis* and *T. idahoensis* (= *T. ishikariensis* var. *idahoensis*), concluded that both taxa were indistinguishable in culture morphology. Smith (1987) and Smith et al. (1989) validated Bruehl's observations. *Typhula canadensis* from Alaska (Anchorage), Canada, Iceland, Norway and Russian Far East (Magadan) has fluffy aerial mycelia on PDA (e.g., Årsvoll & Smith, 1978; Matsumoto et al., 1996; Matsumoto 1997; Hoshino et al., 2004a; Tkachenko, 2013). Especially, strains with small sclerotia of *T. canadensis* produce aerial mycelia abundantly (Årsvoll & Smith,1978). Since, *T. hyperborea* sensu H. Ekstr. also has abundant aerial mycelia under cultural conditions (Ekstrand, 1955). Some strains of *T. hyperborea* from abundant aerial mycelia on PDA even at 0°C (Matsumoto et al., 1996), especially after freezing at -40°C (Hoshino et al., 1998).

Four specimens of *T. ishikariensis* sensu stricto collected by Imai were available from The Tottori Mycological Institute in Japan (Fig. 3A–D). The host plant and locality of the specimen suggested that Imai collected *T. ishikariensis* var. *ishikariensis*. Their sclerotia had turned dark brown (19 bay) due presumably to desiccation (Fig. 3B–D). *Typhula ishikariensis* var. *ishikariensis* strain, MAFF306136 from alfalfa (*Medicago sativa* L.) was similar to Imai's specimen (Fig. 3E). *Typhula ishikariensis* var. *ishikariensis* (represented by biotype A) has brown sclerotia on host surface, while *T. ishikariensis* var *idahoensis* frequently beneath the epidermis (Bruehl & Machtmes, 1980). *Typhula canadensis* (represented by biotype B) has brown to black (12 Fulvous to 36 Fulvous black) sclerotia often buried in plant tissues as does *T. ishikariensis* var. *idahonsis*. Mature sclerotia of *T. canadensis* are almost black (36 Fulvous black) and easily distinguish from brown (15 Brick to 19 Bay) sclerotia of *T. ishikariensis*. *T. canadensis* is highly variable in sclerotium size, strains from Alaska (Anchorage), Canada, Iceland, Norway and Russian Far East (Magadan) produce small sclerotia (0.2–0.5 mm) (e.g., Årsvoll & Smith, 1978; Matsumoto et al., 1996; Matsumoto 1997; Hoshino et al., 2004a; Tkachenko, 2013), and in Japan, populations from localities with deep, persistent snow cover tend to produce large sclerotia more than 2.0 mm in (Matsumoto & Tajimi, 1990). Similar fungus was also collected in Russian Far East (Kamchatka and Sakhalin) and Siberia (Tkachenko, 2013). Sclerotia of *T. hyperborea* formed on PDA are brown (15 Brick to 19 Bay) and indistinguishable from those of *T. borealis* (= *T. ishikariensis* var. *ishikariensis*) in size, shape, and color (Ekstrand, 1955). Our strains

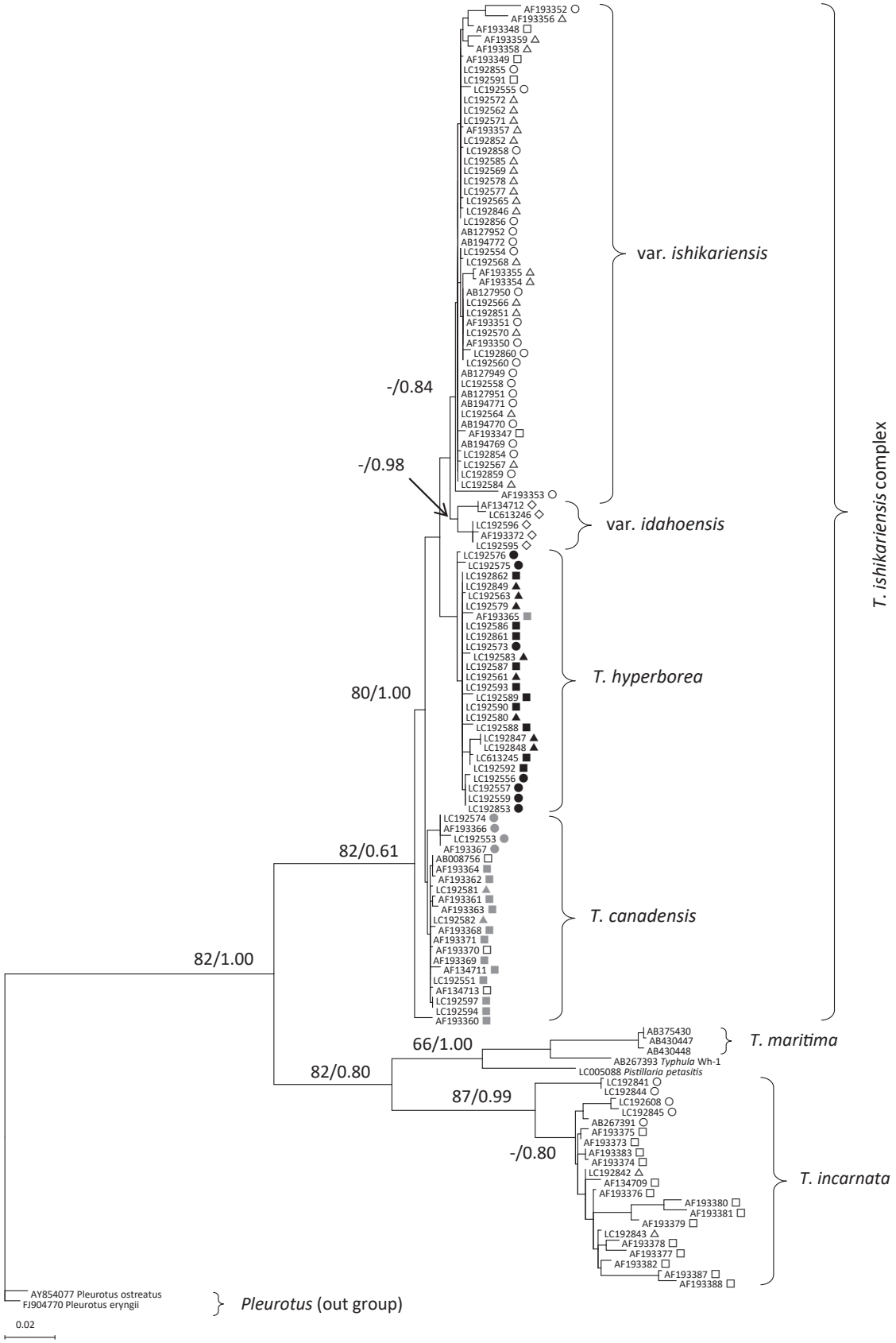

Fig. 1 – A phylogenetic tree of partial ITS sequences of selected typhuloid fungi including *Typhula ishikariensis* complex constructed by the ML method, inferred by using K2 model. Bootstrap values (BS) of maximum likelihood greater than 50% and Bayesian posterior probabilities (PP) above 0.5 are shown along the nodes in the topology (BS/PP). Scale bar indicates the number of substitutions per site.
Circles: localities from Asia including Russian Far East and Siberia; triangle: from Europe; squares: from North America and Greenland. Diamond: specimens or strains annotated in *T. idahoensis* from North America. White: biological group I (A+ B-); grey: II (A- B+); black: III (A- B-).

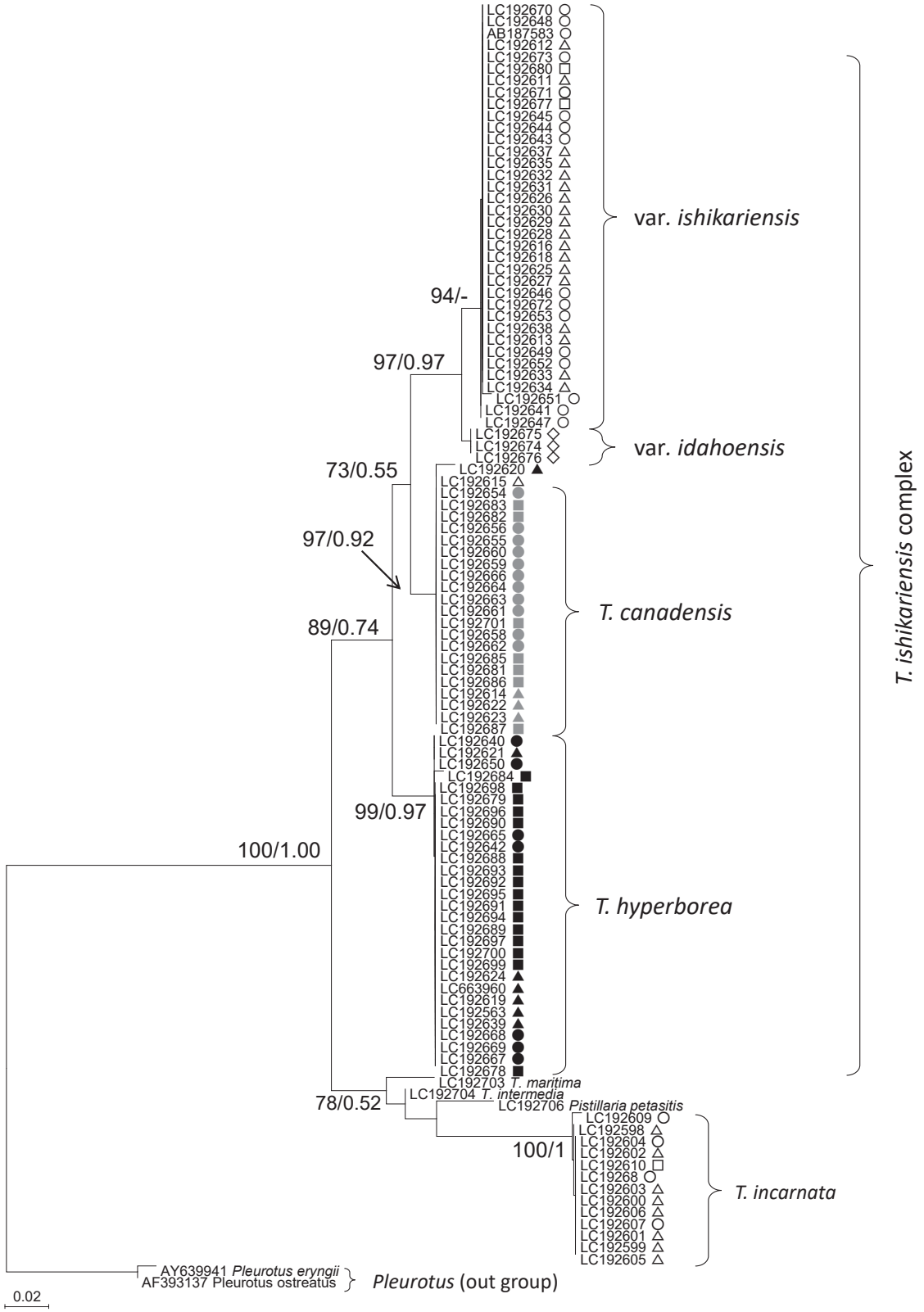

Fig. 2 — A phylogenetic tree of the partial mitochondrial LrRNA sequences of selected typhuloid fungi including *Typhula ishikariensis* complex constructed by the ML method, inferred by using T92+G model. A discrete Gamma distribution was used to model evolutionary rate differences among site [5 categories (+G, parameter = 0.4547)]. Bootstrap values (BS) of maximum likelihood greater than 50% and Bayesian posterior probabilities (PP) above 0.5 are shown along the nodes in the topology (BS/PP). Scale bar indicates the number of substitutions per site. Symbols were same as figure 1.

Table 3 – Summary of taxonomic characteristics of the *Typhula ishikariensis* complex.

Taxonomic species	Mating reaction [a]	Mycelia growth on PDA		Sclerotia		Basidiomata			Host	Distribution
		at 0 c	at 10°C	Formed on host tissues	Color	Size (mm)	Caulocystidia (μm)	Size of spore (μm)		
T. ishikariensis var. *ishikariensis*	A+, B-	less aerial mycelia	normal	easily dislodged	light to dark brown	0.5–2	short (>50)	2.5–5.0×7.5–12.3	monocots, dicots, Gymnosperm	Northern Hemisphere
T. ishikariensis var. *idahoensis*	A-, B+	less aerial mycelia [b]	normal	frequently subepidermal	light brown to almost black	0.5–2	mixed short (>50, main) and long (50-120, rare)	2.5–8.0×6.5–13.0	almost monocots	North America
T. canadensis	A-, B+	aerial mycelia abundant in some strains	normal	frequently subepidermal	light brown to almost black	0.2–2	long (50–120)	2.7–4.5×7.8–12.6	almost monocots	Northern Hemisphere absent from central Asia to middle Europe
T. hyperborea	A-, B-	aerial mycelia abundant	irregular (feather-like) without strains from Greenland [c]	easily dislodged	light to dark brown	0.5–2	short (>50)	3.7–7.1×7.6–14.8	almost monocots	Arctic and Boreal Zone of North Europe and Russia, Alps, North America

[a] see Table 1 for detail. Each taxon but var. *idahoensis* includes strains that showed anomalous mating reaction. For detail, see the test.

[b] Remsberg (1940) and Årsvoll & Smith (1978) suggested abundant aerial mycelia in this species. However, Bruehl & Machtmes (1980) reported that *T. ishikariensis* and *T. idahoensis* were similar in cultural morphology among ca. 400 isolates they tested.

[c] Strains from Nuuk, West Greenland showed normal growth on PDA at 10°C.

of biological species III, *T. hyperborea* from Northern Norway, the Arctic, Alps, European part of Russia (Volga-Ural), Siberia and Russian Far East (Chukotka and Kamchatka) had similar characteristics of the original description of *T. hyperborea* by Ekstrand (1955) (Matsumoto & Tronsmo, 1995; Matsumoto et al., 1996; Hoshino et al., 1997, 1998, 2001; Hoshino, Tronmso, & Saito, 2003; Hoshino, Saito, & Yumoto, 2006). Sclerotial rind cell pattern was considered as important to distinguish *T. ishikariensis* varieties (Årsvoll & Smith, 1978). However, rind cell pattern of strains from Japan and Norway was highly variable and unable to use as a taxonomic criterion (Matsumoto et al., 1996). Our findings agreed with the results of Matsumoto et al. (1996), and regard that rind cell pattern is of limited taxonomic significance (data not shown).

Members of the *T. ishikariensis* complex normally produced clavate basidiomata which consist of fertile heads and stipes, and each basidisium has four sterigmata (Imai, 1930; Remsberg, 1940a; Corner, 1950; Kuznetzova, 1953; Potatosova, 1960c; Parmasto, 1965; Berthier, 1976; Årsvoll & Smith, 1978; Matsumoto & Tajimi, 1991). However, there has been few descriptions of baidioma morphology of *T. hyperborea sensu* H. Ekstr. Some of our strains of *T. hyperborea* developed basidomata with short stems and pointed heads (Fig. 4A). These features are unique to *T. hyperborea*. *Typhula ishikariensis* and *T. canadensis* are indistinguishable in basidoma except the length of caulocystidia; basidiomata of *T. canadensis* have long caulocystidia (ca. 50–120 μm; Fig. 4C and E). The caulocystidia of *T. ishikariensis* var. *idahoensis* are mostly short but rarely long (Fig. 4F and G). Those of *T. ishikariensis* var. *ishikariensis* and *T. hyperborea* are exclusively short (Fig. 4B, D, H and I). Berthier (1976) observed stems of *T. idahoensis*, however, he did not describe the above trait. Other previous reports on basidioma formation by *T. canadensis* (Årsvoll & Smith,1978; Matsumoto & Tajimi, 1991; Kawakami et al., 2004: Hoshino et al., 2004a, 2004b) and other species (Imai, 1930; Remsberg, 1940a; Corner, 1950; Kuznetzova, 1953; Potatosova, 1960c; Parmasto, 1965) did not refer to caulocystidia, either. Further studies, using a number of strains under different environmental conditions, are necessary to evaluate the significance of caulocystidia as a taxonomic criterion.

Basidiospores of *T. hyperborea sensu* H. Ekstr. from northern Scandinavia were short oviform and distinct from those of *T. borealis* (Ekstrand, 1955; Fig. 5A). *Typhula hyperborea* also had similar basidiospores (Fig. 5B). Ekstrand (1955) distinguished between *T. borealis* and *T. hyperborea sensu* H. Ekstr. in Scandinavia based on basidiospore dimension, and we subsequently compared basidiospores drawn in his literature in terms of Q-value (ratio of length and width) to reveal the difference (Fig. 5A). Our strains from various localities in northern Hemisphere were, on the contrary, indistinguishable by basidiospore dimension (Fig. 5B). Spore dimensions of *T. ishikariensis* and *T. canadensis* overlapped each other, and basidiospores of *T. hyperborea* tended to exceed the range of *T. ishikariensis* and *T. canadensis*. If Ekstrand (1955) distinguished *T. hyperborea* from *T. borealis* solely by basidiospore dimension, the separation of both fungi may be arbitrary and is considered to ignore differences in features reflecting genetic background.

3.4. Host range

The members of the *T. ishikariensis* complex differ in host range; *T. ishikariensis* var. *ishikariensis* attacks both mono- and dicots and rarely Gymnosperm (Gulaev, 1948; Potatosova, 1960a, 1960b, 1960c; Hoshino et al., 2004b). Imai by himself or Imai and Tanaka collected three specimens from rotted stalks of red clover in a snowy locality, Sapporo (former Kotoni village), Hokkaido, Japan (Fig. 3A). The original description of *T. borealis* is similar to that of *T. ishikariensis* (Ekstrand, 1955), and the wide host range of *T. borealis*, including winter cereals and grasses, clover, winter rape and beets, overlaps with that of *T. ishikariensis* (Imai, 1930; Tomiyama, 1961; Ylimäki, 1969; Årsvoll & Smith, 1978; Smith, 1989). These results suggest that *T. borealis* (strains from southern Norway) should be regard as *T. ishikariensis* var. *ishikariensis*, and our mating and phylogenic analyses agreed with these results (Table 1, Figs. 1 and 2). The host range of *T. canadensis* and *T. hyperborea*, as well as *T. ishikariensis* var. *idahoensis,* is restricted to monocots (Table 3) except *Stellaria* spp. whose tops get moribund and liable to the attack by fungus before persistent snow cover (Matsumoto & Hsiang, 2016).

3.5. Physiological characteristics of Typhula hyperborea

Typhula hyperborea (= *T. ishikariensis* group III) showed irregular growth as the extension of hyphae was inhibited and formed feather-like colony at 10°C on PDA. Its optimal growth temperature was below 10°C (ca. 0–4°C) (Matsumoto et al., 1996; Hoshino et al., 1997). However, when strains were cultured on corn meal agar (CMA; Difco), mycelial growth was improved in most strains (Fig. 6). Since PDA seemed to contain little radical scavengers and since incubation at high temperatures promoted oxygen uptake in the

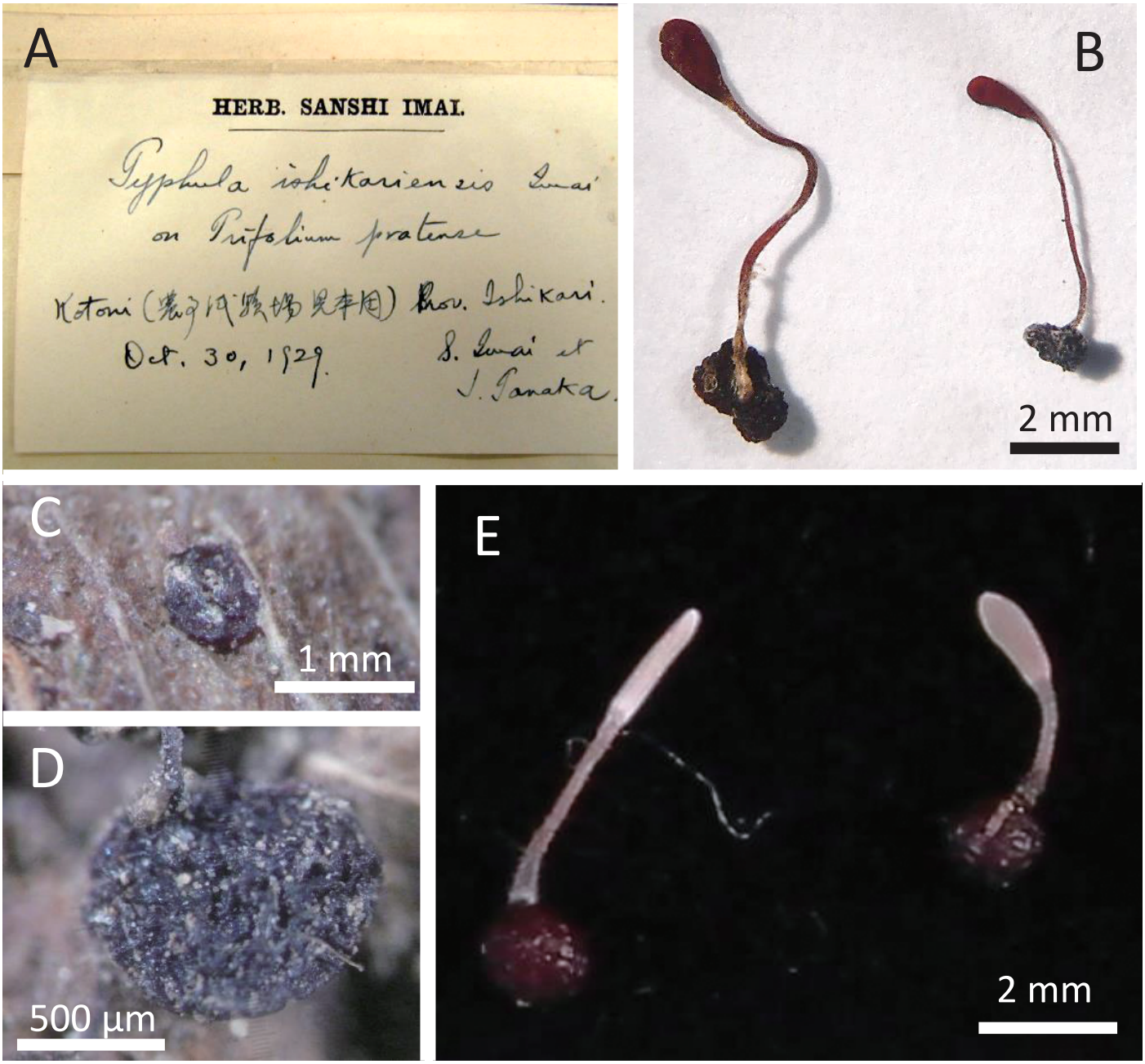

Fig. 3 – Morphological characteristics of *Typhula ishikariensis*.
A–D: lectotype (TNS-F-40457). E: epitype (TNS-F-24716). A: specimen label. B: dried basidiomata. C, D: dried sclerotium on rotted red clover stem. E: fresh basidiomata of *T. ishikariensis* var. *ishikariensis* from alfalfa.

psychrophilic species of the genus *Typhula* (Dejardin & Ward, 1971), PDA was amended with 100 µg/mL β-carotene or 5 mM sodium ascorbate. Consequently, mycelial growth was improved to various extent (Fig. 6), indicating that the injurious effects of active oxygen produced by excess respiration may be alleviated by radical scavengers in most strains of *T. hyperborea*. Its irregular growth at higher temperatures was ascribed to the loss of vital properties of intracellular proteins (Hoshino et al., 1997).

Pathogenisity of *T. hyperborea* was determined, using timothy (Table 4). Strains that showed irregular growth on PDA at 10°C were more virulent than those with normal growth and *T. ishikariensis*. These results may imply that pathogenic strains of *T. hyperborea* are higher in metabolic turnover to exploit host nutrients, which works well exclusively under exposure to stress conditions due to freezing temperatures.

3.6. Taxonomy

Typhula ishikariensis* var. *ishikariensis S. Imai, Trans. Sapporo Nat. Hist. Soc. 11:75, 1930 Figs 3B-E, 4B
Mycobank no. MB 427232.

Basionym: *Typhula ishikariensis* S. Imai, MB 819232
Synonyms: *Typhula humulina* Kusnezowa, Botanicheskie Materialy 9:145 (1953), MB 3027256
Typhula borealis H. Ekstr., Meddn. Växtskyddsant. Stockh. 67:52 (1955), MB 532444
Diagnosis: Sclerotium color of *T. ishikariensis* var. *ishikariensis*,

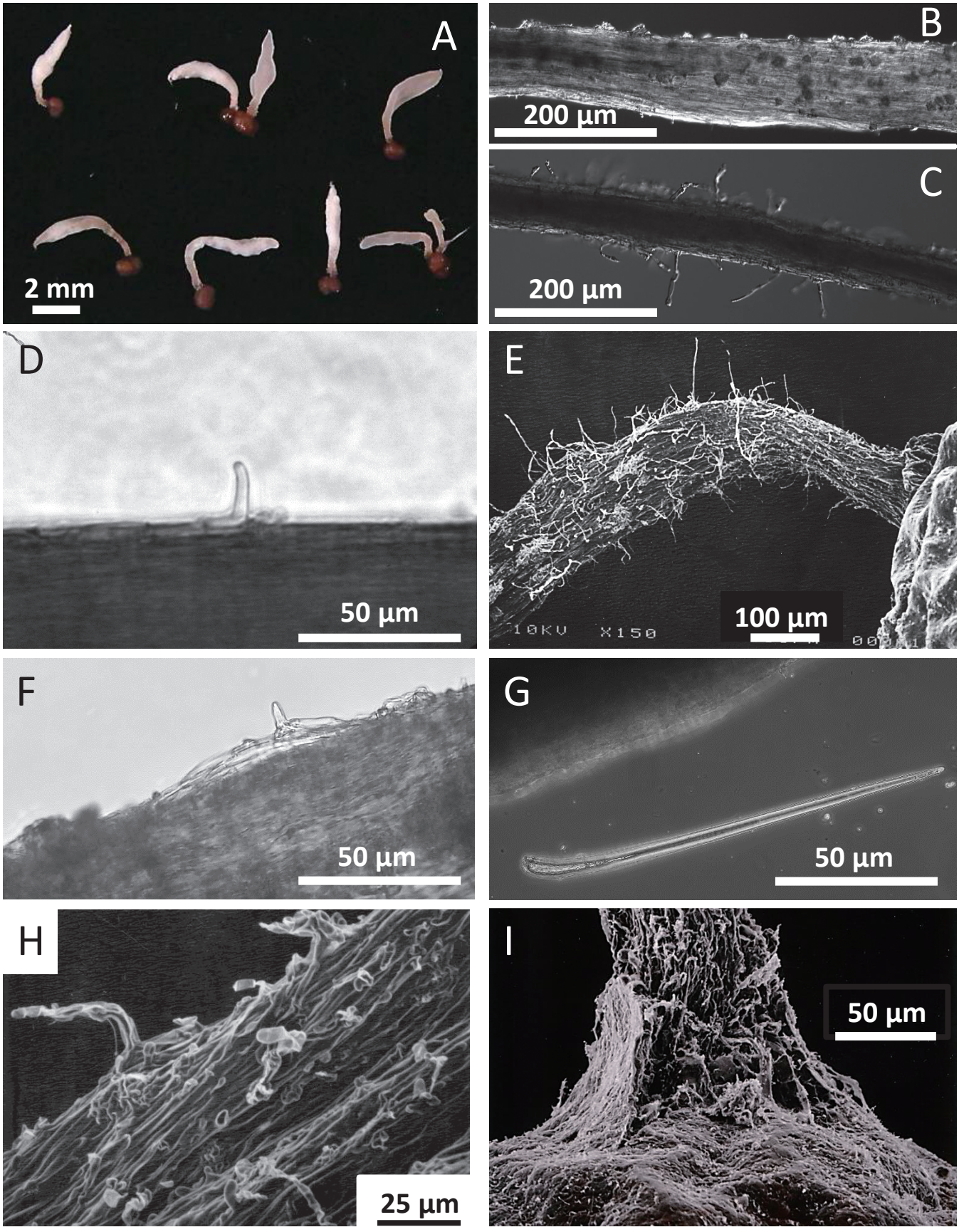

Fig. 4 – Morphological characteristics of *Typhula hyperborea* and their relatives.
A, D, H, I: *T. hyperborea* TNS-F-24687 (epitype). B: *T. ishikariensis* var. *ishikariensis* (epitype TNS-F-24716). C, E: *T. canadensis* MAFF 306142. F, G: *T. ishikariensis* var. *idahoensis* CUP27223 (syntype) A: fresh basidiomata. B–D: stem surface of fresh basidiomata. E–I: stem surface of dried basidiomata.

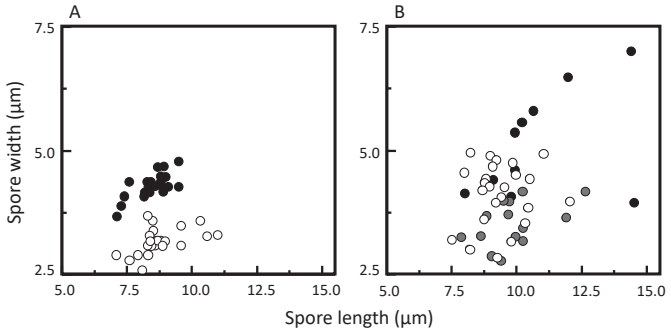

Fig. 5 – Basidiospore dimensions of the *Typhula ishikariensis* complex.
A: White circles: *T. borealis* and black circles: *T. hyperborea sensu* H. Ekstr. in Ekstrand (1955). B: our strains. White circles: *T. ishikariensis*, grey circles: *T. canadensis*, black circles: *T. hyperborea*.

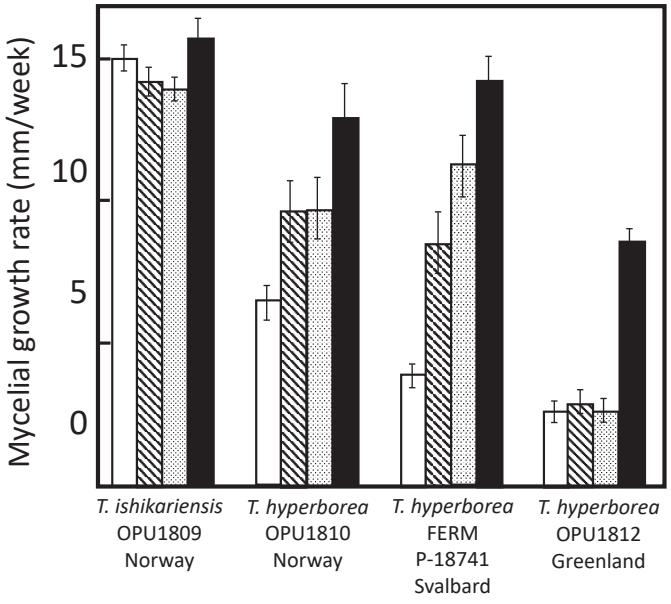

Fig. 6 – Effects of free radical scavengers on mycelial growth of *Typhula ishikariensis* and *T. hyperborea*.
White bars: PDA, shaded bars: PDA amended with 5 mM sodium ascorbate, dotted bars: PDA with 100 μg/mL β-carotene, black bars: corn meal agar. Cultures were incubated at 10˚C.

var. *idahoensis* and *T. hyperborea* were light to dark brown. Those of *T. canadensis* were dark brown almost black. Basidiomata of *T. ishikariensis* var. *ishikariensis* and *T. hyperborea* had short, and *T. canadensis* had long caulocystidia. Less aerial mycelia and normal growth on PDA at 10˚C. *T. canadensis* had abundant aerial mycelia, and *T. hyperborea* showed irregular growth on PDA at 10˚C. Mycelia of *T. ishikariensis* var. *ishikariensis* mainly mated within biological species I.

Type: JAPAN, Hokkaido, Kotoni, leaves of *Trifolium pretense* L., 6 Nov 1929, leg. S. Imai (lectotype, TNS-F-40457; syntype, TNS-F-40455, JAPAN, Hokkaido, Kotoni, leaves of *T. pretense*, 30 Oct 1929, leg. S. Imai & I. Tanaka; epitype, TNS-F-24716, JAPAN, Hokkaido, Sapporo, Hitsujigaoka, leaves of *Medicago sativa* L., May 1976, leg. N. Matsumoto; ex-epitype strain, MAFF306136).

Gene sequences ex-epitype: AB127949 (ITS), LC197671 (mitochondrial gene for large subunit ribosomal RNA).

Description: Sclerotia readily detached from host, globose to subglobose, light to dark brown (15 brick to 19 bay), 0.5–2 mm diam, surface smooth and rough when dry. One or more basidiomata emerging from a sclerotium, clavate or cylindrical, 4–20 mm tall, white to pale yellow (2 B to 6 E). Basidia having four sterigmata (Imai, 1930; Kuznetzova, 1953; Hoshino et al., 2004b). Basidiospores ovoid to ellipsoidal, 7.5–12.3×2.5–5 μm. Caulocystidia short, >50 μm.

Table 4 – Inoculation test of *Typhula ishikariensis* on timothy.

Strains, localities	Biological species	Regrowth after inoculation/ un-inoculated control (g dry weight/plant)	Irregular growth on PDA at 10˚C
OPU1809, Norway	*T. ishikariensis*	0.85	-
OPU1810, Norway	*T. hyperborea*	0.61	+
FERM P-18741, Svalbard	*T. hyperborea*	0.17	+
OPU1811, Greenland	*T. hyperborea*	0.98	-
OPU1812, Greenland	*T. hyperborea*	0.00	+
OPU1813, Greenland	*T. hyperborea*	0.88	-
OPU1814, Greenland	*T. hyperborea*	0.72	-
OPU1817, Greenland	*T. hyperborea*	0.73	-
OPU1816, Greenland	*T. hyperborea*	0.00	+

Host: monocots (grasses, winter cereals, tulip), dicots (beets, forage legumes, hop, rapeseed), and Gymnosperm (pine).

Distribution: widely distributed from Europe through Asian Far East (Matsumoto, 1989; Tkachenko, 2013) to North America (Bruehl & Cunfer, 1975; Årsvoll & Smith, 1978; Millett & Maxwell, 1997; Millett, 1999). This fungus dominates in snowy regions in the Temperate to Frigid Zones of Europe such as Alps (Schmidt, 1976; Årsvoll & Smith, 1978), Scandinavia (Kristinsson & Guðleifsson, 1976; Årsvoll, 1977; Matsumoto & Tronsmo, 1995; Matsumoto et al., 1996; Hoshino et al. 2004a), Baltic countries to European part of Russia and Northern Ukraine (Tkachenko et al., 1997). Similar morphological fungus was recorded from other countries of central Europe such as Germany (Andres, Hindorf, Fehrmann, & Trägner-Born, 1987), Poland (Dynowska, 1983) and former Czechoslovakia (Benada, 1976).

Mating group: *T. ishikariensis* var. *ishikariensis* from North America and Europe, Norwegian group I and Japanese biotype A.

Additional specimens examined: TNS-F-40456, 40458 (Imai also observed these specimens), 17091, 24661, 24663, 14664, 24667, 24670, 24671, 24673, 24675, 24676, 24677, 24678, 24679, 24680, 24681, 24682, 24683, 24684, 24685, 24686, 24695, 24696, 24697, 24704, UPS:BOT:F-707367, PACMA 5487 (*T. borealis*), JHP-549 2643 (*T. borealis*), Herbarium of Norwegian Institute of Bioeconomy Research (NIBIO): 2 packets of *T. ishikariensis*, 19 packets of *T. borealis*, 18 packets of *Typhula "borealis"*, 29 packets of *T.* cf. *borealis*.

Note: The lectotype is in rather poor condition and includes only two basidiomata (Fig. 3B). We did not determine DNA sequences from the lectotype. Thus, we selected an epitype from a prevalent mycelial compatibility group in Hokkaido, Japan in this species. Japanese name: Ishikari-gamanoho-take (Ishikari Province-bulrush-mushroom, Imai, 1930).

Typhula ishikariensis* var. *idahoensis (Remsberg) Årsvoll & J.D. Sm., Can. J. Bot. 56:361 (1978) Figs 4F, G

Mycobank no. MB 348940.

Basionym: *Typhula idahoensis* Remsberg, Mycologia 32:89

(1940), MB 291704

Diagnosis: Sclerotia of var. *idahoensis* and *T. canadensis* were frequently formed subepidermal in host tissues. Those of var. *ishikariensis* and *T. hyperborea* were easily dislodged from host tissues. Morphological characteristics similar to var. *ishikariensis*: however, rarely mates with var. *ishikariensis* (biological specie I) and often with *T. canadensis* (biological species II).

Type: USA, Idaho, Hill City, wheat leaves and stems, May 1939, leg. C.W. Hungerford (lectotype: CUP 27221; syntype, CUP 25153, USA, Montana, Bozeman, 11–24 Mar 1936, *Triticum vulgare*, leg. P.A. Young, CUP 25316, USA, Idaho, Soda Spring, 5 Oct 1936, *Triticum vulgare*, leg. Finch, CUP 27220, USA, Idaho, Felt, May 1930, wheat leaves and stems, leg. C.W. Hungerford, CUP 27222, USA, Idaho, Felt, Apr 1931, wheat leaves and stems, leg. C.W. Hungerford, CUP 27223, USA, Idaho, Tetonia, May 1931, on *Agropyron cristatum*, leg. C.W. Hungerford).

Gene sequences lectotype: LC192550 (ITS); syntype CUP 25153: LC613246 (ITS).

Description: Sclerotia are frequently subepidermal and subspherical in host, globose to subglobose, light to dark brown (15 brick to 19 bay, original description: chestnut-brown to bone-brown: Remsberg, 1940a), 0.5–2 mm diam, surface smooth and rough when dry. One or more basidiomata emerging from a sclerotium, clavate or cylindrical, 4–20 mm tall, white to pale yellow (2 B to 6 E). Basidiospores ovoid to ellipsoidal, 7.5–12.3×2.5–5 μm. Caulocystidia mainly short (>50 μm) and rarely enlonged (<50 μm).

Host: monocots (grasses, winter cereals).

Distribution: North America (Remsberg, 1940a, 1940b; Bruehl et al., 1975; Årsvoll & Smith, 1978)

Mating group: *T. ishikariensis* var. *idahoensis* in North America and *T. canadensis* in North America and Europe, Norwegian group II and biotype B in Japan.

Additional specimens examined: CUP 17507, 17510, 19191, 25316, 27220, 27221a, 37857, UPS:BOT:F-683949 (isosyntype), WSP 12015, 12016, 12017, 12018, 13554, 15993, 16235, 16236, 23669, 35987, 37317, 37322, 46544, 46679, 46711, 46832, 46833, 46834, 46835, 46888, 46890,51550, 71681, TNS-F-24709, 24710, Herbarium of NIBIO: 9 packets of *T. idahoensis*, 55 packets of *Typhula* cf. *idahoensis*

Note: Basidia having 4, 6 or 8 sterigmata (Remsberg 1940a). Cunfer (1974) reported that this fungus had tetrapolar incompatibility with multiple alleles. Japanese name: Hokubei-Ishikari-gamanoho-take (North American-Ishikari Province-bulrush-mushroom, newly named).

Typhula canadensis (J.D. Sm. & Årsvoll) Tam. Hoshino, T. Kasuya, & N. Matsumoto, stat. nov. Figs 4C, E

Mycobank no. MB 819233.

Basionym: *Typhula ishikariensis* var. *canadensis* J.D. Sm. & Årsvoll, Can. J. Bot. 56: 362 (1978), MB 348939

Diagnosis: Sclerotium color of *T. canadensis* was dark brown to almost black. Those of other *T. ishikariensis* complex were light to dark brown. Some strains of Basidiomata of *T. canadensis* and var. *idahoensis* had long caulocystidia, however those of other *T. ishikariensis* complex were short. *T. canadensis* had abundant aerial mycelia and normal growth on PDA at 10˚C. Mycelia of *T. canadensis* mainly mated within biological species II.

Type: CANADA, Saskatchewan, Price Albert National Park, *Poa annus* L. and *Poa pratensis* L., 9 May 1974. leg. J. Drew Smith (holotype, DAOM160550).

Gene sequence holotype: LC192551 (ITS).

Description: Sclerotia highly variable in size: small sclerotia 0.2–0.5 mm often suspended in aerial mycelium, large sclerotia up to 2 mm diam, often produced involving host tissues, globose to subglobose, dark brown (16 cigar brown) almost black (36 fuscous black or 37 olivaceous black), surface smooth and rough when dry. One or more basidiomata emerging from a sclerotium, clavate or cylindrical, 5–25 mm tall, white to pale brown (2 B to 6 F). Basidia with four sterigmata. Basidiospores ellipsoidal, 7.6–12.5×2.5–4 μm. Caulocystidia enlonged, 50–120 μm. Morphological characteristics similar to *T. ishikariensis*; however, rarely mates with var. *ishikariensis* and often mates with var. *idahoensis*.

Host: mainly monocots (grasses, winter cereals, *Cyperaceae*, tubers of Chinese yam) and rarely on a few dicots such as *Stellaria* spp.

Distribution; Far East (Matsumoto, 1989; Matsumoto & Tajimi, 1991; Tkachenko, 2013), Siberia (Tkachenko, 2013), Norway (Matsumoto & Tronsmo, 1995; Matsumoto et al., 1996), Iceland (Kristinsson & Guðleifsson, 1976; Hoshino et al., 2004a), North America (Årsvoll & Smith, 1978; Millett & Maxwell, 1997; Millett, 1999).

Mating group: *T. ishikariensis* var. *canadensis* in North America and Europe, Norwegian group II and biotype B in Japan. *T. ishikariensis* var. *idahoensis* in North America.

Additional specimens examined: TNS-F-24662, 24692, 24693, 24707, 24711

Note: Japanese name: Kuro-tsubu-Ishikari-gamanoho-take (black-specked-Ishikari Province-bulrush-mushroom, newly named).

Typhula hyperborea H. Ekstr., Meddn. Växtskyddsant. Stockh. 67:55 (1955) Figs 4A, D, H, I

Mycobank no. MB 89234.

Diagnosis: *T. hyperborea* had abundant aerial mycelia on PDA at 0˚C and showed irregular growth (feather-like colonies) on PDA at 10˚C. Mycelia of *T. hyperborea* mainly mated within biological species III.

Type: SWEDEN, Norrbotten, Luleå, 1939, (lectotype, strain 7575, Fig. 14 in Meddn. Växtskyddsant. Stockh. 67:49 (1955); epitype, TNS-F-24687, NORWAY, Oppland, Fron, Kvarvet, leaves of *Deschampsia cespitosa* (L.) P. Beauv., May, 1992, leg. N. Matsumoto; ex-epitype: OPU1810).

Gene sequences ex-epitype: LC192579 (ITS), LC663960 (mitochondrial gene for large subunit ribosomal RNA).

Description: Sclerotia readily detached from host, globose to subglobose, light to dark brown (15 brick to 19 bay), 0.5–2 mm diam, surface smooth and rough when dry. One or more basidiomata emerging from a sclerotium, clavate or cylindrical, 5–25 mm tall (under artificial condition), head often tapered, white to pale brown (2 B to 6 F, 30 clay pink). Basidia with four sterigmata. Basidiospores ellipsoidal, 7.6–14.8×3.7–7.1 μm. Caulocystidia short, >50 μm. Mycelium abundant at 0˚C, but growth irregular at 10˚C on PDA (Matsumoto et al., 1996; Hoshino et al., 1997).

Host: monocots (grasses, winter cereals)

Distribution; the Arctic (Hoshino et al., 2003; Hoshino et al., 2006), Kamchatka, Siberia (Hoshino et al., 2001), Ural-Volga in Russia, Alps, Northern Scandinavia (Ekstrand, 1939, 1955; Røed, 1956; Matsumoto & Tronsmo, 1995; Matsumoto et al., 1996), North America (Pouleur, 1988; Pouleur & Couture, 1988). Similar fungus was also recorded in Northern Scandinavia (Jamalainen, 1957; Årsvoll, 1977), Russian Arctic (Petrov, 1983; Shiryaev, 2004, 2006, 2009, 2013a, 2013b) and Yukon in Canada (Lebeau & Longsdon, 1958).

Additional specimens examined: TNS-F-24672, 24674, 24688, 24657, 24658, 24659, 24660, 24665, 24666, 24668, 24669, 24689,

24690, 24691, 24694, 24698, 24699, 246700, 24701, 24702, 24703, 24705, 24706, 24708, 24712, 24713, Herbarium of NIBIO: 18 packets of *Typhula "borealis"*, 29 packets of *T.* cf. *borealis.*

Note: Årsvoll and Smith (1978) suggested that *T. hyperborea* might correspond to *T. ishikariensis* var. *canadensis* or to some intermediate forms but obtained neither exsiccata nor cultures of *T. hyperborea*. We found several specimens labeled *T. "borealis"* or *T.* cf. *borealis* at Herbarium in Norwegian Institute of Bioeconomy Research, Norway (Ekstrand used these names or or *T.* cfr. *borealis* in his publications; Jamalainen, 1954, 1974); however, we were unable to find type specimens of both fungi.

We designated the illustration of basidiospores by Ekstrand (1955) as the lectotype. No other morphological characteristics were shown by Ekstrand (1955). Thus, we selected an epitype from a strain collected from a representative locality in the vicinity of the original description of Ekstrand (1955).

Japanese name: saihate-Ishikari-gamanoho-take (northmost-Ishikari Province-bulrush-mushroom, newly named).

Disclosure

The Authors declare no conflict of interest. All the experiments undertaken in this study comply with the current laws of the countries where they were performed.

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