The Lichenologist **50**(1): 59–75 (2018) doi:10.1017/S0024282917000615

Thamnolia tundrae sp. nov., a cryptic species and putative glacial relict

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Abstract: The lichen species of the genus *Thamnolia*, with their striking wormlike thalli and frequent occurrence in arctic and tundra environments, have often been debated with regard to the use of chemistry in lichen taxonomy. Phylogenetic studies have arrived at different conclusions as to the recognition of species in the genus, but in a recent study based on the analyses of six nuclear markers (genes or noncoding regions) of a worldwide sample of *Thamnolia*, we showed the existence of three well-supported lineages with two different chemistries and geographical distributions. Here, we present two analyses based on ITS and three markers, respectively, which were extended from the study mentioned above to include type specimens and additional *Thamnolia* strains and taxa. In these analyses the same three clades were retrieved. A putative DEAD-box helicase is used here for the first time as an informative phylogenetic marker to provide taxonomic resolution at species level. The distribution of morphological and chemical characters across the phylogeny was analyzed and it was concluded that three morphologically cryptic, but genetically well supported, species occur: *T. vermicularis* s. str., *T. subuliformis* s. str. and *T. tundrae* sp. nov. *Thamnolia vermicularis* s. str. contains individuals with uniform secondary chemistry (producing thamnolic acid) and a rather limited distribution in the European Alps, Tatra Mts and the Western Carpathians, a distribution which might result from glacial survival in an adjacent refugium/refugia. *Thamnolia subuliformis* s. str. is widely distributed in all hemispheres and the samples contain two chemotypes (either with thamnolic or squamatic acids). *Thamnolia tundrae* is described as new; it produces baeomycesic and squamatic acids, and has a distribution limited to the arctic tundra of Eurasia extending to the Aleutian Islands in North America. It may have survived the latest glaciation in coastal refugia near its present distribution. Thus, secondary chemistry alone is not suitable for characterizing species in *Thamnolia*, secondary chemistry and geographical origin are informative, and the ITS region can be confidently used for species recognition. Nomenclatural notes are given on several other names that have been used in *Thamnolia*.

Key words: lichens, molecular phylogeny, new species, nomenclature, secondary chemistry, taxonomy

Accepted for publication 30 *May* 2017

Introduction

Lichens of the genus *Thamnolia* Ach. ex Schaer. are widely distributed in all hemispheres and their wormlike thalli are easy to recognize by the chalk-white colour and gross morphology. They mainly occur in alpine and arctic environments where extensive colonies are often formed. Even if signs of past or infrequent recombination have been found (Onut-Brännström *et al.* 2017), *Thamnolia* has never been found fruiting and it is believed to disperse exclusively by thallus fragments (Andrei *et al.* 2006–2007) or mitospores formed in pycnidia (Lord *et al.* 2013).

Morphology, chemistry and geography have traditionally been used to recognize species in the genus. *Thamnolia vermicularis* (Sw.) Schaer., *T. papelillo* R. Sant. and *T. juncea* R. Sant. were thus recognized by thallus morphology. Variation in secondary chemistry within and between these species has resulted in the recognition of *T. subuliformis* (Ehrh.) W. L. Culb. (syn. *T. subvermicularis* Asahina), differing from *T. vermicularis* (in the above sense) by containing squamatic and baeomycesic acids (reacting UV+ yellow) rather than thamnolic acid (UV−). These two chemistries are hereafter referred to as UV+ and UV−,

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respectively. Sometimes *T. subuliformis* has been considered a variety and rarely a subspecies of *T. vermicularis*, an option supported by the fact that subsp. *vermicularis* and subsp. *subuliformis* on a large scale have different geographical distributions (Satô 1965; Sheard 1977). It is noteworthy that the two chemistries found in *T. vermicularis* s. lat. also occur in both *T. papelillo* and *T. juncea*, and this has also been used as a justification for recognizing infraspecific taxa in these species (Santesson 2004).

Thamnolia has figured prominently in the debate about the recognition of chemotypes as species and previous phylogenetic studies based on molecular data have arrived at different conclusions regarding the use of chemistry in species recognition in this genus. First, Platt & Spatafora (2000) used two specimens of a different chemistry to *T. vermicularis* s. lat. in a phylogenetic study on non-sexual lichens in *Icmadophilaceae*. They concluded that the genetic distance between specimens of the two chemotypes was large enough to warrant the recognition of two different species. In two other studies (Nelsen & Gargas 2009; Lord *et al.* 2013) it was concluded that the chemotypes of *T. vermicularis* s. lat. are not reciprocally monophyletic, although a strongly supported clade composed only of individuals of a UV + chemotype collected from the Aleutian Islands and Norway was demonstrated. In a recent paper (Leavitt *et al.* 2016), the authors performed a multispecies coalescent model analysis based on the dataset of Nelsen & Gargas (2009) and argued for the recognition of the two chemotypes as different species. The authors of the latter study concluded that there is strong evidence for '*T. vermicularis*' (UV−) and '*T. subuliformis*' (UV+) being separate species and it was suggested that the lack of reciprocal monophyly of individuals with different chemistries was due to recent divergence and incomplete lineage sorting.

In our recent study, which was based on the analyses of six nuclear markers (genes or noncoding regions) of a worldwide sample of *Thamnolia* (including the Nelsen & Gargas (2009) dataset), we showed the existence of three well-supported lineages with different chemistries and geographical distributions (Fig. 1) (Onut-Brännström *et al.* 2017). One lineage ('Lineage C', *ibid.*) has a worldwide distribution and is partly sympatric with the other two lineages. It contains individuals of both chemotypes and also specimens of the morphologically characterized *T. papelillo*. The other two lineages ('Lineages A' and 'B') are allopatric and have different chemistries. 'Lineage A' was UV+ and geographically restricted to the tundra region of Eurasia and the Aleutian Islands. Interestingly it contains all the UV+ specimens that were grouping in the well-supported clade of the other two studies mentioned above (Nelsen & Gargas 2009; Lord *et al.* 2013). 'Lineage B' is UV− and has a rather limited distribution in the European Alps, Tatra Mountains and the Western Carpathians. The Nelsen & Gargas dataset of 2009 included both 'Lineage A' (UV+) and 'Lineage C' (both UV− and UV+ specimens), hence they based their conclusion on a heterogeneous sample (Leavitt *et al.* 2016).

This paper aims to formally clarify the complex taxonomic and nomenclatural situation of *Thamnolia*, including the recognition and naming of three distinct clades as species. The aim could alternatively be expressed as it was by Minks (1874): "… eine eingehende und umfassenden, eine befriedigende Schilderung von *Thamnolia vermicularis* zu entwerfen und eine der empfindlichesten Lücken der Lichenologie zu ergänzen." which adequately stressed the importance and complexity in understanding the taxonomy of *Thamnolia*.

Material and Methods

Sampling and phenotype descriptions

Twenty-two samples were newly investigated in this study and analyzed together with data from 65 samples included in the study by Onut-Brännström *et al.* (2017). Information about the samples is given in Table 1. Based on morphological characters, these were previously assigned to the 'phenospecies/taxa': *T. vermicularis*, *T. subuliformis*, *T. papelillo* var. *papelillo*, *T. papelillo* var. *subsolida* (M. Satô) R. Sant., *T. juncea* var. *juncea*, *T. juncea* var. *subjuncea* R. Sant and *T. taurica* (Wulfen) A. Massal. The newly investigated samples included the

https://doi.org/10.1017/S0024282917000615 Published online by Cambridge University Press

TABLE 1. Thamnolia *specimens used in this study together with their original identification and subsequent assignment to one of the three* Thamnolia *species. Sample ID includes the geographical origin and laboratory ID of the specimen. Accession numbers in bold indicate new sequences. Dashes = failed PCR or sequencing reactions; blanks = no attempt made to obtain marker.*

Sample ID	Traditional identification	Thallus morphology	Chemistry*	Origin	GenBank Accession numbers			Collection year	Voucher information and collector
					ITS	Ef	DEAD		
Thamnolia subuliformis s. str.									
571.2_Ts	*T. subuliformis*	cylindrical	UV+	unknown	**MF149087**	–	**MF143800**	XVIII	S, Hb. Swartzii L1596, the right hand side specimen, *Olof Swartz* (?)
AUS_Aul_AggieMt_554.3_Tv	*T. vermicularis*	cylindrical	UV−	Australia, Brindabella Range, Aggie Mts	KY550205			1981	UCONN, ALTA, *Plants of Australia* (D.H. Vitt no 816), *D. H. Vitt*
BO_SamanaPampa_390_Tv	*T. vermicularis*	cylindrical	UV−	Bolivia, Samana Pampa	KY550184			2013	UPS, L-774157, *D. H. Vitt*
CA_Avalon_285_Ts	*T. subuliformis*	cylindrical	UV+	Canada, Newfoundland, Avalon Peninsula, Doe Hills	KY550136	Y654447	KY654355	2007	H (T. Ahti 67988), *Teuvo Ahti*
CA_BritishColumbia_280_Tv	*T. vermicularis*	cylindrical	UV−	Canada, BC, Radio Tower Mt.	KY550132	KY654443	KY654354	2012	UPS, L-774103, *Toby Spribille*
CHL_Navarino_556.3_Tv	*T. vermicularis*	cylindrical	UV−	Chile, Isla Navarino	KY550206			2000	UCONN, *Flora of Chile*, BG 6811, *Bernard Goffinet & Cymion Cox*
DK_Greenland_263_Ts	*T. subuliformis*	cylindrical	UV+	Denmark, Greenland, Qaanaaq	KY550126	KY654440	KY654351	2009	UPS, L-617837, *Eric Steen Hansen*
IC_Skagafjardarsysla_84_Ts	*T. subuliformis*	cylindrical	UV+	Iceland, Skagafjardarsysla	KY550219	KY634078	KY634055	2011	UPS, L-774204, *Starri Heidmarsson*
JA_Kinpu_246.3_Tv	*T. vermicularis*	cylindrical	UV−	Japan, Kai Province, Kinpu Mt.	KY550122	KY654437	KY654397	2012	UPS, L-593493, *Göran Thor*
NE_Dolpo_349_Ts	*T. subuliformis*	cylindrical	UV+	Nepal, Dolpo District	KY550172	KY634097	KY654381	2012	UPS, L-774130, *Steve Behaeghel & Katrijn Van Nieuwenhuyse*
NE_Humla_356_Ts	*T. subuliformis*	cylindrical	UV+	Nepal, Humla, Nyula la Pass	KY550176	KY654478	KY654382	2012	UPS, L-774134, *Steve Behaeghel & Katrijn Van Nieuwenhuyse*
NE_Khumbu_344_Ts	*T. subuliformis*	cylindrical	UV+	Nepal, Khumbu District	KY550170	KY654475	KY654379	2012	UPS, L-774128, *Steve Behaeghel & Katrijn Van Nieuwenhuyse*
NE_Khumbu_345_Tv	*T. vermicularis*	cylindrical	UV−	Nepal, Khumbu District	KY550171	KY654476	KY654380	2012	UPS, L-774129, *Steve Behaeghel & Katrijn Van Nieuwenhuyse*
NE_Moraine_363_Tv	*T. vermicularis*	cylindrical	UV−	Nepal, Moraine, above Thare	KY550177	KY654479	KY654383	2012	UPS, L-774135, *Steve Behaeghel & Katrijn Van Nieuwenhuyse*
NG_StrongMt_586.3_Tjj_HOLOTYPE	*T. juncea var. juncea*	long	UV−	Papua New Guinea, Strong Mt.	**MF149084**	–	–	1971	UPS, L-136871; holotype, *M. Coode & B. Stevens*
NG_WilhelmMt_391.2_Tjs	*T. juncea var. subjuncea*	long	UV+	Papua New Guinea, Wilhelm Mt	**MF149095**	–	–	1968	UPS (not registered), *W. Weber*
NG_WilhelmMt_574.1_Tjs	*T. juncea var. subjuncea*	long	UV+	Papua New Guinea, Wilhelm Mt	**MF149085**	**MF143830**	**MF143803**	1968	UPS (not registered), *W. Weber*
NG_WilhelmMt_574.2_Tjs	*T. juncea var. subjuncea*	long	UV+	Papua New Guinea, Wilhelm Mt	**MF149096**	–	–	1968	UPS (not registered), *W. Weber*
NG_WilhelmMt_585.1_Tjj	*T. juncea var. juncea*	long	UV−	Papua New Guinea, Wilhelm Mt	**MF149083**	**MF143829**	**MF143804**	1966	UPS, *Flora of New Guinea*, not registered, *D. McVean*

TABLE 1 (*continued*).

Sample ID	Traditional identification	Thallus morphology	Chemistry*	Origin	GenBank Accession numbers ITS	Ef	DEAD	Collection year	Voucher information and collector
Thamnolia subuliformis s. str. (cont.)									
NO_Brannsletta_119.1_Tv	*T. vermicularis*	cylindrical	UV−	Norway, Finnmark, loc. Brannsletta	KY550091	KY654413	KY654385	2012	UPS, L-812492, *Anders Larsson*
NO_SorVaranger_116_Tv	*T. vermicularis*	cylindrical	UV−	Norway, Finnmark, Sør-Varanger	KY550088	KY654411	KY634024	2012	UPS, L-774066, *Anders Larsson*
NO_SorVaranger_118_Tv	*T. vermicularis*	cylindrical	UV−	Norway, Finnmark, Sør-Varanger	KY550090	KY654412	KY634024	2012	UPS, L-774067, *Anders Larsson*
PE_AlboDePucarajuPass_496_Tv	*T. vermicularis*	cylindrical	UV−	Peru, Albo de Pucaraju Pass	KY550195	**MF143826**	**MF143809**	2013	UPS, L-774183, *Steve Behaeghel & Katrijn Van Nieuwenhuyse*
PE_Bolognesi_393_Tps	*T. papelillo var. subsolida*	flat	UV+	Peru, Bolognesi	KY550185			1981	UPS (not registered), *R. Santesson & R. Moberg*
PE_HuarazCasma_582_Tps	*T. papelillo var. subsolida*	flat	UV+	Peru, road Huaraz-Casma	**MF14908**			1981	UPS, L-153296, *R. Santesson & R. Moberg*
PE_Jauja_579_Tpp_HOLOTYPE	*T. papelillo var. papelillo*	flat	UV−	Peru, Prov Jauja	**MF149093**	**MF143828**	**MF143806**	1981	UPS, L-136872; holotype, *R. Santesson & R. Moberg*
PE_QuebradaMatara_502_Tv	*T. vermicularis*	cylindrical	UV−	Peru, Quebrada Matara	KY550197			2013	UPS, L-774187, *Steve Behaeghel & Katrijn Van Nieuwenhuyse*
PE_Tarma_392.1_Tps	*T. papelillo var. subsolida*	flat	UV+	Peru, between La Oroya and Junin	**MF149094**	**MF143825**	**MF143805**	1981	UPS, *Flora of Peru* (12th Regnellian Expedition, n. P26.2), *R. Santesson & R. Moberg*
PE_Tarma_392.2_Tps	*T. papelillo var. subsolida*	flat	UV+	Peru, between La Oroya and Junin	KY612985			1981	UPS, *Flora of Peru* (12th Regnellian Expedition, n. P26.2), *R. Santesson & R. Moberg*
PE_Tupatupa_511_Tv	*T. vermicularis*	cylindrical	UV−	Peru, Tupatupa	KY550202			2013	UPS, L-774192, *Steve Behaeghel & Katrijn Van Nieuwenhuyse*
PE_Vientunan_477.1_Tv	*T. vermicularis*	cylindrical	UV−	Peru, Vientunan Pas	**MF149089**	**MF143824**	**MF143808**	2013	UPS, L-812484, *Steve Behaeghel & Katrijn Van Nieuwenhuyse*
PE_Yanashallash_395.1_Tpp	*T. papelillo var. papelillo*	flat	UV−	Peru, Yana-shallash	**MF149090**	**MF143823**	**MF143807**	1981	UPS, L-153301, *R. Santesson & R. Moberg*
PE_Yanashallash_395.2_Tpp	*T. papelillo var. papelillo*	flat	UV−	Peru, Yana-shallash	KY550187			1981	UPS, L-153301, *R. Santesson & R. Moberg*
PO_ Mieguszowiecka_301_Ts	*T. subuliformis*	cylindrical	UV+	Poland, Tatra Mts., Wyżnia Mięguszowiecka pass	KY550147	KY654456	KY654361	2005	KRAM-L 53024, voucher no 78, unknown collector
RO_BucegiMt_239.1_Ts	*T. subuliformis*	cylindrical	UV+	Romania, Bucegi Mts., Caraiman cottage	KY550120	KY654435	KY654395	2012	UPS, L-774094, *Doina Iordachescu*

TABLE 1 (*continued*).

Sample ID	Traditional identification	Thallus morphology	Chemistry*	Origin	GenBank Accession numbers ITS	Ef	DEAD	Collection year	Voucher information and collector
Thamnolia subuliformis s. str. (cont.)									
RO_BucegiMt_239.2_Tv	*T. vermicularis*	cylindrical	UV−	Romania, Bucegi Mts., Caraiman cottage	KY550121	KY654436	KY654396	2012	UPS, L-774095, *Doina Iordachescu*
RU_Bezymyannaya_284.1_Ts	*T. subuliformis*	cylindrical	UV+	Russia, Polar Urals, Bezymyannaya Mt	KY550134	KY654445	KY654399	2012	UPS, L-774105, *S. Ektova, S. Abdulmanova*
RU_Bezymyannaya_284.2_Tv	*T. vermicularis*	cylindrical	UV−	Russia, Polar Urals, Bezymyannaya Mt	KY550135	KY654446	KY654400	2012	UPS, L-774106, *S. Ektova, S. Abdulmanova*
RU_CapeTirol_321_Ts	*T. subuliformis*	cylindrical	UV+	Russia, Polar Urals, Bezymyannaya Mt	KY550156	KY654464	KY654369	2012	UPS, L-774115, *Sergei Kholod*
RU_CapeTrieste_320_Ts	*T. vermicularis*	cylindrical	UV+	Russia, Franz Josef Land, Champ Is., Cape Trieste	KY550155	KY654463	KY654368	2012	UPS, L-774114, *Sergei Kholod*
RU_Litovka_311_Tv	*T. vermicularis*	cylindrical	UV−	Russia, Shkotovskiy district, Litovka (Falaza) Mt.	KY550151	KY654461	KY654366	2012	UPS, L-774110, *Lidia Yakovchenko*
RU_MalayaKhatiparaMt_328_Ts	*T. subuliformis*	cylindrical	UV+	Russia, Caucasus, Malaya Khatipara Mt	KY550161	KY654468	KY654373	2012	UPS, L-774120, *S. Ektova, S. Abdulmanova*
RU_Sabetta_333_Tv	*T. vermicularis*	cylindrical	UV−	Russia, Polar Urals, Yamal Penisula, Sabetta	KY550165	KY654470	KY654374	2012	UPS, L-774124, *S. Ektova, S. Abdulmanova*
RU_Yaroto_334_Ts	*T. subuliformis*	cylindrical	UV+	Russia, Yamal peninsula, Yaroto-2 lake	KY550166	KY654471	KY654375	2012	UPS, L-774125, *S. Ektova, S. Abdulmanova*
RU_Drovyanoy_335_Tv	*T. vermicularis*	cylindrical	UV−	Russia, Yamal peninsula, Drovyanoy	KY550167	KY654472	KY654376	2012	UPS, L-774126, *S. Ektova, S. Abdulmanova, K. Ermokhina*
SE_Abisko_226_Ts	*T. subuliformis*	cylindrical	UV+	Sweden, Abisko, Nuolia peak	KY550116	KY654431	KY634056	2012	UPS, L-774090, *Una Brännström*
SE_Abisko_229_Tv	*T. vermicularis*	cylindrical	UV−	Sweden Abisko, Nuolia peak	KY550117	KY654432	KY634040	2012	UPS, L-774091, *Stela Onut & Gheorghe Onut*
SE_Gotland_04_Ts	*T. subuliformis*	cylindrical	UV+	Sweden, Gotland Isl., Sundre	KY497259	KY634101	KY634042	2010	UPS, L-774054, *Ioana Brännström & Lars Brännström*
SE_Lillulvafjallets_214.1_Ts	*T. subuliformis*	cylindrical	UV+	Sweden, Jämtland, Lill-ulvåfjällets	KY550107	KY654423	KY654388	2012	UPS, L-774082, *Anders Larsson*
SE_Lillulvafjallets_214.2_Tv	*T. vermicularis*	cylindrical	UV−	Sweden, Jämtland, Lill-ulvåfjällets	KY550108	KY654424	KY654389	2012	UPS, L-774083, *Anders Larsson*
SE_LuleLappmark_72_Ts	*T. subuliformis*	cylindrical	UV+	Sweden, Lule Lappmark, Stora Sjöfallet	KY550215	KY634076	KY634053	2009	UPS L-520827, *Anders Nordin*
SE_MoiRana_05_Ts	*T. subuliformis*	cylindrical	UV+	Sweden, Mo i Rana Pass	KY550077	KY634088	KY634059	2010	UPS, L-774055, *Ioana Brännström & Lars Brännström*
SE_Oland_27.3_Ts	*T. subuliformis*	cylindrical	UV+	Sweden, Öland, Stora Alvaret	**MF149086**	**MF143827**	**MF143802**	2010	UPS, L-812487, *Ioana Brännström & Lars Brännström*
SE_Taljstensvalen_217.1.2_Ts	*T. subuliformis*	cylindrical	UV+	Sweden, Jämtland, Täljstensvalen	**MF149091**			2012	UPS, L-774086, *Anders Larsson*

TABLE 1 (*continued*).

Sample ID	Traditional identification	Thallus morphology	Chemistry*	Origin	GenBank Accession numbers ITS	Ef	DEAD	Collection year	Voucher information and collector
Thamnolia subuliformis s. str. (cont.)									
SE_Taljstensvalen_217.2_Tv	*T. vermicularis*	cylindrical	UV−	Sweden, Jämtland, Täljstensvalen	KY550112	KY654427	KY654392	2012	UPS, L-774086, *Anders Larsson*
SE_Tvaradalen_216.1_Tv	*T. vermicularis*	cylindrical	UV−	Sweden, Jämtland, Norder-Tvärådalen	KY550109	KY654425	KY654390	2012	UPS, L-774087, *Anders Larsson*
SW_Valais_113_Ts	*T. subuliformis*	cylindrical	UV+	Switzerland, Valais, Nendaz	KY550086	KY654410	KY634026	2012	UPS, L-774064, *Paul Diederich*
US_AleutianTigalda_291.1_Tv	*T. vermicularis*	cylindrical	UV−	USA, Aleutian Islands, Tigalda Island, Tigalda Bay	KY550142	KY654452	KY654403	2011	H, (*T. Ahti* 700023), *Teuvo Ahti*
US_AleutianUnimak_287.1_Tv	*T. vermicularis*	cylindrical	UV−	USA, Aleutian Islands, Unimak Island, False Pass	KY550138	KY654449	KY654401	2008	H, #TIG003-X-1A, *Teuvo Ahti & Stephan S. Talbot*
US_AleutianUnimak_287.2_Ts	*T. subuliformis*	cylindrical	UV+	USA, Aleutian Islands, Unimak Island, False Pass	KY550139	KY654450	KY654402	2008	H, #TIG003-X-1A, *Teuvo Ahti & Stephan S. Talbot*
US_FingerMt_82_Tv	*T. vermicularis*	cylindrical	UV−	USA, Yukon-Koyukuk Canjon, Finger Mt.	KY550216	KY634093	KY634023	2011	UPS, L-774202, *Sanjia Savic*
US_Galbreat_83.1_Tv	*T. vermicularis*	cylindrical	UV−	USA, Alaska, Wiseman-Antigu Pass and Galbreat Lake	KY550217	KY634094	KY634039	2011	UPS, L-774203, *Sanjia Savic*
US_Galbreat_83.2_Ts	*T. subuliformis*	cylindrical	UV+	USA, Alaska, Wiseman-Antigu Pass and Galbreat Lake	KY550218	KY654482	KY654406	2011	UPS, L-774203, *Sanjia Savic*
US_Selby_210.1_Tv	*T. vermicularis*	cylindrical	UV−	USA, Selby and Narvak Lakes	KY550103	KY654419	KY654386	2012	UPS, L-774078, *Peter Nelson*
US_Selby_210.2_Ts	*T. subuliformis*	cylindrical	UV+	USA, Alaska, Selby and Narvak Lakes	KY550104	KY654420	KY654387	2012	UPS, L-774078, *Peter Nelson*
US_Summit_213_Ts	*T. subuliformis*	cylindrical	UV+	USE, Alaska, Summit Lake	KY550106	KY654422	KY63404	2012	UPS, L-774081, *Peter Nelson*
Thamnolia tundrae									
NO_SorVaranger_117_Ts	*T. subuliformis*	cylindrical	UV+	Norway, Finnmark, Sør-Varanger	KY550089	KY634066	KY634017	2012	UPS, L-774067, *Anders Larsson*
RU_CapeRogatyi_323_Ts	*T. subuliformis*	cylindrical	UV+	Russia, Franz Josef Land, La-Ronser Is., Cape Rogatyi	KY550157	KY634065	KY634016	2012	UPS, L-774116, *Sergei Kholod*
RU_KolguevIs_315.1_Ts	*T. subuliformis*	cylindrical	UV+	Russia, Kolguev Is., Peschanka River	**MF149103**	**MF143821**	**MF143812**	2001	UPS, L-774111, *Sergei Kholod*
RU_KolguevIs_316.1_Ts	*T. subuliformis*	cylindrical	UV+	Russia, Kolguev Is., Peschanka River	**MF149105**			2001	UPS, L-774112, *Sergei Kholod*
RU_Tazovsky_331.1_Ts	*T. subuliformis*	cylindrical	UV+	Russia, Gydan peninsula, Tazovsky district	**MF149107**	**MF143820**	**MF143811**	2012	UPS, L-774122, *S. Ektova, S. Abdulmanova, K. Ermokhina*
RU_Tazovsky_332.1_Ts	*T. subuliformis*	cylindrical	UV+	Russia, Gydan peninsula, Tazovsky district	**MF149106**			2012	UPS, L-774123, *S. Ektova, S. Abdulmanova, K. Ermokhina*
SE_Taljstensvalen_217.1.1_Ts	*T. subuliformis*	cylindrical	UV+	Sweden, Jämtland, Täljstensvalen	**MF149104**	**MF143819**	**MF143810**	2012	UPS, L-812491, *Anders Larsson*
SE_Tvaradalen_216.2.1_Ts	*T. subuliformis*	cylindrical	UV+	Sweden, Jämtland, Norder-Tvärådalen	**MF149092**	**MF143801**	**MF143822**	2012	UPS, L-774085, *Anders Larsson*

TABLE 1 (*continued*).

Sample ID	Traditional identification	Thallus morphology	Chemistry*	Origin	GenBank Accession numbers ITS	Ef	DEAD	Collection year	Voucher information and collector
US_AleutianTigalda_291.2_Ts	*T. subuliformis*	cylindrical	UV+	USA, Alaska, Aleutian Islands, Tigalda Island	KY550143	KY634068	KY634019	2011	H, (T. Ahti 700023) *Teuvo Ahti*
Thamnolia vermicularis s. str.									
569.1_Tv_NEOTYPE	*T. vermicularis*	cylindrical	UV−	unknown	**MF149097**	**MF143817**	**MF143815**	C18th	S, Hb. Swartzii L1596, the middle specimens, neotype
AUS_Wolkerkogel_265.1_Tv	*T. vermicularis*	cylindrical	UV−	Austria, Wölkerkogel	**MF149098**			2012	UPS, L-774097, *Toby Spribille, Walter Obermayer*
AUS_Wolkerkogel_266.1_Tv	*T. vermicularis*	cylindrical	UV−	Austria, Wölkerkogel	**MF149099**	**MF143813**	**MF143818**	2012	UPS, L-774098, *Toby Spribille, Walter Obermayer*
AUS_Wolkerkogel_266.2_Tv	*T. vermicularis*	cylindrical	UV−	Austria, Wölkerkogel	**MF149100**			2012	UPS, L-774098, *Toby Spribille, Walter Obermayer*
PO_Spadowa_306_Tv	*T. vermicularis*	cylindrical	UV−	Poland, Tatra Mts., Wyżnia Spadowa pass	KY550149	KY654459	KY654364	2005	KRAM-L 53027, herbarium voucher no 83, unknown collector
RO_RetezatMt_463.1_Tv	*T. vermicularis*	cylindrical	UV−	Romania, Retezat Mts, Saua Judele	**MF149101**	**MF143816**	**MF143814**	2013	UPS, L-774163, *Anca Dragu*
SW_BotanischerSchynge_433.1_Tv	*T. vermicularis*	cylindrical	UV−	Switzerland, Botanischer Schynige Platteau	**MF149102**			2014	UPS, L-774158, *Sandra Lorena Ament*
SW_Valais_111_Tv	*T. vermicularis*	cylindrical	UV−	Switzerland, Valais, Nendaz	KY550085	KY654409	KY634022	2012	UPS, L-774063, *Paul Diederich*
SW_Valais_115_Tv	*T. vermicularis*	cylindrical	UV−	Switzerland, Valais, Nendaz	KY550087	KY634072	KY634022	2012	UPS, L-774065, *Paul Diederich*
Unidentified									
AUS_588_Tt_NEOTYPE	*T. taurica*	cylindrical	UV−	Austria				late C18th	BM00108967, *Abbé Wulfen*
GER_HartzMts_589_Ts_NEOTYPE	*T. subuliformis*	cylindrical	UV+	Germany, Harz Mts				late C18th	M-0024113, *Ehrhart*
NG_Morobe_587.1_Tjs_ISOTYPE	*T. juncea*	long	UV+	Papua New Guinea, Morobe Prov. Sarawaged Range				1802	UPS, L-136869, *C. Keysser*

*UV− contains thamnolic acid; UV+ contains baeomycesic and squamatic acids.

following types: holotypes of *T. papelillo* var. *papelillo* and *T. juncea* var. *juncea*; neotypes of *T. vermicularis*, *T. subuliformis* and *T. taurica*; an isotype of *T. juncea* var. *subjuncea*. Podetium chemistry was recorded by documenting the fluorescence under UV light (3500 Å): the podetium appears bright yellow when containing squamatic and baeomycesic acids (UV+), and dark red when containing only thamnolic acid (UV−).

DNA extraction, PCR and sequencing

Before DNA extraction, each podetium was investigated for fungal parasites and for each sample we used a 0·3–2·0 cm long part of a single podetium that seemed free of infection. We isolated total genomic DNA using the DNeasy Plant Mini Kit from Qiagen following the manufacturer's recommendations but with the following important modifications. The samples were handled under sterile conditions while they were placed in individual tubes together with two 3 mm tungsten carbide beads after which the samples were dried at 47 °C for 30–40 min. After drying, the tubes were placed in Qiagen TissueLyser II sample holders and kept for 30 min at −80 °C, immediately followed by 1 min of shaking at 27 m s^{-1} in a Qiagen TissueLyser II machine. We incubated the samples for at least 1 h while inverting the tubes every 10 min. Between the last washing and the elution we placed the DNeasy Mini spin column in a new collection tube and centrifuged it for 2 min at 20 000 **g**. Finally, we eluted our sample twice with 60 µl AE buffer. The two eluates were kept in separate tubes.

The Phusion High-Fidelity DNA Polymerase Kit (Thermo Fisher Scientific Inc.) was used to amplify the following nuclear markers of the mycobiont: internal transcribed spacer (ITS), translation elongation factor 1-alpha (Efα), and a putative DEAD-box helicase (DEAD). The latter was introduced as a molecular marker for *Thamnolia* in Onut-Brännström *et al.* (2017). The primers used for each amplified region, their direction and relative positions are presented in Table 2. For herbarium specimens older than 20 years, we used undiluted DNA in the PCR reaction. For old herbarium specimens (>200 years), we amplified regions of a length of *c.* 500 bp using undiluted DNA and *Thamnolia* specific primers. All PCR products were cleaned with Exonuclease I (Thermo Fisher Scientific, SE) and Shrimp Alkaline Phosphatase (Affymetrix), prepared for sequencing with BigDye Teminator v3.1 Cycle Sequencing and BigDye XTerminator Purification Kits (Thermo Fisher Scientific Inc., Waltham, MA, USA) and sequenced on an ABI 3730xl machine. The reads were trimmed and corrected for errors using Geneious R9 (http://www.geneious.com).

Preparing the two datasets

For the phylogenetic analyses, we divided our data into two sets. The first dataset comprised ITS sequences of all samples from which we were able to amplify and sequence the marker (Table 1). The second dataset contained a subsample of the first, for which we had complete information for at least two out of the three nuclear markers: ITS, Efα and DEAD (Table 1). In total, 56 new sequences were identified in this study (Table 1). For the analyses that required the use of outgroups, we used sequences previously obtained from three other species of *Icmadophilaceae*: *Dibaeis baeomyces* (L. f.) Rambold & Hertel, *Icmadophila ericetorum* (L.) Zahlbr., and *Siphula ceratites* (Wahlenb.) Fr. (Onut-Brännström *et al.* 2017). The FASTA files of the nuclear markers obtained for the outgroups are deposited in the TreeBase repository (http://purl.org/phylo/treebase/phylows/study/TB2:S21678).

Phylogenetic analyses

The sequences were visualized with AliView (Larsson 2014) and aligned with MAFFT (Katoh 2013). When necessary, the sequences were trimmed manually and the missing nucleotides were replaced with N. Gaps were treated as phylogenetic information. All alignment files used for the subsequent analyses were deposited in TreeBase repository (http://purl.org/phylo/treebase/phylows/study/TB2:S21678). The nucleotide substitution models for ITS and Efα were selected using jModelTest (Darriba *et al.* 2012) while the amino acid substitution model for DEAD was chosen with MEGA v.06 (Tamura *et al.* 2013).

Three methods were used for phylogenetic analyses: Bayesian inference, maximum likelihood (ML) inference and haplotype networks. Simultaneous Bayesian inference of alignment and phylogeny implemented in Bali-Phy 2.3.8 (Redelings 2014) was used to infer a rooted phylogeny using *D. baeomyces*, *S. ceratites* and *I. ericetorum* as outgroup. For both datasets we chose R07 as the insertion/deletion model. Due to the high computational demand of Bali-Phy, the ITS dataset was collapsed into 33 haplotypes with the web tool FaBOX. For this phylogeny we used the TrNef + G as nucleotide substitution model and started 7 MCMC chains with a total of 70 000 iterations. For the three-gene phylogeny we chose TrNef+G as the nucleotide substitution model for ITS and Efα, and the amino acid substitution model JTT for DEAD. We started 5 MCMC chains accumulating a total of 80 000 iterations. The convergence of chains was detected and confirmed using the program Tracer (Rambaut *et al.* 2014) and the informative parameters calculated by the 'bp-analyze.pl' script of Bali-Phy 2.3.8. After discarding 1500 trees for the ITS dataset and 2000 trees for the three-gene dataset as burn-in, the rest of the iterations were summarized in a 50% majority-rule consensus tree with posterior probabilities (PP) provided. The phylogenies were visualized using FigTree 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). ML phylogenies were obtained from the same datasets but for these analyses we aligned and trimmed both datasets in AliView by removing all the ambiguously aligned sites. The program Garli v.2 was used to construct the ML trees, each with 500 bootstrap iterations. For the ITS ML phylogeny we used K80+G as the nucleotide substitution model. In the case of the concatenated dataset we chose TrNef+I+G for ITS,

TABLE 2. *Primers for the nuclear markers used in this study together with the position of each forward and reverse primer relative to the alignment.*

Primer name	Specificity*	Sequences	Alignment length (bp)**	Position (bp)	Direction
ITS					
ITS 1F	ITS fungal general	CTTGGTCATTTAGAGGAAGTAA	1–1122	1	forward
Thamn_ITSf_for2	ITS fungal *Thamnolia* lineages A, B, C	GAAGCTACCGAAGCCTTTGC		80	forward
Th_ITS_1283_for	ITS fungal *Thamnolia* lineages A, B, C	CAGCCTCGCAAGAGGTGTCC		103	forward
ITS_Icmae_1364_F	ITS fungal *Icmadophilaceae*	AACACRATCGAATTGACGGG		184	forward
Th_anc_F1	ITS fungal *Thamnolia* lineages A, B, C	TGGTATCTCGTGCGAAAGAC		249	forward
Th_anc_F2	ITS fungal *Thamnolia* lineages A, B, C	AGCGAAGGCTCTAAGTCCCA		335	forward
ITS_Icmae_1549_F	ITS fungal *Icmadophilaceae*	GCAACGTTCACAGACTARGTGGT		369	forward
ITS_Icmae_1550_R	ITS fungal *Icmadophilaceae*	CACYTAGTCTGTGAACGTTG		370	reverse
Th_anc_R2	ITS fungal *Icmadophilaceae*	CTTCCGCAGGTTCACCTACG		547	reverse
Th_ITS_1797_rev	ITS fungal *Thamnolia* lineages A, B, C	GCAACCGGGTAACAGTTCGC		617	reverse
Th_anc_F3	ITS fungal *Thamnolia* lineages A, B, C	GAACTGTTACCCGGTTGCTT		619	forward
Th_ITS_1834_for	ITS fungal *Thamnolia* lineages A, B, C	TCCACGCCAGWAGCTCATCA		658	forward
Thamn_ITSf_for1	ITS fungal *Thamnolia* lineages A, B, C	TCCTTCCGCGGTGAAGCTA		68	forward
ITS2	ITS fungal general	GCTGCGTTCTTCATCGATGC		761	reverse
ITS3	ITS fungal general	GCATCGATGAAGAACGCAGC		761	forward
Th_anc_R3	ITS fungal *Icmadophilaceae*	GCATTTCGCTGCGTTCTTCA		768	reverse
Th_ITS_2203_rev	ITS fungal *Thamnolia* lineages A, B, C	ACATCCAGTTCTGGCCAMCG		1024	reverse
Thamn_ITSf_rev2	ITS fungal *Icmadophilaceae*	CCTACCTGATCCGAGGTCAA		1065	reverse
Thamn_ITSf_rev1	ITS fungal *Icmadophilaceae*	ATGCTTAAGTTCAGCGGGTA		1087	reverse
ITS 4	ITS fungal general	TCCTCCGCTTATTGATATGC		1122	reverse
Efα					
Ef_8_Icmae_F	Elongation factor, *Icmadophilaceae*	TTGTCTCCKGGRAGACCCTC	1–726	1	forward
Ef_Th_F1	Elongation factor, general	TCTTGTAGACATCCTGGAGA		155	forward
Ef_343_Th_F	Elongation factor, *Thamnolia* lineages A, B, C	GCCAGAGATTGGCACGAAGG		336	forward
Ef_484_Th_R	Elongation factor, *Thamnolia* lineages A, B, C	CGTACACTCTCGGTGTYAGG		477	reverse
Ef_Th_R2	Elongation factor, general	AGAACATGATTACTGGTACCTCCC		593	reverse
Ef_733_Icmae_R	Elongation factor, *Icmadophilaceae*	ATTGCGCTATGGAAATTCGA		726	reverse
DEAD					
DEAD_Th_363_F	DEAD *Thamnolia*	GAACTCTTCATTCGATCCGC	1–538	1	forward
X-geneFor1	DEAD *Thamnolia*	CGCAATTAGTTGGGGGTA		37	forward
DEAD_Th_605_F	DEAD *Thamnolia*	GACGCAGGCTCAATTGCAGT		243	forward
DEAD_Th_696_R	DEAD *Thamnolia*	CTCGAACTCCAAGTCGACTT		334	reverse
DEAD_Th_863_R	DEAD *Thamnolia*	GGAATTGCCCACAGATCTTG		501	reverse
X-geneRev2	DEAD *Thamnolia*	CGAGGTAGCGATAGACCA		538	reverse

*ITS = Internal Transcribed Spacer, Efα = Elongation Factor alpha, bp = base pairs. DEAD = DEAD-box helicase.
**see Dryad for reference to alignment.

TrNef+G for Efα and K80+G for DEAD region. The Neighbour-Net algorithm (Bryant & Moulton 2004) implemented in SplitsTree v.4 (Huson & Bryant 2006) was used for calculating a non-rooted haplotype network of the ITS dataset.

Results and Discussion

DNA isolation and amplification from *Thamnolia* specimens

DNA extraction and amplification of nuclear markers were successful for the selected specimens of all chemical varieties and morphologies of *Thamnolia* that were collected from 1960 onwards (Table 1). We also succeeded in extracting DNA and amplifying the ITS region from the holotype of *T. juncea* ssp. *juncea*, and all three markers from the holotype of *T. papelillo* var. *papelillo* and from the neotype of *T. vermicularis* (of late 18th century origin). Unfortunately, we failed to amplify DNA from the neotypes of *T. taurica* and *T. subuliformis*, and the isotype of *T. juncea* var. *subjuncea*. Reasons for our failure might be deleterious storage and herbarium treatment practices and/or the small amount of tissue available to us for DNA isolation (fragments of podetia, 3–5 mm long).

Three phylogenetic species

It has previously been shown, based on a six-gene ML analysis (Onut-Brännström *et al.* 2017), that three mycobiont lineages occur in *T. vermicularis* s. lat. (Fig. 1) and furthermore that *T. papellilo* is part of one of these lineages, 'Lineage C'. In this study, we included ITS sequences, and when possible Efα and DEAD markers, from *Thamnolia* type specimens, additional specimens belonging to 'Lineage B' (Onut-Brännström *et al.* 2017), *T. papelillo* and *T. juncea* (Table 1). All tree files resulting from these analyses are deposited in the TreeBase repository (http://purl.org/phylo/treebase/phylows/study/TB2:S21678). The Bayesian and ML analyses together with the haplotype network also showed the three lineages previously mentioned. Furthermore, all phylogenetic analyses revealed that both subspecies of *T. juncea* belonged to 'Lineage C' (Figs 3 & 4). The exclusively UV+ ('Lineage A') and exclusively UV− ('Lineage B') clades, which contained sequences from individuals formerly named *T. vermicularis* s. lat. or *T. subuliformis* s. lat., received strong posterior probabilities (PP) and bootstrap support (BP), both in the ITS (Fig. 3) and the three-gene phylogenies (Fig. 4). Only moderate support was obtained for the third clade ('Lineage C') in both Bayesian and ML analyses. This clade includes samples of both chemistries and different types of morphology, and the earlier analysis based on six nuclear markers (Fig. 1) showed a high BP support for it. Suspecting that the additional samples used in this study might have contributed to the diminished support for this clade, we performed an ML analysis of just three genes (ITS, Ef and DEAD) of the dataset from an earlier study (Onut-Brännström *et al.* 2017). In this we obtained very similar supports to those presented in Fig. 4 (data not shown). Hence, we concluded that 'Lineage C' is monophyletic and the moderate support obtained in this study is due to a more limited selection of markers and not to a disturbance caused by a different sampling.

Given their genetic distinctiveness the three lineages (Fig. 1) shall, in our opinion, be considered separate species. The following names are proposed: 'Lineage A' as *T. tundrae* sp. nov.; 'Lineage B' as *T. vermicularis* and 'Lineage C' as *T. subuliformis*. Recognition of cryptic species in lichenized fungi has become rather uncontroversial in recent years (Crespo & Lumbsch 2010; Lumbsch & Leavitt 2011; Molina *et al.* 2011; Leavitt *et al.* 2015), with Leavitt *et al.* also arguing for justifications for a strong emphasis on genetic data. In the present study the three species are distinct in phylogenies of a comprehensive sampling of 83 (ITS; Fig. 3) and 66 (ITS, Ef, DEAD; Fig. 4) collections of *Thamnolia*. The lineages were morphologically cryptic but they had different distributions (Fig. 2), photobiont specificity and, in part, secondary chemistry.

Morphology and secondary chemistry

No morphological feature was found to be consistently characteristic of any of the three species. This, however, does not preclude the

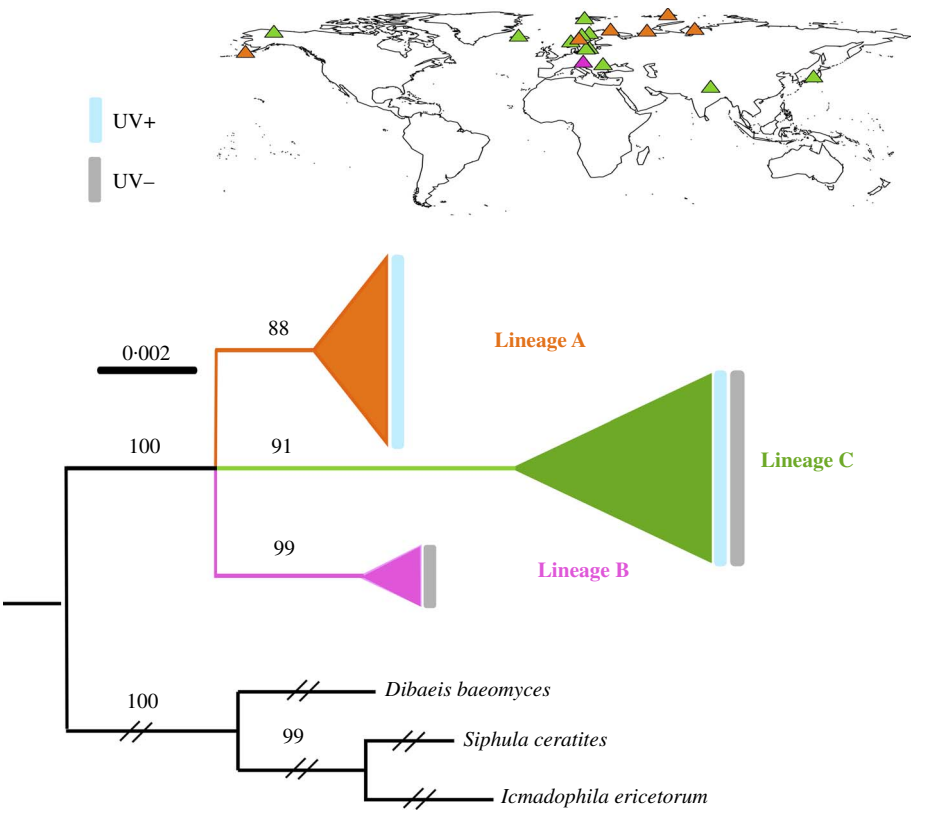


FIG. 1. Phylogenetic relationships in the genus *Thamnolia* based on a six-gene phylogeny (Onut-Brännström *et al.* 2017). The three *Thamnolia* lineages are represented as collapsed clades. Distribution map of the three lineages with triangles corresponding to each lineage: orange = Lineage A, pink = Lineage B, green = Lineage C. Chemistry of lineages represented by rectangles adjacent to triangles: light blue = baeomycesic and squamatic acids (UV+), grey = thamnolic acid (UV−).

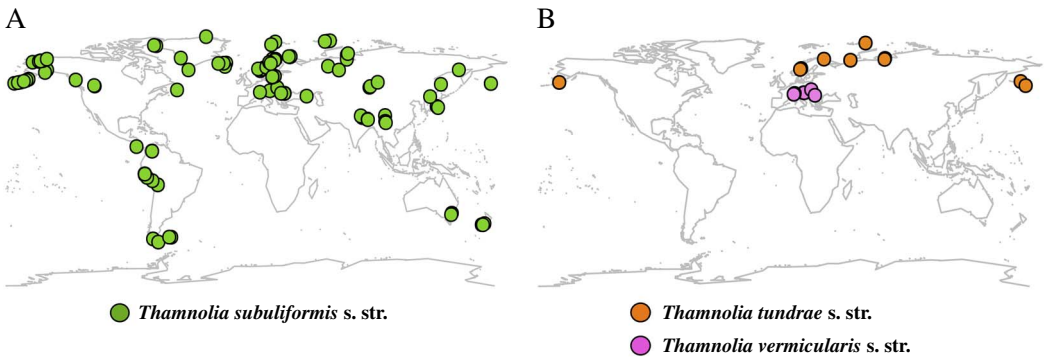
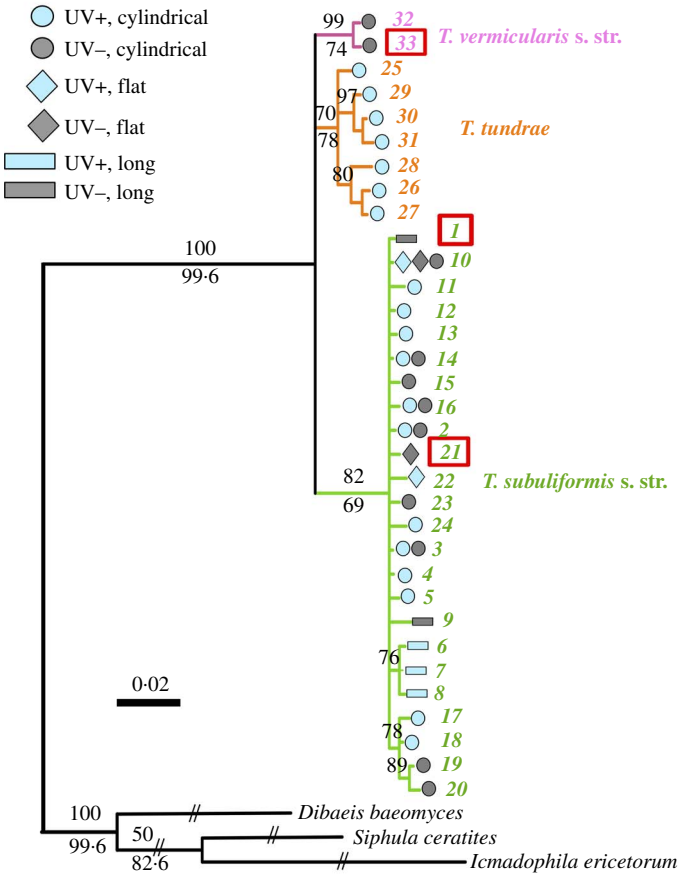


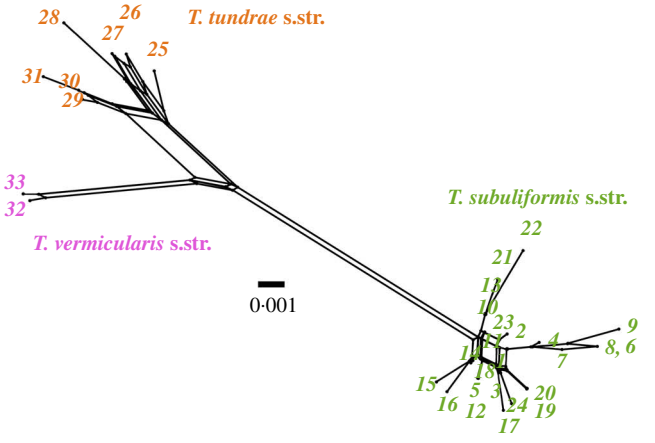
FIG. 2. Distribution maps for the three *Thamnolia* species studied. A, *Thamnolia subuliformis* s. str. is found worldwide and sympatric with the two other species; B, *Thamnolia vermicularis* s. str. and *Thamnolia tundrae* were found only in the Northern Hemisphere and are allopatric with each other and sympatric with *T. subuliformis* s. str. *T. vermicularis* s. str. was recorded in alpine regions of the European Alps, Tatra Mts and Western Carpathians while *T. tundrae* was found only in Sweden, Norway, Siberian Russia and the Aleutian Islands.

https://doi.org/10.1017/S0024282917000615 Published online by Cambridge University Press

A



B



Sample ID	H
NG_StrongMt_586·3_Tjj_HOLOTYPE	1
CHL_Navarino_556·3_Tv	2
SE_Oland_27·3_Ts	2
US_Summit_213_Ts	3
RU_Bezymyannaya_284·1_Ts	3
US_AleutianUnimak_287·2_Ts	3
RU_Yaroto_334_Ts	3
SW_Valais_113_Ts	3
US_Galbreat_83·2_Ts	3
RU_Litovka_311_Tv	3
RO_BucegiMt_239·1_Ts	3
RO_BucegiMt_239·2_Tv	3
SE_Gotland_04_Ts	3
RU_CapeTrieste_320_Ts	4
PO_Mieguszowiecka_301_Ts	4
SE_LuleLappmark_72_Ts	4
RU_CapeTirol_321_Ts	5
NG_WilhelmMt_391·2_Tjs	6
NG_WilhelmMt_574·1_Tjs	7
NG_WilhelmMt_574·2_Tjs	8
NG_WilhelmMt_585·1_Tjj	9
PE_QuebradaMatara_502_Tv	10
PE_Tupatupa_511_Tv	10
AUL_AggieMt_554·3_Tv	10
PE_Bolognesi_393_Tps	10
PE_Vientunan_477·1_Tv	10
PE_AlboDePucarajuPass_496_Tv	10
PE_Yanashallash_395·1_Tpp	10
PE_HuarazCasma_582_Tps	10
PE_Yanashallash_395·2_Tpp	10
571·2_Ts	11
DK_Greenland_263_Ts	12
US_Selby_210·2_Ts	12
SE_Taljstensvalen_217·1·2_Ts	13
SE_Tvaradalen_ 216·2·1_Ts	13
IC_ Skagafjardarsysla_84_Ts	14
SE_ Lillulvafjallets_ 214·1_Ts	14
SE_ Taljstensvalen_ 217·2_Tv	14
SE_ Abisko_ 226_Ts	14
SE_MoiRana_05_Ts	14
RU_Bezymyannaya_284·2_Tv	15
US_AleutianUnimak_287·1_Tv	15
RU_Sabetta_333_Tv	15
US_AleutianTigalda_291·1_Tv	15
RU_ Drovyanoy_335_Tv	15
US_ FingerMt_82_ Tv	15
CA_BritishColombia_280_Tv	15
SE_Tvaradalen_216·1_Tv	15
US_Galbreat_83·1_Tv	15
SE_Lillulvafjallets_214·2_Tv	15
NO_ Brannsletta_ 119·1_Tv	15
NO_SorVaranger_ 116_Tv	15
NO_SorVaranger_ 118_Tv	15
US_ Selby_ 210·1_Tv	15
SE_ Abisko_ 229_Tv	15
CA_ Avalon_285_Ts	16
JA_ Kinpu_246·3_Tv	16
NE_Khumbu_344_Ts	17
NE_Dolpo_349_Ts	18
NE_Humla_356_Ts	18
NE_Moraine_363_Tv	19
NE_ Khumbu_345_Tv	20
PE_ Jauja_ 579_Tpp_ HOLOTYPE	21
PE_Tarma_ 392·2_Tps	22
PE_Tarma_ 392·1_Tps	22
BO_ SamanaPampa_390_Tv	23
RU_ MalayaKhatiparaMt_ 328_Ts	24
US_ AleutianTigalda_ 291·2_Ts	25
NO_SorVaranger_ 117 _Ts	26
RU_Tazovsky_ 331·1_Ts	27
RU_Kolguevls_316·1_Ts	28
RU_Tazovsky_332·1_Ts	28
RU_CapeRogatyi_323_Ts	29
RU_Kolguevls_ 315·1_Ts	30
SE_Taljstensvalen_ 217·1·1_Ts	31
SW_Valais_ 111_Tv	32
SW_Valais_ 115_Tv	32
PO_Spadowa_ 306_Tv	32
SW_ BotanischerSchynge_ 433·1_Tv	33
SW_BotanischerSchynge_433·1 Tv	33
569·1_Tv_NEOTYPE	33
AUS_Wolkerkogel_265·1_Tv	33
AUS_Wolkerkogel_266·1_Tv	33
AUS_Wolkerkogel_266·2_Tv	33
RO_RetezatMt_463·1_Tv	33

https://doi.org/10.1017/S0024282917000615 Published online by Cambridge University Press

possibility that such features might be found in meticulous studies designed for that purpose. An interesting variation in morphology in Siberian *Thamnolia* material was pointed out by Kärnefelt & Thell (1995) and a similar range of variation was also encountered in *T. subuliformis* s. str. in the present study (e.g. Fig. 3). Further studies are thus needed to ascertain a possible connection between the morphological and genetic variation in their material. Secondary chemistry varies among the species in as much as two chemotypes (UV+ with squamatic and baeomycesic acids, and UV− with thamnolic acid) were found only in *T. subuliformis* s. str., whereas *T. vermicularis* s. str. (UV−) and *T. tundrae* (UV+) both had a uniform chemistry (Figs 3 & 4).

The Species

Thamnolia subuliformis (Ehrh.) W. L. Culb.

Brittonia **15:** 144 (1963). —*Lichen subuliformis* Ehrh. Beitr. *Naturk.* **3:** 82 (1788); type: a neotype, not associated with any annotations allowing it to be considered for lectotypification, was designated by Culberson (1963) on a specimen from Ehrhart's exsiccata *Plantae Cryptogamae Linneae* (no. 30, M) from the Harz Mountains in Germany. According to Culberson it contains squamatic and baeomycesic acids (UV+). Unfortunately, we were not able to retrieve any sequence from a small fragment of the neotype. Because of its chemistry it is not likely to belong to 'Lineage B' (Onut-Brännström *et al.* 2017) (i.e. *T. vermicularis* s. str.), and given its origin and secondary chemistry we find it most probably belongs to 'Lineage C' (Onut-Brännström *et al.* 2017), which consequently has to be named *T. subuliformis*.

(Figs 2A & 3)

Chemistry. *Thamnolia subuliformis* has two chemotypes with specimens either containing baeomycesic and squamatic acids (UV+), or thamnolic acid (UV−).

Photobionts. *Trebouxia impressa* Ahmadjian, *T. simplex* Tschermak-Woess 'clade 1', *T. simplex* 'clade 2', *T. vagua* A. Voytsekhovich & A. Beck s. lat. (Onut-Brännström *et al.* 2017).

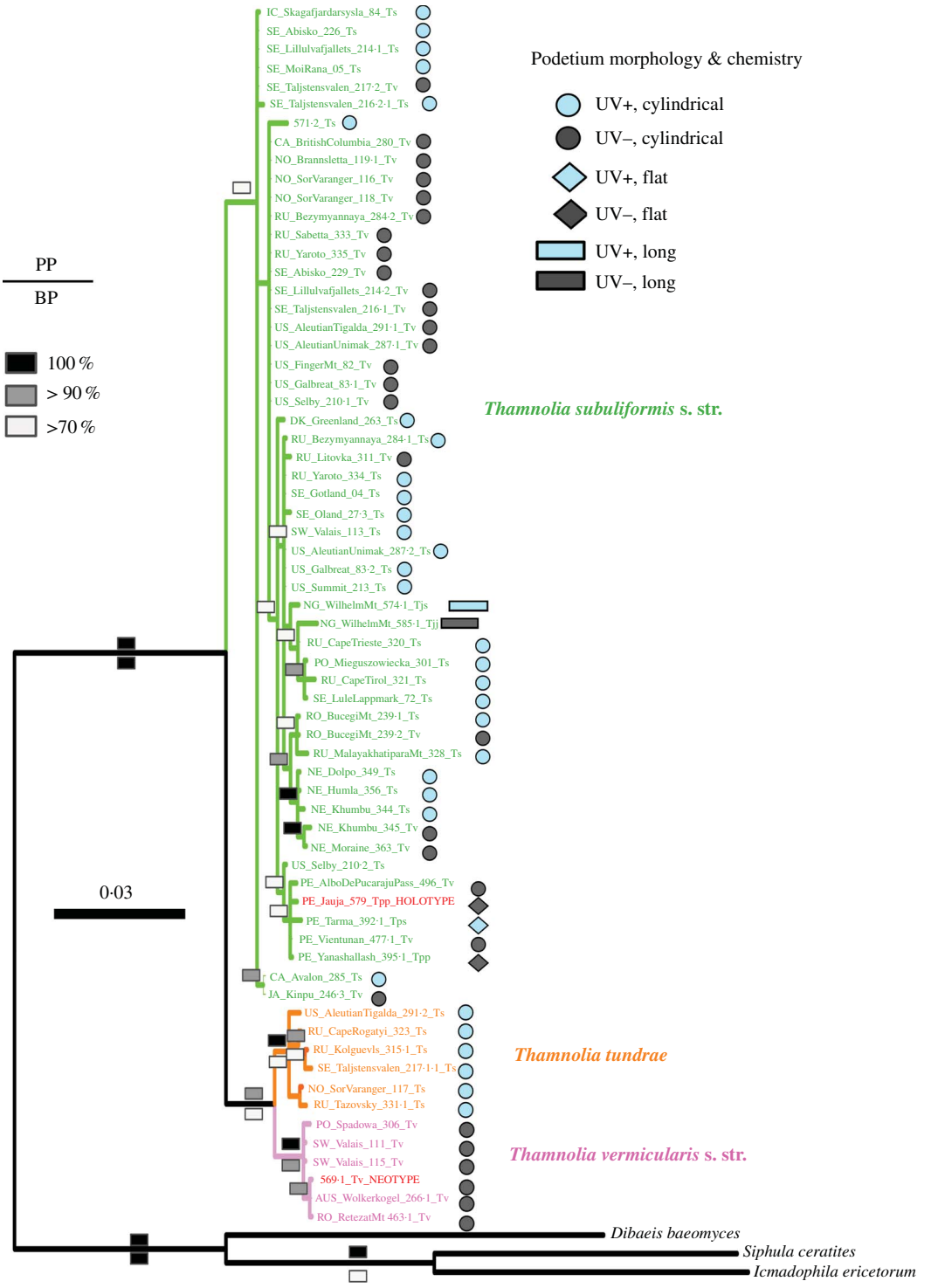
Notes. This species is morphologically cryptic versus *T. tundrae* and *T. vermicularis* s. str., but well characterized by molecular data obtained from six nuclear markers (Onut-Brännström *et al.* 2017). *Thamnolia subuliformis* is a widespread and often locally abundant species, being circumpolar in the Arctic and occurring in many alpine areas of both the Northern and Southern Hemisphere. It is in part sympatric with *T. vermicularis* s. str. and *T. tundrae* (Fig. 2). It is unfortunate that *T. subuliformis* is here given a different interpretation than in most of the 20th century literature, where it has been used to designate UV+ specimens of *T. vermicularis* s. lat. However, Onut-Brännström *et al.* (2017) have shown that this assemblage of specimens is not monophyletic. The nomenclatural status of *T. subuliformis* as conceived by the neotypification could be challenged via an epitypification, but this would be a risky procedure since there is nothing in the protologue that would unequivocally tie the name to an epitype. To be certain about this name one would hope for the discovery and genetic characterization of type material connected to the protologue in such a way that the neotypification could be superseded, but only at the cost of further nomenclatural confusion.

Thamnolia tundrae Brännström & Tibell sp. nov.

MycoBank No.: MB 821537

Thallus white, hollow, cylindrical, sometimes branched and/or tufted, erect or prostrate. Thallus PD + yellow, K+ yellow, UV+ white, containing baeomycesic and squamatic acids. Differs from morphologically similar species in molecular sequences.

FIG. 3. Phylogenetic relationships in *Thamnolia* based on 84 ITS sequences. A, Bali-Phy phylogeny rooted with three outgroups (*Dibaeis baeomyces, Siphula ceratites* and *Icmadophila ericetorum*). Posterior probabilities are above branches, ML bootstrap support ≥65% below branches. Scale bar indicates branch lengths. B, unrooted SplitsTree haplotype network. Scale bar indicates branch length. Numbers signify the assigned haplotype (H) for each sample. Associated information including GenBank numbers can be found in Table 1. Red boxes indicate type specimens.



https://doi.org/10.1017/S0024282917000615 Published online by Cambridge University Press

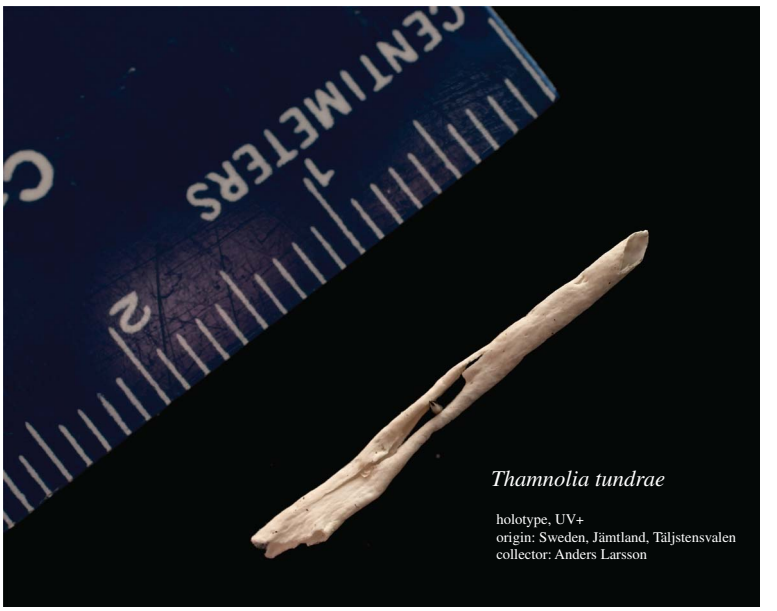


FIG. 5. *Thamnolia tundrae* (holotype, UPS-voucher). The holotype is UV + (baeomycesic and squamatic acids) and has a cylindrical morphology.

Type: Sweden, Jämtland, Åre par., Täljstensvalen, 63°14'36"N, 12°27'06"E, 2012, *A. Larsson* 95 (UPS L-812491—holotype). GenBank numbers: MF149104; MF143819; MF143810.

(Figs 2B, 3 & 5)

Chemistry. Containing baeomycesic and squamatic acids.

Photobionts. *Trebouxia simplex* 'clade 1' and *T. simplex* 'clade 2' (Onut-Brännström *et al.* 2017).

Notes. This species is morphologically cryptic versus *T. subuliformis* s. str. and *T. vermicularis* s. str., but well characterized by molecular data obtained in this study (Figs 3 & 4) and from 6 nuclear markers; it corresponds to 'Lineage A' (Fig. 1) as described in Onut-Brännström *et al.* (2017). It is known mainly from the arctic tundra region of Eurasia reaching the Aleutian Islands. It is partly sympatric with *T. subuliformis* s. str. but allopatric with *T. vermicularis* s. str. *Thamnolia tundrae* (as 'Lineage A' in Onut-Brännström *et al.* 2017) was suggested to have survived the latest glaciation in coastal refugia adjacent to its present distribution. A map of its distribution is given in Fig. 2.

Thamnolia vermicularis (Sw.) Schaer.

Enum. Crit. Lich. Eur.: 243 (1850).

Lichen vermicularis Sw. *Meth. Musc.*: 37 (1781): 'Habitat in alpibus Lapponicis inter gramina et muscos, fruticulis prostratis et diffusis, ascarides primo intuitu referentibus'. Neotype: a neotype was designated by Culberson (1963, Fig. 2) on a specimen from the Swartz herbarium (S-L1596). It has few annotations and no close link to the protologue. Parts of the nuclear markers ITS and DEAD were obtained from the neotype and it belongs to 'Lineage B' (Onut-Brännström *et al.* 2017). GenBank: MF149097; MF143817; MF143815

(Figs 2B & 3, sample 569.1_Tv_ NEOTYPE)

FIG. 4. Phylogenetic relationships in *Thamnolia* based on sequence data using ITS, Efα and DEAD from 52 samples. Type specimens are highlighted in red. Outgroups are *Dibaeis baeomyces*, *Siphula ceratites* and *Icmadophila ericetorum*. PP = posterior probabilities ≥70%; BP = bootstrap values ≥70% obtained by ML analyses. Scale bar indicates branch length.

https://doi.org/10.1017/S0024282917000615 Published online by Cambridge University Press

Chemistry. Contains thamnolic acid.

Photobiont. *Trebouxia simplex* 'clade 2' (Onut-Brännström *et al.* 2017).

Notes. This species is morphologically cryptic versus *T. subuliformis* and *T. tundrae*, but well characterized by molecular data obtained from ITS (Fig. 3), the 3 nuclear markers (Fig. 4) and also in the six-gene phylogeny (Onut-Brännström *et al.* 2017). It is UV− and corresponds to 'Lineage B' (Fig. 1), and is known from the high alpine region of the European Alps, Tatra Mts and the Western Carpathian Mts of Romania. It is partly sympatric with *T. subuliformis* s. str. but allopatric with *T. tundrae*, this is a species suggested to have survived the latest glaciation (Onut-Brännström *et al.* 2017) adjacent to its present distribution (Fig. 2). There is an obvious discrepancy between the protologue of *T. vermicularis* and the neotypification that Culberson was, however, not aware of at the time of neotypification. It was described from 'Lapponia', where *T. vermicularis* as typified by the neotype is so far not known to occur. For nomenclatural purposes, however, Culberson's neotypification has to be followed until type material more closely connected to the protologue has been found and subjected to genetic analysis.

Further Nomenclatural Notes

Thamnolia juncea R. Sant.

Symb. Bot. Upsal. **34**(1)**:** 396 (2004); type: Papua New Guinea, Central Prov., Tapini subdistrict, Mount Strong, summit area, 08°00'S, 147°00'E, *M. Coode & P. Stevens* (UPS L-136871—holotype). GenBank: MF149084.

Notes. The holotype is UV− and has long, solid and unbranched podetia; molecular information based on the ITS region indicates that it belongs to *T. subuliformis* (Fig. 3, haplotype 1). This is supported by the three-gene phylogeny where Efα and DEAD were amplified from another specimen (NG_WilhelmMt_585.1_Tjj) with a similar morphology and collected from the same area as the holotype (Fig. 4, Table 1).

Thamnolia juncea R. Sant. var. subjuncea R. Sant.

Symb. Bot. Upsal. **34**(1)**:** 397 (2004); type: Papua New Guinea, Morobe Prov., Sarawaged Range, *C. Keysser* 67 (W—holotype; UPS L-136869—isotype).

Notes. Thamnolia juncea var. *subjuncea* differs from *T. juncea* var. *juncea* in being UV+. Unfortunately, we were not able to retrieve sequences from the isotype but molecular information based on the ITS region (Fig. 3, haplotypes 6, 7, 8) and three molecular markers (Fig. 4, NG_WilhelmMt_574.1_Tjs) obtained from a morphologically and chemically similar specimen from Papua New Guinea indicates that it belongs to *T. subuliformis* (Table 1).

Thamnolia papelillo R. Sant.

Symb. Bot. Upsal. **34**(1)**:** 394 (2004); type: Peru, Dept. Junín, Prov. Jauja, 30 km NNW of Jauja, *B. & R. Santesson & R. Moberg* P20:1 (UPS L-136872—holotype). GenBank: MF149093; MF143828; MF143806.

Notes. The holotype is UV− and has flattened, solid and dichotomously branched podetia; molecular information from this study (Figs 3 & 4, sample PE_Jauja_579_Tpp_HOLOTYPE) indicates that it is a taxonomic synonym of *T. subuliformis*.

Thamnolia papellilo R. Sant. var. subsolida (Satô) R. Sant.

Symb. Bot. Upsal. **34**(1)**:** 395 (2004).—*Thamnolia subuliformis* var. *subsolida* Satô, *Bryologist* **71:** 50 (1968); type: Peru, Junín, between Tarma and Jauja, 1965, *F. Maekawa* (TNS—holotype).

Notes. According to Santesson (2004) *T. papellilo* var. *subsolida* contains squamatic and baeomycesic acids. The holotype was not studied, but molecular data obtained from Peruvian samples identified by Santesson as *T. papelillo* var. *subsolida* in this study (Table 1, Figs 3 & 4) indicate it to be a synonym of *T. subuliformis*.

Thamnolia subvermicularis Asahina

J. Jap. Botany **13:** 317 (1937). Typification was suggested by Kashiwadani & Kurokawa (2003) but there is no reference to this material in the protologue and thus it cannot be accepted. The alleged type not seen by us.

Notes. Characterized by having squamatic and baeomycesic acids. There are no sequence data available for the type but, given its origin and secondary chemistry, we consider it most probably to be a taxonomic synonym of *T. subuliformis*.

Lichen tauricus Wulfen in Jacquin

Collectanea Bot. **2:** 177 (1789); type: Austria, Carinthia; a neotype ('Lichen Tauricus. alijo subuliformis, alijo etiam vermicularis') was designated by Culberson (1963) on a specimen from the Dawson Turner herbarium in London (BM). The specimen has few annotations, all however seemingly in the same hand (Wulfen's?), but it is not otherwise closely connected to the protologue as both collecting date and locality are missing. The neotype, according to Culberson, contains thamnolic acid. We were not able to retrieve sequences from a fragment of the neotype but, given its origin and secondary chemistry, we find it most likely to be a taxonomic synonym of *T. vermicularis*.

We thank Nahid Heidari and Markus Hiltunen for their help with DNA isolation, PCR amplification and sequencing of the old and recent herbarium material. We are grateful to the curators of the following herbaria for providing specimens for study: BM, M, and UPS (both types and recent collections). We would also like to thank all the collectors who helped to obtain *Thamnolia* specimens from different parts of the world, and in particular Anders Larsson, Mats Thulin and Alexander Paukov, who collected specimens of *T. tundrae* from Sweden and Russia, and Anca Dragu who collected specimens of *T. vermicularis* from Romania. Our great appreciation goes to two anonymous reviewers for their useful comments and corrections.

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