

Standard Paper

A new lichen and lichenicolous fungus from *Larix laricina* in patterned fens of boreal North America

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Abstract

Calicium poculatum and *Ramboldia canadensis* are described as new species occurring on *Larix laricina*. *Calicium poculatum*, currently known from four Canadian provinces and the US state of Minnesota, is characterized by its short-stalked black ascomata, short ascospores and occurrence as a parasite on *Lecanora caesiurubella* subsp. *saximontana*. Based on DNA sequence data, its nearest relative is the likewise parasitic *Calicium episcalaris*. *Ramboldia canadensis*, currently known only from dead wood of *Larix laricina* snags in Canada, engages in a fully developed lichen symbiosis with *Trebouxia simplex* and is characterized *in statu symbiotico* by having a rimose to verrucose-areolate, greyish creamy sorediate thallus with dark brown to blackish soredia that begin on the margins, and the occurrence of a secondary metabolite similar to barbatolic acid. Phylogenetic analysis recovers it as sibling to a clade of the genus heretofore known only from the Southern Hemisphere and the lower latitudes of the Northern Hemisphere. We also report *Lecidella xylophila* as new to North America.

Keywords: calicioid fungi; *Calicium*; kelo trees; *Lecanoromycetes*; phylogenetics; *Ramboldia*; saproxylic species; taxonomy; *Trebouxia*

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Introduction

Peatlands cover vast areas of the central boreal plain of North America, and are widely referred to by their Nêhiyawêwin (Cree) name ᐱᓄᓄᓄ or muskeg, anglicized as muskeg. Many are wooded and usually dominated by tamarack (*Larix laricina*) and/or black spruce (*Picea mariana*), but the term muskeg has also been applied to sparsely treed or open peatlands. Muskeg has been widely studied for its physiographic properties and vegetation composition, and its different component peatland types have been compared in detail to the nomenclature of peatlands elsewhere in the Northern Hemisphere (Stanek 1977). Several of these peatland types can be rich in lichen biomass, particularly on hummocks. In muskegs that approach the northern treeline, *Cladonia* mats become the dominant ground cover over large areas (Harris *et al.* 2018). Although these habitats have been included in regional surveys (e.g. Piercey-Normore *et al.* 2016), little attention has been dedicated to the ecological niches that muskegs offer to ecologically specialized lichens and their associated fungi.

Many different kinds of boreal peatland have been described, and some of the more striking physiographic manifestations are patterned fens, which include ‘string fens’ in which water flow and slope interact to produce striking landscape features such as string patterning (Foster *et al.* 1983; Zoltai & Pollett 1983), as well as ‘palsa fens’ or ‘palsa bogs’ in which mounded patterning emerges as a consequence of localized pockets of permafrost (Zoltai 1972). Depending on the local climate and surrounding fire history, such fens can be largely open with only isolated, old trees, or they can be completely grown in, especially, in North America, with *Larix laricina* (Fig. 1). It was originally in such habitats that different members of our author team independently discovered what we recognized to be an undescribed species of lichenicolous *Calicium* in 2015 in Ontario (Canada), in 2017 in Minnesota (USA) and 2018 in Manitoba (Canada), as well as an undescribed sorediate lichen in Canadian string fens that we ultimately recognized to be a new species of *Ramboldia*. Here, we describe these species and report on their occurrence, ecology and importance for conservation.

Materials and Methods

The two species described here were collected during fieldwork by RTM in Ontario, OG in Minnesota and by GT and TS in Manitoba, Saskatchewan and Alberta. Once we became aware of the species, we began to search for them in numerous fens across

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Figure 1. Isolated *Larix laricina* trees in string fens. A, at the type locality of *Ramboldia canadensis* near Niton Junction, Alberta. B, at the type locality of *Calicium poculatum* near the hamlet of Hudson Bay, Saskatchewan. In colour online.

the region, as well as in other boreal forest habitats including upland *Picea mariana*, *P. glauca* and *Pinus banksiana* forest.

Specimens were examined using dissecting and light microscopes. Measurements of ascospores and soredia were taken in water mounts with a 1 μm precision. Secondary lichen compounds were investigated with HPTLC (Arup *et al.* 1993), using the most stable solvent C (below as TLC). The colour reaction of the thallus was tested using common household bleach (C), 10% aqueous potassium hydroxide (K), Steiner's solution (PD) and short-wave (254 nm) and long-wave (365 nm) ultraviolet light. Calcium oxalate crystals were searched for by applying 10% sulphuric acid to squash preparations of thallus samples. Ascospore and soredia measurements are given as (min.–) $\bar{x} \pm \text{SD}(\text{–max.})$, n = number of measurements. Specimens were also examined using scanning electron micrography (SEM) with a Zeiss Sigma 300 VP-FESEM.

DNA extraction and sequencing

We acquired DNA from both species using material from multiple localities (Table 1). For extracting *Calicium* DNA, ascomata including stalks were broken off and placed in a 1.5 μl Eppendorf tube. For placing the fungus we later realized was a *Ramboldia*, we removed thallus fragments using sterile razor blades and transferred them to water droplets on a microscope slide, where we removed visible contaminants by transferring the target material to new, clean water drops. This procedure was repeated as often as necessary until a clean piece of agglutinated material was obtained, the excess water wicked away with fine-tipped forceps and placed against the inner wall of an Eppendorf, where it was allowed to air dry. A sterile 3 mm carbide bead was then added and the tube frozen at -80°C for > 1 h in a Qiagen TissueLyser rack. The deep-frozen rack was then subsequently shaken at 27 shakes per second for 25 s, optimizing cell lysis by manual pulsing (5 s on, 5 s off). The pulverized samples were used for a Qiagen Investigator forensic DNA extraction using the Blood & Tissue protocol.

For placement of the species in their respective groups, we amplified four DNA loci from both species using polymerase chain reaction (PCR) and compared these to data from available loci in public databases (Table 1). For *Calicium*, we decided to target ITS rRNA, 28S rRNA, protein-coding mini-chromosome maintenance complex 7 (*Mcm7*) and mitochondrial 12S rRNA as loci were already available for numerous taxa in the genus, using primers ITS1F, ITS4, LRlecF, LRlecR, MCM7-709for, MCM7-1348rev, mrssu1 and mrssu3R (White *et al.* 1990; Gardes & Bruns 1993; Zoller *et al.* 1999; Schmitt *et al.* 2009; Schneider *et al.* 2015). Since we suspected that the sorediate crust we later identified as a *Ramboldia* could be placed in the order *Lecanorales*, we targeted the same four loci as used for *Calicium* as these were broadly informative based on our previous work in that group (Spribille *et al.* 2020). For *Trebouxia*, we used ITS1T and ITS4T (Kroken & Taylor 2000) with the following modified PCR protocol: an initial denaturation at 95 $^\circ\text{C}$ for 5 min, 10 touchdown cycles of 95 $^\circ\text{C}$ for 30 s, 66–58 $^\circ\text{C}$ for 30 s, and 72 $^\circ\text{C}$ for 1 min, followed by 35 cycles of 95 $^\circ\text{C}$ for 30 s, 57 $^\circ\text{C}$ for 30 s, and 72 $^\circ\text{C}$ for 1 min, with a final extension of 72 $^\circ\text{C}$ for 7 min. All PCR reactions were run on a Veriti 96-Well Fast Thermal Cycler (Applied Biosystems, Waltham, Massachusetts) where 2 μl dilutions of DNA extract were used in a 22 μl total reaction volume with KAPA3 G Plant PCR Kit (Millipore Sigma, Burlington, Massachusetts) following the manufacturer's instructions.

For placement of the species, we downloaded from NCBI available sequences from the genus *Calicium* covered by the four loci mentioned above as of March 2023, as well as outgroup taxa from the genera *Heterodermia*, *Physcia* and *Phaeophyscia*. For the placement of *Ramboldia*, we included sequences of *Ramboldia* available at the same time plus a selection of representative sequence sets in *Lecanoraceae*, *Miriquidicaceae*, *Parmeliaceae*, *Ramalinaceae*, *Sphaerophorus* and *Psoraceae*, with *Rhizocarpon oederi* as outgroup. We aligned and concatenated the sequences using MAFFT v. 7.380 (Katoh & Standley 2013) implemented in phyloscripts (Reisl 2015). Vertical alignment positions with

Table 1. Taxa, voucher information and DNA sequences used for the analyses. The new species are shown in bold.

Isolate ID	Used in	Name	Voucher/isolate and repository, where known	ITS+5.8S	28S	MtSSU	Mcm7
T638	Figure 3	<i>Alectoria sarmentosa</i>	Spribille s. n., 2009 (GZU)	JN009706	–	KR017393	–
X386	Figure 3	<i>Bacidia rosella</i>	Ekman 3117	AF282086	AY300829	AY300877	–
X1178	Figure 3	<i>Bryonora curvescens</i>	voucher Svensson 3490 (UPS)	OM423662	–	OM417204	OM417051
X1177	Figure 3	<i>B. rhypariza</i>	voucher Svensson 3397 (UPS)	OM423666	–	OM417208	OM417050
X1140	Figure 3	<i>Bryoria furcellata</i>	isolate L147	HQ402722	KR995380	HQ402667	KR995539
X1036	Figure 2	<i>Calicium abietinum</i>	Tibell 25061 (UPS)	–	KX512872	KX512971	KX529041
X1037	Figure 2	<i>C. adpersum</i>	Prieto 3037 (S)	–	KX512895	KX512949	KX529055
X1038	Figure 2	<i>C. chlorosporum</i>	Tibell 25012 (UPS)	–	KX512892	KX512956	KX529059
X1039	Figure 2	<i>C. chlorosporum</i>	Thor 20859 (UPS)	–	–	KX512955	–
X1040	Figure 2	<i>C. corynellum</i>	Prieto (S)	KX512908	KX512855	KX512985	KX529048
X1041	Figure 2	<i>C. denigratum</i>	Prieto (S)	KX512909	KX512878	KX512965	KX529044
X1058	Figure 2	<i>C. episcalaris</i>	27-XI-2013 Knutsson s. n. (UPS)	KX228552	–	–	–
X1183	Figure 2	<i>C. episcalaris</i>	Vondrák 24110 (PRA)	OQ717763	–	OQ646151	–
X1042	Figure 2	<i>C. glaucellum</i>	Wedin 8563 (S)	KX512910	KX512864	KX512980	KX529032
X1077	Figure 2	<i>C. indicum</i>	Tibell 21908	DQ789085	–	–	–
X1078	Figure 2	<i>C. indicum</i>	Tibell 222064	DQ789084	–	–	–
X1075	Figure 2	<i>C. indicum</i>	Tibell 23250	DQ789087	–	–	–
X1074	Figure 2	<i>C. indicum</i>	Tibell 23282	DQ789088	–	–	–
X1073	Figure 2	<i>C. indicum</i>	Tibell 23304	DQ789089	–	–	–
X1072	Figure 2	<i>C. indicum</i>	Tibell 23381	DQ789090	–	–	–
X1076	Figure 2	<i>C. indicum</i>	Tibell 29234	DQ789086	–	–	–
X1071	Figure 2	<i>C. laevigatum</i>	Tibell 21868	DQ789091	–	–	–
X1070	Figure 2	<i>C. laevigatum</i>	Tibell 23337	DQ789092	–	–	–
X1043	Figure 2	<i>C. lecideinum</i>	Prieto (S)	KX512911	KX512882	KX512961	KX529046
X1044	Figure 2	<i>C. lenticulare</i>	Tibell 23284 (UPS)	KX512912	–	KX512979	KX529033
X1045	Figure 2	<i>C. montanum</i>	van den Boom 23445 (UPS)	KX529069	KX512853	–	–
X1046	Figure 2	<i>C. nobile</i>	Tibell 21968 (UPS)	KX512913	KX529070	KX512988	KX529060
X1047	Figure 2	<i>C. nobile</i>	Tibell 23396 (UPS)	KX512914	KX529071	KX512987	KX529061
X1068	Figure 2	<i>C. nobile</i>	Tibell 21936a	DQ789095	–	–	–
X1069	Figure 2	<i>C. nobile</i>	Tibell 21974	DQ789094	–	–	–
X1048	Figure 2	<i>C. notarisii</i>	Prieto 3007 (S)	KX512915	KX512883	KX512960	KX529047
X1059	Figure 2	<i>C. pinastri</i>	Palice 4044 (Hb. Palice)	AY450575	–	–	–
X1049	Figure 2	<i>C. pinicola</i>	Lendemmer & Knudsen 14982 (UPS)	KX512916	KX512871	KX512972	KX529040
X1050	Figure 2	<i>C. pinicola</i>	Thor 19856 (UPS)	KX512917	KX512887	KX512991	KX529066
T1955	Figure 2	<i>C. poculatum</i>	Spribille 45911 (type, ALTA)	PP760145	PP756442	PP756438	PP828769
X1155	Figure 2	<i>C. poculatum</i>	Gockman 5756 (CANL, isolate MCM 251)	PP760146	–	–	–
X1156	Figure 2	<i>C. poculatum</i>	Gockman 5756 (CANL, isolate MCM 252)	PP760147	–	–	–

(Continued)

Table 1. (Continued)

Isolate ID	Used in	Name	Voucher/isolate and repository, where known	ITS+5.8S	28S	MtSSU	Mcm7
X1084	Figure 2	<i>C. pyriforme</i>	Tibell 23333	DQ812126	–	–	–
X1051	Figure 2	<i>C. quercinum</i>	Tibell 22287 (UPS)	KX512918	KX512854	–	–
X1060	Figure 2	<i>C. robustellum</i>	Tibell 16689 (UPS)	AY450577	–	–	–
X1052	Figure 2	<i>C. salicinum</i>	Prieto (S)	KX512919	KX512861	KX512982	KX529027
X1063	Figure 2	<i>C. sequoiae</i>	isolate T648	EU635979	–	–	–
X1062	Figure 2	<i>C. sequoiae</i>	isolate T649	EU635980	–	–	–
X1061	Figure 2	<i>C. sequoiae</i>	isolate T715	EU635981	–	–	–
X1053	Figure 2	<i>C. tigillare</i>	Prieto 3038 (S)	JX000104	JX000088	JX000123	JX000162
X1054	Figure 2	<i>C. trabinellum</i>	Wedin 8517 (S)	KX512920	KX512858	–	KX529026
X1055	Figure 2	<i>C. trachyloides</i>	Nordin 4002 (UPS)	KX512933	KX529072	KX512959	KX529058
X1064	Figure 2	<i>C. tricolor</i>	Tibell 16640 (UPS)	AY450580	–	–	–
X1056	Figure 2	<i>C. verrucosum</i>	Tibell 23198 (UPS)	–	–	–	KX529030
X1065	Figure 2	<i>C. victorianum</i>	K(M)122465	EU010389	–	–	–
X1057	Figure 2	<i>C. viride</i>	Wedin 24/4 2000	HQ650703	AY340538	AY584696	JX000153
X1066	Figure 2	<i>Calicium</i> sp.	Palice 4047	DQ789083	–	–	–
X1082	Figure 2	<i>Calicium</i> sp.	Tibell 23196	DQ812137	–	–	–
X1080	Figure 2	<i>Calicium</i> sp.	Tibell 23197	DQ812139	–	–	–
X1081	Figure 2	<i>Calicium</i> sp.	Tibell 23198	DQ812138	–	–	–
X1079	Figure 2	<i>Calicium</i> sp.	Tibell 23225	DQ812140	–	–	–
X1083	Figure 2	<i>Calicium</i> sp.	Tibell 23374	DQ812136	–	–	–
X1067	Figure 2	<i>Calicium</i> sp.	Tibell 23396	DQ789096	–	–	–
T639	Figure 3	<i>Cetraria sepincola</i>	Spribille & Wagner 32131 (GZU)	JN009715	–	KR017394	–
X1139	Figure 3	<i>C. sepincola</i>	Bjerke 29-9-2010 (TROM)	KR995274	KR995388	KR995320	KR995559
X1138	Figure 3	<i>Cladonia mitis</i>	L526 voucher L. Myllys 240513-1 (H)	KJ947957	–	–	KJ948081
X391	Figure 3	<i>C. mitis</i>	von Brackel 6034	KT792799	–	KT792824	–
X1142	Figure 3	<i>C. stipitata</i>	isolate AFTOL-ID 1657	–	DQ973026	DQ972975	–
X381	Figure 3	<i>Frutidella caesioatra</i>	Andersen 91 (BG)	–	AY756349	AY567765	–
X726	Figure 3	<i>F. caesioatra</i>	isolate 5760	MG925971	–	MG925872	–
X1143	Figure 3	<i>Haematomma flexuosum</i>	Lücking 15097h (F)	EU075560	EU075555	EU075551	–
X392	Figure 3	<i>Hertelidea botryosa</i>	AFTOL 4929	–	KJ766568	KJ766403	–
T636	Figure 2	<i>Heterodermia speciosa</i>	Spribille 26300 (KLGO)	KR017141	KP794975	–	–
X1133	Figure 2	<i>H. speciosa</i>	Wetmore s. n. (S), isolate MP34	KX512927	KX512868	KX512975	KX529037
T764	Figure 3	<i>Japewia subaurifera</i>	Spribille & Wagner s. n., 2009 (GZU)	JN009716	KR017230	–	–
T532	Figure 3	<i>J. tornoensis</i>	Spribille 28417 (GZU)	MN483113	–	MN508270	–
X385	Figure 3	<i>Lecania cyrtella</i>	Ekman 3017	AF282067	AY300840	AY567720	–
X279	Figure 3	<i>Lecanora caesiorubella</i> subsp. <i>glaucomodes</i>	Lumbsch 19094a	JN943722	JN939506	JQ782666	JN992710
X1175	Figure 3	<i>L. cavicola</i>	KRAM: Rodriguez-Flakus 29582	OL604041	OL663901	OL604121	OK665503
X288	Figure 3	<i>L. flavopallida</i>	Lumbsch 20031a	JN943724	JN939514	JQ782674	JN992721

(Continued)

Table 1. (Continued)

Isolate ID	Used in	Name	Voucher/isolate and repository, where known	ITS+5.8S	28S	MtSSU	Mcm7
X289	Figure 3	<i>L. formosa</i>	20129045-2/XL0031-2	KT453771	KT453773	KT453819	–
X290	Figure 3	<i>L. formosa</i>	YN0188/YN0190	KT453769	KT453774	KT453820	KT453877
X291	Figure 3	<i>L. garovaglii</i>	Leavitt 089 (BRY-C)	KT453728	KT453775	KT453818	KT453867
X295	Figure 3	<i>L. hybocarpa</i>	Lumbsch s. n.	EF105412	EF105421	EF105417	–
X297	Figure 3	<i>L. intricata</i>	U166/U510	AF070022	DQ787345	DQ787346	–
X1174	Figure 3	<i>L. intricata</i>	KRAM: Rodriguez-Flakus 3757	OL603995	OL663863	OL604076	OK665493
X305	Figure 3	<i>L. polytropa</i>	AFTOL-ID 1798	HQ650643	DQ986792	DQ986807	–
X309	Figure 3	<i>L. tropica</i>	Papong 6440	JN943720	JN939518	JQ782699	JN992714
X310	Figure 3	<i>Lecidella</i> aff. <i>elaeochroma</i>	not provided in original paper	KT453753	KT453778	KT453825	KT453884
X313	Figure 3	<i>L. carpathica</i>	not provided in original paper	KT453741	KT453784	KT453831	–
X315	Figure 3	<i>L. effugiens</i>	not provided in original paper	KT453747	KT453786	KT453833	KT453883
X317	Figure 3	<i>L. elaeochromoides</i>	not provided in original paper	KT453750	KT453790	KT453836	–
X318	Figure 3	<i>L. enteroleucella</i>	not provided in original paper	KT453757	KT453792	KT453838	–
X319	Figure 3	<i>L. euphorea</i>	AFTOL-ID 1374	HQ650596	–	DQ986784	–
X321	Figure 3	<i>L. patavina</i>	not provided in original paper	–	KT453800	KT453846	–
X324	Figure 3	<i>L. stigmatea</i>	not provided in original paper	KT453766	KT453803	KT453849	–
X326	Figure 3	<i>L. stigmatea</i>	not provided in original paper	KT453765	KT453806	KT453850	KT453881
X329	Figure 3	<i>L. tumidula</i>	not provided in original paper	KT453737	KT453809	KT453853	–
X331	Figure 3	<i>Letharia columbiana</i>	not provided in original paper	KT453735	KT453811	KT453855	KT453878
X332	Figure 3	<i>Mirioidica complanata</i>	Szczepanska 935	KF562187	KF562179	–	KF562169
T1196	Figure 3	<i>M. gyrizans</i>	Fryday 10175 (MSC)	MN483126	MN460217	MN508282	–
T852	Figure 3	<i>M. instrata</i>	Spribile s. n., 2010 (GZU)	JN009720	MN460241	MN508311	JN009746
X334	Figure 3	<i>M. leucophaea</i>	Kossowska 448	KF562189	KF562181	–	KF562170
T77	Figure 3	<i>Mycoblastus sanguinarius</i>	Spribile 30127-A (GZU)	JF744910	KR017231	KR017396	JF744742
X717	Figure 3	<i>Palicella glaucopa</i>	PRF2672	KJ152481	KJ152462	KJ152477	–
X397	Figure 3	<i>P. schizochromatica</i>	Hauck s. n.	HQ650652	HQ660532	HQ660555	–
X1134	Figure 2	<i>Phaeophyscia ciliata</i>	Prieto s. n. (S), isolate MP8	KX512929	KX512886	KX512958	KX529051
X1132	Figure 2	<i>Physcia aipolia</i>	Wedin 6145 (UPS), isolate MP77	KX512931	AY300857	AY143406	KX529052
X282	Figure 3	<i>Polyzozia contractula</i>	AFTOL-ID 877	HQ650604	DQ986746	DQ986898	–
T1185	Figure 3	<i>P. schofieldii</i>	Spribile 39188 (MSC)	MN483119	MN460213	MN508274	–
X395	Figure 3	<i>Protoblastenia calva</i>	AFTOL-ID 992	HQ650618	JQ301601	DQ986904	–
X1146	Figure 3	<i>Protoparmelia</i> aff. <i>badia</i>	Holien 11762, L-12476 (TRH)	KP822218	KP796268	KP822410	KP822323
X1141	Figure 3	' <i>Protoparmelia</i> ' <i>cupreobadia</i>	Fryday 863 (MSC)	KF562192	KF562184	KR995365	KF562175

(Continued)

Table 1. (Continued)

Isolate ID	Used in	Name	Voucher/isolate and repository, where known	ITS+5.8S	28S	MtSSU	Mcm7
X336	Figure 3	<i>P. picea</i>	Haugan 9612 (O)	KF562194	KF562186	–	KF562177
X302	Figure 3	<i>Protoparmeliopsis muralis</i>	Vondrak 9413	KT453730	KT453777	KT453823	KT453875
X355	Figure 3	<i>P. peltata</i>	not provided in original paper	KT453722	KT453815	KT453860	KT453873
T611	Figure 3	<i>Pseudevernia cladonia</i>	Spribille s. n., 2009 (GZU)	KR017139	–	KR017392	–
X1135	Figure 3	<i>Pulvinora pringlei</i>	isolate ED787acp	MW257114	MW257114	MW257153	–
X274	Figure 3	<i>Puttea margaritella</i>	M149	EU940187	EU940111	EU940261	–
X1136	Figure 3	<i>Pyrrhospora querneae</i>	Ekman 3019 (BG)	AF517930	AY300858	AY567712	–
T990	Figure 3	<i>Ramalina almqvistii</i>	Talbot AML008-X-01A (GZU)	MT041620	MT039416	MT039418	MT041632
T770	Figure 3	<i>R. dilacerata</i>	voucher Spribille 30671B (GZU)	MN483142	KP794953	MN508294	–
X1176	Figure 3	<i>Ramalinora glaucolivida</i>	voucher CANB: Rogers 11908	–	HM441298	HM441297	HM441296
X338	Figure 3	<i>Ramboldia arandensis</i>	Elix 28721(CANB)	EU075541	DQ431919	EU075527	–
X339	Figure 3	<i>R. brunneocarpa</i>	Elix 36756(F)	EU075542	EU075520	EU075528	–
T1910	Figure 3	<i>R. canadensis</i>	Spribille 43597 (PMAE)	–	PP756443	PP756439	PP828770
T1956	Figure 3	<i>R. canadensis</i>	Spribille 45910 (ALTA)	PP760148	PP756444	PP756440	PP828772
T2002	Figure 3	<i>R. canadensis</i>	Spribille 45087 (ALTA)	PP760149	PP756445	PP756441	PP828771
T624	Figure 3	<i>R. cinnabarina</i>	Spribille 21549 (GZU)	KR017140	KR017229	–	MN437630
X340	Figure 3	<i>R. elabens</i>	H: Lichenotheca Graecensis	–	KJ766648	KJ766482	–
X341	Figure 3	<i>R. gowardiana</i>	UBC: Bjork 9447	–	KJ766649	KJ766483	–
X1144	Figure 3	<i>R. haematites</i>	voucher Frisch (M)	EU075543	DQ431918	EU075529	–
X342	Figure 3	<i>R. laeta</i>	Elix 31817 (F)/36817	EU075544	DQ431920	EU075530	–
X343	Figure 3	<i>R. petraeoides</i>	Elix 36816(F)	EU075545	EU075521	EU075531	–
X1145	Figure 3	<i>R. quaesitica</i>	Elix 28837	EU075546	EU075525	EU075532	–
X344	Figure 3	<i>R. russula</i>	Luecking 17640(F)	EU075547	EU075524	EU075533	–
X345	Figure 3	<i>R. sanguinolenta</i>	Elix 28835 (F)	EU075548	EU075523	EU075534	KT453887
X346	Figure 3	<i>R. stuartii</i>	Elix 28664(F)	EU075549	EU075522	EU075535	–
T1071	Figure 3	<i>Rhizocarpon oederi</i>	Spribille 36629 (MSC)	MN483144	MN460228	MN508296	MN437622
X718	Figure 3	<i>R. oederi</i>	isolate AFTOL 1372	–	DQ986804	DQ986788	–
X348	Figure 3	<i>Rhizoplaca chrysoleuca</i>	BRY 55000	HM577233	KT453812	KT453856	HM577385
X349	Figure 3	<i>R. haydenii</i>	Leavitt 727 (BRY-C)	HM577304	KT453813	KT453857	HM577438
T1649	Figure 3	<i>Sphaerophorus globosus</i>	Spribille 41201 (GZU)	MN483149	MN460232	MN508300	MN437626
KS122	Figure 3	<i>Stereocaulon</i> sp.	Resl s. n. (2014) (GZU)	MN483154	MN460234	MN508303	MN437627
X363	Figure 3	<i>Thalloidima sedifolium</i>	AFTOL-ID 1283	KT232214	KJ766666	KJ766503	–

data in $\leq 10\%$ (in *Calicium*) and $\leq 20\%$ (in *Lecanorales*) of sequences were culled for the final analysis. We calculated maximum likelihood trees using IQ-TREE v. 1.6.12 (Nguyen *et al.* 2015; Minh *et al.* 2020), inferring substitution models for each locus partition and within *Mcm7* by codon position using ModelFinder (Chernomor *et al.* 2016; Kalyaanamoorthy *et al.* 2017). The trees were calculated using 1000 standard, non-parametric bootstrap replicates followed by the generation of a

single consensus tree. We only display support values on branches if they have a bootstrap value $\geq 70\%$.

Results and Discussion

We generated new sequences from six specimens. The final alignment for the *Calicium* tree minus sites excluded owing to excessive missing data, contained 57 tips and 3469 nucleotide positions divided

into six partitions (1–619, 620–1964, 1965–2801 and each codon position of 2802–3469). The result of the substitution model inference for all six partitions in this alignment was TNe + R3, TIM2e + R2, TPM2u + F + I + G4, K2P + I, K2P + I + G4 and TIM2 + F + I. A total of 923 sites were parsimony-informative. The maximum likelihood tree had a log-likelihood value of –20191.621. For the *Lecanorales* tree for placement of *Ramboldia*, the final alignment minus sites excluded owing to excessive missing data included 83 tips and 3437 nucleotide positions likewise divided into six partitions (1–582, 582–1860, 1861–2790 and each codon position of 2791–3437), with the following inferred substitution models: TIM2 + F + I + G4, TIM2 + F + R3, TVM + F + R4, TIM2 + F + G4, TPM3 + F + R2 and HKY + F + I + G4. A total of 1366 sites were parsimony-informative. The maximum likelihood tree had a log-likelihood value of –44167.4735. Both trees are unrooted but graphically displayed with distantly related taxa drawn as roots.

The consensus tree for *Calicium* (Fig. 2) recapitulates the main groups of the genus recovered by Prieto & Wedin (2017), including their Clade A (including *Calicium abietinum* Pers., *C. glaucellum* Ach., *C. pinicola* (Tibell) M. Prieto & Wedin, *C. trabinellum* (Ach.) Ach.) and their Clade B with its three respective subclades: 1 (including *C. adpersum* Pers., *C. nobile* Tibell, *C. chlorosporum* F. Wilson), 2 (including *C. lenticulare* Ach., *C. notarissii* (Tul.) M. Prieto & Wedin, *C. tigillare* (Ach.) Pers., *C. trachylioides* (Nyl. ex Branth & Rostr.) M. Prieto & Wedin), and 3 (including *C. corynellum* (Ach.) Ach., *C. lecideinum* (Nyl.) M. Prieto & Wedin, *C. viride* Pers.). In addition, our tree recovers *C. sequoiae* C. B. Williams & Tibell (Williams & Tibell 2008) in its own clade within Prieto & Wedin's (2017) Clade B, though with poor bootstrap support, and places several species from the Indian subcontinent described by Tibell (2006), or yet undescribed but deposited in NCBI, in Clades A and B. The inclusion of sequences from Tibell (2006) and Tibell & Knutsson (2016), which include only one locus (ITS), probably contributes to lower bootstrap support for relationships among species, although the main groups and numerous lower-level clades receive bootstrap support > 80%. The new species *Calicium poculatum* is recovered in Prieto & Wedin's (2017) Clade A in a subclade with 100% bootstrap support, together with *C. episcalaris* Tibell & Knutsson, *C. pinastri* Tibell and *C. montanum* Tibell. Its sibling relationship with *C. episcalaris* carries 91% bootstrap support. Here, too, the low support is likely related to missing data in the compared taxa: the absence of sequence data for loci other than ITS and mtSSU in *C. episcalaris*, other than ITS in *C. pinastri* and other than ITS and LSU in *C. montanum*.

The consensus tree for placing *Ramboldia* in the *Lecanorales* (Fig. 3) has a weakly supported backbone as is characteristic of recent phylogenies of *Lecanorales*, with unsupported sibling relationships in much of *Lecanora* s. lat. The new species *Ramboldia canadensis* is, however, recovered within a highly supported clade representing the genus *Ramboldia*, in a subclade together with species with dark apothecia found in Australasia (*R. stuartii* (Hampe) Kantvilas & Elix, *R. brunneocarpa* Kantvilas & Elix, *R. petraeoides* (Nyl. ex C. Bab. & Mitt.) Kantvilas & Elix) and species with the cinnabar-red apothecial pigment russulone, found in the Southern Hemisphere (Australia: *R. arandensis* (Elix) Kalb *et al.*, *R. sanguinolenta* (Kremp.) Kalb *et al.*; Australia and New Zealand: *R. laeta* (Stirt.) Kalb *et al.*; Australia and Brazil: *R. quae-sitica* Elix & Kalb; Kalb *et al.* 2008) and pantropical regions of both hemispheres (*R. haematites* (Fée) Kalb *et al.*, *R. russula* (Ach.) Kalb *et al.*). The relationships among these subgroups of

Ramboldia are, however, poorly supported, and the nearest sibling of *R. canadensis*, of the taxa sampled, could not be established with certainty. It should also be noted that more than half of the species in the genus *Ramboldia* have yet to be sampled using molecular methods. However, such an analysis is beyond the scope of the present study.

The new species

Calicium poculatum G. Thor, McMullin & Gockman sp. nov.

Mycobank No.: MB 853516

Similar to *Calicium episcalaris* in being parasitic, but occurring on *Lecanora caesiorubella* Ach. subsp. *saximontana* Imsh. & Brodo and having shorter stalks (up to 0.2 mm tall) and shorter ascospores (8–13 µm).

Type: Canada, Saskatchewan, 41 km by air ENE of village of Hudson Bay, large fen along Silica Sands Road, 52.928°N, 101.788°W, on *Lecanora caesiorubella* subsp. *saximontana* on decorticated branches of *Larix laricina* at edge of large fen dominated by *Carex lasiocarpa*, in marginal transition zone to *Picea mariana* muskeg with *Andromeda polifolia*, 303 m, 26 June 2020, T. Spribille 45911 (CANL—holotype; ALTA, UPS—isotypes).

(Fig. 4)

Thallus immersed in the host, associated with a zone of discoloration.

Ascomata parasitic on *Lecanora caesiorubella* subsp. *saximontana*, both on the disc and margin of apothecia or sometimes on nearby thallus, often occurring in aggregations of up to five ascomata on a single host apothecium, shining black, ±sessile or on stalks up to 0.2 mm tall and up to 0.2 mm diam.; stalks consisting of dark brown, sclerotized hyphae; capitulum ±doliiform, up to 0.3 mm diam.; excipulum formed by dark brown, sclerotized hyphae; excipulum edge sometimes thinning out and pale, thus mimicking a narrow whitish pruina; hypothecium dark brown, consisting of dark brown, sclerotized hyphae. *Asci* cylindrical, c. 32 × 7 µm, with uniseriately or some biseriately arranged ascospores. *Ascospores* ellipsoidal, brown, with a minutely verrucose surface and deep irregular cracks, 1-septate, (8–)9–12(–13) × (5–)5–7(–8) µm (length: \bar{x} = 10.6 µm, SD = 1.2 µm, n = 120; width: \bar{x} = 5.9 µm, SD = 0.7 µm, n = 120).

Photobiont not seen; unknown if the fungus interacts with the host photobiont.

Conidiomata not seen.

Chemistry. All parts of the ascomata I–, K–, K/I–.

Etymology. From the Latin *poculum* (cup, goblet), a reference to the shape of the ascomata.

Notes. Characterized by its parasitic, short-stalked, shining black ascomata and short ascospores. The only other obligately parasitic *Calicium* described is *C. episcalaris*, described from Sweden (Tibell & Knutsson 2016) and recently reported for the north-eastern USA (Selva *et al.* 2023) and the Czech Republic (published along with the release of Vondrák *et al.* (2023)). However, this species grows on *Hypocnomyces scalaris* (Ach.) M. Choisy, and has more distinctly stalked ascomata (up to 0.37 mm tall) and longer ascospores (10–14 × 6–8 µm). Based on the two DNA isolates of *C. episcalaris* and the three from

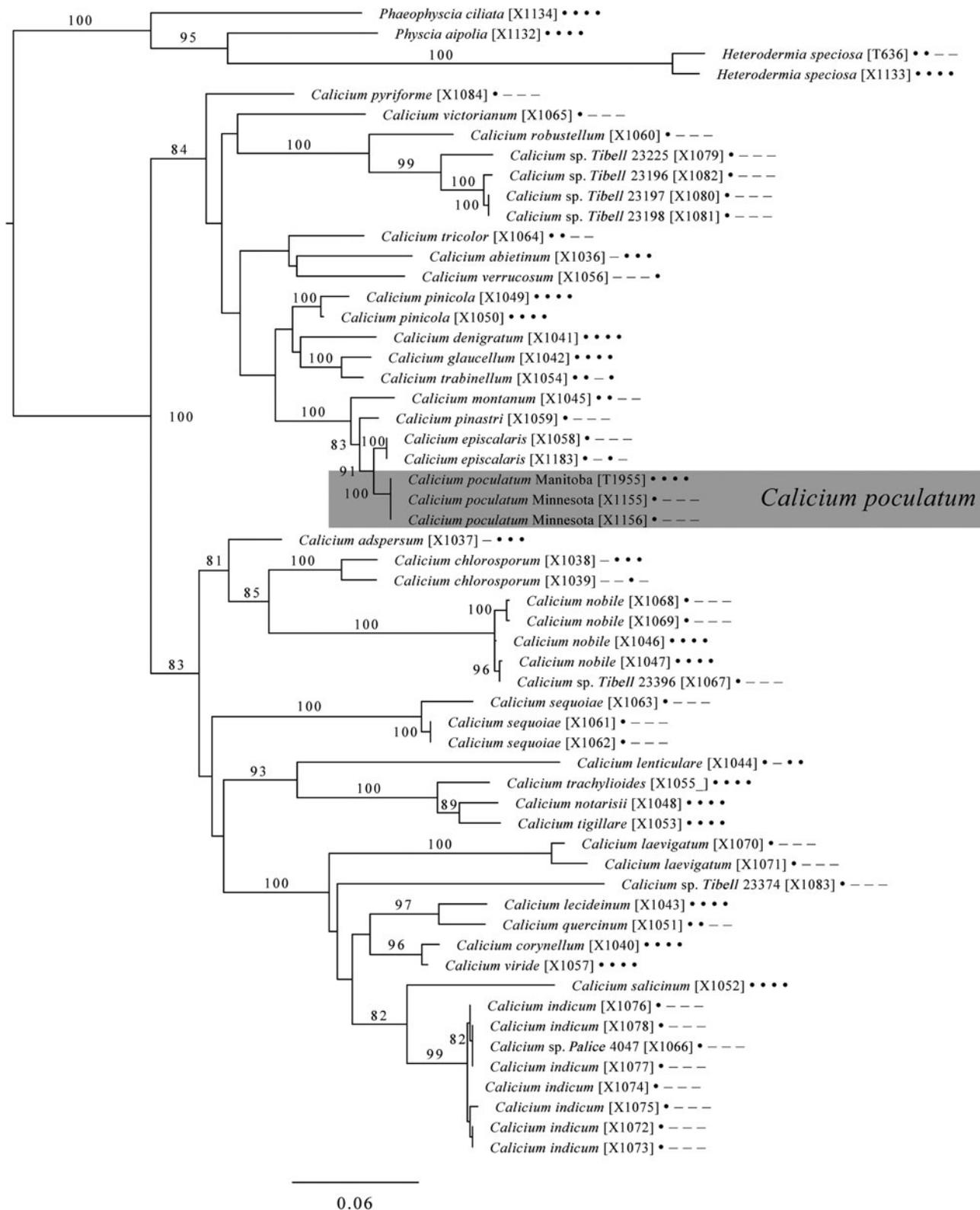


Figure 2. Position of the new species, *Calicium poculatum* within the genus *Calicium* based on a maximum likelihood tree of concatenated ITS, 28S and *Mcm7* nuclear genes and the mitochondrial SSU gene. Numbers after the taxon names indicate single specimen isolates (Table 1); dots and dashes indicate presence and absence (respectively) of the loci for that sample in the order of loci given above.

C. poculatum, the two species differ in seven positions in ITS1, one in 5.8S, and six in ITS2. It is likely an ambiguous base call (N) at one of these positions in *C. poculatum* isolate X1155 that leads to a bootstrap value of 91% (instead of 100%) between

C. episcalaris and *C. poculatum*. The branch lengths among the species *C. montanum*, *C. pinastri*, *C. episcalaris* and *C. poculatum* are shorter than those of other species in the tree, suggesting a set of relatively recent speciation events. *Chaenothecopsis kalbii* Tibell

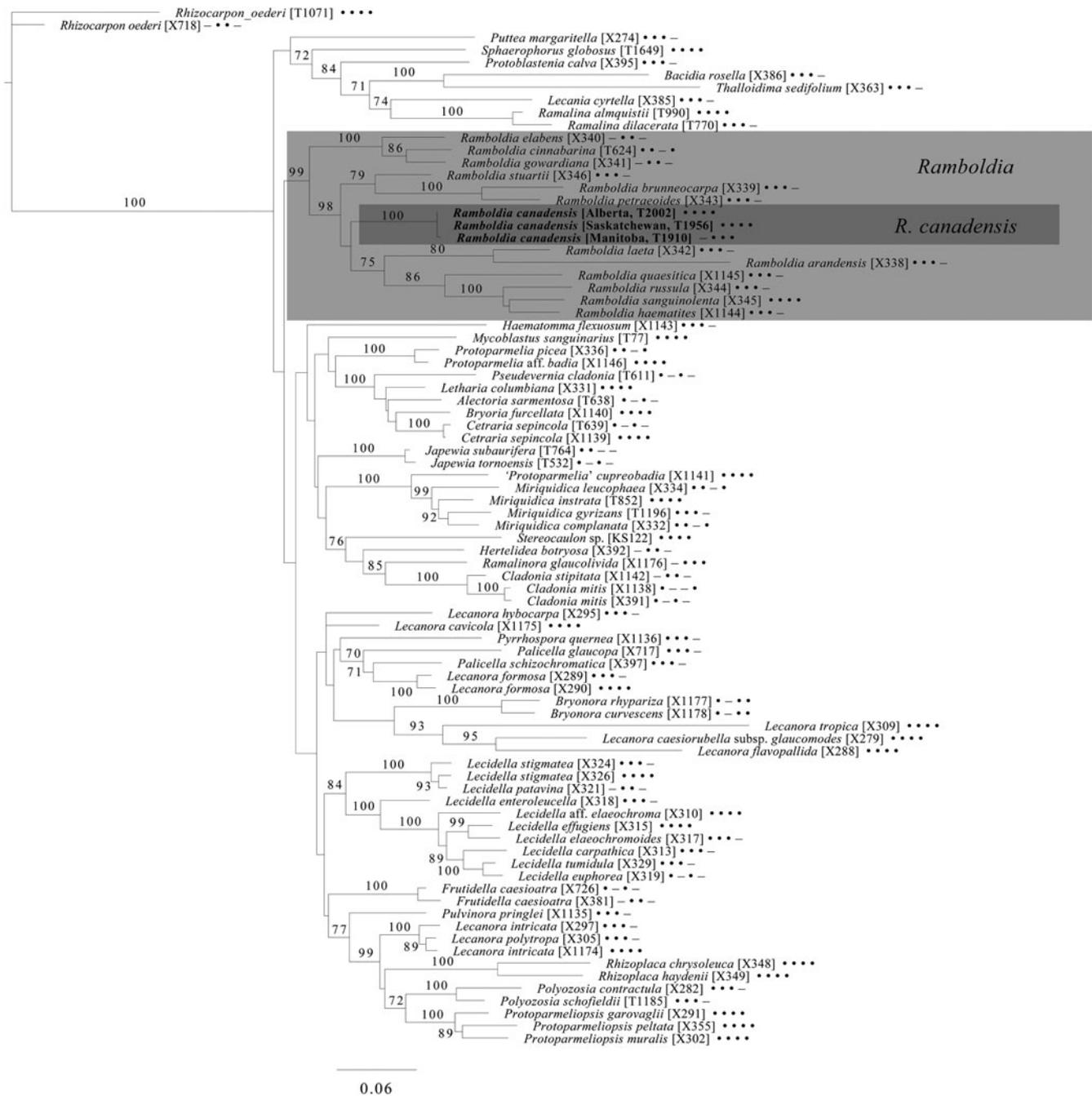


Figure 3. Position of the new species *Ramboldia canadensis* within the order Lecanorales based on a maximum likelihood tree of concatenated ITS and 28S nuclear ribosomal genes, the mitochondrial SSU gene, and the nuclear *Mcm7* gene. Numbers after the taxon names indicate single specimen isolates (Table 1); dots and dashes indicate presence and absence (respectively) of the loci for that sample in the order given above.

& K. Ryman is another species of calicioid fungus growing on *Lecanora caesiorubella* (Tibell & Ryman 1995), but this species differs by the apothecia not being mazaediate, the stalk being pale at the base, and by having narrower spores (4–5.5 µm) with pointed ends. In addition, *Ch. kalbii* is described from Brazil and is also known to occur in Australia, north-western Mexico and the south-eastern USA, as well as on the island of Réunion (Tibell & Ryman 1995; Lendemer et al. 2008).

Habitat and distribution. The species appears to be parasitic, causing necrosis and finally the death of the host tissue near

the *Calicium* ascomata. In the Canadian populations, the host, *Lecanora caesiorubella* subsp. *saximontana*, was growing on wood in the upper part of *Larix laricina* snags (up to 2 m high) with some branches still remaining, as well as on the decorticated branches of live trees. The snags are certainly not covered by snow in wintertime. In Manitoba it was intermixed with, for example, *Bryoria* spp., *Buellia arborea* Coppins & Tønsberg, *Calicium tigillare* (Ach.) Pers., *Elixia flexella* (Ach.) Lumbsch, *Evernia mesomorpha* Nyl., *Flavopunctelia flaventior* (Stirton) Hale, *Parmeliopsis ambigua* (Wulfen) Nyl., *Pycnora sorophora* (Vain.) Hafellner and *Ramboldia canadensis* (see below), as well

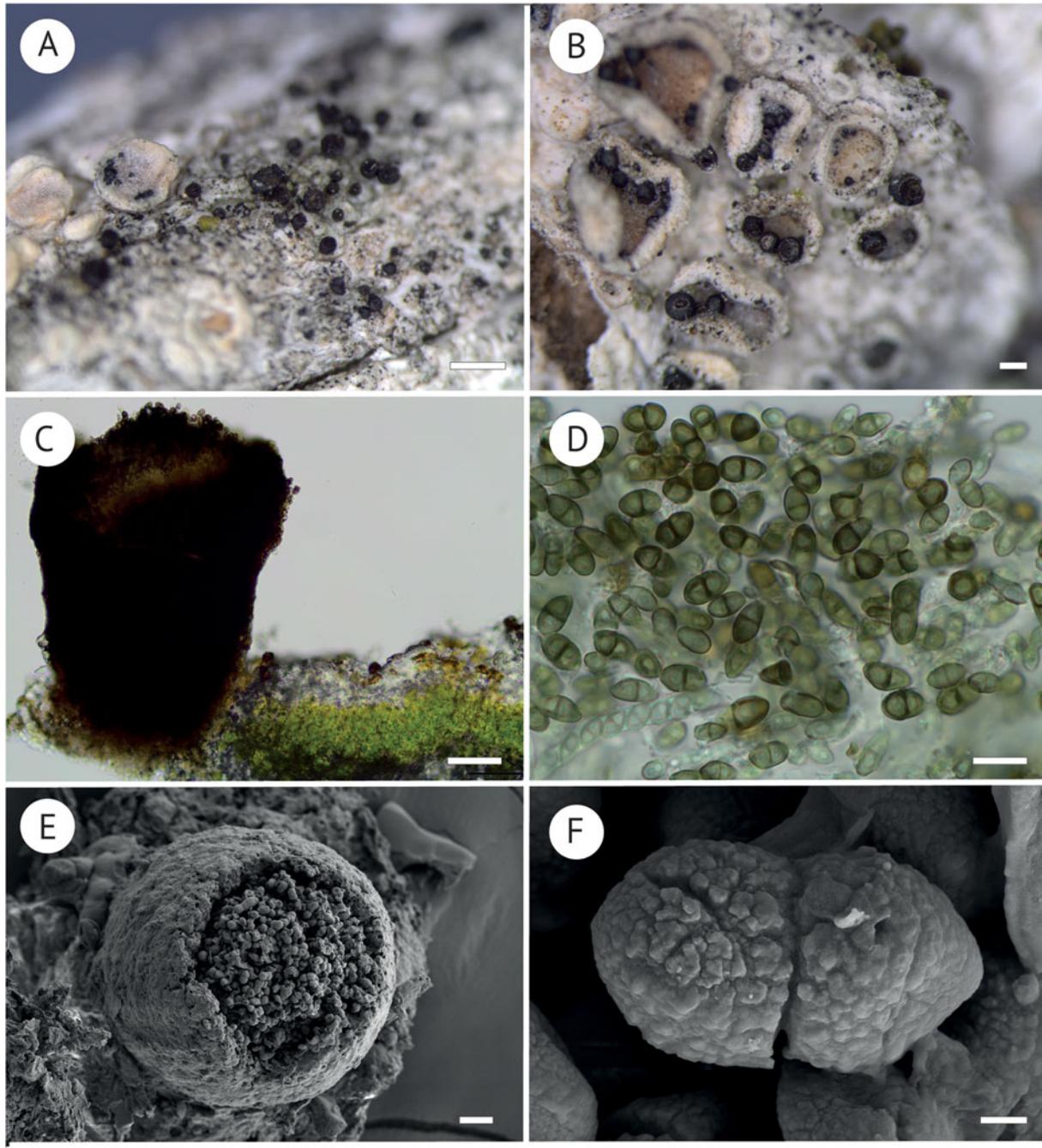


Figure 4. *Calicium poculatum* (holotype). A & B, habit in and around the apothecia of *Lecanora caesiorubella* subsp. *saximontana*. C, apothecium and associated parasitized thallus (in water). D, ascospores (in water). E, apothecium (SEM). F, ascospore (SEM). Scales: A = 500 μm ; B = 200 μm ; C = 50 μm ; D = 10 μm ; E = 20 μm ; F = 1 μm . In colour online.

as *Lecidella xylophila* (Th. Fr.) Knoph & Leuckert which is reported here as new to North America (HPTLC: atranorin; G. Thor 37390, UPS). This might be neglected as a species-rich lichen habitat in Canada and should be studied further. The discovery of a small specimen on *Picea mariana* twigs suggests the species may have a wider ecology. The Minnesota population is found on the bark of living *Larix laricina* and *Picea mariana* in a large peatland complex. At this location, the species and its host were primarily observed in a band of intermixed *Larix laricina* and *Picea mariana* situated between a poor, *Picea mariana*-dominated system in the centre of the peatland and a

rich, wet forest dominated by *Fraxinus nigra* located adjacent to the upland.

Additional specimens examined. **Canada:** **Alberta:** Sand River area N of Glendon, 54.541493°N, 111.25033°W, on *Lecanora caesiorubella* subsp. *saximontana* on decorticated *Larix laricina* branch in string fen, 580 m, 2019, T. Spribille 45043 (PMAE). **Manitoba:** 152 km N of the village Grand Rapids, W of Highway 6, open bog with a few small trees, on 2 m high *Larix laricina* snag, 54.51352°N, 99.04272°W, 255 m, 2018, G. Thor 37385 (UPS); *ibid.*, T. Spribille 43463 (CANL); Division No. 19,

Lake Winnipeg, Long Point, end of Long Point Rd at lake, 52° 56.783'N, 98°47.664'W, on *Picea mariana* twigs in dense forest, 224 m, 2018, *T. Spribille* 43506 (PMAE). Ontario: Kenora District, Hudson Bay Lowlands, Ring of Fire, 28 km SE of Kitchie Lake, 19 km WNW of Streatfeild Lake, 14 km SW of Armes Lake, 52.2371°N, 86.1974°W, on *Lecanora caesiorubella* subsp. *saximontana* on decorticated *Larix laricina*, fen, dominant species cover includes *Carex chordorrhiza*, *Chamaedaphne calyculata*, *Larix laricina*, *Picea mariana*, *Trichophorum alpinum* and *T. cespitosum*, 183 m, 2015, *McMullin* 17450 (CANL). Saskatchewan: 53 km NNW of Hudson Bay, Pasquia Bog, 53.27211°N, 102.00159°W, on branch of *Larix laricina* in falsa bog, 336 m, *T. Spribille* 45811 (CANL).—USA: Minnesota: Aitkin County, 7.8 km north-west of McGrath and 15.8 km E of Malmo, on the bark of living *Larix laricina* and *Picea mariana* in a large acid peatland system, 46°18.151'N, 93°19.150'E, 385 m, 2017, *Gockman* 5477 (MIN); 8.1 km NW of McGrath and 15.35 km E of Malmo, on the bark of living *Larix laricina* and *Picea mariana* in a large acidic peatland system, 46°18.181'N, 93°19.502'E, 385 m, 5 xii 2018, *Gockman* (MIN).

***Ramboldia canadensis* G. Thor & T. Sprib. sp. nov.**

MycoBank No.: MB 853517

Similar to, for example, *Ramboldia elabens* (c.sp., Northern Hemisphere), *R. farinosa* and *R. sorediata* (both with soralia,

Australia), but with a rimose to verrucose-areolate, greyish creamy sorediate thallus with dark brown to blackish, mucilage-encrusted soredia.

Type: Canada, Alberta, Yellowhead County, c. 9.7 km NNE of Niton Junction, c. 10 km NW of Mackay, 53.69486°N, 115.70927°W, on decorticated *Larix laricina* branch at the edge of a floating string fen, 815 m, 5 August 2021, *A. Huereca Delgado* 993 & *T. Spribille* (UPS—holotype [fertile]; CANL—isotype [sterile]).

(Fig. 5)

Ascomata apothecia, seen only once, lecideine, shining black, to 0.6 mm diam.; excipulum indistinct and thin, concolorous with the disc, composed of conglutinated, radiating, brown hyphae; hypothecium pale brown; hymenium of sparsely branched paraphyses, pale brown, partly K/I+ blue, K–; paraphyses 1 µm diam.; apices to 4 µm wide, brown. *Asci* of *Lecanora*-type, only seen filled with epiplasm, I and K/I+ blue. *Ascospores* not seen (only one apothecium checked).

Symbiotic thallus crustose, to c. 50 mm wide and to c. 0.5 mm thick, rimose to verrucose-areolate; individual areoles irregular, scattered to confluent, flat to convex, to 1 mm wide or sometimes diffusely delimited, greyish creamy and shiny; soralia excavating from both the upper surface and the sides of areoles, soon ±flat, 0–3 per areole, sometimes confluent; soredia dark brown to blackish contrasting with the white medulla exposed

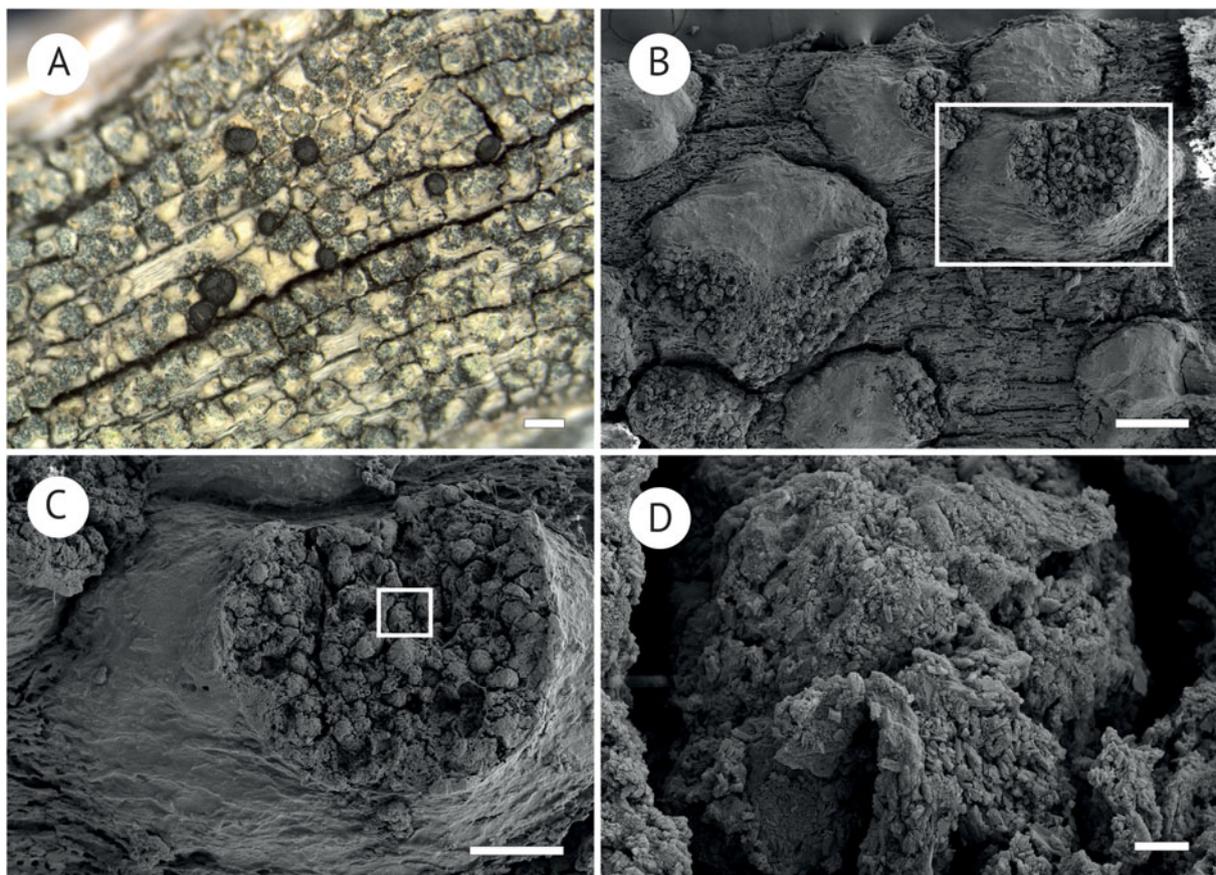


Figure 5. *Ramboldia canadensis*. A, habit of thallus and apothecia (holotype). B–D, detail of an areole and its soredia using scanning electron microscopy (Spribille 45910). D is a magnification of the inset of C, which in turn is a magnification of the inset in B. Scales: A = 500 µm; B = 200 µm; C = 100 µm; D = 3 µm. In colour online.

in the soralia, encrusted with secondary metabolite crystals, globose, (20–)26–47(–71) μm diam. (\bar{x} = 36.3 μm , SD = 10.8 μm , n = 120).

Photobiont Trebouxia simplex Tscherm.-Woess based on ITS rRNA from isolates T1956 (type, Saskatchewan, GenBank PP756436) and T2002 (Alberta, GenBank PP756437); cells globose (8–)9–13(–16) μm diam. (\bar{x} = 11.3 μm , SD = 2.1 μm , n = 120).

Conidiomata not seen.

Chemistry. Medulla C–, I–, K+ yellow, K/I–, Pd+ yellow, UV–, without calcium oxalate. TLC (solvent system C): two unknown substances, R_f 15 and R_f 20, both yellow-orange and UV+ green with brown halo. The spots might refer to substances similar to barbatolic acid.

Etymology. Named after its occurrence in Canada.

Notes. *Ramboldia* is a genus of lecideoid fungal symbionts of lichens that form ascomata that can be black to crimson red, reflecting a rich diversity of pigments (Kalb et al. 2008). Most species are described from Australia (Kantvilas 2016; Elix & McCarthy 2017, 2018). The new species is characterized by having a rimose to verrucose-areolate, greyish creamy sorediate thallus with dark brown to blackish corticated soredia. A small number of apothecia were observed once and are apparently rare. It can be confused with *Buellia arborea* with which it is intermixed (in Manitoba) but *B. arborea* has a thin or immersed thallus, concave soralia and soredia which are brownish to blackish with a bluish tint. *Buellia arborea* also has atranorin and placodiolic acid. The only other sorediate *Ramboldia* species reported in the boreal biome are *R. cinnabarina* (Sommerf.) Kalb et al. and *R. subcinnabarina* (Tønsberg) Kalb et al., but these species have a rimose coherent thallus, whitish to greenish soredia, and atranorin and fumarprotocetraric acid or fatty acids as secondary metabolites.

Habitat and distribution. Similar to *Calicium poculatum* but perhaps extending further west: it was found at several localities in the western part of the boreal forest where *C. poculatum* could not be found. So far known from central Alberta to central Manitoba. The type locality in Yellowhead County, Alberta, lies just outside the perimeter of a major wildfire that burned several thousand hectares in May 2023.

Additional specimens examined. **Canada:** **Alberta:** Sand River area 33 km north of Glendon, 54.541493°N, 111.25033°W, on decorticated *Larix laricina* branches in string fen, 2019, T. Spribille 45044 (ALTA); between Lac La Biche and Conklin, 55.25558°N, 111.31361°W, on decorticated *Larix laricina* twigs in large string fen, 670 m, 2019, T. Spribille 45087 & D. Díaz Escandón (ALTA); 11 km S of Conklin, 55.530044°N, 111.076118°W, on decorticated *Larix laricina* branches in string fen, 655 m, 2019, T. Spribille 45249 (to be issued in *Dupla Graecensia Lichenum*); Westlock County, fen near Vega, 54.44116°N, 114.27664°W, 17 vii 2021, A. Huereca Delgado s. n. & T. Spribille (ALTA). **Manitoba:** 152 km N of the village Grand Rapids, W of Highway 6, open mire with a few small trees, on 2 m high *Larix laricina* snag, 54.51352°N, 99.04272°W, 255 m, 2018, G. Thor 37396 (UPS, CANL); *ibid.*, T. Spribille 43585 (CANL), 43597 (PMAE). **Saskatchewan:** 53 km NNW of Hudson Bay, Pasquia Bog, 53.27211°N, 102.00159°W, on

decorticated branch of *Larix laricina* in falsa bog, 336 m, T. Spribille 45810 (CANL); 41 km by air ENE of village of Hudson Bay, large fen along Silica Sands Road, 52.928°N, 101.788°W, on decorticated branches of *Larix laricina* at edge of large fen dominated by *Carex lasiocarpa*, in marginal transition zone to *Picea mariana* muskeg with *Andromeda polifolia*, 303 m, 2020, T. Spribille 45910 (ALTA, CANL, GZU, H).

Significance of the habitat

The two newly described species are the first lichen and lichenicolous fungus to be described with a high habitat specificity for the tamarack tree (*Larix laricina*), one of the marquee species of muskeg vegetation. However, some differences in the micro-habitat amplitude are apparent based on the localities discovered so far. *Ramboldia canadensis* has so far been found only on hard wood of isolated *L. laricina* snags. *Calicium poculatum*, by contrast, has been found in the same habitats in Canada and in more densely grown wooded *L. laricina* fens in Minnesota, as well as once on its *Lecanora caesiorubella* host on *Picea mariana* in western Manitoba.

The fact that the majority of findings of *Calicium poculatum* and all records of *Ramboldia canadensis* to date are on isolated *Larix laricina* snags in string or falsa fens suggests that these habitats play a special role in supporting populations of the new lichen and lichenicolous fungus. What distinguishes these specific trees from the millions of other trees in the boreal forest is likely their special landscape position and associated propensity to escape destruction during wildfires. The central Canadian boreal forest has a relatively dry, continental climate with mean annual precipitation ranging from 418 mm (e.g. Fort McMurray, Alberta) to 491 mm (Grand Rapids, Manitoba) and is an ecosystem shaped by recurrent fire (Larsen 1980). In this region, one of the overarching attributes of the habitats where the new lichens described here occur is that the trees or clumps of trees are relatively isolated (Fig. 1). The ages of *L. laricina* snags in string fens in Canada has not been systematically investigated, but local studies have found living trees up to 337 years old (see Table S1 in Caners et al. (2024)). One branch in a Manitoba specimen of *R. canadensis* (Thor 37385) measuring 15 mm in diameter had c. 30 annual rings. We suspect that the isolation of the trees increases the likelihood that they escape the cyclical fires that burn surrounding contiguous forests, and may contribute to tree ages otherwise anomalous in this region and climate type. In more humid regions, it is possible such ages can be achieved without the need for trees to be isolated, as natural fire return intervals may be longer. This may account for *Calicium poculatum* occurring in Minnesota in a fen complex in which the host trees grow densely together, in a region with higher mean annual precipitation (c. 780 mm).

The occurrence of ancient trees on fen strings or hummocks recalls key lichen habitats known as kelo trees in Fennoscandia. Like the *Larix laricina* snags we sampled, kelo trees can occur in string fens known regionally as flark-string aapa mires (Laitinen et al. 2007) or string mires in aapa fen complexes (Malmer 1985), where they grow if hummocks are sufficiently elevated (up to 0.5 m) above the water table. Coniferous trees in this habitat are slow growing and die before reaching a specific height. The cause of the death of the trees is not well explained in the literature but one possibility is that trees are killed by rapidly increasing temperatures in spring, causing the trees to start photosynthesizing, while the ground where the roots are located is still

frozen. In Manitoba, Saskatchewan and Alberta the main coniferous tree occurring on strings in string fens is *Larix laricina*. The snags of this species resemble kelo trees in having old, hard, silvery grey and decorticated trunks (Niemelä *et al.* 2002). However, they differ from kelo trees, which in Europe are formed from *Pinus sylvestris*, in usually being less impregnated with resin. Formation and decay of kelo trees are slow processes but under certain conditions, which probably include repeated wildfires, wood of *P. sylvestris* becomes hard and resin-impregnated and is then very long lasting (Santaniello *et al.* 2017). *Pinus sylvestris* can reach up to 800 years of age in Fennoscandia, and its transformation into kelo trees takes *c.* 40 years after tree death (Sirén 1961; Leikola 1969). Kelo trees can remain standing for over 700 years (Niemelä *et al.* 2002). *Larix laricina* snags in string fens can be considered to constitute a North American counterpart to kelo trees, and the discovery of a lichen and lichenicolous fungus largely specialized to this type of structure again highlights the importance of dead wood for lichen diversity (Spribille *et al.* 2008; Santaniello *et al.* 2017).

Larix laricina snags in patterned fens clearly represent an overlooked and important habitat for lichen specialists in North American muskegs. Notwithstanding their resilience to natural fire regimes, such habitats are on the decline owing to the increasing intensity of wildfires under anthropogenic climate change, and locally are threatened by peat mining and the disruption of water flow from construction of roads and seismic lines for oil and gas exploration. It is too early to say whether *Calicium poculatum* and *Ramboldia canadensis* are rare, as only a tiny fraction of string fens in Canada and the boreal part of the United States have been surveyed, and both species appear to have a rather wide range. However, they were found only in a fraction of the fens surveyed, and the specialized habitats are a tiny area of the overall boreal forest. Furthermore, other specialists can be expected to be discovered with increased survey attention, and *L. laricina* snags should be treated as priority habitats for survey and stewardship.

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