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Gremmeniella balsamea sp. nov. on balsam fir in Canada

Gaston Laflamme and Edgar Smerlis

Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, 1055 du P.E.P.S., Québec, QC,
Canada G1V 4C7.

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Corresponding author: Gaston Laflamme, gaston.laflamme@nrca-nrcan.gc.ca.

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Abstract: *Gremmeniella balsamea* sp. nov. is described from specimens collected on balsam fir (*Abies balsamea*) in eastern Canada. This is a fungal pathogen specific to balsam fir. Until now, it was considered to be *G. abietina* var. *balsamea*, this variety including also a *Gremmeniella* fungus on spruces. Consequently, this new species causes changes in the taxonomy within the genus *Gremmeniella*.

Key words: *Gremmeniella*, *Abies balsamea*, cankers.

Introduction: The genus *Gremmeniella* was proposed by Morelet (1969) and is now widely accepted. The type species, *Gremmeniella abietina* (Lagerb.) Morelet, is found mainly on stems and shoots of pines. A taxonomic re-evaluation of *Gremmeniella* by Petrini et al. (1989) recognized *G. abietina* var *balsamea* on balsam fir (*Abies balsamea* (L.) Mill.) and spruces (*Picea* spp.) in the North American boreal forest as being distinct from var. *abietina*. However, Petrini et al. (1989) decided not to create new species for these fungi on balsam fir and spruces because they lacked well-defined morphological differences.

Since that reappraisal, it is now known that *Gremmeniella* found on balsam fir is a pathogen specific to that host (Laflamme et al. 1996) and this has been confirmed by completing Koch's postulates (Smerlis and Laflamme, 2011). This fungus causes cankers on stems and branches of seedlings and saplings. It is relatively common in the boreal forest and was first collected in the Réserve faunique des Laurentides, north of Quebec City, Canada, by the second author in 1956.

Our objectives are to underline morphological differences between *Gremmeniella* species found on balsam fir, spruces and pines, and to give a detail description of the proposed new *Gremmeniella* species on balsam fir. We are providing the GenBank accession numbers of two sequences of this new proposed species that we obtained from a molecular study in progress in our laboratory.

Materials and Methods: For morphological measurements, thirty four fungal specimens representing *G. abietina* from pines, and *Gremmeniella* sp. from spruce were obtained from the René Pomerleau Herbarium (QFB) of the Laurentian Forestry Centre, the Forest Research Station, Wageningen, The Netherlands, the ETH, Zurich, Switzerland, and the Norwegian Forest Research Institute, Aas, Norway. All the

specimens from the René Pomerleau Herbarium (QFB) are listed here in numerical order; these numbers are followed by the host species, the locality, the county (Co), the date of collection and the collector. Samples from other herbarium are at the end of the list:

QFB 7977, on *Picea glauca*, Lac Jacques-Cartier, Co. Montmorency, 12 VII 1965, coll. E. Smerlis and M. Saint-Laurent; **QFB 7978**, on *Picea mariana*, Rivière Montmorency, Co. Montmorency, 5 VII 1968, coll. E. Smerlis; **QFB-9291**, on *Pinus sylvestris*, Hedmark, Norway, 12 VI 1961, coll. F. Roll-Hansen; **QFB-17141**, on *Pinus strobus*, Valcartier, Co. Québec, 14 VI 1967, coll. E. Smerlis and M. Saint-Laurent; **QFB 17142**, on *Picea glauca*, Parc des Laurentides, Co. Montmorency, 3 VIII 1964, coll. E. Smerlis and M. Saint-Laurent; **QFB-17143**, on *Pinus contorta*, Valcartier, Co. Québec, 14 VI 1967, coll. E. Smerlis and M. Saint-Laurent; **QFB-17144**, on *Pinus resinosa*, Valcartier, Co. Québec, 14 VI 1967; coll. E. Smerlis and M. Saint-Laurent; **QFB-17145**, on *Pinus banksiana*, Saint-Urbain, Seigneurie du Gouffre, 9 VI 1966, coll. E. Smerlis and M. Saint-Laurent; **QFB 17146**, on *Picea glauca*, Parc des Laurentides, 3 VIII 1964, coll. E. Smerlis and M. Saint-Laurent; **QFB 17147**, on *Picea glauca* x *Picea sitchensis*, Valcartier, Co. Québec, 15 VII 1975, coll. E. Smerlis; **QFB 17148**, on *Picea abies*, Valcartier, Co. Québec, 15 VII 1975, coll. E. Smerlis; **QFB 17149** on *Picea rubens*, Valcartier, Co. Québec, 15 VII 1975, coll. E. Smerlis; **QFB-17150**, on *Pinus sylvestris*, Valcartier, Co. Québec, 14 VI 1967, coll. E. Smerlis and M. Saint-Laurent; **QFB-18512**, on *Pinus banksiana* Saint-Siméon, Co. Callières, 10 VI 1981, coll. E. Smerlis; **QFB-18525**, on *Pinus banksiana*, Sainte-Marguerite, Co. La Verendrye, 8 VI 1982, coll. E. Smerlis; **QFB-18526**, on *Pinus banksiana*, Saint-Jean-de-Cherbourg, Co. Cherbourg, 12 VI 1984, coll. E. Smerlis; **QFB-18533**, on *Pinus banksiana*, Saint-Hilarion, Co. Settrington, 8 VI 1983, coll. E. Smerlis; **QFB 25601**, on *Pinus banksiana*, Rivière York, Co. Fletcher, 9 VII 1982, coll. E. Smerlis; **QFB**

25602, on *Pinus banksiana*, Rivière Malbaie, Co. Montmorency, 6 VII 1977, coll. E. Smerlis; **QFB 25603**, on *Pinus banksiana* Pellegrin, Co. Pellegrin, 10 VII 1984, coll. E. Smerlis; **QFB 25604**, on *Pinus banksiana*, Les Méchins, Co. Dalibaire, II VI 1981, coll. E. Smerlis; **QFB 25605**, on *Pinus banksiana*, Lac Waconichi, Co. Roy, 10 VI 1970, coll. E. Smerlis; **QFB,25606**, on *Pinus banksiana*, Lac Albanel, Co. Saint-Pierre, 22 VI 1968, coll. E. Smerlis; **QFB 25607**, on *Pinus banksiana*, La Croche, Co. Langelier, 12 VI 1974, coll. E. Smerlis; **QFB 25608**, on *Pinus banksiana*, Girardville, Co. Girard, 26 VI 1969, coll. E. Smerlis; **QFB 25609**, on *Pinus banksiana*, Cap-de-la-Madeleine, Co. Champlain, 7 VI 1973, coll. E. Smerlis; **QFB 25610**, *Pinus resinosa*, Sainte-Paule, Co. Matapedia, 15 VII 1982, coll. E. Smerlis; **QFB 25611**, *Pinus sylvestris*, Saint-Jean-de-Cherbourg, Co. Cherbourg., 13 VI 1984, coll. E. Smerlis; **QFB 25612**, on *Pinus resinosa*, Lac aux Iroquois, Co. Ross, 3 VI 1982, coll. E. Smerlis; **QFB 25613**, *Pinus resinosa*, Les Méchins, Co. Dalibaire, 15 VII 1982, coll. E. Smerlis; **QFB 25614**, *Pinus resinosa*, Saint-Jean-de-Cherbourg, Co. Cherbourg, 12 VI 1984, coll. E. Smerlis; on *Pinus* sp., Apelscha State Forest, Netherlands, XI 1956, coll. J. Gremmen; on *Pinus* sp., Smilde State Forest, Netherlands, 4 IV 1957, coll. J. Gremmen; on *Pinus* sp., Davos, Switzerland, 14 VIII 1940, coll. L. Ettliger.

Measurements of fungal specimens were performed with an image analysis system (Software from Bioquant Image Analysis Corporation, Nashville, TN, USA) connected to a research microscope (Polyvar, Reichert-Jung, Wien, Austria) at 400 x for ascospores and conidia. Length and width of thirty ascospores and/or conidia were measured for each specimen. Minimum and maximum lengths and widths for each element of all specimens were used to characterize these fungi.

For comparison of *G. abietina* var. *balsamea* on balsam fir in cultures with two other

Gremmeniella species, we used the isolates CF-79-0620 on balsam fir, CF-85-0168 of *G. abietina* from *Pinus resinosa* Ait., and CF-82-419 of *Gremmeniella* sp from *Picea mariana* (Mill.) B.S.P. Isolates of *G. abietina* var. *balsamea* from balsam fir used for sequence analysis are CF-79-0620, CF-87-0061 and CF-87-0063. These cultures are deposited in the collection and stored in liquid nitrogen at the Laurentian Forestry Centre.

The DNA extraction protocol used was according to Zolan and Pukkila (1986), Gardes and Bruns (1993), and Sokolski et al. (2006) with some modifications. A sample of approximately 0.5-1 cm² plug of mycelium of each fungal culture was transferred into a sterile 1.5 mL Eppendorf tube and stored at -20°C until processing. The tissue was disrupted by homogenization with a micropestle in 600 µL of extraction buffer as described by Gardes and Bruns (1993) (2% CTAB, 100 mM Tris [pH 8], 20 mM EDTA, 1.4 M NaCl, and 0.2% beta-mercaptoethanol) and incubated for 1 h at 65°C. To extract the DNA, 600 µL of phenol-chloroform-isoamyl alcohol (25:24:1) was added and the tube was mixed by inversion for 2 min. The emulsion was centrifuged at room temperature at 13,000 rpm (18327 g) for 10 min. The upper aqueous phase was transferred to a clean tube and the genomic DNA was precipitated with 600 µL of ice cold isopropanol. The tubes were incubated at -20°C for 30 min and centrifuged at 10,000 rpm (10844 g) for 10 min at 4°C. The pellet was rinsed twice with 300 µL of ice cold 70% ethanol followed each time by a centrifugation at 10,000 rpm for 10 min, dried, dissolved in 50 µL of storage buffer TE [pH 8.0], and stored at -20°C.

The ITS region of rDNA was amplified using the universal primer ITS4 (White *et al.* 1990) and the universal fungal primer ITS1F (Gardes and Bruns 1993). The amplification was done according to Sokolski *et al.* (2006) with minor modifications. Briefly, 1 µL of DNA diluted 100-fold was added to the PCR mixture containing 1 X PCR buffer

supplied with the enzyme (20 mM Tris-HCl [pH 8.4], 50 mM KCl, and 3 mM MgCl₂), 0.4 mM each of dATP, dCTP, dGTP and dTTP, 0.125 μM of each PCR primer, 1 U of recombinant Taq DNA Polymerase (Invitrogen Life Technologies, Grand Island, NY, USA), and molecular biology-grade water to a final volume of 25 μL. The amplification was performed on a thermal cycler (PTC 200, MJ Research, Waltham, MA, USA) programmed with an initial denaturation at 94°C for 3 min followed by 40 cycles at 94°C for 30 s, 56°C for 1 min, and 72°C for 1 min and 30 s. The amplification was terminated by strand completion at 72°C for 10 min. The PCR products were analyzed on a 1.5% agarose gel in 1X TAE buffer (Invitrogen, 24710030). The gels were stained 10 min in an aqueous solution of 1% ethidium bromide and photographed under UV light using the GeneSnap Gel/Blot Imaging Software from Syngene, Frederick, MD, USA. Sequencing of the PCR fragments was done by the Sequencing and Genotyping Platform of the CHUL (CHUQ) Research Centre, Québec City, Canada, where the samples were analyzed by capillary electrophoresis using the ABI 3730/XL sequencing apparatus (Applied Biosystems). The sequence analyses were performed with BioEdit version 7.0.5.2, designed by Hall (1999). The sequences were aligned with Clustal W (Thompson *et al.* 1994) and compared with those in GenBank (NCBI) by BLAST.

Results: The ascospores of *G. balsamea* measured 15.4 – 40.6 x 3.5 – 4.9 μm and conidia 24.5 – 56.0 x 2.1 – 3.5 μm (Table 1). In specimens of *G. abietina* collected on pine in Québec, the ascospores measurements are 10.5 – 27.3 x 3.5 – 6.3 μm and conidia measures 11.2 – 49.0 x 2.8 – 3.5 μm (Table 1). In specimens of *Gremmeniella* sp. collected on spruce in Québec, ascospores measures 14 – 26.1 x 3 – 5 μm and conidia 24 – 48 x 1.8 – 4 μm (Table 1). Illustrations of ascus, ascospores, secondary spores and conidia are presented (Figure 1-4) as well as apothecia (Figure 5). *G. balsamea* is well distributed in the boreal forest (Figure 6).

Cultures of all three species, *G. balsamea*, *G. abietina* and *Gremmeniella* sp. on spruces, are slow growing, reaching 22 to 28 mm on 3% malt agar at 15°C in 4 weeks and the underside is dark brown or black. Culture color of *G. balsamea* is yellow, yellowish green or yellowish brown (Figure 7), while culture color of *G. abietina* isolates from pine are dark green and of *Gremmeniella* sp. isolates from spruce are bright yellow green.

ITS sequences were obtained from two isolates (CF-87-0063 and CF-79-0620) of *G. balsamea* and were deposited in GenBank to be accessible to all (Table 2). These sequences are 100% identical to those already present of *Gremmeniella* fungi on *A. balsamea* in GenBank and obtained from one isolate (CF-87-0061) and four herbarium specimens. The specimens from the René Pomerleau Herbarium (QFB) at the Laurentian Forestry Centre are QFB 15131, QFB 17317, QFB 17333 and QFB 19956 (Table 2).

All the specimens listed under the descriptions of the new fungal species are deposited in the René Pomerleau Herbarium (QFB) at the Laurentian Forestry Centre, Québec City, Canada.

Gremmeniella balsamea Laflamme & Smerlis sp. nov. [Figs. 1-5] (Mycobank 564037)
= *Gremmeniella abietina* var. *balsamea*
O. Petrini, L. Petrini, G. Laflamme, G.B.
Ouellette, Canadian Journal of Botany 67: 2805-2814. 1989.

In caulibus et ramis Abies balsamea. Apothecia erumpentia, solitaria vel subcaespitosa, brevistipitata, cupulata, fusco-brunnea, coriacea, margine involuto, glabra vel tenuiter squamosa, rotunda, oblonga vel triangula, 300 – 1 500 μm dia., 350 – 1000 μm latit.; stipes 160 – 300 μm latit. et 160 – 280 μm crassis; excipulum 50 – 240 μm crassum ad basim et 25 – 120 μm ad apicem; stipes et excipulum medulosum textura angularis, e cellulis fusco-brunneis et crasse

Table 1: Comparison of ascospores and conidia (length and width in μm) measured by the authors and reported in literature for the following three fungal species: *Gremmeniella balsamea* on balsam fir, *Gremmeniella* sp. on spruces and *G. abietina* on pines.

Fungal species and references	Ascospores	Conidia
<i>Gremmeniella balsamea</i>	15.4–40.6 x 3.5–4.9	24.5 – 56 x 2.1 – 3.5
<i>Gremmeniella</i> sp. on spruces	14–26.1 x 3–5	24–48 x 1.8–4
Laflamme 1988	11.2–24.3 x 2–4.8	15– 56 x 1.8–3.7
<i>Gremmeniella abietina</i>	10.5–27.3 x 3.5–6.3	11.2–49.0 x 2.8–3.5
Morelet 1980*	12–24 x 3–6	19–55 x 2.5–4
Ettlinger 1945	10.5–25.8 x 3.6–6.5	12.3–63.3 x 2.4–4.0
Van Vloten and Gremmen 1953	15.2–16.1 x 3.8–4.7	26.0–47.7 x –
Roll-Hansen 1964	12–23 x 3.5–5.0	30–43 x 2,7–3,6
Schläpfer-Bernhard 1969	14–20 x 3.5–5.0	24–50 x 2.5–3

* The description of the type species *Gremmeniella abietina* by Morelet (1969) does not include any measurement of the fungus elements.

Table 2: Information including GenBank accession numbers obtained from *Gremmeniella balsamea* isolates, or directly from herbarium material (QFB) collected on *Abies balsamea*. Numbers between brackets are included as references only.

Isolate Number	QFB Number	Locality	Collector	Date	GenBank Accession Number
CF-79-0620	(17321)	Secteur Launière	A. Carpentier	31-07-1979	JN896311
CF-87-0063	(19957)	Secteur Jumeau	G. Laflamme et al.	30-06-1987	JN896310
(CF-87-0063)	19957	Secteur Jumeau	G. Laflamme et al.	30-06-1987	JN131825
-	17317	Secteur Jumeau	R. Paquet	04-07-1979	JN131823
-	17333	Lac Ball	A. Carpentier	12-06-1979	JN131824
-	15131	Secteur Jumeau	G. Laflamme et al.	30-06-1987	JN131822
CF-87-0061	(19956)	Secteur Launière	G. Laflamme et al.	30-06-1987	GAU72259

tunicatis; excipulum ectalum e textura angularis ad basim et textura oblita ad apicem; discus concavus, flavidus; hypothecium 30 – 50 µm crassum, hyalinum e textura intricata, e cellulis elongatis et implicatis; asci octospori, cylindraceuti, brevi-stipitati, tenuiter tunicati, rotundatis ad apicem, poro in iodo non coerulescente, 75 – 140 x 7 – 9 µm; ascosporae biseriatae, hyalinae, non septatae vel 1-5-septatae, fusioideae, ellipsoideae vel fere cylindraceutae, rectae, curvatae vel sigmoideae, 15.4 – 40.6 x 3.5 – 4.9 µm; sporae secundariae hyalinae, non septatae, ellipsoideae, oblongae vel globosae, 2.0 – 4.9 x 1.4 – 2.1 µm; paraphyses filiformes, septatae, simplices vel interdum ramosae, ad apicem leviter incrassatis, epithecium formantus.

Pycnidia erumpentia, solitaria vel gregaria, coriacea, subglobosa vel patellaria, rotunda vel ellipsoidea, fusco-brunnea vel atra, glabra vel rugosa, uni- vel plurilocula, sine ostiolo; peridium e textura angularis ad basim et textura intricata ad apicem, e cellulis fusco-brunneis, crasse tunicatis; conidia hyalina, elongato-fusioidea, cylindraceuta vel aciculare, recta, curvula vel sigmoidea, 1-6-septata, 24.5 – 56.0 x 2.1 – 3.5 µm; conidiophora septata, simplica vel composita.

Holotype: **QFB 18989**, on stems and shoots of *Abies balsamea*, Lac Chartier (47° 27' N; 71° 17' W) Montmorency Co., Québec, Canada, 9 VIII 1963, E. Smerlis and M. Saint-Laurent.

On stems and shoots of *Abies balsamea*.
apothecia innate-erumpent, single or cespitose, short-stipitate, cup-shaped, dark brown, coriaceous, inrolled and brittle when dry, smooth or somewhat scabrous, circular, ellipsoid or triangular, 300 – 1 500 µm in dia., 350 – 1 000 µm in height; stipe 160 – 300 µm long, 160 – 280 µm wide; excipulum 50 – 240 µm thick at the base, tapering to 15 – 30 µm in the middle part and flaring to 25 – 120 µm at the top; stipe and medullary excipulum of textura angularis,

composed of thick-walled, isodiametric or somewhat elongated brownish cells 3 – 16 µm in dia.; lower part of the ectal excipulum of textura angularis, composed of thick-walled, reddish brown, isodiametric cells 3 – 8 µm in dia. which are gradually transformed into textura oblita consisting of dark brown, parallel hyphae 2 – 5 µm in dia. in the upper part; disc concave, pale yellowish; hypothecium 30 – 50 µm thick, of textura intricata, composed of hyaline, interwoven hyphae; asci 8-spored, thin-walled, cylindrical, short-stipitate, obtuse at the apex, the pore not staining blue in iodine, 75 – 140 x 7 – 9 µm; ascospores hyaline, aseptate to 5-septate, fusiform, narrowly ellipsoid or nearly cylindrical, straight, curved or sigmoid, smooth or slightly constricted at the septa, granular and pluriguttulated, irregularly biseriata, 15.4 – 40.6 x 3.5 – 4.9 µm; secondary spores, formed after the discharge of ascospores on minute phialides of each cell, hyaline, aseptate, ellipsoid, oblong or globular, 2.0 – 4.9 x 1.4 – 2.1 µm; paraphyses hyaline, filiform, simple or occasionally dichotomously branched, septate, 1.4 µm wide, enlarged at the apex up to 2.8 µm in dia. and forming an epithecium.

Pycnidia innate-erumpent, single or gregarious, subglobose to short-cylindrical, circular or ellipsoid in outline, dark brown to black, glabrous, smooth or rough, unilocular or plurilocular, opening irregularly at the top, 200 – 650 µm in height, 250 – 1 200 µm in dia.; wall 20 – 180 µm thick, composed at the base of textura angularis which merge through textura oblita into textura intricata at the summit of the fruiting body; cells of the wall thick-walled and dark coloured, isodiametric or somewhat elongated, 3 – 15 µm in dia. in the lower part, cylindrical, 2 – 5 µm wide in the upper part and the summit; conidia acrogenous, hyaline, acicular, narrowly cylindrical or narrowly fusiform, straight or curved, 1- to 6-septate, occasionally slightly constricted at the septa, uniform in diameter or occasionally wider in one of the cells, 24.5 – 56.0 x 2.1 – 3.5 µm; conidiophores septate,

simple or compound with 2 to 3 phialides, 10 – 30 x 2 – 3 µm, lining the base and sides of the cavity. The anamorph is a *Brunchorstia* type.

Cultures of *G. balsamea* are slow growing, reaching 23 to 26 mm on 3% malt agar at 15°C in 4 weeks. Advancing zone white, margin even or irregular, raised. Mat white, tuft-like, 3 to 4 mm in height during the first week, becoming yellow, yellowish green or yellowish brown and felt-like, except for a raised centre in older cultures (Figure 7). Reverse dark brown or black. Aerial hyphae 1.4 to 4.2 µm in dia., relatively straight and infrequently branched, thin-walled, hyaline or golden yellow with hyaline or brown oil globules and frequently incrustated. Submerged mycelium extensive, hyphae 1.4 to 6.3 µm in diameter, thin-walled, hyaline or golden yellow and containing large oil globules. Medium and fine hyphae characterized by a) terminal or intercalary hyaline to golden brown swellings up to 25 µm in dia. and b) by terminal, lateral or intercalary dark segments smaller in diameter than the supporting or adjacent cells.

Other specimens examined, all on *Abies balsamea* located in eastern Canada (Figure 6), are listed here in the numerical order used in the René Pomerleau Herbarium (QFB) of the Laurentian Forestry Centre; these numbers are followed by the locality, the county (Co), the date of collection and the collector.

QFB 7625, Secteur Launière, Réserve faunique des Laurentides, Co. Montmorency, 18 VII 1985, coll. A. Carpentier; **QFB 9003**, Réserve faunique des Laurentides, Co. Montmorency, 8 VII 1987, coll. E. Gaillard; **QFB 9329**, Barrière Laterrière, Réserve faunique des Laurentides, Co. Chicoutimi, 23 VII 1973, coll. M. Bolduc; **QFB 9449**, Parc de la Gaspésie, Co. Gaspé Ouest, 5 VIII 1994, coll. G.B. Ouellette; **QFB 14669**, Lac Chartier, Secteur Jumeau, Réserve faunique des Laurentides, Co. Montmorency, 7 VIII 1987, coll. A. Carpentier; **QFB 15014**, Secteur Jumeau, Réserve faunique des Laurentides, Co.

Montmorency, 31 VII 1984, coll. G. Laflamme; **QFB 15131**, Secteur Jumeau, Réserve faunique des Laurentides, Co. Montmorency, 25 VII 1988, coll. G. Laflamme; **QFB 15227**, Réserve faunique des Laurentides, Co. Montmorency, 10 VII 1956, coll. E. Smerlis; **QFB 15247**, Lac Drucilla, Z.E.C. Batiscan-Neilson, Co. Québec, 8 VI 1988, coll. A. Carpentier; **QFB 15292**, Lac Dufresne, Z.E.C. Batiscan-Neilson, Co. Québec, 21 VI 1988, coll. A. Carpentier; **QFB 15310**, Lac Lozon, Z.E.C. Batiscan-Neilson, Co. Québec, 8 VI 1988, coll. A. Carpentier; **QFB 15331**, Lac Poses, Z.E.C. Batiscan-Neilson, Co. Québec VI 1988, coll. A. Carpentier; **QFB 16563**, Secteur Grands Jardins, Co. Charlevoix, 19 VI 1984, coll. A. Carpentier; **QFB 16571**, Secteur Grands Jardins, Co. Charlevoix, 19 VI 1984, coll. A. Carpentier; **QFB 16593**, Secteur Launière, Réserve faunique des Laurentides, Co. Montmorency, 12 VII 1984, coll. A. Carpentier; **QFB 16594**, Secteur Jumeau, Réserve faunique des Laurentides, Co. Montmorency, 14 IX 1983, coll. A. Carpentier; **QFB 17059**, Lac Barrette, Secteur Jumeau, Réserve faunique des Laurentides, Co. Montmorency, 31 VIII 1981, coll. A. Carpentier; **QFB 17067**, Secteur Jumeau, Réserve faunique des Laurentides, Co. Montmorency, 31 VIII 1981, coll. A. Carpentier; **QFB 17068**, Lac Chartier, Secteur Jumeau, Réserve faunique des Laurentides, Co. Montmorency, 31 VIII 1981, coll. A. Carpentier; **QFB 17069**, Lac Chartier, Secteur Jumeau, Réserve faunique des Laurentides, Co. Montmorency, 31 VIII 1981, coll. A. Carpentier; **QFB 17075**, Secteur Jumeau, Réserve faunique des Laurentides, Co. Montmorency, 31 VIII 1981, coll. A. Carpentier; **QFB 17202**, Lac Rousseau, Réserve de Portneuf, Co. Portneuf, 15 VII 1982, coll. A. Carpentier; **QFB 17206**, Lac Eau claire, Z.E.C Batiscan-Neilson, Co. Québec, 6 VIII 1982, coll. A. Carpentier; **QFB 17209**, Lac Orignal, Réserve de Portneuf, Co. Portneuf, 15 VII 1982, coll. A. Carpentier; **QFB 17210**, Lac Gautron, Réserve de Portneuf, Co. Portneuf, 15 VII 1982, coll. A. Carpentier; **QFB 17220**, Lac Absolon, Réserve faunique des Laurentides, Co. Montmorency, 26 VI 1982, coll. A. Carpentier;

- QFB 17223**, Lac Chartier, Secteur Jumeau, Réserve faunique des Laurentides, Co. Montmorency, 14 IX 1982, coll. A. Carpentier; **QFB 17224**, Lac Beaudin, Secteur Jumeau, Réserve faunique des Laurentides, Co. Montmorency, 9 VI 1982, coll. A. Carpentier; **QFB 17234**, Lac Chartier, Secteur Jumeau, Réserve faunique des Laurentides, Co. Montmorency, 29 VI 1982, coll. A. Carpentier; **QFB 17239**, Lac L'Espérance, Secteur Jumeau, Réserve faunique des Laurentides, Co. Montmorency, 29 VI 1982, coll. A. Carpentier; **QFB 17310**, Lac Beauséjour, Secteur Launière, Réserve faunique des Laurentides, Co. Montmorency, 31 VII 1979, coll. A. Carpentier; **QFB 17311**, Secteur Launière, Réserve faunique des Laurentides, Co. Montmorency, 27 VII 1979, coll. A. Carpentier; **QFB 17312**, Lac Gabriella, Réserve faunique des Laurentides, Co. Montmorency, 12 VI 1979, coll. A. Carpentier; **QFB 17313**, Lac Toosey, Réserve faunique des Laurentides, Co. Montmorency, 12 VI 1979, coll. A. Carpentier; **QFB 17314**, Lac Corbin, Co. Québec, 12 VI 1979, coll. A. Carpentier; **QFB 17315**, Lac à la Chute du Lac Vachon, Réserve faunique des Laurentides, Co. Montmorency, 27 VI 1979, coll. A. Carpentier; **QFB 17316**, Lac Boyer, Réserve faunique des Laurentides, Co. Montmorency, 28 VI 1979, coll. A. Carpentier; **QFB 17317**, Barrière, Secteur Jumeau, Réserve faunique des Laurentides, Co. Montmorency, 4 VII 1979, coll. R. Paquet; **QFB 17318**, Chemin Launière, Réserve faunique des Laurentides, Co. Montmorency, 31 VII 1979, coll. A. Carpentier; **QFB 17319**, Secteur Jumeau, Réserve faunique des Laurentides, Co. Montmorency, 27 VI 1979, coll. A. Carpentier; **QFB 17320**, Secteur Launière, Réserve faunique des Laurentides, Co. Montmorency, 27 VI 1979, coll. A. Carpentier; **QFB 17321**, Lac Chat, Secteur Launière, Réserve faunique des Laurentides, Co. Montmorency, 31 VII 1979, coll. A. Carpentier; **QFB 17322**, Lac Guay, Réserve faunique des Laurentides, Co. Montmorency, 27 VI 1979, coll. A. Carpentier; **QFB 17323**, Lac Chavaudray, Secteur Launière, Réserve faunique des Laurentides, Co. Montmorency, 31 VII 1979, coll. A. Carpentier; **QFB 17324**, Lac Vachon, Réserve faunique des Laurentides, Co. Montmorency, 27 VI 1979, coll. A. Carpentier; **QFB 17325**, Secteur Launière, Réserve faunique des Laurentides, Co. Montmorency, 27 VI 1979, coll. A. Carpentier; **QFB 17326**, Lac Grégory, Co. Québec, 3 VII 1979, coll. R. Paquet; **QFB 17327**, Lac Fiset, Secteur Launière, Réserve faunique des Laurentides, Co. Montmorency, 31 VII 1979, coll. A. Carpentier; **QFB 17328**, Lac Nordêt, Réserve faunique des Laurentides, Co. Montmorency, 27 VI 1979, coll. A. Carpentier; **QFB 17329**, Ancienne barrière, Secteur Jumeau, Réserve faunique des Laurentides, Co. Montmorency, 27 VI 1979, coll. A. Carpentier; **QFB 17330**, Lac Frazie, Réserve faunique des Laurentides, Co. Montmorency, 30 VIII 1979, coll. R. Paquet; **QFB 17331**, Chute Rivière Noire, Forêt Montmorency, 20 VI 1978, coll. A. Carpentier; **QFB 17332**, Lac Bec Croche, Réserve faunique des Laurentides, Co. Montmorency, 27 VI 1979, coll. A. Carpentier; **QFB 17333**, Lac Ball, Réserve faunique des Laurentides, Co. Montmorency, 12 VI 1979, coll. A. Carpentier; **QFB 17334**, Lac Noir, Réserve faunique des Laurentides, Co. Montmorency, 27 VI 1979, coll. A. Carpentier; **QFB 17335**, Lac Valois, Secteur Launière, Réserve faunique des Laurentides, Co. Montmorency, 27 VI 1979, coll. A. Carpentier; **QFB 17336**, Barrière Laterrière, 23.3 milles sud, Secteur Jumeau, Réserve faunique des Laurentides, Co. Montmorency, 23 VII 1973, coll. M. Dubuc; **QFB 17337**, 5 milles de l'ancienne barrière, Réserve faunique des Laurentides, Co. Montmorency, 4 VII 1966, coll. R. Blais; **QFB 17338**, Ancienne Barrière, 5 milles au nord de l'Étape, Réserve faunique des Laurentides, Co. Montmorency, 4 VII 1966, coll. R. Blais; **QFB 18422**, Lac Georgiana, Réserve faunique des Laurentides, 18 VI 1961, coll. R. Huard; **QFB 18567**, Lac Lanoraye, Co. Montmorency, 26 VII 1963, coll. E. Smerlis and M. Saint-Laurent; **QFB 18568**, Lac Régis, Co. Montmorency, 30 VII 1963, coll. E. Smerlis and M. Saint-Laurent; **QFB 18575**, Petit Lac à l'Épaula, Co. Montmorency,

19 VIII 1983, coll. E. Smerlis; **QFB 18577**, Seigneurie Lac Mitis, Co. Matapédia, 28 VI 1961, coll. M. Saint-Laurent; **QFB 18579**, Lac Pamouscachiou, Co. Dubuc, 14 VIII 1968, coll. W. Smirnoff; **QFB 18583**, Lac Albanel, Baie James, 21 VII 1970, coll. E. Smerlis; **QFB 18584**, Mont Berry, Co. Matapédia, 28 VII 1961, coll. E. Smerlis; **QFB 18585**, Lac du Chapeau-de-Paille, Co. Champlain, 22 VII 1960, coll. E. Smerlis and M. Saint-Laurent; **QFB 18589**, Lac Jalobert, Co. Chicoutimi, 28 VII 1975, coll. E. Smerlis; **QFB 18592**, St-Jean-de-Cherbourg, Co. Cherbourg, 31 VII 1979, coll. E. Smerlis; **QFB 18596**, Lac Sainte-Anne, Co. Gaspé Ouest., 5 VIII 1980, coll. E. Smerlis; **QFB 18597**, Lac Sainte-Anne, Co. Québec, 19 VII 1967, coll. E. Smerlis; **QFB 18600**, Valcartier, Co. Québec, 4 VII 1969, coll. E. Smerlis; **QFB 18602**, Mont-Apica, Co. Montmorency, 16 VII 1964, coll. E. Smerlis; **QFB 18605**, Lac Pomart, Co. Montmorency, 23 VII 1958, coll. E. Smerlis and M. Saint-Laurent; **QFB 18609**, Lac Fossambault, Co. Montmorency, 19 VII 1963, coll. E. Smerlis; **QFB 18610**, Lac Valois, Co. Montmorency, 5 VIII 1961, coll. E. Smerlis and M. Saint-Laurent; **QFB 18620**, Lac Beauséjour, Co. Montmorency, 19 VII 1963, coll. E. Smerlis and M. Saint-Laurent; **QFB 18641**, Lac Simard, Co. Montmorency, 14 VIII 1964, coll. E. Smerlis; **QFB 18642**, Lac Malbaie, Co. Montmorency, 31 VII 1964, coll. E. Smerlis; **QFB 18650**, Lac Jacques-Cartier, Co. Montmorency, 19 VIII 1983, coll. E. Smerlis; **QFB 18651**, Lac Quatre-Jumeaux, Co. Montmorency, 27 VII 1958, coll. E. Smerlis and M. Saint-Laurent; **QFB 19956**, (holotype of *G. abietina* var. *balsamea*, not much material) Chemin Launière, Réserve faunique des Laurentides, Co. Montmorency, 30 VI 1987, coll. G. Laflamme, L. Petrini and O. Petrini; **QFB 19957**, Secteur Jumeau, Réserve faunique des Laurentides, Co. Montmorency, 30 VI 1987, coll. G. Laflamme, L. Petrini and O. Petrini.

Discussion: It is possible to differentiate the following three species, *Gremmeniella balsamea*, *G. abietina* and *Gremmeniella* sp. on spruces on

the basis of their color in culture. Cultures of *G. balsamea* are yellowish green to yellowish brown (Figure 7), while cultures of *G. abietina* on pine and *Gremmeniella* sp. on spruce are respectively dark green and bright yellow green. *G. balsamea* has also longer ascospores than the two other species reaching 40 µm compared to 20 – 27 µm for the two other species measured by the authors, as well as five different authors (Table 1). On the Figure 4 in Petrini *et al.* (1989), the graph shows clearly longer ascospore measurements on balsam fir than the ones from pines and spruces. However there is some overlaps between the ascospore longer length of *G. abietina* and the ascospore shorter length of *G. balsamea*. The shapes of *G. balsamea*'s ascospores are slightly different than the ones of *G. abietina*. The ascospores of *G. balsamea* are generally fusiform, occasionally narrowly ellipsoid or nearly cylindrical as compared with the ascospores of *G. abietina* which are ellipsoid (Ettlinger 1945, van Vloten and Gremmen 1954) or ellipsoid to slightly clavate (Schlöpfer-Bernhard 1969). However, the secondary spores formed by the ascospores of *G. balsamea* are indistinguishable from those of *G. abietina* from pines and *Gremmeniella* from spruces collected in Québec. They all are globose, oblong to nearly cylindrical, 2.1 – 4.2 x 2.1 – 2.8 µm in size. These secondary spores are formed after the discharge of ascospores, or, in older apothecia, after the release from ruptured asci. Secondary spore formation by *G. abietina* was reported earlier by Petrini *et al.* (1989).

The length of conidia cannot differentiate the three species. While Figure 5 in Petrini *et al.* (1989) shows slightly longer conidia for some specimens of *G. balsamea*, conidia measurements are indistinguishable in size from those of *G. abietina* and *Gremmeniella* sp. from spruce (Table 1). Nevertheless, there is a slight difference in conidial shape between *G. balsamea* and *G. abietina*. Typical conidia of *G. balsamea* are acicular or narrowly cylindrical and occasionally narrowly fusiform. The conidia of *G.*

abietina have been described as fusoid (Ettlinger 1945, van Vloten and Gremmen 1954, Schläpfer-Bernhard 1969).

Finally, *G. balsamea* is specific to its host, *A. balsamea*. This has been demonstrated by two pathogenicity tests performed in Canada, where the respective authors inoculated isolates from *Picea* spp., *Pinus* spp. and *Abies*, which confirmed both host preference (Laflamme *et al.* 1996) and host specificity (Smerlis and Laflamme 2011) for each sample of these three groups of isolates to their respective hosts.

The preliminary work on the ITS sequences provided in this paper strongly support our morphological and microscopic data. Further investigations are in progress with more *Gremmeniella* species.

In conclusion, in light of this information, the var. *balsamea* should no longer exist. *Gremmeniella* found on balsam fir is distinct from the type species *G. abietina*, and thus the new species *Gremmeniella balsamea* on *A. balsamea* is introduced. Another distinct *Gremmeniella* species on spruces in eastern Canada remains yet to be described, and a further revision of the genus *Gremmeniella* should be undertaken.

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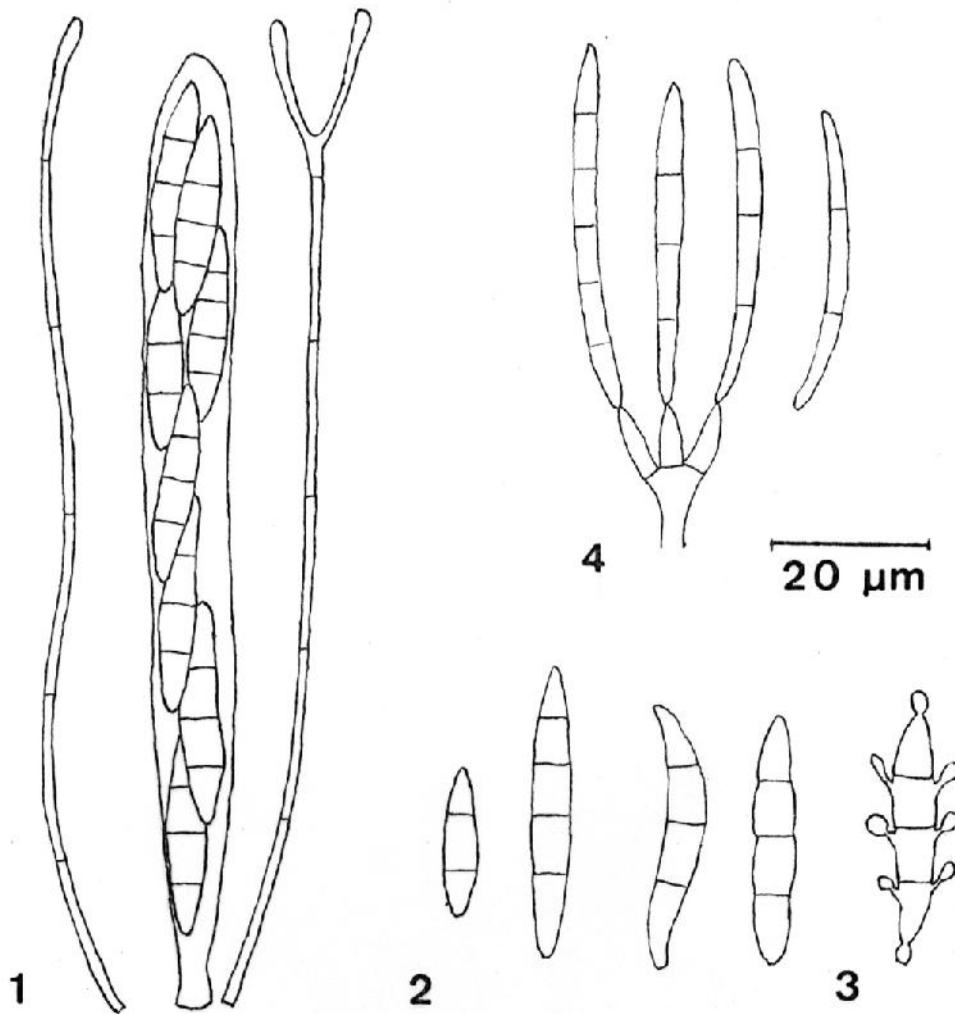
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Figures 1-4: *Gremmeniella balsamea*. Fig. 1. Ascus and paraphyses. Fig. 2. Ascospores. Fig. 3. Ascospore with secondary spores. Fig. 4. Conidia.

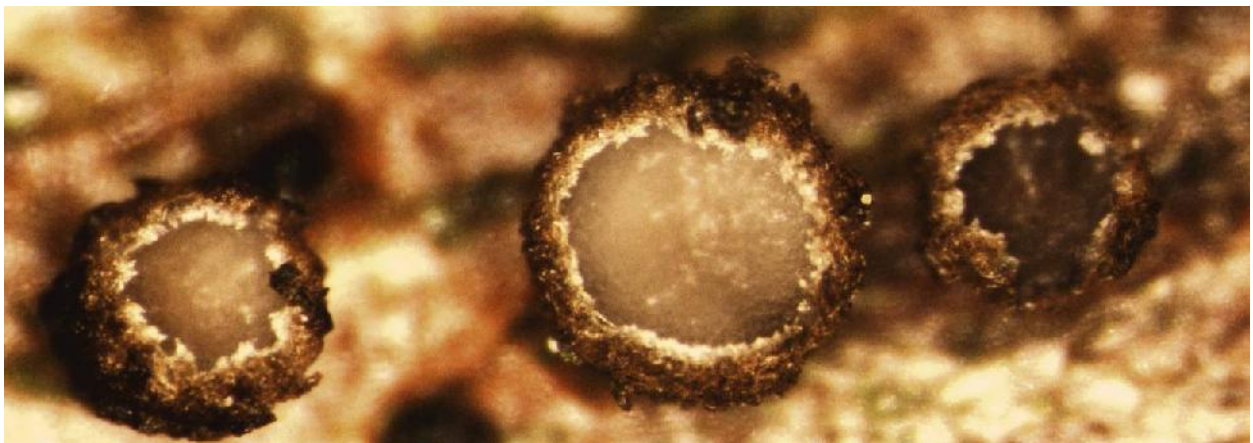


Figure 5: Apothecia of *Gremmeniella balsamea* on a twig of *Abies balsamea*.

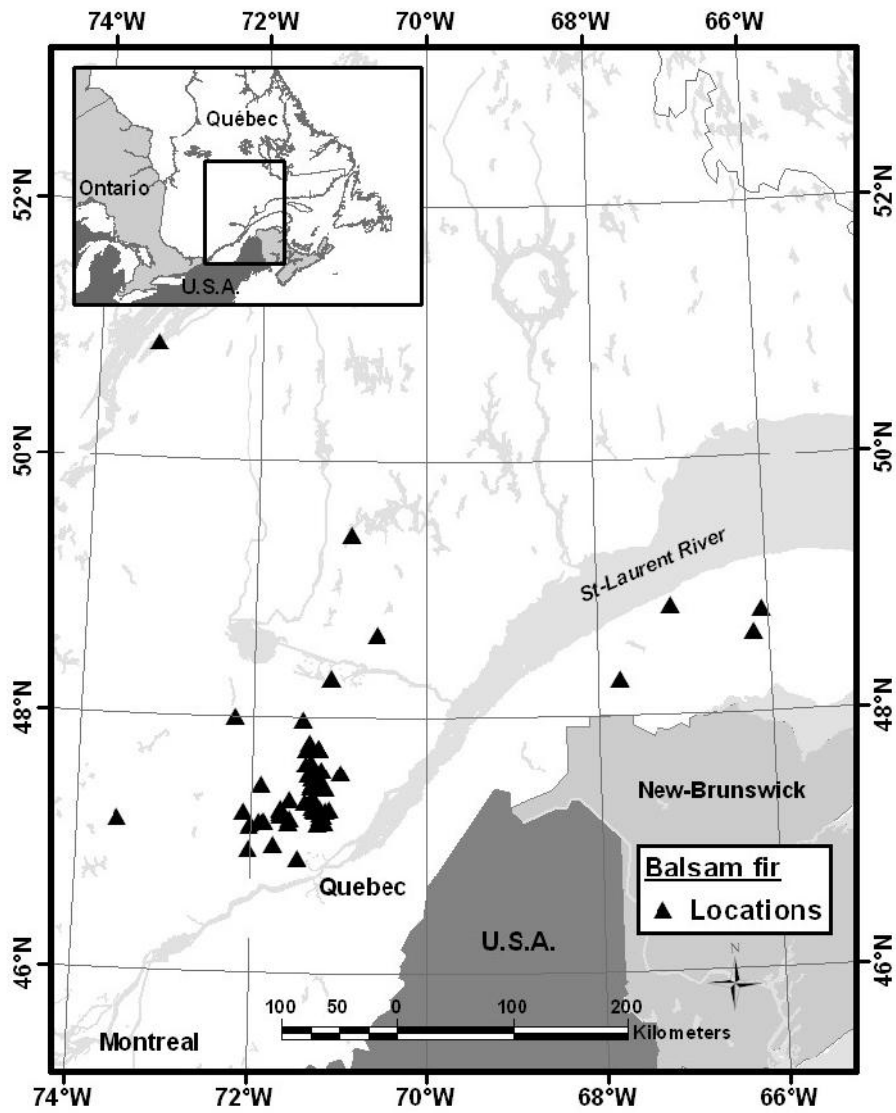


Figure 6: Known distribution of *Gremmeniella balsamea* in Canada (Map by J. Morissette).

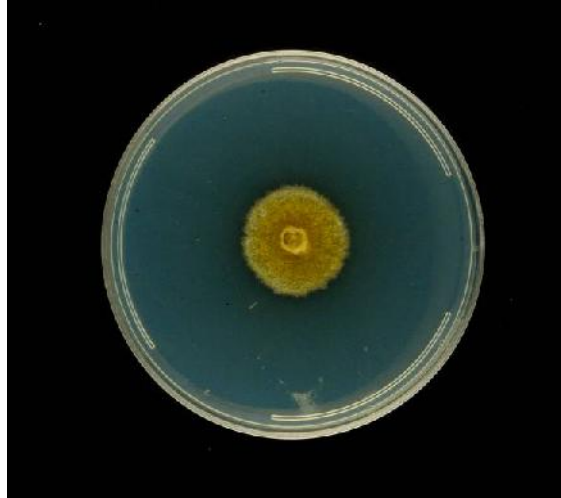


Figure 7: Culture of *Gremmeniella balsamea*, isolate 79-620, on PDA medium after 6 weeks of growth.