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First report of *Nemania serpens* var. *hydnicola* in Canada, and production of the teleomorph in culture

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Abstract: *Nemania serpens* (Pers.: Fr.) S.F. Gray var. *hydnicola* (Schwein.) Y.-M. Ju & J.D. Rogers is reported for the first time in Canada, fruiting on a decaying *Fomitopsis pinicola* sporocarp collected in Victoria, BC. Freshly ejected ascospores were germinated in culture, and produced both a *Geniculosporium* anamorph and the teleomorph on oatmeal agar. The isolate failed to produce teleomorphic stromata on scratch malt extract medium.

Key words: *Nemania serpens* var. *hydnicola*, *Geniculosporium serpens*, Xylariaceae, *Fomitopsis pinicola*, oatmeal agar culture, fungicolous fungi, British Columbia, Canada.

Introduction: The genus *Nemania* Gray was recently monographed by Ju and Rogers (2002), and is distinguished from *Hypoxylon* Bull. by dull – coloured carbonaceous pulvinate stromata with interiors that are initially soft and whitish, no release of coloured pigments in 10% potassium hydroxide, ascospores without gelatinous sheaths, and a *Geniculosporium* Chesters & Greenh. anamorph. The type species of the genus, *Nemania serpens* (Pers.:Fr.) S.F. Gray, previously known to most mycologists as *Hypoxylon serpens* (Pers.: Fr.) J. Kickx fil., is described in detail by Ju and Rogers (2002). *Nemania serpens* var. *hydnicola* (Schwein.) Y.-M. Ju & J.D. Rogers differs from the typical variety in that its ascus rings are amyloid, the ascospores have abruptly narrowed or "pinched" ends, and it is frequently fungicolous on *Fomitopsis pinicola*. It is known from Norway, Sweden, and Finland [Granmo et al., 1999, as *N. reticulata* (P. Karst.) Granmo], and was collected in France in 2004 (Fournier and Magni, n.d.). It is also represented in North America by two herbarium specimens from New Jersey and North Carolina, but these were collected on unusual hosts (*Rhus toxicodendron* L. and *Hydnum* sp. respectively), over 100 years ago (Ju and Rogers, 2002).

In the spring of 2006, a freshly picked *F. pinicola* conk was brought to the author for identification by the co-collector James Brannigan, who later placed it on a partially shaded rocky outcrop covered by a thick layer of moss near their home. More than a year later, the author flipped the rotting conk over, and discovered dark pyrenomycete stromata on its hymenial layer. Based on morphological features of the stromata, asci, and ascospores, plus the unusual substrate, the pyrenomycete was identified as *Nemania serpens* var. *hydnicola*. A culture of the fungus was initiated from germinating ascospores, and resulted in a *Geniculosporium* anamorph. The culture, described below, also produced the teleomorph.

Materials and Methods: Microscopic observations were made with a Nikon Optiphot II compound microscope equipped with differential interference contrast (DIC) optics. Microscopic measurements and photomicrographs were taken from material mounted in water, and measured at 400x. All photographs were taken with a Nikon Coolpix 4500 digital camera. A drop of Melzer's reagent (1.5 g potassium iodide, 0.5 g iodine, 20 g chloral hydrate saturated solution, dissolved in 20 ml distilled water) was added to water mounts of asci to determine if the apical rings stained blue. Cultures were grown on scratch malt extract agar [herein abbreviated as "SME", made with 20 g malt extract, 15 g agar, 1000 ml water, slightly modified from the formulation developed by Kenerley and Rogers (1976), which called for 17.7 g agar] and Difco oatmeal agar (OA), in 100 mm x 15 mm polystyrene Petri plates. To initiate cultures, freshly ejected ascospores were removed from the stromatal surface with fine-tipped forceps wetted with sterile distilled water. The resulting droplet of ascospore suspension was placed in the centre of the plate, and then streaked over the agar surface with a sterile bacterial loop. All cultures were initiated on April 12, 2007, and incubated at 20° C with 12 h alternating fluorescent light and darkness, supplemented by natural daylight from a nearby window.

Results: The stromata fruiting on the *F. pinicola* conk (Fig. 1) closely match descriptions in the literature by Granmo et al. (1999), differing only in that the perithecial mounds are distinct rather than faint (Fig. 2). Masses of ascospores crusted on the surface of the stroma appeared black or slightly greenish under standard indoor fluorescent lighting, but under intense light used to illuminate the dissecting microscope stage, they had a striking greenish iridescence (Fig. 3). Ascus apical rings were nearly square when viewed from the side, and stained blue in Melzer's reagent (Fig. 4). Individual ascospores were ellipsoid-inequilateral, brown, often with

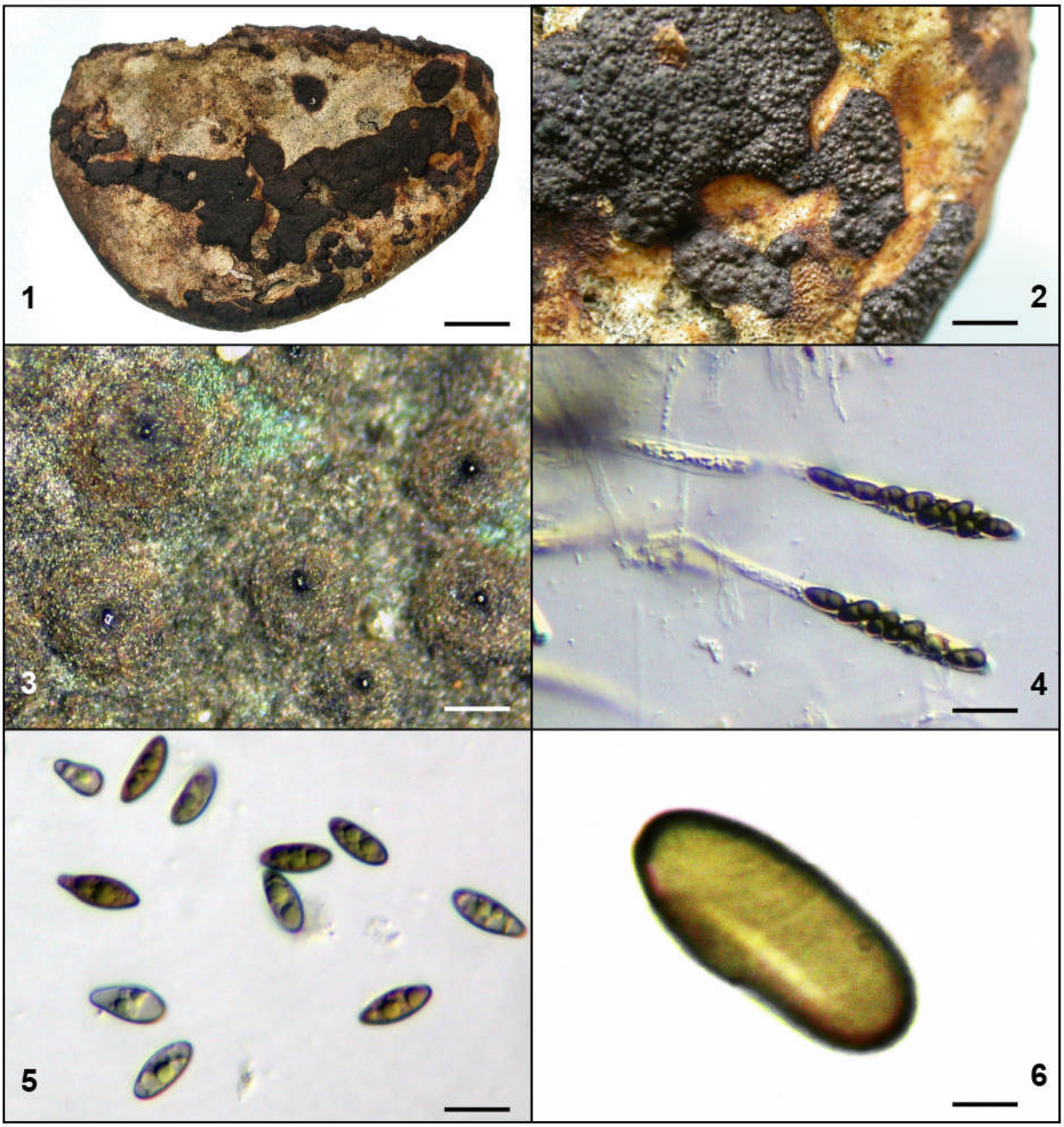
"pinched ends" (Fig. 4), ranged in size from 10-12 x 4-5 µm, and bore an easily visible germ slit that extended approximately half the length of the spore on the least convex side (Fig. 6).

Cultures were initiated from ascospores on April 12, 2007. Ascospores germinated within 4 days after streaking onto SME plates. Colonies on SME were diffuse, at first hyaline, then pale tan, covered with sparse cottony surface hyphae bearing conidiophores typical of *Geniculosporium serpens*. Colonies developed small dark globose knots, ca. 1 mm in diameter, composed of thick-walled hyphae, but did not produce mature perithecia.

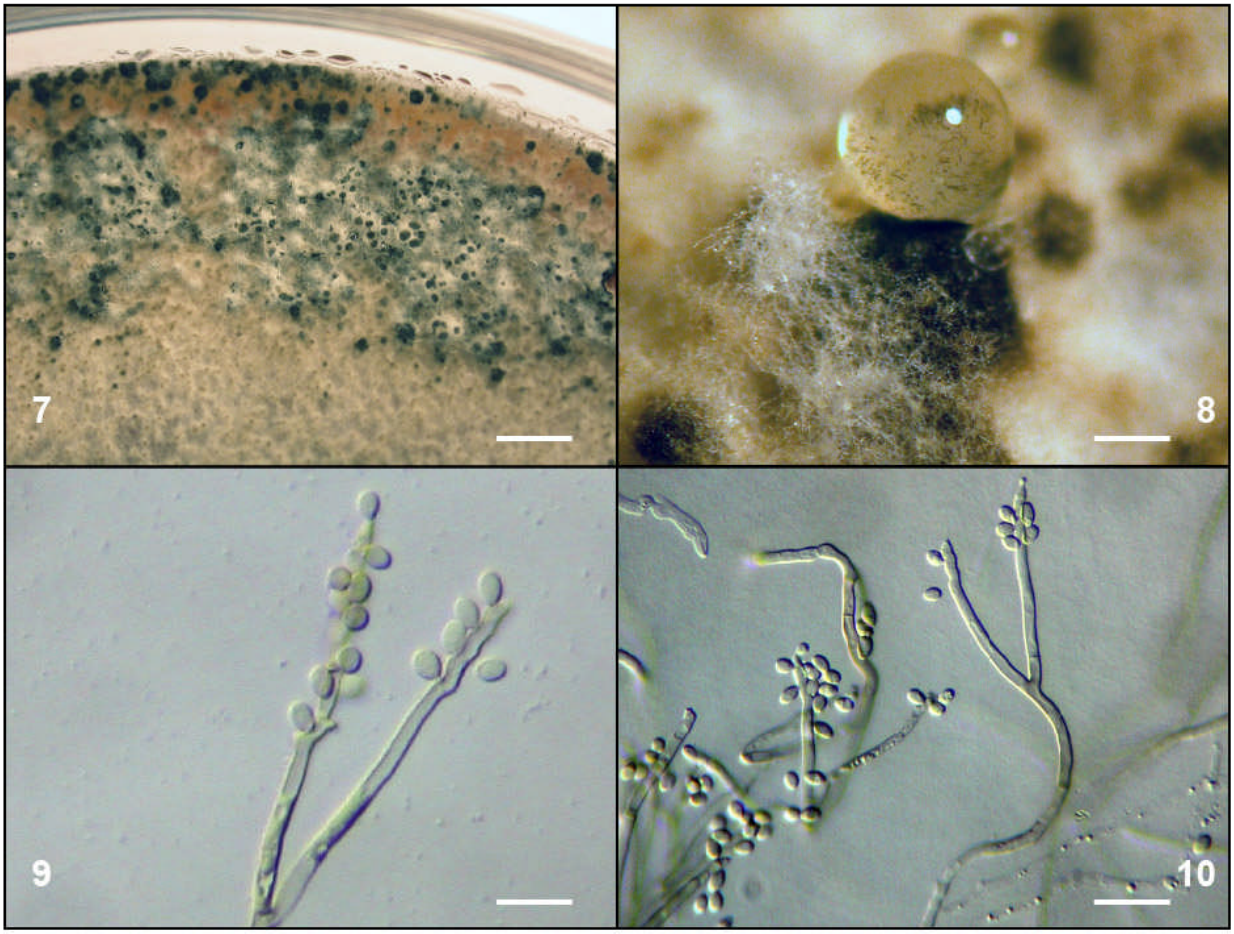
Colonies were transferred on to OA on June 21, which promoted more vigorous growth and a denser layer of surface hyphae. The colony surface was at first felty, white, then became more coarsely floccose at the centre and darkened to tan or olivaceous-grey. The anamorph was readily found in these cultures, but was not dense enough to have given them a dusty appearance. Agar viewed from the underside of the Petri dish stained grayish pink (dusky rose) after a few days, eventually darkening to a deep gray. The colony surface reached the edge of the Petri plate (9 cm diameter) after 14 days of growth and small hemispherical perithecial mounds started to differentiate, often topped with a dot of coarser dark hyphae. These mounds were initiated over the entire colony surface, but only matured at the periphery of the Petri plate (Fig. 7). Colonies occasionally developed olivaceous to brownish grey dusty wedge-shaped sectors where conidia sporulated more profusely. These sectors did not produce perithecial initials. Ascus initials were first observed on August 12, and by August 20, had elongated to approximately 1/3 mature size, with tips staining blue in Melzer's reagent. Mature ascospores were first observed in culture on September 10, 2007, 82 days after the cultures were initiated. There was no evidence of forcible ascospore ejection in culture. The asci were observed to be extruded intact within a moisture droplet at the apex of each perithecium (Fig. 8). Dimensions of ascospores in culture were (12-) 13-14(-18) x 4-5(-6) µm, noticeably larger than ascospores obtained from stromata on the natural substrate. The ascospores from culture were also frequently malformed, with elongated ends (Fig. 5). The *Geniculosporium* anamorph produced conidiophores with terminal or intercalary geniculated conidiogenous regions reaching from 30 -40 µm in length in young (2 week) cultures, but exceeding 200 µm in older cultures before collapsing prior to perithecial development. Conidiogenous regions bore hyaline obovoid conidia ranging from 2-6 x 2-3 µm. The conidia had flattened bases at the former point of attachment to the conidiogenous cell.

Material examined: *Nemania serpens* (Pers.: Fr.) S.F. Gray var. *hydnicola* (Schwein.) Y.-M. Ju & J.D. Rogers: **Canada**. British Columbia: Highlands District, Victoria BC. 681 Blacktail Road. 48° 30' 26.13" N, 123° 30' 53.5" W, April 12, 2007, James Brannigan and Brenda Callan, DAVFP 29311, on the pore layer of a rotting *Fomitopsis pinicola* (Sw.:Fr.) P. Karst. conk placed hymenium-side down on a mossy shaded rock outcrop approximately one year earlier. The *F. pinicola* conk came from a nearby decaying *Pseudotsuga menziesii* (Mirb.) Franco log; DAVFP 29319. B.Callan, Dec. 1, 2007, dried OA culture initiated from DAVFP 29311, bearing telemorphic stromata. A culture initiated from DAVFP 29311 is also deposited in the Pacific Forestry Centre Herbarium culture collection, No. 69.

Discussion: The dull colour and cryptic and unusual habitat of this pyrenomycete has doubtless contributed to the infrequency of its detection. The specimen described herein is the first North American collection on a polypore host, which is the most common substrate for



Figs. 1-4. *Nemania serpens* var. *hydnicola* from natural substrate (DAVFP 29311). All photomicrographs were taken using DIC unless otherwise noted. Fig. 1. Hymenium of *Fomitopsis pinicola* bearing perithecial stromata. Bar = 1.2 cm. Fig. 2. Stromata enlarged to show perithecial mounds. Bar = 0.43 mm. Fig. 3. Perithecial ostioles on a freshly collected stroma, with nearby dried masses of ascospores (iridescent green areas). Bar = 0.25 mm. Fig. 4. Asci with apical ring stained blue by Melzer's reagent, bearing mature ascospores. Bar = 14 µm. Fig. 5. Ascospores from OA culture illustrating irregular shape and pinched ends. Bar = 11 µm. Fig. 6. Ascospore from DAVFP 29311 with germ slit evident (Brightfield). Bar = 1.8 µm.



Figs. 7-10. *Nemania serpens* var. *hydnicola* on OA. All photomicrographs were taken using DIC. Fig. 7. 82-day-old colony on OA with mature perithecia evident at the periphery. Bar = 4 mm. Fig. 8. Single mature perithecium extruding asci and ascospores in a droplet of liquid at the ostiole. Bar =0.3 mm. Figs. 9,10. *Geniculosporium* anamorph from young cultures. Fig. 9 Bar = 7.5 µm. Fig. 10 Bar = 15 µm.

this fungus in Europe. It is the first Canadian record, and also represents the first link of a naturally occurring collection of this variety to a teleomorph-producing culture. Until now, agar cultures from only three *Nemania* species fruiting on natural substrates have been induced to produce the teleomorph: *N. gwyneddii* (Whalley, R. L. Edwards & S. Francis) Pouzar, *N. minutula* (Penz. & Sacc.) Y.-M. Ju & J. D. Rogers, and *N. maritima* Y.-M. Ju & J. D. Rogers. *Nemania serpens* var. *hydnicola* is easily differentiated from these taxa by ascospore size, culture morphology, and substrate (Ju and Rogers, 2002). The *N. serpens* var. *hydnicola* isolate appears to be distinct from the three *N. serpens* isolates obtained from soil and plant tissue (as endophytes) which produce teleomorphs in culture (Petrini and Rogers, 1986). These isolates were not derived from stromata produced on natural substrates in nature, and unlike them, the var. *hydnicola* isolate failed to produce mature perithecia on SME, and took several months, instead of a few weeks, to produce ascospores in perithecia on OA. It would be interesting to compare growth and morphology of all four isolates on OA, and also compare DNA sequences to determine how closely they are related to each other.

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Literature cited

Ju, Y.-M., and J.D. Rogers. 2002. The genus *Nemania* (Xylariaceae). Nova Hedwigia 74: 75-120. http://dx.doi.org/doi:10.1127/0029-5035/2002/0074-0075

Fournier, J., and J.-F. Magni. n.d. *Nemania serpens* var. *hydnicola*. In: Pyrenomycetes from southwestern France. http://pyrenomycetes.free.fr/nemania/html/N_hydnicola.htm

Granmo, A., T. Læssøe and T. Schumacher. 1999. The genus *Nemania* s.l. (Xylariaceae) in Norden. Sommerfeltia 27: 1-96.

Kenerley, C.M., and J.D. Rogers. 1976. On *Hypoxylon serpens* in culture. Mycologia 68: 688-691. http://dx.doi.org/doi:10.2307/3758993

Petrini, L.E., and J.D. Rogers. 1986. A summary of the *Hypoxylon serpens* complex. Mycotaxon 26:401-436.