Supplementary observations and notes on microepiphytes

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The discovery of *Hyphodiscus* sp. as both an endophyte and an apparent saprotroph producing apothecia on healthy *Picea* twigs suggests additional endophytes may also sporulate on live twigs or branches. A fungus tentatively identified as *Pocillopycnis* sp. (NB-531) producing falcate conidia from pycnidia on *Picea rubens* twigs was isolated and resulting ITS sequences show it is closely related to sequences of two unidentified *Picea mariana* isolates accessioned in GenBank [KT289599: Identities = 568/575 (99%), gaps = 3/575 (0%); DQ979632: Identities = 565/574 (98%), gaps = 3/574). This close phylogenetic relationship suggests that some endophytes may also exist as epiphytes (e.g.: Osono and Mori 2004), although studies typically show a distinction between epiphyte and endophyte communities (Legault et al. 1989, Santamaría and Bayman 2005, Kharwar et al. 2010, Osono 2014).

Living *Picea* branches provide a rich habitat for microepiphytic fungi and a substrate or surface for itinerant spores (Supplementary Figure S1). Conidia, fruiting bodies, or thalli of cf. *Acremonium* sp., (twigs, petioles, needles), *Aureobasidium pullulans* (twigs, trichomes, petioles, needles), *Cladosporium* spp. (twigs, needles), *Dictyopolyschema pirozynskii* (twigs, trichomes, petioles), *Epicoccum nigrum* (twigs, petioles, needles), cf. *Phaeomoniella* sp. (trichomes), *Pocillopycnis* sp., *Pseudophacidium piceae* (twigs, trichomes, petioles), *Metacapnodium* sp. (twigs), *Setomelanomma holmii* (twigs), *Seuratia millardetii* (twigs), *Stigmina lautii* (needles), and various unidentified coelomycetes and hyphomycetes were frequently observed on living *Picea* branches. Isolation of *D. pirozynskii*, cf. *Phaeomoniella* sp., and several other unidentified fungi was unsuccessful. An unknown coelomycete (NB-568) found

on living *Picea rubens* twigs often in association with algae on twig and petiole surfaces was isolated and sequences show it is closest related to *Anungitea eucalyptorum* (incertae setae, Sordariomycetes) based on ITS sequences, but could not be satisfactorily placed within a genus [NR132904: Identities = 547/593 (92%), gaps = 14/593 (2%)] and LSU [KJ869176: Identities = 853/867 (98%), gaps = 2/867 (2%)]. In New Brunswick, microepiphytes, especially algae and *Seuratia millardetii*, were more abundant on *Picea rubens* needles in humid microclimates such as low depressions or in coastal boreal rainforests (Clayden et al. 2011).

Microepiphytic fungi and algae on perennial substrates such as conifer needles accumulate over time. For example, Peveling et al. (1992) reported that the microepiphyte layer on *Picea abies* needles was <30 µm thick and covered <10% of the total surface in 1-year- old needles and increased to 90–130 µm thick covering 65–80% of the surface in 4-year-old needles. Søchting (1997) found that the microepiphyte crust on *Picea abies* needles reached a maximum thickness after the first three years then remained stable, similar to the findings of microbial biomass increasing with needle age for first three years in *Pseudotsuga menziesii* needles (Carroll 1979). Microepiphyte biomass may be a more significant carbon source than realized, for example Søchting (1997) estimated that microepiphyte biomass represented 11.6% of *Picea abies* needle weight in Denmark. Carroll (1979) estimated that the microbial cell volume of a single *Ps. menziesii* tree was 1093 cm^3 and extrapolated the biomass to 38–60 kg at the stand level. Needle and twig epiphytes might account for approximately 600 kg/ha and 300 kg/ha, respectively,

per year in primary production that would otherwise be unaccounted for in conventional carbon-budget analyses (Carroll 1979, Carroll et al. 1980). Fungal biomass in turn provides a food resource for grazing mycophagous invertebrates such as collembola, oribatid mites, and nematodes, which were also commonly observed on living twig and needle surfaces in this study.

Microepiphytic fungi can obtain nutrients from a variety of sources including parasitism of co-occurring microorganisms and invertebrates, degradation of dead organic material that accumulates on the needle surface over time, excreta from invertebrates including honeydew from Hemiptera, organic and inorganic exudates dissolved in leachates and through fall from the above canopy, and plant exudates (Stadler and Müller 1996, Søchting 1997, Søchting and Sutton 1997, Stadler and Müller 2000). Microepiphytic fungi may in turn benefit the host plant by reducing disease and pest incidence and severity through parasitism, competition for resources, and production of bioactive secondary metabolites, and by playing an important role in nutrient cycling and concentrating dilute organic nutrients (Stone and Carroll 1996). The combined positive effects might compensate for their potential reduction in host photosynthetic capabilities (e.g.: 4–10 % in colonized *Picea abies* needles; Burg 1990).

Several taxa observed on *Picea* in Eastern Canada were also reported in other studies of conifer needle and twig epiphytes, including acremonium-like species, *Aureobasidium pullulans*, *Cladosporium*, *Epicoccum*, *Metacapnodium*, and *Seuratia millardetii* (Sherwood and Carroll 1974, Carroll 1979, Carroll et al. 1980, Legault et al. 1989, Ranta 1990). Most commonly observed epiphytic species, such as

Aureobasidium pullulans and *Cladosporium*, are not host specific but are found on various plant surfaces or litter, although some species, such as *Dictyopolyschema pirozynskii* and *Metacapnodium* sp., appeared to have narrower host and substrate preferences. Microepiphyte distribution is not random; for example coverage is usually denser on the upper needle surfaces and stomata zones and midrib grooves of *Ps. menziesii* needles accounted for more than 70% of the total microbial cell volume (Carroll 1979). Trichomes were frequently colonized by fungi different than those occurring on twigs,menziesii needles accounted for more than 70% of the total microbial cell volume (Carroll 1979). Trichomes were frequently colonized by fungi different than those occurring on twigs,substrate preferences. Microepiphyte distribution is not random; for, example coverage is usually denser on the upper needle surfaces and stomata zones and midrib grooves of *Ps. menziesii* needles accounted for more than 70% of the total microbial cell volume (Carroll 1979). Trichomes were frequently colonized by fungi different than those occurring on twigs, suggesting some substrate preference and a possible source for novel diversity (e.g.: Pereira-Carvalho et al. 2009).

Epiphytes observed in this study provided examples of named-but-unsequenced or putatively undescribed taxa and a close relative to an unidentified *Picea mariana* endophyte (*Pocillopycnis* sp.). Future endeavors should include epiphytic fungi associated with *Picea* twigs and needles to generate cultures, reference sequences, and potential endophyte connections. Microepiphytes in Canadian conifer forests are overlooked despite their important ecological roles in mature forests

(Stone and Carroll 1996); describing epiphyte diversity will provide a better understanding of epiphyte communities and function. Generating epiphyte reference sequences will also provide tools to better compare epiphyte and endophyte communities using next generation sequencing (NGS) and identify possible contamination sources in endophyte studies.

Fig. S1. Fungi commonly encountered on living *Picea rubens* twigs. A. Dead aphid colonized by *Lecanicillium attenuatum*. B. Immature Mycosphaerellaceae sp. perithecia. C–E. *Dictyopolyschema pirozynskii* on trichomes. F. cf. *Phaeomoniella* sp. on trichome. G–H. *Mzetacapnodium* sp. I. Unknown conidia, possibly associated with *Metacapnodium* sp. J. *Prosthemium* sp. conidium probably originating from nearby *Betula* trees. K–L. *Monodictys* sp. on petiole. M. Overmature fruiting bodies. N. *Setomelanomma* cf. *holmii* perithecia. O–P. *Stigmina lautii* on stomata. Q. *Pseudophacidium piceae* ascoma on petiole of dead needle. R. *Rhizosphaera* sp. pycnidia erumpent through stomata and bearing waxy stomatal plugs. S–T. *Phyllactinia* cf. *guttata* chasmothecia probably originating from nearby *Betula* trees. Scale bars: D–F, L = 10 µm.