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Dedicated to Professor Barbara Gumińska on the occasion of her eighty-fifth birthday

Robillarda sessilis, a rare coelomycete isolated from Scots pine seedlings

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A coelomycete with appendage-bearing conidia, *R. sessilis*, was isolated three times from stems of living healthy *Pinus sylvestris* seedlings of the 1st year growing in a nursery in central Belarus. Macroscopic and microscopic morphology of the fungus in culture is described.

Key words: Belarus, nursery, *Pinus sylvestris*, stem

INTRODUCTION

Robillarda sessilis (Sacc.) Sacc. was found during studies of the fungi inhabiting stems of healthy *Pinus sylvestris* seedlings in a sample nursery in central Belarus. This species is a remarkable fungus with conidia bearing three long hair-like appendages. It was selected as a generic type for *Robillarda*, an anamorphic genus with unknown teleomorph (Nag Raj 1993). Altogether 35 species were published under this generic epithet, 12 of which were referred, according to Nag Raj (1993), to seven other genera.

MATERIAL AND METHODS

The inventory of the fungi was carried out by sampling plants in 1 m^2 plots on a 800 m^2 plantation situated in central Belarus. A first sample (taken 2 VI 2009) included 5 well-developed seedlings from a single plot located in the center of plantation. A second sample (taken 22 VI 2009) included 25 equally well-developed seedlings collected from 5 plots (5 plants per plot). Four plots were situated near corners of

the plantation, one plot in the plantation center (this central plot position did not coincide with the plot in previous sampling).

For isolation of the fungi, the seedlings were washed 3 min under a strong stream of tap water on a sieve. Each seedling stem, from the root neck to the foot of leaf whorl, was cut using a sterile razor blade into *ca* 2 mm long segments. The segments were put on malt extract agar (MEA: 1% malt extract, Amresco, USA, and 1.5% agar) with addition of tetracycline 30 μg/mL (Amresco), and incubated for 10 days at 26°C. For the control that the propagules did not originate from tap water, 1.5 mL of the water was poured in Petri dish with solid 2% MEA, in 3 replicates. Mycelia growing from stem pieces and producing pycnidia, were transferred for storing on MEA slants under mineral oil. For describing cultural morphology, isolates were taken from storage and cultured on MEA (2% malt extract, 1.5% agar) at 26°C in the dark.

For describing micromorphology, preparations were mounted in 3% KOH water solution. Dry cultures were deposited in V.F. Kuprevich Institute of Experimental Botany Herbarium (MSK-F).

RESULTS

It the first sample from the plantation center (2 VI 2009), one isolate of *R. sessilis* was obtained. In the second sample (22 VI 2009) two isolates were obtained from 2 of 25 studied plants, one from central, one from a corner plot. The morphological diagnosis of *R. sessilis*, based on cultures, is given below.

Morphological description. After 1 week: *mat* 25 mm in diam, more or less rounded, colorless, low felty, near 1 mm high, more or less fasciculate or tufted in periphery zone and just near the point of inoculation; *margin* somewhat wavy, abrupt, with scarce cilia-shaped hyphae; *reverse* pale apricot yellow. After 2 weeks: *mat* about 50 mm in diam, very pale pinkish cream, in central and middle zone floccose-felty; periphery zone clearly delimited, *ca* 10 mm wide, low velvety, with slight radial depressions; *margin* without aerial growth, transparent; *reverse* in central and middle zone brownish yellow with orange tint, with blackish or dark olive spots due to the presence of conidiomata. *Conidiomata* occurring about 1.5–2 weeks post inoculation, especially developing when the medium dries up, in mat periphery and middle zone scattered, but at the border between these zones abundant and often aggregated, forming a ring, having dark brown color in reverse (Fig. 1).

Aerial hyphae moderately branched, somewhat wavy, but with even walls, smooth, hyaline or subhyaline, narrower ones (2-3.5 μm) long-celled and small-guttulate, wider ones (3.5-7.7 μm) with short swollen cells and rich-guttulate; thin lateral branches 0.8-1 μm wide, tapering. *Repent and immersed hyphae* moderately branched, with short more or less swollen cells, rich-guttulate, often disintegrating at septa, 1.7-8.7 μm wide. *Marginal hyphae* rather richly branched, divided into axial hyphae and their lateral branches; axial hyphae 4.3-8.2 μm wide, with short, more or less swollen, somewhat guttulate cells; lateral branches 3-4 μm wide, having blunt apices 2-3.2 μm wide (Fig. 2).

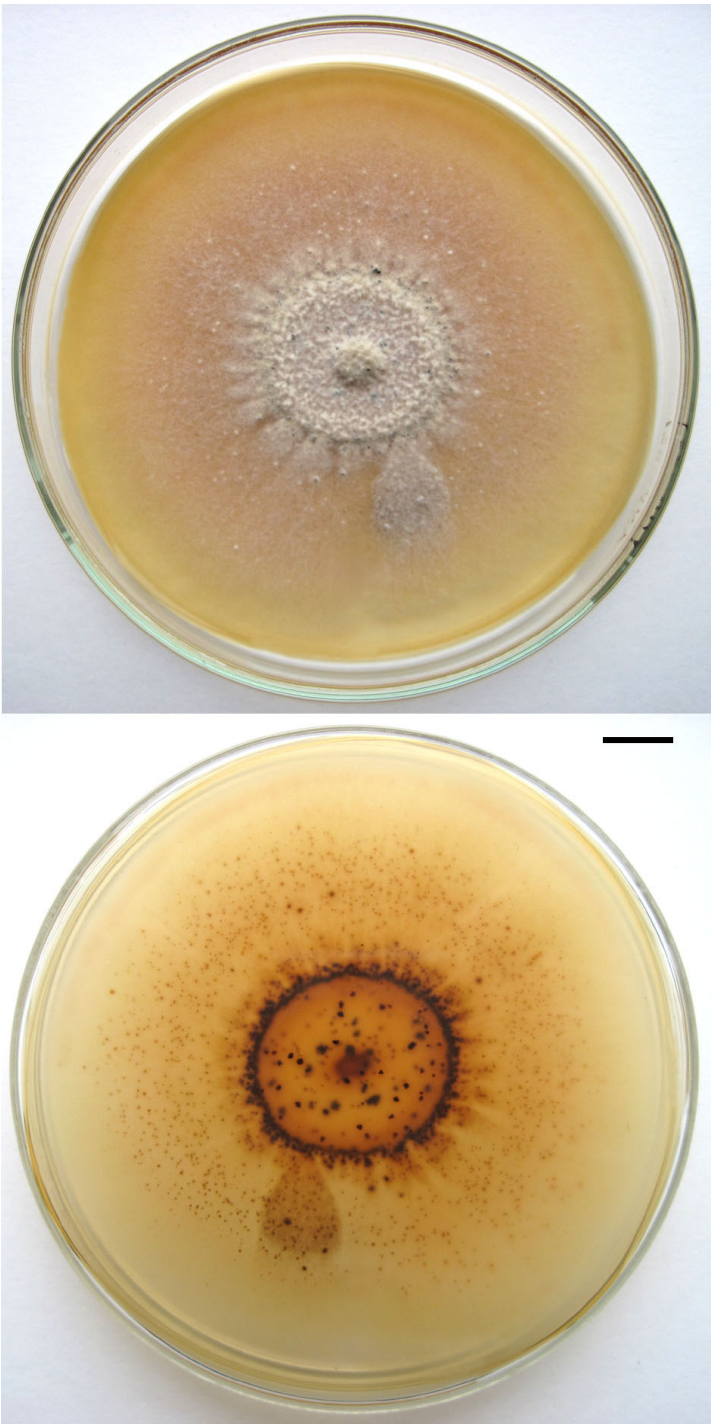

Fig. 1. Two-weeks old culture of *Robillarda sessilis* on 2% MEA: face of the mat (above) and reverse (below). Scale bar = 1 cm.

Conidiomata pycnidioid, solitary, 100-150(-500) μm in diam, or aggregated 3-10 and more and partly confluent (aggregations to 1-1.5 mm in diam), globose or irregular, flattened when large, fully or partly immersed, black, glabrous, glossy, with uneven surface, ostioles not visible; conidiomatal wall *ca* 25-35 μm thick, of pseudoparenchymatic cells, outer layer dark brown, 7-10 μm thick, mainly of *textura angularis* (partly also of narrow *textura prismatica*) with somewhat thick-walled cells 4-13(-17.5)×(1.5-)2.2-7 μm, turning into a hyaline inner layer of thin-walled and guttulate cells 7-30×2.5-5 μm. *Conidiophores* reduced, mostly one-celled, in shape of somewhat elongated conidiogeneous cells, originating from pseudoparenchyma lining the inner surface of the locule and indistinctly differring from pseudoparenchymatic cells. *Conidiogeneous cells* ampulliform to subcylindrical, hyaline, thin-walled, smooth, 6-11×2-3 μm. *Conidiogenesis* holoblastic with limited sympodial proliferation (sometimes two conidia simultaneously developing on the same cell); *conidia* originating at apices of conidiogeneous cells, often sitting on very small protuberances or denticles, at early developmental stages without appendages; in a layer with many developing conidia, appendages situated parallel or divergent at a very sharp angle. *Conidia* 2-septate, consisting of a two-celled conidium body and an apical cell with appendages; conidium body medianly 1-euseptate, fusiform, straight or a little curved, often very little constricted at the septum, basally more or less truncated, subhyaline to pale brown, usually yellowish, smooth, thin-walled, 9.3-12(-14.2)×2.7-3.5 μm; apical cell hyaline, very thin-walled, short cylindrical basally for 1-2 μm, divided into 3 hyaline, smooth, hair-like, divergent, straight or curved, more or less apically attenuated appendages 17-26 μm long and 0.5-0.6 μm wide in the middle part; older conidia in preparation often loosing apical cell (Fig. 3).

Substrata and general distribution. *R. sessilis* was documented from quite variable hosts and substratum types: on stems (bark), dead branches, leaves (causing leaf spot), seeds of *Bischofia*, *Cocos*, *Ficus*, *Fragaria*, *Fumana*, *Ludwigia*, *Magnolia*, *Paeonia*, *Quercus*, *Randia*, *Rosa*, *Rubus*, *Vitis*. The fungus has been recorded in Europe (Hungary, Italy), Asia (India), North America (USA), Caribbeans, Africa (Angola), and on the plant material imported from Japan. The majority of records are from India (Nag Raj 1993). In 1970 this species was described from *Northea* seeds imported to St-Petersburg, Russia, from Indonesia, under the name *Mycohypallage northeae* (Melnik 1970). Gasich (1995) collected this fungus in Saratov oblast, Russia, where it caused leaf spot disease of *Eryngium* (see also Melnik 1997). So far, five localities, including the type locality in Italy and our find, but excluding the find on imported seeds, are know from Europe today. In Nebraska, USA, *R. sessilis* was collected on *Pinus ponderosa*, but details about the age of the plants or the kind of organs are unknown (Nag Raj 1993). The fungus was also isolated from soil in Australia (Matsushima 1989) and from a soil sample collected in mountain pine forest in Pakistan (Matsushima 1993).

Our case indicates that *R. sessilis* is capable of infecting stem tissues of healthy, vigorous *Pinus sylvestris* seedlings as either an endophyte or phylloplane component. The fungus can be named more widely 'associated with seedling' because of used method of sterilization. Conidiomata were not observed on this host. Only three plants colonized by the fungus, were detected among 30 ones subjected to cultural experiment, and thus the species can be regarded as rare in the studied plantation.

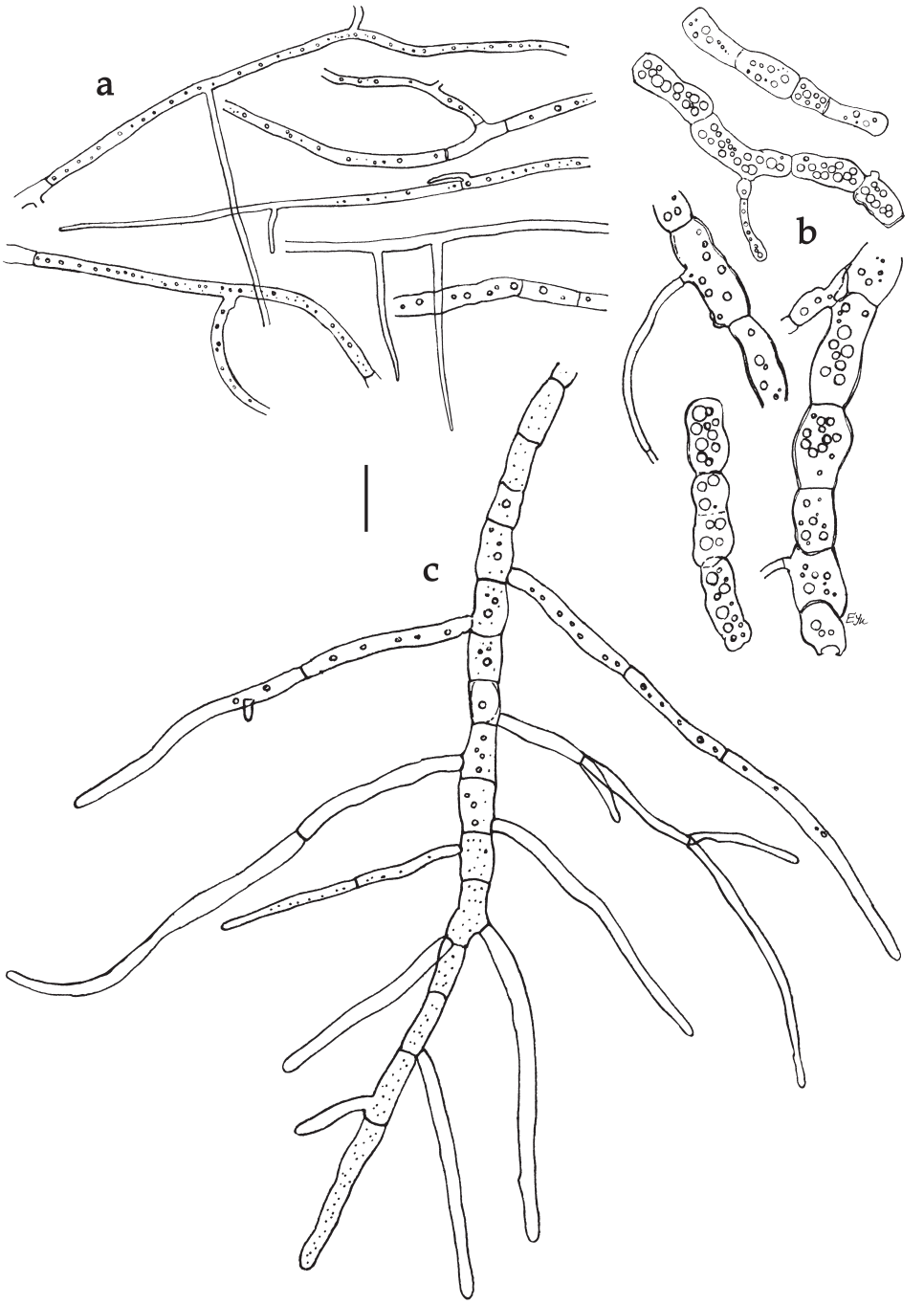

Fig. 2. *Robillarda sessilis* hyphae in culture: a – aerial, b – repent and immersed, c – marginal. Scale bar = 10 μm.

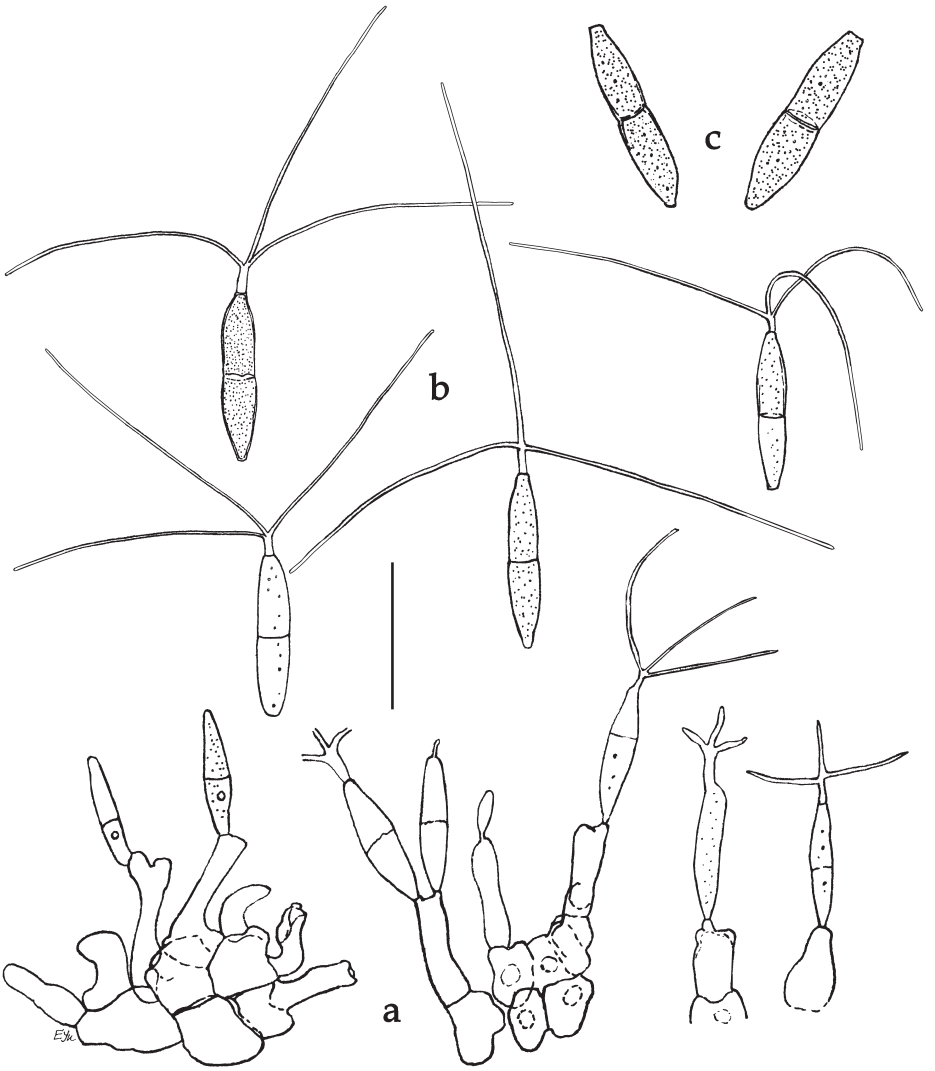

Fig. 3. *Robillarda sessilis*: a – conidiophores and developing conidia, b – mature conidia with appendages, c – conidia that lost apical cell MSK 7065. Scale bar = 10 μm.

Material examined: Belarus, Minsk oblast, Dzyarzhynsk district, near Enerhetykau settlement, Basic Forest Nursery of Belarusian State Technological University, plantation of 1st year *Pinus sylvestris*, coll. E. Yurchenko 2 VI 2009 (MSK 7065); *ibid*., coll. E. Yurchenko 22 VI 2009 (MSK 7138a, b).

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