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Raffaelea lauricola, a new ambrosia beetle symbiont and pathogen on the *Lauraceae*

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Abstract — An undescribed species of *Raffaelea* earlier was shown to be the cause of a vascular wilt disease known as laurel wilt, a severe disease on redbay (*Persea borbonia*) and other members of the *Lauraceae* in the Atlantic coastal plains of the southeastern USA. The pathogen is likely native to Asia and probably was introduced to the USA in the mycangia of the exotic redbay ambrosia beetle, *Xyleborus glabratus*. Analyses of rDNA sequences indicate that the pathogen is most closely related to other ambrosia beetle symbionts in the monophyletic genus *Raffaelea* in the *Ophiostomatales*. The asexual genus *Raffaelea* includes *Ophiostoma*-like symbionts of xylem-feeding ambrosia beetles, and the laurel wilt pathogen is named *R. lauricola* sp. nov.

Key words — *Ambrosiella*, *Coleoptera*, *Scolytidae*

Introduction

A new vascular wilt pathogen has caused substantial mortality of redbay [*Persea borbonia* (L.) Spreng.] and other members of the *Lauraceae* in the coastal plains of South Carolina, Georgia, and northeastern Florida since 2003 (Fraedrich et al. 2008). The fungus apparently was introduced to the Savannah, Georgia, area on solid wood packing material along with the exotic redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (*Coleoptera: Curculionidae: Scolytinae*), a native of southern Asia (Fraedrich et al. 2008, Rabaglia et al. 2006). As in the case of many ambrosia beetles (Beaver 1989, Harrington 2005), *X. glabratus* has mycangial pouches for carrying fungal symbionts, and the redbay pathogen lives as a budding yeast phase within the mycangium (Fraedrich et al. 2008). Spores of the fungal symbiont ooze out of the mycangium and inoculate the

xylem as the adult female constructs her tunnels and lays eggs. The pathogen moves systemically through the vessels of the host and causes a vascular wilt disease similar to Dutch elm disease, which is caused by *Ophiostoma novo-ulmi* Brasier (Fraedrich et al. 2008).

Symbionts of ambrosia beetles are asexual fungi that typically produce small conidiophores in tight clusters (sporodochia) in beetle galleries, and larvae and adults feed on the conidia (Batra 1967). Most ambrosia beetle symbionts have been described in the anamorph genera *Raffaelea* Arx & Hennebert emend. L.R. Batra or *Ambrosiella* Brader emend. L.R. Batra. The two genera were distinguished by sympodial proliferation of the conidiogenous cell in the former, leaving inconspicuous scars along the side, and percurrent profileration, leaving rings at the tip of the conidiogenous cell, in the latter (Batra 1967). However, these distinctions are difficult to discern and are questionable criteria for distinguishing the anamorph genera associated with *Ophiostoma* Sydow & P. Sydow (Gebhardt & Oberwinkler 2005, Harrington 1993), to which most ambrosia beetle symbionts seem to be related (Harrington 2005).

Previously, phylogenetic analyses of rDNA sequences suggested that *Raffaelea* species and some other ambrosia beetle symbionts comprise a monophyletic group arising within *Ophiostoma* (Gebhardt et al. 2005, Jones & Blackwell 1998, Rollins et al. 2001). However, three *Ambrosiella* species, including the type species, *A. xylebori* Brader ex Arx & Hennebert, have phialidic conidial development and are relatives of *Ceratocystis* Ellis & Halst. (Cassar & Blackwell 1996, Gebhardt et al. 2005, Paulin & Harrington 2000). *Dryadomyces* is closely related to the other *Ophiostoma*-like symbionts of ambrosia beetles and appears to fall into the *Raffaelea* "clade," but its conidiogenous cells differ from those of *Raffaelea* spp. because the conidiogenous cells have denticles at the point of conidial dehiscence (Gebhardt et al. 2005). Otherwise, *D. amasae* fits ecologically and phylogenetically with the other *Ophiostoma*-like symbionts (Gebhardt et al. 2005). The monophyletic and asexual genus *Raffaelea* would appear to be the best available name for all ambrosia beetle symbionts with affinities to *Ophiostoma*, at least until the taxonomy of the large and diverse genus *Ophiostoma* is better resolved.

The laurel wilt pathogen was shown by analyses of rDNA sequences (EU123076, EU123077) to occur in the monophyletic group of species with *Raffaelea* and other asexual fungi associated with ambrosia beetles (Fraedrich et al. 2008). The laurel wilt pathogen, like *Ophiostoma* species and related anamorphs, tolerates cycloheximide (Cassar & Blackwell 1996, Harrington 1981). Also, the pathogen produces conidiophores and conidia similar to those of other ambrosia beetle symbionts that are related to the *Ophiostoma* clade (Kubono & Ito 2002). The new species appears to be most appropriately placed in the anamorph genus *Raffaelea*.

Materials & methods

Cultures of the new species were obtained from dead or dying trees or isolated directly from adult females of *X. glabratus.* Representative cultures are maintained in the collection of the senior author. The holotype specimen was deposited in the U.S. National Fungus Collections, USDA, Beltsville (BPI), and the ex-type was deposited in the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Center, the Netherlands (CBS).

Morphological descriptions were made from cultures on malt extract agar (MEA, 1.5% Difco malt extract and 1.5% agar) at 25 C in the dark. Mycelial colors are described using terminology from Rayner (1970). Growth at 10, 15, 25, 30 and 35 C after 10 days was also determined on MEA.

Taxonomy

Raffaelea Arx & Hennebert **emend**. T.C. Harr.
= *Dryadomyces* Gebhardt, Mycol. Res. 109: 693. 2005.

Condiophores single to aggregated in sporodochia, hyaline, unbranched or sparingly branched, one-celled to septate, producing terminal conidia holoblastically. Conidiogenous cells proliferating percurrently or sympodially, leaving denticles, inconspicuous scars or annelations. Conidia small, hyaline, elliptical to ovoidal to globose, slimy, secession schizolytic, producing yeast-like growth through budding. Tolerating cycloheximide in culture. Associated with ambrosia beetles.

Type Species —***Raffaelea ambrosiae*** Arx & Hennebert, Mycol. Mycopathol. Appl. 25: 310. 1965.

Raffaelea lauricola T.C. Harr., Fraedrich & Aghayeva **sp. nov.** Figure 1
MycoBank MB 511590

Coloniae in agaro (MEA) post 10 dies ad 25 C, 65 mm diam, cremae-bubalinae, mucosae. Conidia blastosporae, oblongatae vel ovatae, 3.5–4.5 × 1.5–2.0 µm. Socius cum Xyleborus glabratus.

Holotype—**UNITED STATES. South Carolina**: Hunting Island State Park, from *Xyleborus glabratus*, June 2006, S. Fraedrich, BPI 878183, from isolate C2339 (= CBS 121567).

Maximum growth on malt extract agar at 25 C, colony diameter 60 mm diam. at 25 C, 10 mm diam. or less at 10 and 30 C. Colony at 10 days cream-buff, smooth, but later mucilaginous in the center, margins of colony uneven, side branches of submerged hyphae at advancing front producing conidia and tight clusters of blastoconidia; 2 week old colonies cottony, honey yellow, and with a yeasty odor. Conidiophores hyaline, usually aseptate and unbranched but sometimes septate at branches, terminal or arising as a side branch from

hyphae, variable in length but mostly (8.5)13–60(120) × (l.0)2.0(2.5) µm wide. Conidia produced holoblastically, at the tip of the conidiogenous cell, but not leaving conspicuous scars or annelations, primary conidia oblong to obovoid, sometimes flattened at the point of attachment, hyaline, thin walled, (3.0)3.5–4.5(8.0) × (1.0)1.5–2.0(3.5) µm; budding new cells, the blastospores forming in a cluster at the tip of the conidiophore and a slimy mass over the central part of the colony.

Cultures examined—**UNITED STATES. South Carolina**: Hunting Island State Park, from *Persea borbonia*, September 2005, S. Fraedrich, C2214; **Georgia**: Pembroke, from *P. borbonia,* December 2005, S. Fraedrich, C2245; **Florida**: Fort George Island, from *P. borbonia,* December 2005, S. Fraedrich, C2258.

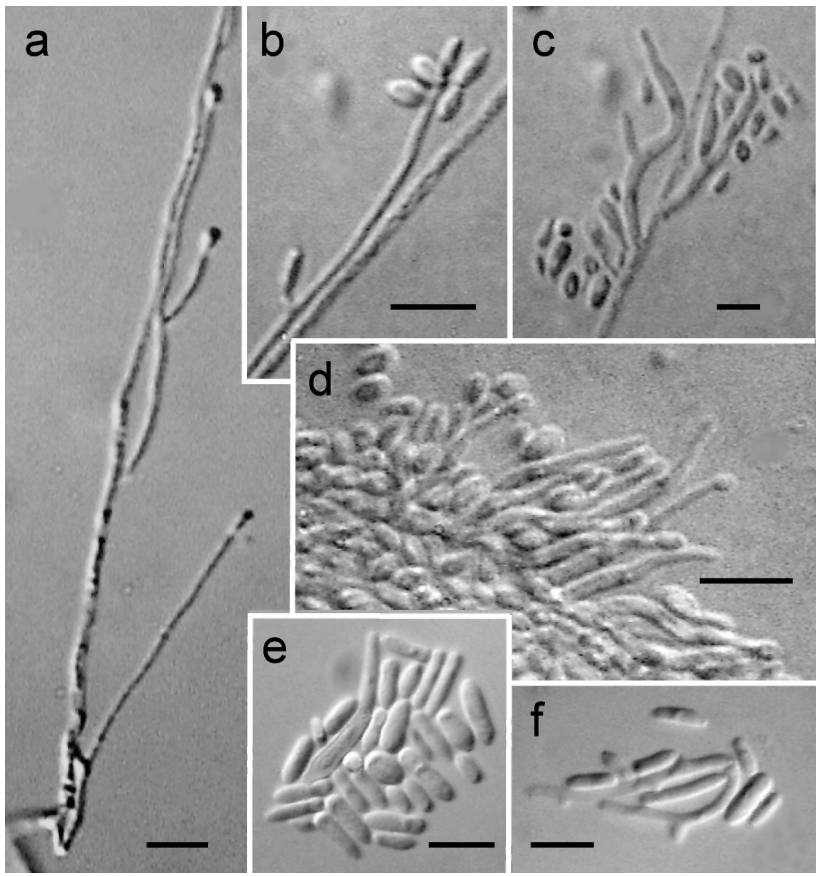

Fig. 1. Conidiophores, conidia and budding yeast cells of *Raffaelea lauricola*. Bars in all figures are 10 µm.

Comments — In cultures initiated from spores, *R. lauricola* forms colonies that are initially highly mucilaginous, but then submerged hyphae grow from the colony margin, and clusters of yeast-like conidia form on the short side branches of the hyphae. The conidiophores are small and hyaline, conidiogenous cells lack conspicuous scars or annelations at the point of conidial dehiscence, similar to the generic concept of the anamorph *Hyalorhinocladiella*, a common anamorph of *Ophiostoma* spp. (de Hoog 1993). The anamorph genus *Raffaelea* includes ambrosia beetle symbionts that produce conidiophores similar to *Hyalorhinocladiella*, but in *Raffaelea* the conidiophores are grouped into sporodochia (Batra 1967). Clumping of conidiophores is sometimes seen in vitro, which may represent incompletely formed sporodochia. Like other species of *Raffaelea*, the new taxon tolerates high concentrations of cycloheximide (Cassar & Blackwell 1996).

Earlier work based on rDNA sequences placed *R. lauricola* among species of *Ophiostoma* and their anamorphs (Fraedrich et al. 2008). The SSU rDNA sequences placed the fungus near *Ambrosiella brunnea*, which has been associated with *Monarthrum* spp. in the eastern USA (Batra 1967). The conidia of *A. brunnea* are larger than those of *R. lauricola*, and *A. brunnea* produces yellow to brown mycelium in culture, while *R. lauricola* has only hyaline hyphae.

Conidia and conidiophores of *R. lauricola* are similar to those of the recently-described *R. quercivora* Kubono & Shin. Ito, though *R. quercivora* has a faster growth rate and pale-olive to brown-olive cultures at 2 weeks, and its conidia are broader (Kubono & Ito 2002). No rDNA sequences of *R. quercivora* are available. *Raffaelea quercivora* is associated with *Platypus quercivorus* (Murayama), which attacks living oaks in Japan. The fungus is capable of causing lesions in inoculated seedlings but does not cause a vascular wilt disease (Kinura & Kobayashi 2006, Murata et al. 2005, Murata et al. 2007). To date, *R. lauricola* is the only known ambrosia beetle symbiont that moves systemically throughout the host and causes a true vascular wilt disease (Fraedrich et al. 2008).

Raffaelea lauricola is readily isolated from the mycangia of the exotic beetle *X. glabratus*, and we assume that the fungus was brought to the USA from Asia with its vector in solid wood packing material (Fraedrich et al. 2008). The fungus has been isolated from diseased redbay in South Carolina, Georgia, and Florida in association with *X. glabratus*, and there has been serious redbay mortality wherever the fungus and beetle vector have been encountered (Fraedrich et al. 2008). In addition it also causes a vascular wilt disease in native *Sassafras albidum* (Nutt.) Nees, *Lindera melissifolia* (Walter) Blume, *Litsea aestivalis* (L.) Fernald, and avocado (*Persea americana* Mill.) (Fraedrich et al. 2008), all new world members of the family *Lauraceae*. Perhaps the *Lauraceae* native to Asia are more resistant to the pathogen, but this has not been tested.

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