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SHORT COMMUNICATION

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# *Raffaelea quercivora* sp. nov. associated with mass mortality of Japanese oak, and the ambrosia beetle (*Platypus quercivorus*)

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**Abstract** A hyphomycete consistently isolated from dead oak trees (*Quercus serrata* and *Q. mongolica* var. *grosseserrata*) attacked by the ambrosia beetle *Platypus quercivorus* in Japan is described and illustrated as *Raffaelea quercivora* sp. nov. The new species is characterized by having small obovoid to pyriform sympodioconidia and slender, long conidiophores that taper to a point. The fungus has been isolated from the body surfaces and mycangia of the beetle. It is likely that the fungus was transferred to oak trees by *P. quercivorus*.

**Key words** Hyphomycetes · *Platypus quercivorus* · *Quercus mongolica* var. *grosseserrata* · *Quercus serrata* · *Raffaelea quercivora* sp. nov.

Mass mortalities of oak trees (*Quercus serrata* Thunb. ex Murray and *Q. mongolica* Fisch. ex Ledeb. var. *grosseserrata* (Blume) Rehder ex Turcz. et E.H. Wilson) have been occurring since 1990 in Honshu, Japan, predominantly in Yamagata, Fukui, Kyoto, and Tottori Prefectures, which all front on the Sea of Japan (Ito and Yamada 1998). Consistent association between tree deaths and attacks by the ambrosia beetle *Platypus quercivorus* (Murayama) (Kinuura 1995; Ito et al. 1998; Urano 2000) has been reported. Because oak trees with diameter at breast height (dbh) of 20–50 cm and height of 20–30 m generally wilt within 2–3 months following a major attack by *P. quercivorus*, this mass mortality of oak trees was hypothesized as a vascular wilt syndrome caused by a pathogenic fungus vectored by *P. quercivorus* (Kuroda and Yamada 1996). A project to determine the causal pathogen commenced in 1996. Fungal isolation tests frequently revealed a moniliaceous fungus from the discolored sapwood, inner bark, and beetle galleries in dead oak trees and from the body surfaces and mycangia of the ambrosia beetles (Ito et al. 1998, 2000). These observations strongly suggested that this fungus could be closely linked to the wilt of the oak trees. As some smaller trees (18 years old, dbh 8 cm) of *Q. mongolica* var. *grosseserrata* planted in the nursery were killed in inoculation tests with this fungus (Ito et al. 1998, 2000), it was suggested that this fungus might play a causal role in the mass mortality syndrome in Japanese oak trees. In this article, we discuss the taxonomic position of the fungus based mainly on its morphological characteristics.

Infected materials were collected from 1996 to 1998. Pieces about 3 × 3 × 3 mm in size were obtained from the discolored sapwood, inner bark, and galleries of *P. quercivorus* bored in dead oak trees in Asahi-mura in Yamagata Prefecture, northern Honshu, and the discolored sapwood of dead oak trees in Fukui, Siga, Wakayama, Kyoto, and Tottori Prefectures in central Honshu. The pieces were sterilized with 80% ethanol and 0.1% solution of mercuric chloride, washed in two changes of sterilized water, and inoculated to potato dextrose agar (PDA) plates.

Male and female adults of *P. quercivorus* were extracted from dead oak trees, washed in two changes of sterilized water, then inoculated onto PDA. Mycangia of female *P. quercivorus* were excised from their bodies using a sharp scalpel and inoculated onto PDA directly. These plates were incubated at 18°C for about 1 month in the dark. Then, the most frequently occurring fungus from all materials, i.e., dead oak trees in each region and the body surfaces and mycangia of *P. quercivorus* was examined.

Conidium-producing structures formed on PDA were observed with a light microscope and a scanning electron microscope (SEM). For SEM observations, agar disks 5 mm in diameter were cut from the colonies and fixed in 5% glutaraldehyde for 24 h at 4°C followed by 0.1% osmium tetroxide for 90 min at 4°C, dehydrated through a graded ethanol series, and dried with a Hitachi Critical Point Dryer

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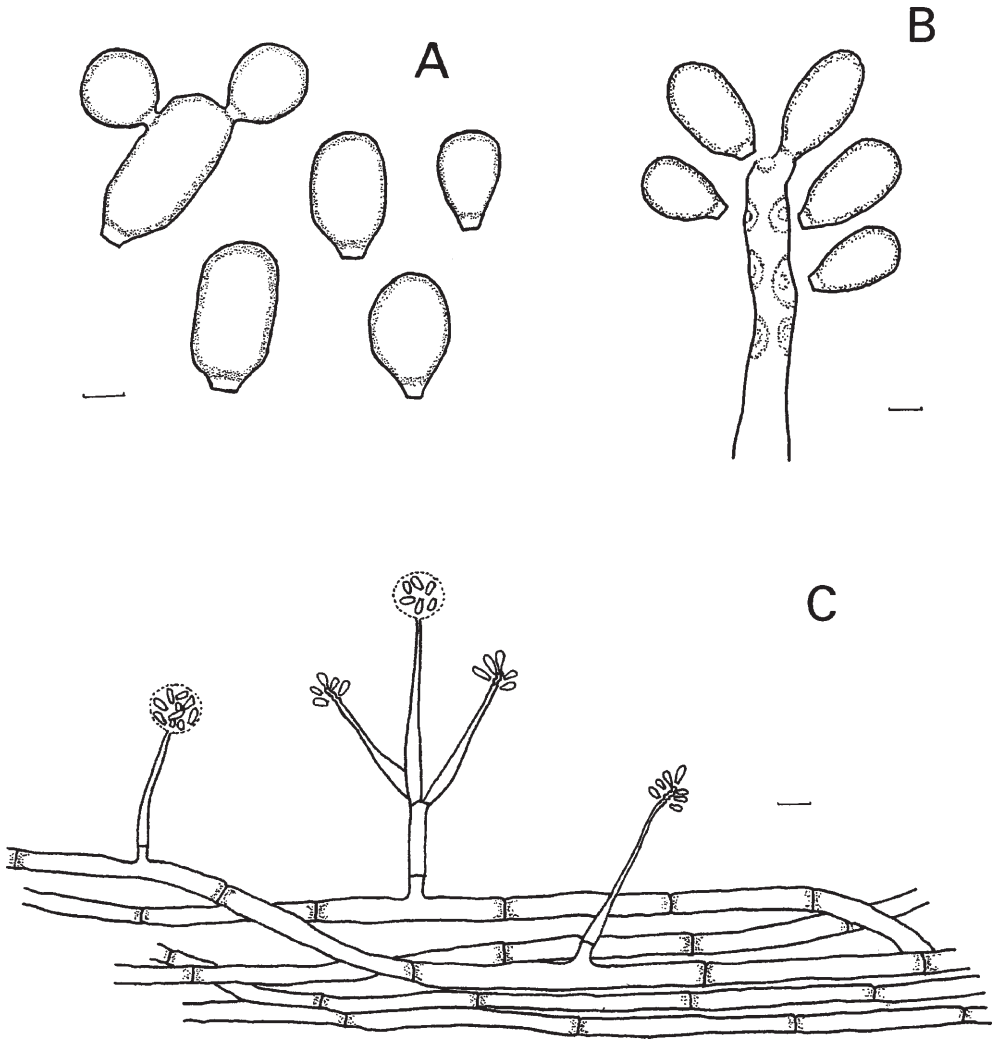



**Fig. 1.** *Raffaelea quercivora.* **A** Conidia. **B** Conidiogenous cell with holoblastic proliferation and cicatricial scars. **C** Conidia and conidiophores; one at the *left* and one at the *center*, showing conidia in droplet. *Bars* **A**, **B** 1 μm; **C** 5 μm

(Hitachi, Ibaraki, Japan). They were coated with gold using an IB-3 Ion Coater (Eiko, Ibaraki, Japan), and observed in a JSM-5310 LV scanning electron microscope (JEOL, Tokyo, Japan) operating at 20 kV.

The present fungus was characterized by the presence of sporodochia, obovoid to pyriform sympodioconidia, and the conidiogenous cells bearing cicatricial scars (Figs. 1, 6–11), and its habitats. Because of the combination of mycological characters already described, the present fungus was placed in the genus *Raffaelea* erected by von Arx and Hennebert (1965).

So far, ten species have been described in the genus (Jones and Blackwell 1998; Morelet 1998). Of these species, *Raffaelea variabilis* B.C. Sutton does not appear to be associated with beetles because the species was isolated from a vegetable oil plant, *Lannea grandis* (Sutton 1975). *Raffaelea variabilis* is also clearly distinguished from the present fungus in having larger conidia (Sutton 1975). The remaining nine species are all associated with ambrosia beetle tunnels in wood and with mycangia of the beetle (Table 1). *Raffaelea santoroi* Guerrero clearly differs from the present fungus in the presence of catenate conidia (Guerrero 1966) (Table 2). The present fungus is readily distinguished from the other seven species (*R. albimanens* D.B. Scott et J.W. duToit , *R. ambrosiae* Arx et Hennebert, *R. arxii* D.B. Scott et J.W. duToit, *R. canadensis* L.R. Batra, *R. montetyi* M. Morelet, *R. sulcati* A. Funk, and *R. tritirachium* L.R. Batra) on conidiophore width and conidial size. The present fungus has smaller conidia and more slender conidiophores compared to other *Raffaelea* species, and most closely resembles *R. hennebertii* D.B. Scott et J.W. duToit in size and shape of conidia, but differs in having distinctly slender conidiophores (Table 2; Figs. 1, 4). The present fungus does not correspond to any *Raffaelea* species hitherto described. We, therefore, describe the present fungus as a new member of *Raffaelea*.

***Raffaelea quercivora*** Kubono et Shin. Ito, sp. nov.

Figs. 1–11

Coloniae in PDA inter ad 20°–25°C effusae, celeriter crescentes, post 5 dies 80 mm diamattingentes, margine indistincto et albido, humefacto-nitidae, mucosae, odore

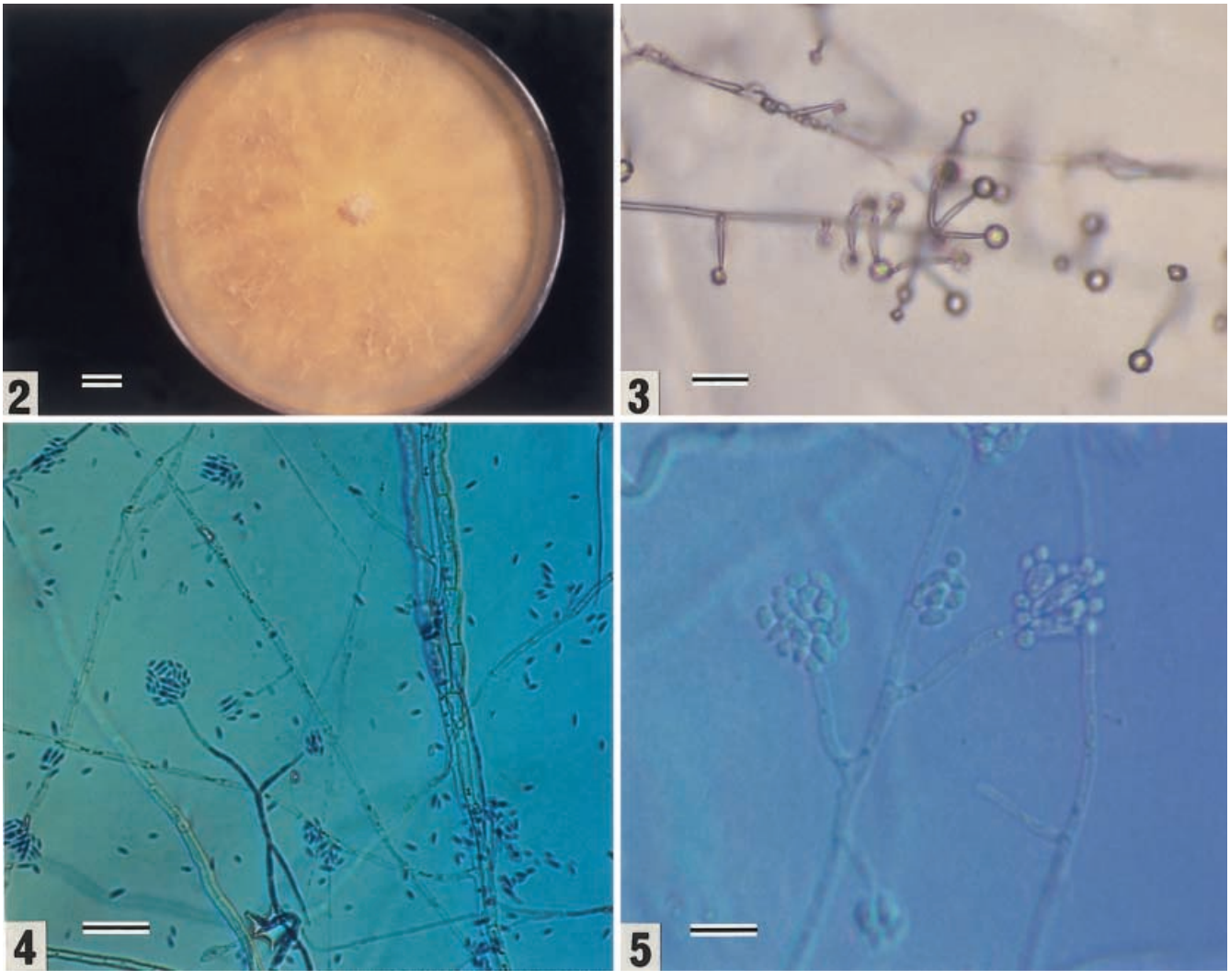



**Fig. 2.** Colony with sporulation on potato dextrose agar (PDA) at 20°C after 10 days. *Bar* 1 cm
**Fig. 3.** Conidia in droplet and conidiophores produced on PDA. *Bar* 20 μm
**Figs. 4,5.** Conidiophores and conidia produced on PDA. *Bars* **4** 20 μm; **5** 10 μm

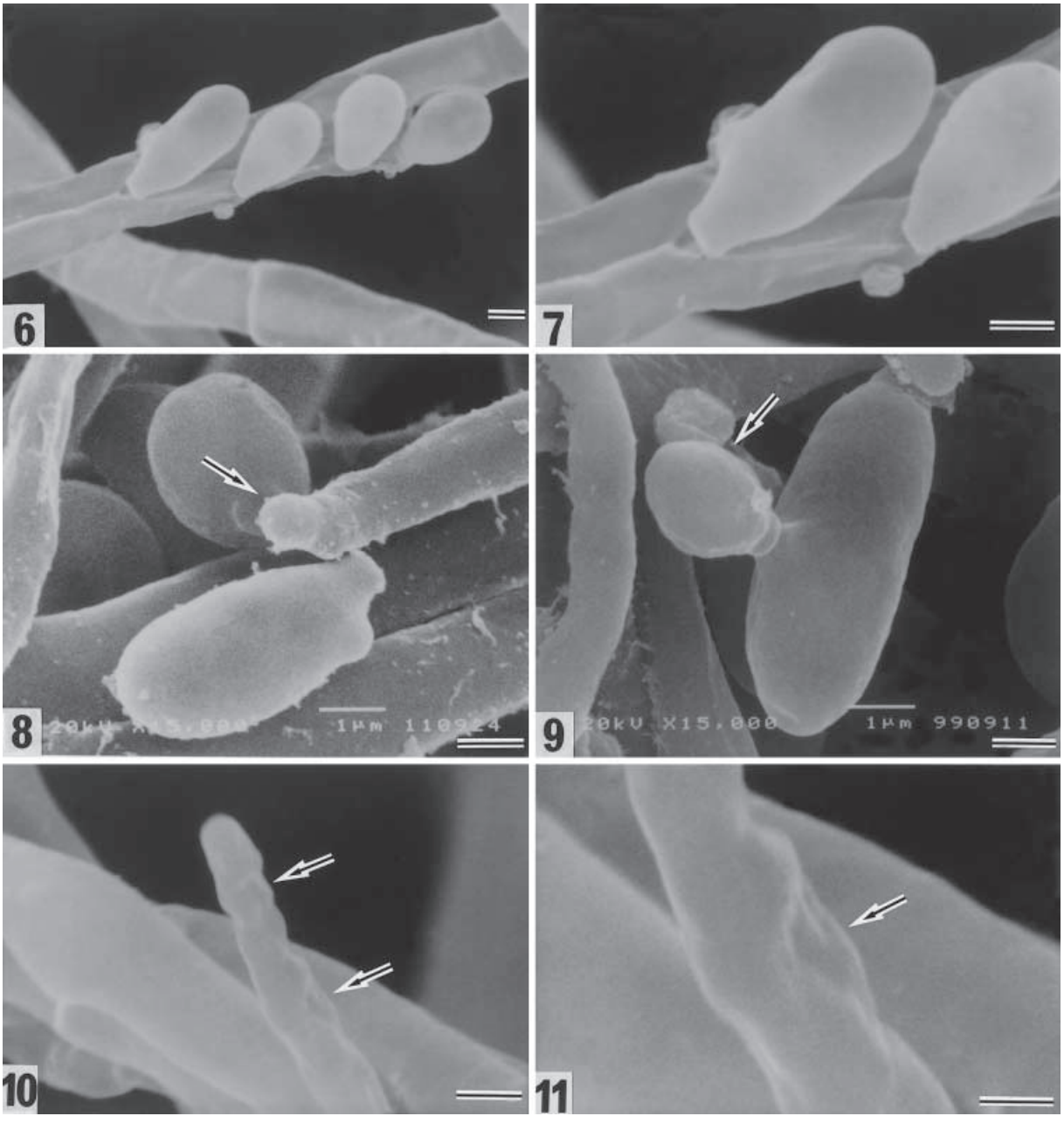
alcoholico; reversum post 14 dies olivacescens vel olivaceo-brunnescens. Hyphae aeriae abundantes, floccosae, ramosae, septatiae, hyalinae, laestdves, ad centrum coloniarum in fasciculos usque ad 1 cm altus formantes. Diffusum et coloniis Sporodochia in fasciculos discretos formata, confluentia, mucosa. Conidiophora macronematosa, in sporodochiis aggregata vel separata, simplicia, recta, hyalina, septata, laevia, 16.5–22.5 × 0.9–1.5 μm. Cellulae conidiogenae sursumgradatim attenuatae, polyblasticae, sympodiales, indeterminatae, discretae vel integratae, terminales, hyalinae, laeves, prope apicem cicatricibus incrassatis planis vix protuberentibus versus formantes. Conidia mucosa, acropeta, hyalina, aseptata, recta, laevia, eguttulata, obovata vel pyriformia, versus basim truncatam valde decrescentia, apice obtuso, 3.1–4.7 × 2.0–2.4 μm, saepe in guttula conglomerato-circumnexta, frequenter cellulas gemmationes pullulantia.

Holotype: TFM:FPH-7587 (Herbarium of Forest Pathology, Forestry and Forest Products Research Institute, Japan), a dried culture, isolated from discolored sapwood in dead *Quercus mongolica* Fisch. ex Ledeb. var. *grosseserrata* (Blume) Rehder ex Turcz. et E.H. Wilson, Asahi-mura, Yamagata Prefecture, Japan, Oct. 1998, T. Kubono.

Ex holotype culture: JCM 11526 (Japan Collection of Microorganisms) (= MAFF 410918) (National Institute of Agrobiological Resources, Tsukuba, Japan).

Other isolates examined: Isolates from the discolored sapwood in dead *Q. mongolica* var. *grosseserrata*. MAFF 410919, Asahi-mura, Yamagata Prefecture, Japan, Oct. 1998, T. Kubono in National Institute of Agrobiological Resources, Tsukuba (MAFF); MAFF 410920 Imajyo-machi, Fukui Prefecture, 18 Oct. 1995, Y. Takahata; MAFF 410921, Makino-cho, Shiga Prefecture 7 Nov. 1997, Y. Takahata; MAFF 410922, Iwami-cho, Tottori Prefecture, Sept. 1995, S. Nishigaki.

Colonies on PDA at 20°–25°C effuse, spreading rapidly, reaching 80 mm diameter in 5 days with an indistinct white




**Figs. 6–11.** Conidia, conidiogenous cells, and cicatricial scars of *R. quercivora* on PDA observed by SEM. **6,7** Conidiogenous cell with holoblastic proliferation and conidia. *Bars* 1 µm. **8** Forming conidiogenous cell (*arrow*) and conidia. *Bar* 1 µm. **9** Conidia and sprout cell (*arrow*) produced from conidia. *Bar* 1 µm. **10,11** Conidiogenous cell with a series of flat cicatricial scars (*arrows*). *Bars* **10** 1 µm; **11** 0.5 µm

margin, appearing water-soaked and mucilaginous; aerial mycelium abundant, floccose, composed of branched, septate, hyaline, smooth hyphae, arranged in fascicles and simulating coremia, reaching 1 cm high; color diffusing and turning pale olive to brown-olive after 2 weeks; odor fragrant, resembling that of ethyl alcohol. Sporodochia of several fascicles, becoming confluent and mucilaginous. Conidiophores macronematous, mononematous, formed in sporodochia or produced separately, simple or branched, straight, hyaline, septate, smooth, 16.5–22.5 × 0.9–1.5 µm. Conidiogenous cells gradually narrowed toward the apex, polyblastic, sympodial, indeterminate, discrete or integrated, terminal, hyaline, smooth, with a series of flat, scarcely protruding scars situated toward the apex. Conidia short-clavate slimy, borne in acropetal order, hyaline, aseptate, straight, smooth, eguttulate, obovoid to pyriform, tapered markedly toward the truncate base, apex obtuse, frequently yielding sprout cells, formed in droplets, 3.1–4.7 × 2.0–2.4 µm.

Taking into consideration that *R. quercivora* was isolated from the discolored sapwood in dead oak trees and from the bodies and mycangia of *P. quercivorus* in these isolation tests, it is likely that this fungus was transferred to oak trees by *P. quercivorus*.

**Table 1.** Relationship between *Raffaelea* spp. and the isolated specimens

| Species | Source of isolation | Insect species | Tree species | Damage characteristics of attacked tree | Locale |
|---|---|---|---|---|---|
| *Raffaelea quercivora*[a] | Gallery, mycangia | *Platypus quercivorus* | *Quercus serrata*<br>*Q. mongolica* var. *grosseserrata* | Dead<br>Dead | Japan |
| *R. albimanens*[b] | Gallery | *P. externedentatus* | *Ficus sycomorus* | Infesting | South Africa |
| *R. ambrosiae*[c] | Gallery | *P. cylindrus*<br>*P. compositus* | *Quercus* spp. | Infesting | England, USA |
| *R. arxii*[b] | Gallery | *Xyleborus torguatus* | *Cussonia umbellifera* | Infesting | South Africa |
| *R. canadensis*[d] | Gallery | *P. wilsoni* | *Pseudotsuga menziesii* | Infesting | Canada |
| *R. hennebertii*[b] | Gallery | *P. externedentatus* | *Ficus sycomorus* | Infesting | South Africa |
| *R. montetyi*[e] | Gallery, mycangia | *P. cylindrus* | *Q. suber* | Infesting | France |
| *R. santoroi*[f] | Gallery | *P. sulcatus* | –[i] | –[i] | Argentine |
| *R. sulcati*[g] | Gallery, mycangia | *Grathotrichus sulcatus* | Conifers | Infesting (attack weakened tress) | Canada |
| *R. tritirachium*[d] | Gallery | *Monarthium mali* | *Quercus* spp. | Infesting | USA |
| *R. variabilis*[h] | –[i] | –[i] | *Lannea grandis* | –[i] | Malaysia |

[a] The authors
[b] Scott and du Toit (1970)
[c] von Arx and Hennebert (1965)
[d] Batra (1967)
[e] Morelet (1998)
[f] Guerrero (1966)
[g] Funk (1970)
[h] Sutton (1975)
[i] –, no description

**Table 2.** Comparison of morphological characters among *Raffaelea* spp.

| Species | Morphology and volume of sporodochia | Conidiophore (μm) | Conidia (μm) | Conidia shape |
|---|---|---|---|---|
| *Raffaelea quercivora*[a] | Fascicles | 16.5–22.5 × 0.9–1.5 | 3.1–4.7 × 2.0–2.4 | Obovoid–pyriform |
| *R. albimanens*[b] | Abundant | (−600) × 2.5 | 4–6 × 3–4 | Obovoid |
| *R. ambrosiae*[c] | Superficial | 70–150 × 5–9 | 5–9 × 4–6 | Obovoid–triangular |
| *R. arxii*[b] | Confluent | (−750) × 3–4 | 8–12 × 4–6 | Obovoid–pyriform |
| *R. canadensis*[d] | Rare | 30–110 × 1.5–3.0 | 5–6.5 × 8–13 | Pyriform–globose |
| *R. hennebertii*[b] | Fascicles | 18–36 × 2.5–3.8 | 3–5 × 2–4 | Obovoid–elongate |
| *R. montetyi*[e] | –[i] | –[i] | 6.6–13 × 3–6.6 | Obovoid–claviform |
| *R. santoroi*[f] | Conidia produced with chain structure | –[i] | –[i] | |
| *R. sulcati*[g] | –[i] | 150–275 × 4–6 | 4–7 × 2–4 | Obovoid–conicotruncate |
| *R. tritirachium*[d] | Fascicles | 6–15 × 3–5 | 5–10.4 × 1.5–2.5 | Narrowly globose |
| *R. variabilis*[h] | Confluent | 19–32 × 2.5–4.5 | 4–16 × 2.5–7.5 | Turbinate–cuneiform |

[a] The authors
[b] Scott and du Toit (1970)
[c] von Arx and Hennebert (1965)
[d] Batra (1967)
[e] Morelet (1998)
[f] Guerrero (1966)
[g] Funk (1970)
[h] Sutton (1975)
[i] –, no description

No *Raffaelea* species has been known to kill woody plants (see Table 1). As the next stage of continuous investigations, it is an important requirement to confirm the pathogenicity of *R. quercivora* to oak trees based on a wider range of materials. Also, the effects on tree physiology resulting from the proliferation of the fungus within the internal components of the trees should be clarified.

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