Esteya floridanum sp. nov.: An Ophiostomatalean Nematophagous Fungus and Its Potential to Control the Pine Wood Nematode

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ABSTRACT

The nematophagous fungal genus *Esteya* is reported as a natural enemy of the pine wood nematode *Bursaphelenchus xylophilus*, which causes pine wilt in Asia and Europe. During a survey of fungi associated with ambrosia beetles in Florida, an undescribed *Esteya* species was found. A phylogenetic analysis based on nuclear large subunit and β -tubulin DNA sequences supported this isolate as a new species, *E. floridanum*. Morphological and phylogenetic characteristics and a species description are provided here. The fungus was observed to kill the pine wood nematode in vitro. To evaluate the ability of *E. floridanum* to protect trees against the pine wood nematode in vivo, the effect of prophylactic

inoculation was tested on *Pinus koraiensis* and *Larix olgensis* in Liaoning, China. The results suggest that the fungus is not a plant pathogen and that it delays wilt and postpones death of two conifer trees. This presents a potential new avenue for research on biocontrol of pine wilt disease and stresses the value of research on pest organisms in their native regions.

Keywords: biological control, biotechnology, disease control, endophytic, forest pathology, invasive, *Larix*, nematodes, Ophiostomatalean fungi, parasite, pest management, *Pinus*, phylogeny

Pine wilt is a dramatic disease of susceptible conifers caused by the pine wood nematode (PWN) *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae). PWN is not considered an economic pest in North American forests where it originates from (Bergdahl 1988). This parasite is widespread, causing pine wilt disease in Eastern Asia, first identified as the causal agent of this devastating disease on Asian pine in 1969 (Mamiya 1983; Tokushige and Kiyohara 1969). The ability of this nematode to rapidly spread in newly invaded habitats is attributed to its insect vectors, longhorn beetles of the genus *Monochamus* (Wang et al. 2014; Zhang et al. 2007). Once within the tree tissues, the nematodes proliferate and move actively throughout the tree until they eventually encounter a new beetle vector (Kobayashi et al. 1984).

The wilting disease of pine trees has been increasing since the 2000s in a wide area of temperate and subtropical regions of Asia and Europe (Abelleira et al. 2020; Mota et al. 1999; Zhang et al. 2010). The results of regular management measures, such as direct cutting and burning of infested trees and fumigation of logs by pesticides, have not been satisfactory. Introductions of natural enemies of the PWN have been attempted, including the nematode endoparasitic fungus *Esteya vermicola*. This fungus was reported as a natural enemy of the PWN in Taiwan (Liou et al. 1999), showing

significant potential as a biological agent against the PWN in laboratory and field evaluations (Wang et al. 2018).

The genus *Esteya* was described more than 20 years ago but still includes only a single species, *E. vermicola* (Liou et al. 1999; Wang et al. 2014). *E. vermicola* is the only known nematophagous fungus in the order Ophiostomatales (Dreaden et al. 2014), which represent a polyphyletic group of tree- or wood-infecting fungi and are commonly vectored by bark and ambrosia beetles (Chang et al. 2017; Jankowiak et al. 2019). Phylogenetically, the genus *Esteya* is sister to the *Raffaelea sulphurea* complex, which accommodates fungi specifically associated with ambrosia beetles, especially beetles in the subfamily Platypodinae (Dreaden et al. 2014; Simmons et al. 2016; Wang et al. 2019). So far, eight strains of *E. vermicola* are known to science and are distributed widely across the globe, including in Brazil, Czechia, Italy, the United States, Taiwan island, Japan, Korea, and mainland China (Chu et al. 2015; Kobayashi et al. 1984; Li et al. 2018; Wang et al. 2016, 2019). All of these strains have demonstrated the ability to endoparasitize the PWN and thus bear potential for biological control applications (Wang et al. 2008, 2018).

During a survey of fungi associated with pine ambrosia beetles in Florida in March 2017, we isolated an unusual *Esteya* fungus from the head of a *Myoplatypus flavicornis* ambrosia beetle in loblolly pine trees in Gainesville (Li et al. 2018). However, since there was no information on the fungal identity, its relationship with PWN was unknown. Its identity and biological and ecological characterization are important because the fungus could potentially antagonize with the PWN and thus deserves attention for several reasons. First, PWN is native to North America, sympatric with the new *Esteya* species (Figueiredo et al. 2013; Mallez et al. 2013). All *Esteya* strains previously tested to control PWN were found in Eurasia and South America, where there is no natural population of PWN (Wang et al. 2014, 2019). Second, the fungal species described here was isolated from one of the most common wood borers on the local pine plantation. Third, building a broader phylogenetic framework including ecologically distinct taxa is crucial to understand the biology and evolution

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*The e-Xtra logo stands for “electronic extra” and indicates there are supplementary materials published online.

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of ophiostomatalean fungi. Therefore, the objectives of this study were to designate a new species for the genus *Esteya* based on morphological, ecological, and molecular data and to test its potential for use as a control agent for the PWN in North China.

MATERIALS AND METHODS

Fungus isolation. A sample was isolated from the head of a male adult of *Myoplatypus flavicornis* on loblolly pine in Austin Cary Forest, Gainesville, Florida (Li et al. 2018). A culture was made on potato dextrose agar (PDA) media (Becton, Dickinson and Company, Sparks, MD) amended with 0.05 g/liter of cycloheximide (Harrington 1981). The fungal strains were shipped from the University of Florida to the Shenyang Institute of Technology (SIT) under a memorandum of understanding between the two institutions. Pure cultures of the fungus were deposited in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute at the University of Pretoria in South Africa.

Nematode isolation. The PWN for testing was isolated from a sick Korean pine (*Pinus koraiensis*) in Qingyuan, Fushun, Liaoning, China. PWN was identified in a previous study (Yu et al. 2019) and reared on the PDA media or potato dextrose water media.

DNA extraction, PCR amplification, and sequencing. Genomic DNA was extracted from the fungal culture grown on PDA, following the Extract-N-Amp Plant PCR kit (Sigma-Aldrich, St. Louis, MO) with the modification of using 3% bovine serum albumin in the place of a dilution solution. Primer combinations used for PCR amplifications were as follows: (i) LR0R/LR5 (Hopple 1994; Vilgalys and Hester 1990) for nuclear large subunit (LSU) ribosomal DNA (rDNA), (ii) Bt2a/Bt2b (Glass and Donaldson 1995; O'Donnell and Cigelnik 1997) for β -tubulin (β -tub), and (iii) ITS1F/ITS4 (Gardes and Bruns 1993; White et al. 1990) for portions of the internal transcribed spacer ITS1-5.8S-ITS2 (ITS) rDNA locus. PCR reactions contained the following: 1 μ l of template DNA, 1 μ l of forward and reverse primers (10 mM), 12.5 μ l of Premix Taq polymerase (Takara ExTaq), 1 μ l of dimethyl sulfoxide, and 9.5 μ l of molecular-grade DNA-free water. The PCR cycling conditions for ITS and β -tub were as follows: 1 min at 95°C; 35 cycles of 94°C for 30 s, 53°C for 45 s, and 72°C for 90 s; and finally, 72°C for 5 min. PCR cycling conditions for the LSU gene were as follows: 1 min at 95°C; 35 cycles of 95°C for 30 s, 55°C for 45 s, and 72°C for 90 s; and finally, 72°C for 10 min. Amplified products were purified and Sanger sequenced by GENEWIZ (South Plainfield, NJ). Sequence chromatograms were edited and assembled in Geneious 9.0.5 (<https://www.geneious.com/>). Representative sequences for suspected new species were uploaded to GenBank (Supplementary Table S1, accession numbers LC363546, MT811023, and MT858361).

Alignment and phylogenetic analysis. The concatenated LSU plus β -tub (introns 3/4/5 removed) dataset was analyzed in

Geneious for phylogenetic reconstruction. The ITS sequences were not included in the phylogenetic analyses, as these sequences were only used for molecular identification (Supplementary Table S1). The phylogenetic analysis included the suspected new species in this study and reference isolates representing *E. vermicola* and other ophiostomatalean fungi related to the genus *Esteya* (Li et al. 2018; Musvuugwa et al. 2015; Simmons et al. 2016; Supplementary Table S1). The alignments were conducted using MAFFT (Kato et al. 2019) and deposited in TreeBASE (treebase.org; Study ID: S26477). The Akaike information criterion in jModeltest 2.1.10 (Guindon and Gascuel 2003; Posada 2008) was used to select the nucleotide substitution model GTR+I+G for both genes. Bayesian inference analyses were performed using MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003), and maximum likelihood (ML) analyses were performed using the RAxML2.0 (Stamatakis 2014) plug-in in Geneious. ML phylogenetic analyses were conducted with bootstrap support (BS) values from 500 search replicates. Bayesian posterior probabilities (BPPs) were estimated with two chains, and each was executed simultaneously for 1,000,000 generations, sampling every 200 generations, and a burn-in of the first 100,000 trees. The best ML tree was edited in FigTree 1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree>) and Adobe Photoshop CS4 (<https://www.adobe.com>).

Growth trials and morphological characterization. To determine the optimal growth conditions of the new *Esteya* species, mycelial plugs (4 mm in diameter) were plated on new fresh PDA plates (90 mm in diameter) and incubated in the dark at 15, 20, 25, and 30°C for 8 days. For each temperature, five replicates were used. Colony diameter was measured after 5 and 8 days of incubation. Two colony diameters perpendicular to each other were averaged and means $\pm$ standard deviations were calculated. Morphological features were examined by inoculating sterile slide mounts of malt extract agar (MEA; Becton, Dickinson and Company) with propagules collected by running a sterile needle along the surface of cultures growing on MEA. Once taxonomically informative structures were observed using a dissecting microscope (24 to 48 h), slides were mounted and examined on a Leica DM2500 LED optical microscope using LAS X software (<https://www.leica-microsystems.com>). Measurements of conidiophores ($n = 30$) and two types of conidia ($n = 30$) were made and means $\pm$ standard deviations were calculated.

Assay of controlling PWN on the media. To prepare the fungal inoculum, fungal isolates were cultured onto MEA plates at 25°C until fully colonized (approximately 2 weeks), then the fungal spores and hyphae on the surface of media were scraped with 5 ml of water and transferred to a clean Petri dish. One milliliter of PWN in water (approximately 1,500 individuals) was also added to this Petri dish and to an empty Petri dish as a negative control. Then, the Petri dishes were preserved in an incubator at 25°C for 30 days. Sterile water was added to each plate every few days to maintain humidity. The mortality

TABLE 1. Inoculation of two conifer trees

Species	Treatment no.	Seedlings (n)	Treatment ^a		Mortality (%)	
			First inoculum	Second inoculum	14 weeks	Ultimate
<i>Larix olgensis</i>	1	20	<i>Esteya floridanum</i>	N/A	0	0
	2	20	Sterile water	N/A	0	0
	3	20	<i>E. floridanum</i>	PWN	90	100
	4	20	N/A	PWN	100	100
<i>Pinus koraiensis</i>	5	20	<i>E. floridanum</i>	N/A	0	0
	6	20	Sterile water	N/A	0	0
	7	20	<i>E. floridanum</i>	PWN	95	100
	8	20	N/A	PWN	100	100

^a N/A = no second inoculum and PWN = pine wood nematode.

rate of adult PWN was counted from 100 randomly selected individuals under an inverted biological microscope (Optec BDS400).

Assay of controlling PWN within conifer trees. Seedlings of healthy Korean pine (*P. koraiensis*) and Olga Bay larch (*L. olgensis*) were provided by Cangshi Forest Farm in Fushun, Shenyang, China. We performed our nematophagous tests on small, containerized trees (1-gallon pot) with an initial height of approximately 0.3 to 0.4 m of 4-year-old Korean pine and 0.4 to 0.5 m of 2-year-old Olga Bay larch. Individual trees within each species were of similar size. These two tree species were selected because (i) Korean pine and Olga Bay larch are dominant tree species in Northeastern China and are of ecological as well as economic significance and (ii) PWN causes serious damage to both tree species (Pan et al. 2019; Yu et al. 2019). Eighty seedlings of each species were grown and tested in a quarantine greenhouse facility at the SIT. In the greenhouse, trees were individually inspected and monitored for 2 weeks to test for the absence of any existing pathogens or pests prior to inoculation. The trees were maintained under natural light, 40% RH, and at an average temperature of 8 to 10°C at night and 20 to 25°C in the daytime in the winter. The trees were watered every 2 days without additional treatments (e.g., fertilization).

Forty randomly chosen individuals of each tree species were prophylactically inoculated with the new *Esteya* species on 11 October 2019 (Table 1). A wound at a 80° downward angle on the phloem of each seedling was cut with a sterile scalpel. Wounds were made within the basal 5 cm of the stem and were up to 1 cm deep. Then, the wounds were filled with a sterile folded gauze (2 × 2 cm) inoculated with 1 ml of spore suspension by pipette (approximately 10⁶ spores). Wounds and gauze were wrapped in parafilm immediately to avoid cross-contamination.

Two weeks after inoculation with the fungus, half of the fungal-inoculated trees ($n = 20$) and noninoculated trees ($n = 20$) were inoculated with the PWN solution (approximately 1,000 nematodes per tree) following the same method as above except the wounds were made within the basal 7 cm of the stem. Controls ($n = 20$) were treated in the same manner as the inoculated trees, except that only sterile water was inoculated.

Seedlings were monitored for 12 weeks after fungal inoculation in the greenhouse. All signs and symptoms of pathogenic development (including resin bleeding, wilt, and mortality) were recorded every week. To confirm the survival of the fungal inoculum, fungi were reisolated from the branch and trunk surrounding the inoculation site on 10 randomly selected pine trees, if the tree was surviving. The sampled wood tissues were rinsed in sterile water, blotted dry, and plated onto PDA plates incubated at 25°C. Subcultures were plated onto PDA and identified using morphology.

RESULTS

The phylogenetic analyses of the combined LSU rDNA and β -tub data matrices by ML and Bayesian inference were highly similar and led to the same conclusion. The placement of *E. floridanum* sp. nov. as a sister species to *E. vermicola* was strongly supported (BS = 99, BPP = 100). Our results also strongly suggested (BS = 99, BPP = 100) that the genus *Esteya* is sister to the *R. sulphurea* complex (Fig. 1, Supplementary Figure S1).

Taxonomy. *E. floridanum* Y. Li, Araújo & Hulcr, sp. nov. *E. floridanum* Y. Li, Araújo & Hulcr, sp. nov. was deposited in MycoBank (MB 835374; Fig. 2). Colonies were initially white, turning gray after 5 days; reverse initially white, aging to brown on PDA and beige on MEA. Colonies grew on PDA at an average rate

Fig. 1. Phylograms obtained from maximum likelihood (ML) analysis of nuclear large subunit and β -tubulin sequences of the genus *Esteya* with the genus *Aureovirgo* as the outgroup. Novel sequences obtained in this study are shown in bold. ML bootstrap support values $\geq 70\%$ are indicated with numbers. Bold branches indicate Bayesian posterior probability support ≥ 0.95 . The photograph of the beetle is from A. J. Johnson.

of 0.51 ± 0.16 mm/day at 10°C , 1.53 ± 0.30 mm/day at 15°C , 2.91 ± 0.34 mm/day at 20°C , 2.73 ± 0.68 mm/day at 25°C , and 3.33 ± 0.33 mm/day at 30°C ; there was no growth at 35°C .

The mycelium produced two types of conidiophores: conidiogenous cells and conidia (see Fig. 2E and G). The first type of conidiophores were macronematous, mononematous, unbranched, erect, ampulliform, septate, subhyaline to light brown, asymmetrical, smooth. Conidiogenous cells were terminal, $(11-17) \times (-35) \times (-3) 3.5 (4.4) \mu\text{m}$, swollen at the base tapering upward into a neck, 4.5 to 7×1.5 to $2 \mu\text{m}$ phialidic, usually supported by a short-swollen cell. Conidia were solitary, one-celled, lunate, apiculate ends, hyaline, smooth-walled, 6.5 to 10.5×2.3 to $4.9 \mu\text{m}$, containing an endospore-like structure 4 to 5×2 to $2.5 \mu\text{m}$, adhesive.

The second type of conidiophores were macronematous, mononematous, simple or branched, erect, irregular, subhyaline to light brown, septate, swollen at the base irregularly tapering toward the apex, smooth to roughened, $(22-30) (43-) \times (3.2-) 3.8 (-4.2) \mu\text{m}$. Conidiogenous cells were phialidic, enteroblastic, often terminal but also intercalary, usually swollen at the base tapering toward the apex, 14 to 20×3 to $4.5 \mu\text{m}$. Conidia were solitary, one-celled, cylindrical, ellipsoidal to hemispherical, hyaline, smooth, $(3.4-) 5.2 (-7.1) \times (-1.9) 2.8 (-3.6) \mu\text{m}$, commonly dividing by budding after release.

No teleomorphic (sexual) phase was observed.

Etymology. Etymology refers to the locality of the holotype, Florida.

Type. USA: Florida: Gainesville, Austin Cary Forest (29.717321 N, -82.241621 W), from the head of ambrosia beetle *Myoplatypus flavicornis* (Coleoptera: Curculionidae: Platypodinae) in a loblolly pine *P. taeda*. Collector: Y. Li. Date: 5 March 2017 (CMW55942-ex-holotype).

In vitro assay of PWN control. After *E. floridanum* was released into the PWN culture in the plate, 97% of PWNs were

parasitized (Fig. 3) and ultimately killed by *E. floridanum* after 30 days, whereas 69% of PWNs survived in the controls.

Both tree species inoculated only with *E. floridanum* (Table 1, treatments 1 and 5) or sterile water (Table 1, treatments 2 and 6) but no PWN survived and displayed no symptoms of poor health. In the pine trees inoculated with *E. floridanum*, the strains were reisolated from the three trees after 12 weeks.

All seedlings inoculated with PWN showed serious wilt before ultimately dying after 14 weeks (treatments 3 to 4 and 7 to 8; Figs. 4C, F, I, and L and 5). Representative characteristics of external symptoms are shown in Figure 4. Wilt started earlier and progressed more rapidly on Korean pine than on Olga Bay larch. Seventy-five percent of Korean pine seedlings wilted 2 weeks after inoculation with PWN. Leaf drooping was not observed on Korean pine, but defoliation occurred rapidly in larch after wilt (Fig. 4E and F).

For both conifer seedlings, treatment with *E. floridanum* deferred wilt for 2 to 4 weeks and death for 4 to 6 weeks, respectively. For instance, all seedlings untreated with *E. floridanum* and then inoculated with PWN died within the experimental period, whereas

Fig. 2. *Esteya floridanum* morphological features in pure culture on **A, B, E, F, G, and H**, malt extract agar (MEA) and **C and D**, potato dextrose agar (PDA) and two types of conidia. **A** and **B**, Upper and reverse of culture growth after 14 d at 25°C on MEA. **C** and **D**, Upper and reverse of culture growth after 14 d at 25°C on PDA. **E** and **F**, Hyphae bearing long, flask-shaped conidiogenous cells with conidia at the apex and occasional sessile lateral conidia. **G** and **H**, Hyphae bearing long, flask-shaped conidiogenous cells with lunate conidia at the apex.

Fig. 3. Pine wood nematode killed by *Esteya floridanum*.

only 50% of larch and 35% of Korean pine died within 8 weeks after inoculation of PWN and *E. floridanum* (Fig. 5).

DISCUSSION

E. floridanum is the second described species in the genus *Esteya*. This genus has remained monotypic since its discovery (Liou et al.

1999). Our discovery of this new species suggests that the true diversity of nematophagous fungi, especially within Ophiostomatales, is still unknown and the genus *Esteya* could potentially harbor a reservoir of undocumented species with potential to become effective agents against pine wilt disease.

Similar to *E. vermicola*, *E. floridanum* also produces two types of conidia, both observed to attach to nematodes (Fig. 3). This

Fig. 4. Conifer seedlings inoculated with the pine wood nematode (PWN). A, D, G, and J, Two weeks later. B, E, H, and K, Four weeks later. C, F, I, and L, Twelve weeks later.

characteristic is unique to the genus *Esteya* among ophiostomatalean fungi and supports the placement of the new fungal species within the genus *Esteya* from an ecological perspective. Regarding morphology, both *Esteya* species are morphologically very similar. However, while conidiogenous cells of *E. vermicola* are formed directly on the main vegetative hyphae, conidiogenous cells of *E. floridanum* are often supported by a short, swollen cell, which connects it to the main hyphae (Fig. 2G and H). Both *E. vermicola* and *E. floridanum* produce two types of similar conidia, but type 2 of *E. floridanum* is wider (see Table 2). Our molecular data also strongly support *E. floridanum* as an *Esteya* species, sister to *E. vermicola* (Fig. 1).

The results of our phylogenetic analyses were in agreement with analogous studies by Li et al. (2018) and Simmons et al. (2016), which showed the genus *Esteya* and the *R. sulphurea* complex as sister clades within *Leptographium* sensu lato (Fig. 2). Interestingly, the genus *Esteya* represents a drastic ecological shift within the

order Ophiostomatales, an interkingdom host switch from plant-based nutrition, which is the most common ecology across ophiostomatalean species, to become a nematophagous fungus (Fig. 3). It is parsimonious to propose that such a transition occurred as a result of niche overlap, since the nematodes live inside the trees and thus encounter of both organisms is likely to occur. In addition, both organisms use beetles for transport from one host tree to another, considerably increasing the likelihood of encounter. This example could be framed from the perspective of the “host habitat hypothesis,” which suggests that microhabitats and feeding habitats promote the encounter of both organisms, facilitating an eventual transition from one host to another (Araújo and Hughes 2019; Nikoh and Fukatsu 2000).

Our results also showed that *E. floridanum* could infect the PWN in culture (Fig. 3). *E. floridanum* showed a similar infection process as that of the *E. vermicola* strain (Wang et al. 2014). Only lunate conidia were available to attach to nematode and germinate from cadavers.

Considering that the new species is not known to occur in Asia and that numerous members in the *R. sulphurea* complex and genus *Leptographium* are tree pathogens, it was essential to test the biological security of a potential introduction of this fungus in Northern China before evaluating the inhibiting effect on PWN. None of the pine and larch exhibited any health impacts or death after inoculation. This indicates that *E. floridanum* is not a pathogen to conifer trees in North China. Because *E. floridanum* was recovered after 14 weeks, it may grow as an endophytic fungus within living trees.

E. floridanum presented limited ability to inhibit the PWN in pine and larch. Almost all inoculated trees were finally killed by the nematode. We observed that the tree inoculated with *E. floridanum* in advance was wilted a few weeks later than the noninoculated treatment tree. Death of seedlings may be accelerated as a result of their young stage, at which they usually are more sensitive to pathogens (Rabiey et al. 2019). Delayed wilt and death was more obvious in larch than pine. These data suggested that *E. floridanum* took effect on the plant. Before this study, the only field example of applying *E. vermicola* to combat PWN was conducted on large pine tree (Wang et al. 2018). This type of live tree test is essential for the assessment of the protective effects of the genus *Esteya* on conifers. Further work is being conducted in this area on expanding the trials to mature trees and with varied environmental conditions.

The discovery of a new *Esteya* species suggests that there may be additional nematophagous fungi in Ophiostomatales. About half of the recorded *Esteya* strains were isolated from bark and ambrosia beetle microhabitats. Those habitats have been cited repeatedly as a main source for fungal diversity and fungi with various symbiotic or biocidal effects (Blackwell 2011; Hawksworth and Lücking 2017; Wijayawardene et al. 2020), of which microhabitats such as bark and ambrosia microhabitats are part.

Fig. 5. Survival of conifer seedlings inoculated with the pine wood nematode (PWN; treatments 3 to 4 and 7 to 8) and *Esteya floridanum*.

TABLE 2. Comparison of two *Esteya* fungi

Characteristic	<i>E. vermicola</i>	<i>E. floridanum</i>
Type 1 (μm)		
Conidiogenous cells	(10–) 16–29 (–62) × (1.5–) 3–4.4	(11–) 17 (–35) × (–3) 3.5 (–4.4)
Conidia	8.2–11.1 × 3.5–3.7	6.5–10.5 × 2.3–4.9
Type 2 (μm)		
Conidiogenous cells	(22.2–) 34.1–43 × 3–4.4	(22–) 30 (–43) × (3.2–) 3.8 (–4.2)
Conidia	(–3) 4.4–7.4 × 1.5–1.9	(3.4–) 5.2 (–7.1) × (–1.9) 2.8 (–3.6)
Beetle (vector)	<i>Oxoplatypus quadridentatus</i> , <i>Scolytus intricatus</i> , <i>Tomicus yunnanensis</i>	<i>Myoplatypus flavicornis</i>
Host plant	<i>Olea eropaea</i> , <i>Pinus thunbergii</i> , <i>P. yunnanensis</i> , <i>Quercus petraea</i> , <i>Q. polycarpa</i> , <i>Q. robur</i>	<i>P. taeda</i>
Distribution	Brazil, China, Czechia, Italy, Japan, Korea, Taiwan, and United States (Virginia)	United States (Florida)

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