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Fusarium oligoseptatum sp. nov., a mycosymbiont of the ambrosia beetle *Euwallacea validus* in the Eastern U.S. and typification of *F. ambrosium*

T. Aoki[1], M.T. Kasson[2], M.C. Berger[2], S. Freeman[3], D.M. Geiser[4], K. O'Donnell[5]

[1]Genetic Resources Center, National Agriculture and Food Research Organization, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan
[2]Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506, USA
[3]Department of Plant Pathology and Weed Research, ARO, The Volcani Center, Bet Dagan 50250, Israel
[4]Department of Plant Pathology & Environmental Microbiology, The Pennsylvania State University, University Park, PA 16802, USA
[5]Mycotoxin Prevention and Applied Microbiology Research Unit, National Center for Agricultural Utilization Research, US Department of Agriculture, Agricultural Research Service, 1815 North University Street, Peoria, IL 61604, USA

*Corresponding author: taoki@affrc.go.jp

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Abstract: *Fusarium oligoseptatum* sp. nov. was isolated from the invasive Asian ambrosia beetle *Euwallacea validis* (Coleoptera, Scolytinae, Xyleborini) and from the galleries that females had constructed in dying *Ailanthus altissima* (tree-of-heaven) symptomatic for Verticillium wilt in south-central Pennsylvania, USA. This ambrosia fungus was cultivated by *Euwallacea validis* as the primary source of nutrition together with a second symbiont, *Raffaelea subfusca*. Female beetles transport their fungal symbionts within and from their natal galleries in paired pre-oral mycangia. *Fusarium oligoseptatum* was distinguished phenotypically from the 11 other known members of the Ambrosia *Fusarium* Clade (AFC) by uniquely producing mostly 1–2 septate clavate sporodochial conidia that were swollen apically. Phylogenetic analysis of multilocus DNA sequence data resolved *F. oligoseptatum* as a genealogically exclusive species-level lineage but evolutionary relationships with other members of the AFC were unresolved. Published studies have shown that *F. oligoseptatum* can be identified via phylogenetic analysis of multilocus DNA sequence data or a PCR multiplex assay employing species-specific oligonucleotide primers. In addition, to provide nomenclatural stability, an epitype was prepared from an authentic strain of *F. ambrosium* that was originally isolated from a gallery constructed in Chinese tea (*Camellia sinensis*) by *E. fornicatus* in India, together with its lectotypification based on a published illustration.

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INTRODUCTION

Ambrosia beetles (Coleoptera, Curculionidae: Scolytinae and Platypodinae) are obligate mutualistic mycetophagous insects that cultivate ambrosia fungi as a source of nutrition typically in dead but occasionally in healthy woody hosts (Hulcr & Stelinski 2017). Most ambrosia beetles studied to date carry specific symbiotic ambrosia fungi within their mycangia, which are disseminated by females when they leave their natal galleries to establish new colonies (Hulcr & Cognato 2010, Hulcr & Dunn 2011). Genera in the tribe Xyleborini (Scolytinae) are considered to be the most ecologically successful ambrosia beetles (Hulcr & Stelinski 2017). Several well-studied fungus-farming beetles, including representatives of several tribes, have recently caused significant mortality of trees. Notable examples include the invasive Asian ambrosia beetle *Xyleborus glabratus* and its nutritional symbiont *Raffaelea lauricola* on redbay (*Persea borbonia*) in the southeastern United States (Fraedrich *et al.* 2008), and *Platypus quercivorus* and its symbiont *Raffaelea quercivora* on Japanese oak (*Quercus serrata* and *Q. mongolica* var. *grosseserrata*) in Japan (Kubono & Ito 2002, Seo *et al.* 2012).

Compared to their beetle partners, relatively few fungal symbionts have been formally described. Most of the ambrosia fungi described to date are ascomycetous fungi in the *Ophiostomatales*, including members of *Afroraffaelea*, *Ceratocystiopsis*, *Dryadomyces* and *Raffaelea* (von Arx & Hennebert 1965, Upadhyay & Kendrick 1975, Gebhardt *et al.* 2005, Harrington *et al.* 2008, 2010, Alamouti *et al.* 2009, Dreaden *et al.* 2014, Bateman *et al.* 2016, Hulcr & Stelinski 2017). The *Microascales* also include multiple groups of ambrosia fungi, some of which are important and widespread: *Ambrosiella*, *Meredithiella*, and *Phialophoropsis* (Mayers *et al.* 2015). Less common are symbionts belonging to the *Polyporales* (Li *et al.* 2015, Kasson *et al.* 2016, Simmons *et al.* 2016), *Hypocreales* (i.e., *Geosmithia*) (Kolařik & Hulcr 2009, Kolařik & Kirkendall 2010), and *Saccharomycetales* (van der Walt 1972, Hulcr & Stelinski 2017).

In addition to the symbionts mentioned above, *Fusarium ambrosium* (*Hypocreales*, *Nectriaceae*) is cultivated by *Euwallacea fornicatus* (formerly *Xyleborus fornicatus*) as a source of nutrition (Gadd & Loos 1947, Norris & Baker 1967, Brayford 1987, Nirenberg 1990). The taxonomic history of *F. ambrosium*, however, is complicated because the species was originally misclassified and established in *Monacrosporium*, as *M. ambrosium*. This fungus

was isolated originally and described from galleries of the tea shot-hole borer, *E. fornicatus*, in *Camellia sinensis* (Chinese tea) and *Ricinus communis* (caster-oil tree) stems in Sri Lanka (Gadd & Loos 1947). Subsequently, *F. bugnicourtii* was described based on collections from galleries in Chinese tea in India, borer-damaged *Hevea brasiliensis* (rubber tree) and *Theoborma cacao* (cacao) in Sabah, Malaysia (Brayford 1987). Nirenberg (1990) synonymized *F. bugnicourtii* with *M. ambrosium* and recombined the latter as *F. ambrosium* based on nomenclatural priority. Brayford (1987) considered *F. bugnicourtii* to be conspecific with *F. tumidum* var. *coeruleum* (Bugnicourt 1939), but distinct from *F. tumidum*. Although the type of *F. tumidum* var. *coeruleum* based on a collection from *H. brasiliensis* appears to be phylogenetically distinct from *F. bugnicourtii*, the holotype of *F. bugnicourtii* selected by Brayford (IMI 296597 = NRRL 20438) is conspecific with *F. ambrosium* (Kasson *et al.* 2013).

Kasson *et al.* (2013) conducted an extensive multilocus molecular phylogenetic study on the ambrosial fusaria, based on isolates from beetles, their galleries, or from trees showing extensive borer damage and dieback. These included *Camellia sinensis*, *Persea americana* (avocado), *Ailanthus altissima* (tree-of-heaven), *Acer negundo* (box elder), and *Hevea brasiliensis* from natural and cultivated ecosystems, and avocado in the United States, Israel and Australia. Seven different *Fusarium* species lineages were reported to be associated with *Euwallacea* ambrosia beetles within the Ambrosia *Fusarium* Clade (AFC) and one other species (i.e., *Fusarium* sp. AF-9) with *Xyleborus ferrugineus* in Costa Rica. The monophyletic AFC is nested within Clade 3 of the *F. solani* species complex (FSSC; O'Donnell 2000), which contains 60 plus phylogenetic species based on genealogical concordance phylogenetic species recognition (GCPSR; Taylor *et al*. 2000). The AFC comprises two strongly supported clades: the four species within Clade A typically produce curved fusiform septate macroconidia, which are typical of *Fusarium*, whereas nine of the 10 species within Clade B produce clavate macroconidia (Kasson *et al*. 2013, Aoki *et al.* unpubl.), described as 'dolphin-shaped' by Brayford (1987). O'Donnell *et al.* (2015) conducted a multilocus phylogenetic analysis of the AFC and *Euwallacea* and found evidence of repeated host shifts rather than strict co-evolution of this mutualism.

Freeman *et al.* (2013) described a new species, *F. euwallaceae*, based on isolates corresponding to the ambrosia species symbiotic with the *Euwallacea* sp. #1 *sensu* O'Donnell *et al.* (2015), which causes serious damage to avocado production in Israel and California, USA (Mendel *et al.* 2012, Eskalen *et al.* 2013). *Fusarium euwallaceae* is closely related morphologically to *F. ambrosium*, but it can be distinguished from the latter by the abundant production of bluish to brownish sporodochial conidia that form greenish masses on PDA after 1 mo in culture, together with hyaline conidia. To date only three of the 16 species within the AFC have been described formally (Kasson *et al.* 2013, O'Donnell *et al.* 2015, Na *et al*. 2018). Similar to *E. validus*, *Euwallacea* sp. #1 also carries additional symbiotic fungi, *Graphium euwallaceae* and *Paracremonium pembeum* (Freeman *et al.* 2016, Lynch *et al.* 2015). Recently, PCR multiplexes were developed to discriminate *Fusarium* symbionts of invasive *Euwallacea* ambrosia beetles that inflict damage on numerous tree species throughout the United States, including *F. euwallaceae* and *F. kuroshium* along with four unnamed AFC species-level lineages: AF-3, AF-4, AF-6 and AF-8 (Short *et al.* 2017). One of the undescribed species, which was informally referred to as *Fusarium* sp. AF-4, is cultivated by the ambrosia beetle *E. validus* primarily in Verticillium wilt-stressed and dying stands of *A. altissima*, as well as from Verticillium wilt-stressed *Acer pensylvanicum* (striped maple), *Aralia spinosa* (devils walkingstick) and *Rhus typhina* (staghorn sumac) in south-central Pennsylvania, USA (Schall & Davis 2009, Kasson *et al.* 2013, 2015). In the present study, this species is described as *F. oligoseptatum sp. nov.* based on a comparison with *F. ambrosium* (AF-1) and *F. euwallaceae* (AF-2) (Kasson *et al.* 2013, Freeman *et al.* 2013). In addition, because type material for *F. ambrosium* was not designated (Gadd & Loos 1947), or appears to have been lost (Nirenberg 1990), a line-drawing of a clavate conidium of the species from Gadd & Loos (1947) was selected as the lectotype. Furthermore, an epitype was prepared from an authentic strain of this species to stabilize its taxonomy, according to the International Code of Nomenclature for algae, fungi and plants (ICN, the Melbourne Code; McNeill *et al.* 2012).

MATERIALS AND METHODS

Fungal isolates and type specimens

Fusarium strains examined in this study (Table 1) are stored in the Agriculture Research Service Culture Collection (NRRL), National Center for Agricultural Utilization Research (NCAUR), U.S. Department of Agriculture in Peoria, Illinois, USA. These strains were originally isolated from *Euwallacea* ambrosia beetles and their galleries, or from host trees showing extensive borer damage (Kasson *et al.* 2013). The Pennsylvanian strains of *F. oligoseptatum* were isolated from *E. validus* ambrosia beetles that had colonized *A. altissima*. Beetles were surface disinfested for 15 s in 70 % ethanol and then washed three times in sterile deionized water. Whole beetles or their heads were macerated using sterile Tenbroek homogenizers (Pyrex, Corning, NY), or pellet pestles (Fisher Scientific, Hampton, NH), suspensions were diluted 1:10 and 1:100, and then spread evenly over half-strength Potato Dextrose Agar (PDA, BD-Difco™, Thermo Fisher Scientific, Waltham, MA) amended with 100 ppm streptomycin sulfate (Sigma-Aldrich, St. Louis, MO) as described in Kasson *et al.* (2013). Other related ambrosia fusaria or close relatives within the *F. solani* species complex (O'Donnell *et al*. 2008) were obtained from culture collections (Table 1). Isolates used in this study are available upon request from NRRL (http://nrrl.ncaur.usda.gov/cgi-bin/usda/), NARO Genebank, Microorganisms Section (MAFF), Genetic Resources Center, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan (http://www.gene.affrc.go.jp/about-micro_en.php), and the Westerdijk Institute (formerly CBS-KNAW Fungal Biodiversity Center), Utrecht, the Netherlands (http://www.westerdijkinstitute.nl/). Isolates of four novel Taiwanese AFC species discovered very recently, i.e. AF-13 to AF-16 (Na *et al*. 2018), were not included in this study.

Holotype and epitype specimens newly prepared from the selected strains were deposited in BPI, US National Fungus Collection (https://nt.ars-grin.gov/fungaldatabases/specimens/specimens.cfm).

Incidence of *Fusarium oligoseptatum* and other fungi from *Euwallacea validus* mycangia across recently confirmed tree hosts

Mycangial fungal communities were characterized as previously described by Kasson *et al.* (2013) for adult female

Table 1. Strains of Ambrosia *Fusarium* Clade (AFC) species examined in present study. Bold text is used to identify ex-holotype of *Fusarium oligoseptatum* and ex-epitype of *F. ambrosium*.

Species	AFC clade #	NRRL strain # [a]	Equivalent nos. in other collections [b]	Host beetle [c]	Host plant	Origin	Country	Date	Collector [d]	Remark
F. oligoseptatum	AF-4	62578	FRC S-2576	*Euwallacea validus*	*Ailanthus altissima*	Dauphin Co., Pennsylvania	USA	30-Jan-10	M. Kasson Bh17	Studied only phylogenetically
	AF-4	**62579**	**FRC S-2581 = MAFF 246283 = CBS 143241**	***Euwallacea validus***	***Ailanthus altissima***	**Dauphin Co., Pennsylvania**	**USA**	**30-Jan-10**	**M. Kasson Bh24**	**Ex-HOLOTYPE**
	AF-4	62580	FRC S-2594 = MAFF 246284 = CBS 143242	*Euwallacea validus*	*Ailanthus altissima*	Franklin Co., Pennsylvania	USA	9-Mar-10	M. Kasson Ch19	
	AF-4	62581	FRC S-2616 = MAFF 246285 = CBS 143243	*Euwallacea validus*	*Ailanthus altissima*	Huntington Co., Pennsylvania	USA	27-Feb-10	M. Kasson Dh24	
	AF-4	62582	FRC S-2627 = MAFF 246286 = CBS 143244	*Euwallacea validus*	*Ailanthus altissima*	Mifflin Co., Pennsylvania	USA	1-Jul-09	M. Kasson Eh11	Degenerated strain
F. ambrosium	AF-1	20438	IMI 296597 = MAFF 246291	*Euwallacea fornicatus*	*Camellia sinensis*	Chinchona, Maharashtra	India	17-Jul-85	anonymous	Ex-holotype of *F. bugnicourtii*
	AF-1	22345	BBA 65389 = MAFF 246288	*Euwallacea fornicatus*	*Camellia sinensis*	Upari Tea Institute	India	9-May-90	V. Agnihothrudu	
	AF-1	**22346**	**BBA 65390 = CBS 571.94 = MAFF 246287**	***Euwallacea fornicatus***	***Camellia sinensis***	**Upari Tea Institute**	**India**	**9-May-90**	**V. Agnihothrudu**	**Ex-EPITYPE**
	AF-1	36510	BBA 65390 = MAFF 246289	*Euwallacea fornicatus*	*Camellia sinensis*	Upari Tea Institute	India	9-May-90	V. Agnihothrudu	Duplicate of NRRL 22346
	AF-1	46583	IMI 339338 = MAFF 246290	*Euwallacea fornicatus*	*Camellia sinensis*	High Forest Estate, Anamallais, Coimbatore District, Tamil Nadu	India	26-Mar-90	anonymous	Received as *F. bugnicourtii*

Table 1. (Continued).

Species	AFC clade #	NRRL strain # [a]	Equivalent nos. in other collections [b]	Host beetle [c]	Host plant	Origin	Country	Date	Collector [d]	Remark
	AF-1	62605		*Euwallacea fornicatus*	*Camellia sinensis*	Tea Research Institute of Sri Lanka, St. Coombs, Talawakelle	Sri Lanka	Mar-12	S. Freeman 31.14	
F. euwallaceae	AF-2	54727	MAFF 243816 = CBS 135859	*Euwallacea* sp. #1	*Persea americana*	Volcani	Israel	17-Feb-10	S. Freeman 5-4	
	AF-2	62626		*Euwallacea* sp. #1	*Persea americana*	California	USA		A. Eskalen 1854	
Fusarium sp.	AF-3	62606		*Euwallacea interjectus*	*Acer negundo*	Gainesville, Florida	USA		J.A. Smith PL1499	
	AF-3	62628		*Euwallacea interjectus*	*Acer negundo*	Gainesville, Florida	USA		J.A. Smith 1190	
	AF-5	22231	IMI 110107	unknown	*Hevea brasiliensis*	Agriculture Research Centre Tuaran, Sabah, Borneo	Malaysia	19-Nov-64		Received as *F. bugnicourtii*
	AF-5	46518	FRC S-2075	unknown	*Hevea brasiliensis*		Malaysia			
	AF-6	62590		*Euwallacea* sp. #2	*Persea americana*	Miami, Florida	USA		R.C. Ploetz AF9	
	AF-6	62591		*Euwallacea* sp. #2	*Persea americana*	Miami, Florida	USA		R.C. Ploetz AF10	
	AF-7	62610		*Euwallacea* sp. #3	*Persea americana*	Queensland	Australia		A.D.W. Geering 1	
	AF-7	62611		*Euwallacea* sp. #3	*Persea americana*	Queensland	Australia		A.D.W. Geering 2	
	AF-8	62584		*Euwallacea* sp. #2	*Persea americana*	Miami, Florida	USA		R.C. Ploetz Amb2	
	AF-8	62585		*Euwallacea* sp. #2	*Persea americana*	Miami, Florida	USA		R.C. Ploetz AF4	
	AF-9	22643	ATCC 44215	*Xyleborus ferrigineus*			Costa Rica		E.B. Smalley	
	AF-9	66088		unknown	*Delonix regia*	Florida	USA			
	AF-10	62941	IMI 351954	unknown			Singapore			
	AF-11	62943		*Euwallacea* sp. #4	*Camellia sinensis*		Sri Lanka		P. Liyanage	
	AF-11	62944		*Euwallacea* sp. #4	*Camellia sinensis*		Sri Lanka		P. Liyanage	
F. kuroshium	AF-12	62945		*Euwallacea* sp. #5	*Platanus racemosa*	San Diego, CA	USA		A. Eskalen	
	AF-12	62946		*Euwallacea* sp. #5	*Platanus racemosa*	San Diego, CA	USA		A. Eskalen	

Table 1. (Continued).

Species	AFC clade #	NRRL strain #[a]	Equivalent nos. in other collections[b]	Host beetle [c]	Host plant	Origin	Country	Date	Collector[d]	Remark

[a] NRRL: ARS Culture Collection, NCAUR-ARS-USDA, Peoria, IL, USA.

[b] ATCC: American Type Culture Collection, Manassas, VA, USA; BBA: Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Mikrobiologie (currently Julius-Kühn-Institut), Berlin, Germany; CBS: Westerdijk Institute (formerly CBS-KNAW Fungal Biodiversity Center), Utrecht, the Netherlands; FRC: Fusarium Research Center, The Pennsylvania State University, State College, PA, USA; IMI: CABI Biosciences, UK Centre, Egham, Surrey, UK; MAFF: NARO Genebank, Microorganisms Section, Genetic Resources Center, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan.

[c] The five unnamed *Euwallacea* spp. are distinguished by #1–#5 (O'Donnell *et al*. 2015). AF-12 was later described as *F. kuroshium* (Na *et al*. 2018).

[d] V. Agnihothrudu, Upari Tea Institute, India; A. Eskalen, Department of Plant Pathology and Microbiology, University of California, Riverside, CA, USA; S. Freeman, Department of Plant Pathology and Weed Research, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel; A. Geering, University of Queensland, Brisbane, Australia; M. Kasson, Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV, USA; R. C. Ploetz, University of Florida, Homestead, FL, USA; J. A. Smith, University of Florida, Gainesville, FL, USA.

beetles that harbor paired pre-oral mycangia. In addition to *F. oligoseptatum*, described in this paper, previous studies revealed that *E. validus* harbors a second less abundant symbiont, *Raffaelea subfusca* (Kasson *et al.* 2013). To determine if these trends held across other tree hosts and geographic locations, *E. validus* from *Ailanthus* and 16 other confirmed tree hosts across seven states were sampled. When available, a minimum of 10 adult females were included. Log-extracted females were processed as previously described (Kasson *et al.* 2013). Following serial dilution plating of head macerates, individual fungal colony forming units (CFUs) were quantified by morphotype and representatives of each morphotype retained for molecular characterization using the ITS barcoding gene. Unlike the other fungal morphotypes, representative fusaria were subjected to an AF-3 / *Fusarium oligoseptatum* (AF-4) multiplex PCR recently developed by Short *et al.* (2017) to discriminate known *Fusarium* symbionts of *E. interjectus* and *E. validus* in the eastern U.S.

When comparing the CFUs recovered from individual beetle heads between the primary symbionts of *E. validus*, *F. oligoseptatum* and *R. subfusca*, a chi-squared test was performed across all tree species. To examine if there were differences in the relative amount of colony forming units (CFUs) recovered from individual beetle heads between the primary symbionts of *E. validus*, *F. oligoseptatum* and *R. subfusca* within individual species, a second chi-squared test was performed for each individual species. Results of the tests were deemed significant if $p < 0.05$.

Molecular systematics and biology

Methods for culturing mycelium, DNA extraction, PCR amplification, DNA sequencing and phylogenetic analyses followed published protocols (Kasson *et al.* 2013, O'Donnell *et al*. 2015). DNA sequence data included in this study were deposited in GenBank as JQ038007–JQ038034.

Phenotypic characterization

Strains were grown on PDA and synthetic low-nutrient agar (SNA; Nirenberg 1990, Nirenberg & O'Donnell 1998) in the dark, under continuous black light (Black light blue fluorescent tubes, FL8BL-B 8W/08, Panasonic, Osaka, Japan), or under an ambient daylight photoperiod. Strains were cultured on PDA in 9 cm Petri dishes at 20 °C in the dark to characterize colony color, odor and morphology. Kornerup & Wanscher (1978) was used as the color standard. PDA cultures were also used for determining mycelial growth rates in the dark at eight temperatures (5–40 °C) at 5 °C increments (Aoki *et al.* 2015). Culture plates were examined at 1 and 4 d post inoculation, and radial growth was calculated as arithmetic mean values per day by measuring 16 radii around the colony. Measurements of growth rate at different temperatures were replicated twice, and the data averaged for each strain. Cultures on SNA were used for examination of microscopic characters as described by Aoki *et al.* (2015). Conidia and conidiophores were examined in water mounts after culturing on SNA under continuous black light. Phenotypic characters were compared with data from the related AFC species, *F. ambrosium* (published as *Monacrosporium ambrosium*; Gadd & Loos 1947), *F. bugnicourtii* (synonymized as *F. ambrosium*; Brayford 1987, Nirenberg 1990), and *F. euwallaceae* (Freeman

et al. 2013). To compare the number of conidial septa in strains of *F. oligoseptatum* and *F. ambrosium*, they were incubated on SNA at 25 °C under continuous black light for one to two weeks and the number septa in the clavate sporodochial conidia were counted.

RESULTS

Incidence of *Fusarium oligoseptatum* and other fungi from *Euwallacea validus* mycangia across recently confirmed tree hosts

Mycangial communities were characterized from adult female beetles extracted from sixteen native host trees and *Ailanthus* (Fig. 1). Overall, *F. oligoseptatum* and *Raffaelea subfusca* comprised 84 % of all fungal CFUs from female heads across all plant hosts with *F. oligoseptatum* yielding significantly more CFU's (10 992) compared to *R. subfusca* (7 014; $p < 0.0001$). The remainder included miscellaneous yeasts and other fungi including *Paracremonium* sp. and a putatively novel *Graphium* sp. (Freeman *et al.* 2016, Lynch *et al.* 2016), as well as a variety of singleton taxa that were not further characterized. Incidence of the two primary symbionts from the heads of female *E. validus* was compared across and within plant hosts. Overall, significant differences were detected across hosts indicating that the relative proportion of the two symbionts varied across hosts with a majority of beetles from a majority of plant hosts yielding higher counts of *F. oligoseptatum* (Fig. 1). Of these, beetles from 11 of the 16 plant hosts, including *Ailanthus*, had significantly higher total CFU counts of *F. oligoseptatum* compared to *R. subfusca*. Only five species had a mean percent incidence of *F. oligoseptatum* below 50 %: *Fraxinus americana* (white ash), *Pinus virginiana* (Virginia pine), *Populus grandidentata* (bigtooth aspen), *Quercus montana* (chestnut oak), and *Amelanchier arborea* (serviceberry) (Fig. 1). Of these, white ash, Virginia pine, and chestnut oak had significantly higher total CFU counts of *R. subfusca* compared to *F. oligoseptatum* (Fig. 1).

Molecular phylogenetics

A 4914 bp 31-taxon 4-locus dataset was constructed that included the internal transcribed spacer region and domains D1 and D2 of the nuclear ribosomal large subunit (ITS+LSU rDNA: 1004 bp alignment, 26 parsimony informative characters (PIC)), and portions of translation elongation factor 1-α (*TEF1*: 687 bp alignment, 53 PIC), DNA-directed RNA polymerase II largest (*RPB1*: 1588 bp alignment, 164 PIC) and second largest subunit (*RPB2*: 1635 bp alignment, 165 PIC) from 12 AFC species. Molecular phylogenetic analyses were conducted using maximum parsimony (MP) with PAUP v. 4.0b10 (Swofford 2003) and maximum likelihood (ML) with GARLI 2.01 (Zwickl 2006). Sequences of *Fusarium neocosmosporiellum* (≡ *Neocosmospora vasinfecta*; Geiser *et al.* 2013) NRRL 22468 and 43467 were used to root the phylogenies based on more inclusive analyses (O'Donnell *et al.* 2013). *Fusarium oligoseptatum* (AF-4) was poorly supported (MP and ML bootstrap = 63–56 %) as sister to a clade that included *F. euwallaceae* (AF-2) from Israel and California and *Fusarium* sp. (AF-3) from Florida. With the exception of *F. ambrosium*, the other AFC species represented by two or more strains received moderate to strong monophyly bootstrap support. The putative triparential hybrid strain *F. ambrosium* NRRL 62605 from Sri Lanka (Kasson *et al*. 2013), however, did not form a genealogically exclusive group with *F. ambrosium* from India. As reflected by poor bootstrap support along the backbone on the phylogeny, relationships among the species were generally unresolved (Fig. 2). The analyses did support monophyly of the AFC and the early diverging subclades designated Clade A and B (Fig. 2).

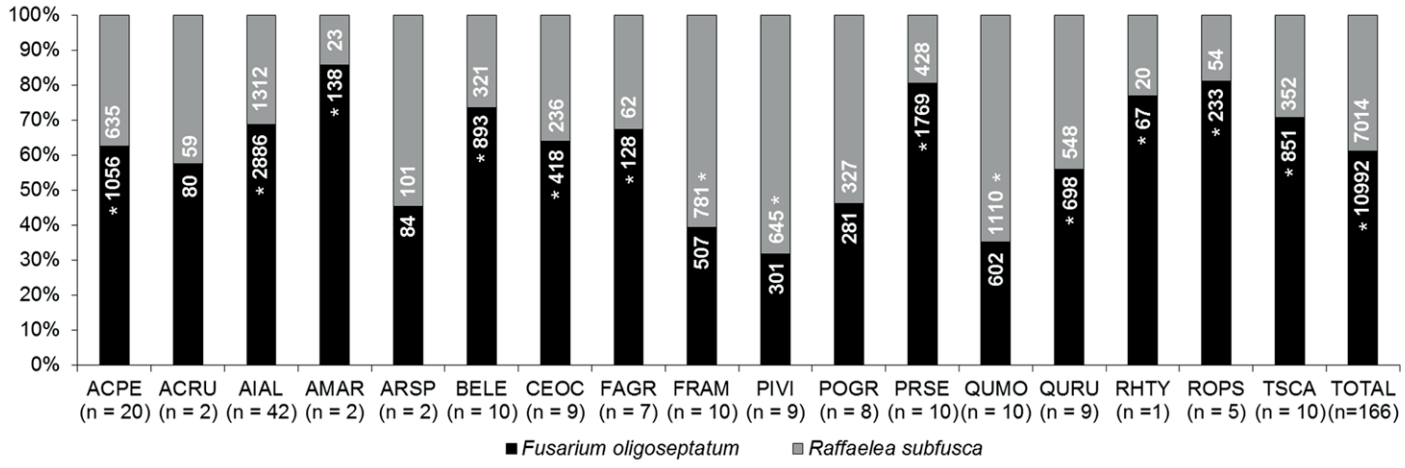

Fig. 1. Total number of *F. oligoseptatum* and *Raffaelea subfusca* CFUs recovered from macerated heads of adult female *E. validus* from 17 tree hosts. A significant difference between the two fungal CFUs within a specific host is indicated with an asterisk on the side corresponding to the higher count. Host plant IDs are based on USDA PLANTS Database (https://plants.usda.gov/java/) abbreviations, which are derived from the first two letters of the genus and species of the Latin binomial. Abbreviations are as follows: ACPE: *Acer pensylvanicum*, ACRU: *Acer rubrum*, AIAL: *Ailanthus altissima*, AMAR: *Amelanchier arborea*, ARSP: *Aralia spinosa*, BELE: *Betula lenta*, CEOC: *Celtis occidentalis*, FAGR: *Fagus grandifolia*, FRAM: *Fraxinus americana*, PIVI: *Pinus virginiana*, POGR: *Populus grandidentata*, PRSE: *Prunus serotina*, QUMO: *Quercus montana*, QURU: *Quercus rubra*, RHTY: *Rhus typhina*, ROPS: *Robinia pseudoacacia*, and TSCA: *Tsuga canadensis*.

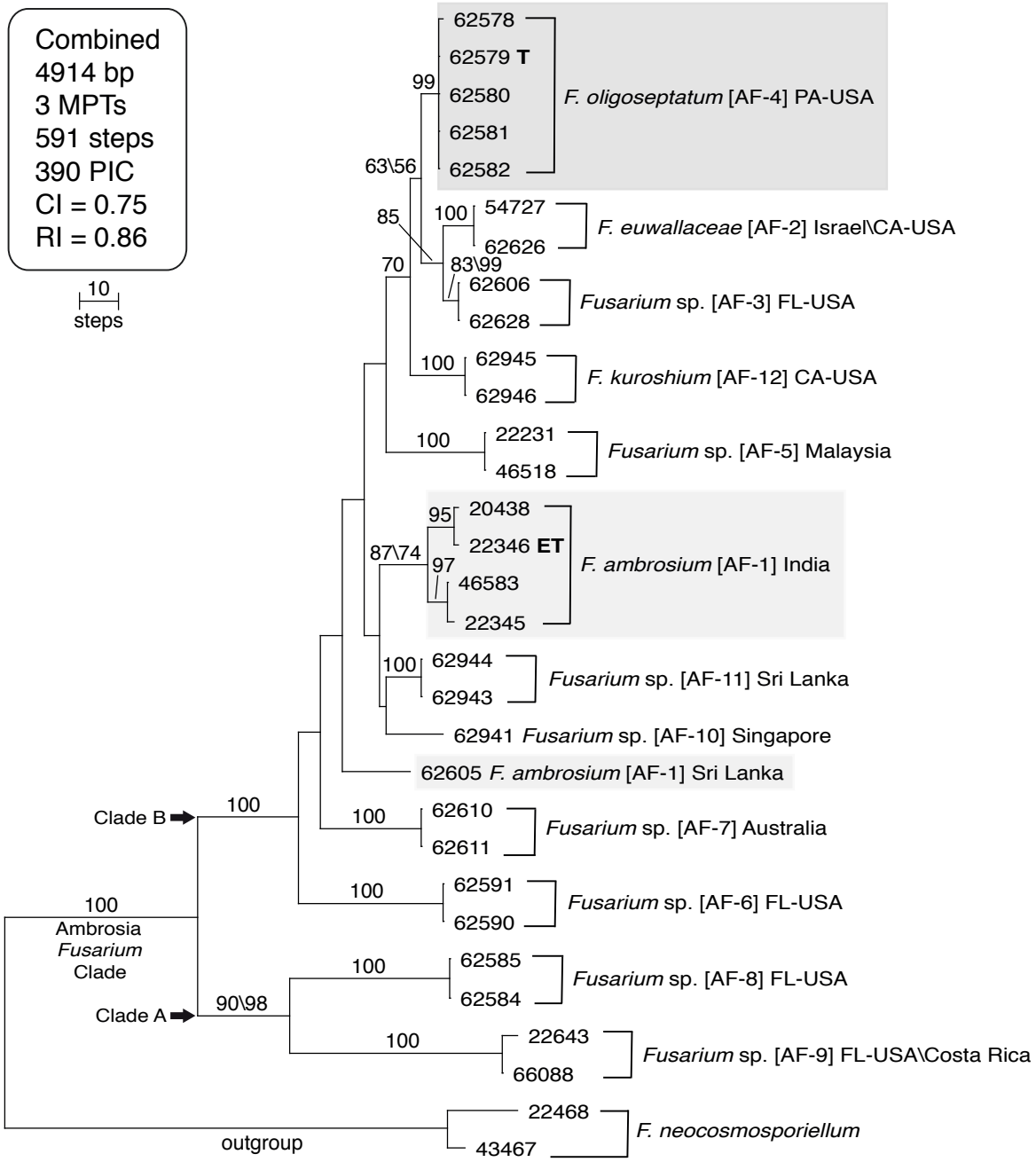

Fig. 2. One of three most-parsimonious trees (MPTs) inferred from a combined 4-gene dataset (ITS + 28S rDNA, *RPB1*, *RPB2* and *TEF1*) comprising 4914 bp of aligned DNA sequence data. The phylogram was rooted on sequences of *Fusarium neocosmosporiellum* NRRL 22468 and 43367 based on more inclusive analyses (O'Donnell *et al*. 2013). The 12 species within the Ambrosia *Fusarium* Clade (AFC) are identified as AF-1 through AF-12 using an ad hoc nomenclature (Kasson *et al*. 2013). Two early diverging monophyletic sister clades are identified as Clade A and B. Numbers above nodes represent maximum parsimony (MP) and maximum likelihood (ML) bootstrap based on 1000 pseudoreplicates of the data (MP-BS\ML-BS). The ML-BS value is only shown when it differed by ≥5 % of the MP-BS value. Note that 10 of the 11 AFC species with two or more strains received strong monophyly bootstrap support. However, *Fusarium ambrosium* highlighted in light gray was resolved as non-monophyletic because the interspecific hybrid strain NRRL 62605 from Sri Lanka did not group with the other strains of this species. The five strains of *F. oligoseptatum*, formally described herein, are identified using dark gray highlight. CI, consistency index; ET, ex-epitype; PIC, parsimony informative characters; RI, retention index; T, ex-holotype.

TAXONOMY

Fusarium oligoseptatum T. Aoki, M.T. Kasson, S. Freeman, Geiser & O'Donnell, ***sp. nov.*** MycoBank MB822305. Figs 3–5.

Etymology: oligo- + septatum, based on frequent production of sporodochial conidia with only 1–2 septa.

Diagnosis: Distinguished from *F. ambrosium* and *F. euwallaceae* by three times as many 0–2-septate sporodochial conidia (i.e., ≥75 % compared with values less than 25% in *F. ambrosium* and *F. euwallaceae*).

Type: **USA**, *Pennsylvania:* Dauphin Co., a dried specimen from a culture of NRRL 62579, isolated from a live female ambrosia beetle, *Euwallacea validus*, extracted from a gallery in a tree-

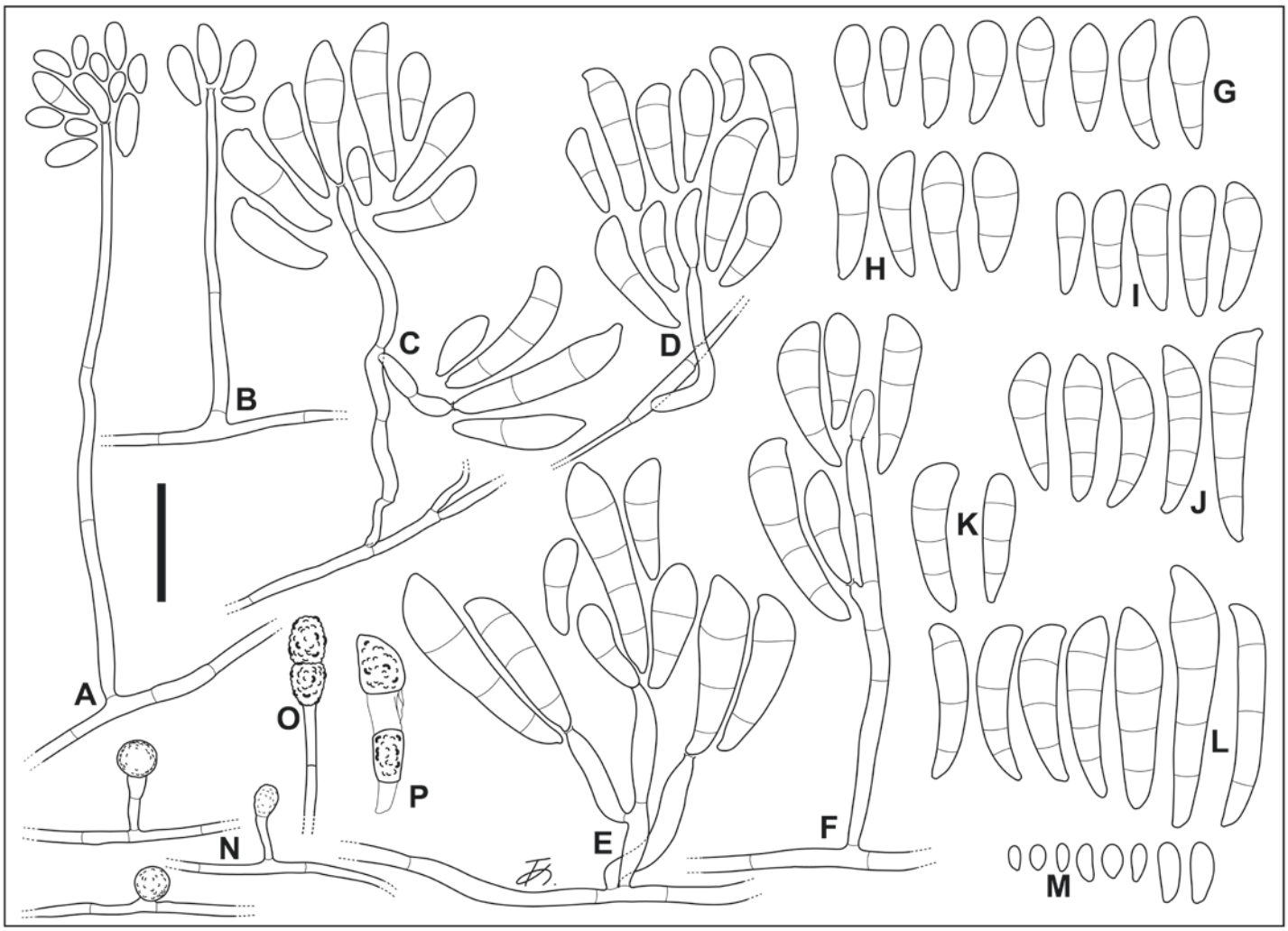

Fig. 3. *Fusarium oligoseptatum* cultured on SNA under black light. **A, B.** Tall and short aerial conidiophores forming mostly 0-septate conidia. **C, D.** Short aerial conidiophores forming conidia with relatively few septa. **E, F.** Sporodochial conidiophores forming clavate conidia with different septation. **G–L.** Septate conidia formed on sporodochial conidiophores, with some large clavate conidia (G–I: Short clavate conidia with 1–2 septa). **M.** 0-septate conidia formed on aerial conidiophores. **N–P.** Chlamydospores in hyphae (N, O) and in conidia (P). A–D, G, J, N from NRRL 62579 (ex-holotype); E, H, L, P from NRRL 62580; F, I, K, M, O from NRRL 62581. Bar = 25 µm.

of-heaven, *Ailanthus altissima*, 30 Jan. 2010, *Matthew T. Kasson (Kasson Bh24)* (BPI 910525 – holotype, designated in this study; NRRL 62579 = FRC S-2581 = MAFF 246283 = CBS 143241 – ex-holotype cultures).

Additional strains examined: **USA**: *Pennsylvania*: Franklin Co., isolated from a live *E. validus* female infesting an *A. altissima* tree, 9 Mar. 2010, *Matthew T. Kasson (Kasson Ch19)* (NRRL 62580 = FRC S-2594 = MAFF 246284 = CBS 143242); *Pennsylvania:* Huntingdon Co., isolated from a live *E. validus* female infesting an *A. altissima* tree, 27 Feb. 2010, *Matthew T. Kasson (Kasson Dh24)* (NRRL 62581 = FRC S-2616 = MAFF 246285 = CBS 143243; *Pennsylvania:* Mifflin Co., isolated from a live *E. validus* female infesting an *A. altissima* tree, 1 July 2009, *Matthew T. Kasson (Kasson Eh11)* (NRRL 62582 = FRC S-2627 = MAFF 246286 = CBS 143244; morphologically degenerated strain).

Description: *Colonies* on PDA showing radial mycelial growth rates of 2.2–3.6 mm/d at 20 °C and 3.3–4.6 mm/d at 25 °C in the dark. Colony color on PDA white (1A1) to yellowish-white (4A2) or orange white (5A2) in the dark, white (1A1) to yellowish-white (3–4A2) or pale yellow (3–4A3) under black light. Aerial mycelium white (1A1), sparsely formed or floccose in the dark, more abundantly formed and covering entire surface of colonies under black light. Colony margin entire to undulate. Reverse pigmentation absent or yellowish-white (3–4A2) or pale yellow (3–4A3) in the dark and under black light. *Exudates* absent. *Odor* absent, or slightly moldy or sweet in some strains. *Hyphae* on SNA 1.5–7.5 µm wide. *Chlamydospores* present but formation delayed in hyphae and in septate sporodochial conidia, mostly subglobose to round ellipsoidal, intercalary or terminal, mostly single, sometimes in chains, ordinary hyaline to very pale-yellow, wall smooth or often minutely roughened, 6–23.5 × 4.5–9 µm. *Sclerotia* absent. *Sporulation* on SNA and PDA generally rapid and abundant under black light, delayed in the dark, sometimes less sporulation on PDA in the dark; light-colored on SNA and PDA under black light or under daylight; sporodochia formed sparsely on SNA, rare on PDA. *Aerial conidiophores* formed abundantly on SNA under black light, less frequently in the dark, erect, short or tall and narrow, mostly unbranched, rarely branched sparsely, up to 130 µm long, 3–5.5 µm wide at base, thin-walled, forming monophialides integrated in the apices. *Phialides* on aerial conidiophores simple, subcylindrical to subulate, tapering towards apex, often with a minute collarette at the tip, 10–62.5 × 2.5–5.5 µm. *Aerial conidia* mostly (1) elliptical, oblong-elliptical,

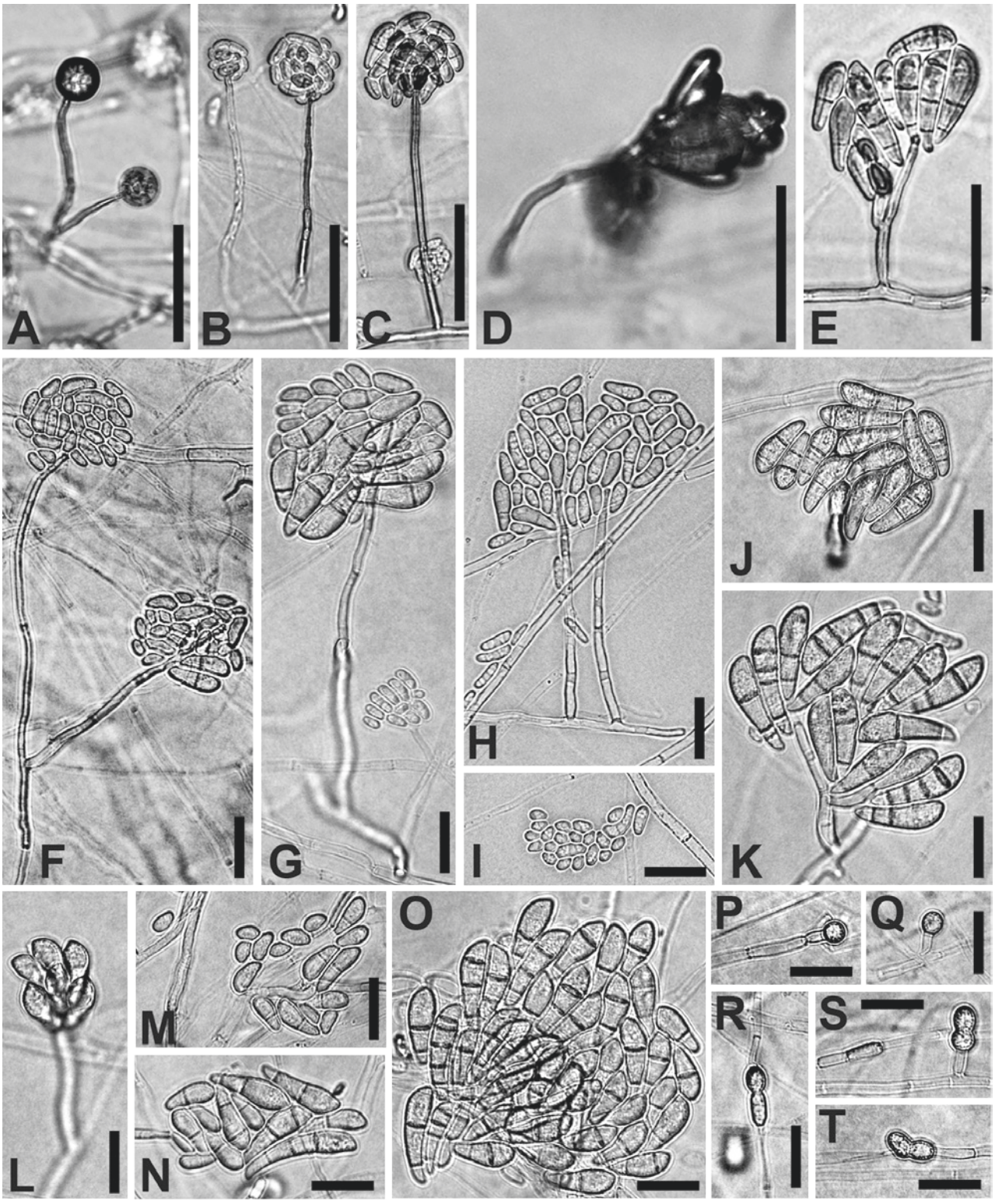

Fig. 4. *Fusarium oligoseptatum* cultured on SNA under black light. **A–C, F–H.** Aerial conidiophores forming 0–1(–2)-septate conidia. **D, E, J–L.** Sporodochial conidiophores forming clavate conidia, often swollen apically with 1–4 septa. **I.** Round to obovate 0-septate aerial conidia. **M–O.** Mostly clavate conidia formed on sporodochial conidiophores, including some 0-septate that are oblong or short-clavate. **P–T.** Chlamydospores formed in hyphae. A–I, K, M, N, Q–S from NRRL 62579 (ex-holotype); J, O, P, T from NRRL 62580; L from NRRL 62581. (A–E: Aerial view without a cover slip; F–T: Mounted in water with a cover slip). Bars: A–E = 50 µm, F–T = 20 µm.

fusiform-elliptical to short clavate, occasionally reniform, some obovate to subglobose, 0–1(–2)-septate; 0-septate on SNA in the dark: 3–13 × 2–5.5 µm in total range, 5.3–8.5 × 2.8–3.9 µm on average [ex type (NRRL 62579): 3.5–12 × 2–4 µm in total range, 6.9±2.0 × 2.8±0.5 µm on average ± S.D.]; 0-septate on SNA under black light: 3–17 × 2–6.5 µm in total range, 6.0–9.0 × 2.8–3.8 µm on average [ex type (NRRL 62579): 4–17 × 2.5–6.5 µm in total range, 9.0±2.8 × 3.8±0.9 µm on average ± S.D.]; 1-septate on SNA under black light: 7.5–26 × 2.5–8 µm in total range, 14.5–15.3 × 4.6–4.9 µm on average [ex type: 10.5–21.5 × 2.5–6 µm in total range, 15.1±2.7 × 4.6±0.7 µm on average ± S.D.]; sometimes with (2) larger, falcate to clavate, or curved cylindrical,

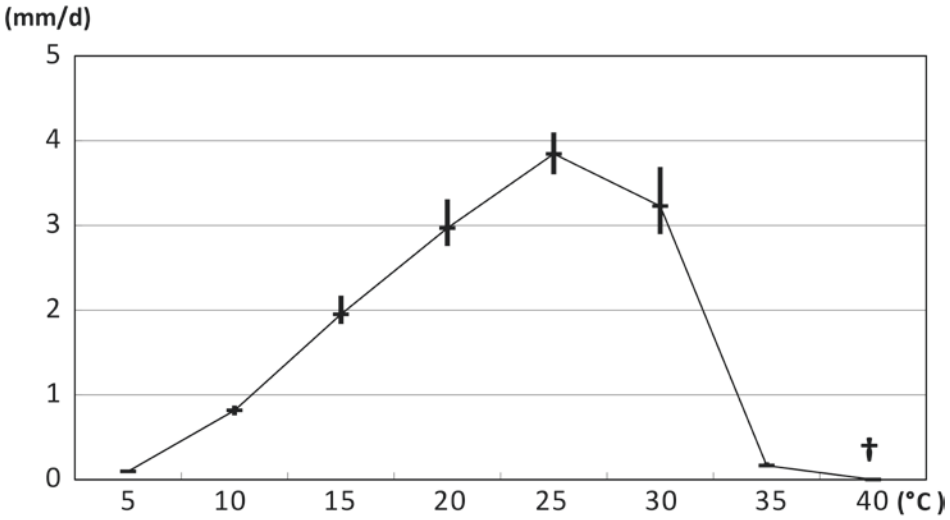

Fig. 5. Radial mycelial growth rate of *Fusarium oligoseptatum* per day on PDA cultured at eight different temperatures. Thick horizontal and vertical bars indicate means and total ranges, respectively, of the 4 isolates analyzed. All isolates failed to grow and died at 40 °C.

(1–)2(–3)-septate conidia, morphologically continuous with falcate sporodochial conidia. *Sporodochial conidiophores* generally shorter and thicker than aerial conidiophores, unbranched or sometimes sparsely branched, contorted, forming monophialides integrated apically, 20–145 × 3–6 µm, or sometimes adelophialides. *Sporodochial phialides* simple, subulate, lanceolate or subcylindrical, often with a conspicuous collarette at the tip, 9.5–44 × 2.5–5.5 µm. *Sporodochial conidia* hyaline, mostly falcate to long clavate, sometimes curved cylindrical, often swollen slightly or conspicuously in their upper part, tapering towards base, often with a rounded or papillate apical cell, and an indistinct foot-like or rounded basal cell, (0–)1–3(–5)-septate; swollen conidia sometimes 'dolphin-like' (Brayford 1987) or comma-shaped when 1- to 2-septate, formed on SNA frequently under black light, less frequently in the dark, very rarely formed on PDA under black light; 1-septate on SNA under black light: 11–32.5 × 4–10 µm in total range, 18.2–20.1 × 6.1–7.2 µm on average [ex type: 13.5–30 × 4.5–8.5 µm in total range, 19.3±3.9 × 6.2±1.0 µm on average ± S.D.]; 2-septate on SNA under black light: 15.5–39.5 × 5.5–12 µm in total range, 24.8–26.5 × 7.4–8.0 µm on average [ex type: 22–34 × 5.5–12 µm in total range, 26.1±3.2 × 7.4±1.2 µm on average ± S.D.]; 3-septate on SNA under black light: 20–60 × 5.5–12.5 µm in total range, 31.4–35.7 × 8.3–8.7 µm on average [ex type: 23–60 × 5.5–12.5 µm in total range, 35.7±7.2 × 8.7±1.3 µm on average ± S.D.]; 4-septate on SNA under black light: 28.5–67.5 × 7–11 µm in total range, 38.7–47.9 × 8.8–8.9 µm on average [ex type: 28.5–67.5 × 7–10 µm in total range, 47.9 × 8.9 µm on average]. Together with multiseptate sporodochial conidia, often forming (0-)1(-2)-septate, oblong to naviculate or short-clavate, straight or curved conidia with a rounded apex and truncate base.

Clade-based diagnosis: Distinguished by phylogenetic analysis of multilocus DNA sequence data (Kasson *et al*. 2013, O'Donnell *et al*. 2015).

Substrates or hosts: All ex-holotype and authentic strains were isolated from *E. validus* in the galleries of *A. altissima* in Pennsylvania (PA), USA. *Fusarium oligoseptatum* has also been confirmed using multilocus sequence typing from Ohio (OH), Virginia (VA) and Maryland (MD) (O'Donnell *et al*. 2015), and from Tennessee (TN) and (West Virginia) WV using the AF-3 / *F. oligoseptatum* (AF-4) multiplex PCR assay (Short *et al.* 2017). Currently known from 16 additional plant hosts, all of which have been confirmed molecularly as *F. oligoseptatum* using AF-3 / *F. oligoseptatum* (AF-4) multiplex PCR (Short et al. 2017): *Acer pensylvanicum* (PA, USA), *Acer rubrum* (PA, USA), *Amelanchier arborea* (VA, USA), *Aralia spinosa* (PA, USA), *Betula lenta* (PA, USA), *Celtis occidentalis* (WV, USA), *Fagus grandifolia* (OH, USA), *Fraxinus americana* (WV, USA), *Populus grandidentata* (PA, USA), *Prunus serotina* (GA, USA), *Quercus montana* (PA, USA), *Quercus rubra* (PA, USA), *Rhus typhina* (PA, USA), *Robinia pseudoacacia* (PA, USA), *Tsuga canadensis* (OH, USA), and *Pinus virginiana* (VA, USA).

Distribution: Presently confirmed from GA (Georgia), MD, OH, PA, TN, VA, and WV, USA.

Notes: Morphological data on sporodochial conidia was based mainly on NRRL 62579, 62580 and 62581. Strain NRRL 62582 appears degenerated and produced only 1-septate sporodochial conidia after 1 mo on SNA under continuous black light. Strains of this species were all isolated from female *E. validus* ambrosia beetles infesting *A. altissima* that were collected in different counties in Pennsylvania, USA. The most distinctive morphological feature of this fungus is the frequent production of sporodochial conidia with 1–2 septa (Table 2, Figs. 3E–I, 4E, J–O). This species formed sporodochial conidia with more than two septa, but the percentage of 0–2-septate conidia (76.5–81 %) was much higher than observed in *F. ambrosium* (3.7–24.5 %) and *F. euwallaceae* (Freeman *et al.* 2013), where more than 75 % of the conidia were 3–5-septate. Cultures appear whitish to yellowish-white when aerial mycelium is sparse on PDA.

Fusarium ambrosium (Gadd & Loos) Agnihothr. & Nirenberg, *Stud. Mycol.* **32**: 98. 1990. MycoBank MB130225. Figs 6–8.
Basionym: *Monacrosporium ambrosium* Gadd & Loos, *Trans. Brit. Mycol. Soc.* **31**(1 & 2): 13. 1947. MB288427.
Synonyms: *Dactylella ambrosia* (Gadd & Loos) K.Q. Zhang, Xing Z. Liu & L. Cao, *Mycosystema* **7**: 112. 1995. MB447506.
Neocosmospora ambrosia (Gadd & Loos) L. Lombard & Crous, *Stud. Mycol.* **80**: 227. 2015. MB810957.
Fusarium bugnicourtii Brayford, *Trans. Brit. Mycol. Soc.* **89** (3): 350. 1987. MB133337.

Type: **India**, Upari Tea Institute, a dried specimen from culture of NRRL 22346, isolated from a gallery of *Euwallacea fornicatus* infesting a tea tree, *Camellia sinensis*, 9 May 1990, *V. Agnihothrudu*

Table 2. Percentage of clavate sporodochial conidia of *Fusarium oligoseptatum* and *F. ambrosium* with different numbers of septa cultured on SNA under black light at 25 °C.

	Percentage of conidia with different numbers of septa								
Species/strain	**0-septate**	**1-septate**	**2-septate**	**3-septate**	**4-septate**	**5-septate**	**6-septate**	**7-septate**	**Total number of conidia counted**
F. oligoseptatum[a]									
NRRL 62579 (ex-holotype)	**9.5**	**41.0**	**30.5**	**15.2**	**1.9**	**1.9**	**0**	**0**	**105**
NRRL 62580	12.1	46.8	20.6	16.8	3.7	0	0	0	107
NRRL 62581	3.8	47.1	25.5	18.9	3.8	0.9	0	0	106
F. ambrosium									
NRRL 20438	0	3.4	5.0	44.5	38.7	6.7	1.7	0	119
NRRL 22345	0	5.9	7.8	25.5	23.5	29.5	4.9	2.9	102
NRRL 22346 (ex-epitype)	**0**	**0**	**3.7**	**40.2**	**46.8**	**8.4**	**0.9**	**0**	**107**
NRRL 36510	0	0.9	12.3	30.2	43.4	13.2	0	0	106
NRRL 46583	0	4.4	5.3	28.1	44.6	12.3	5.3	0	114
NRRL 62605	1.0	4.9	18.6	52.0	18.6	4.9	0	0	102

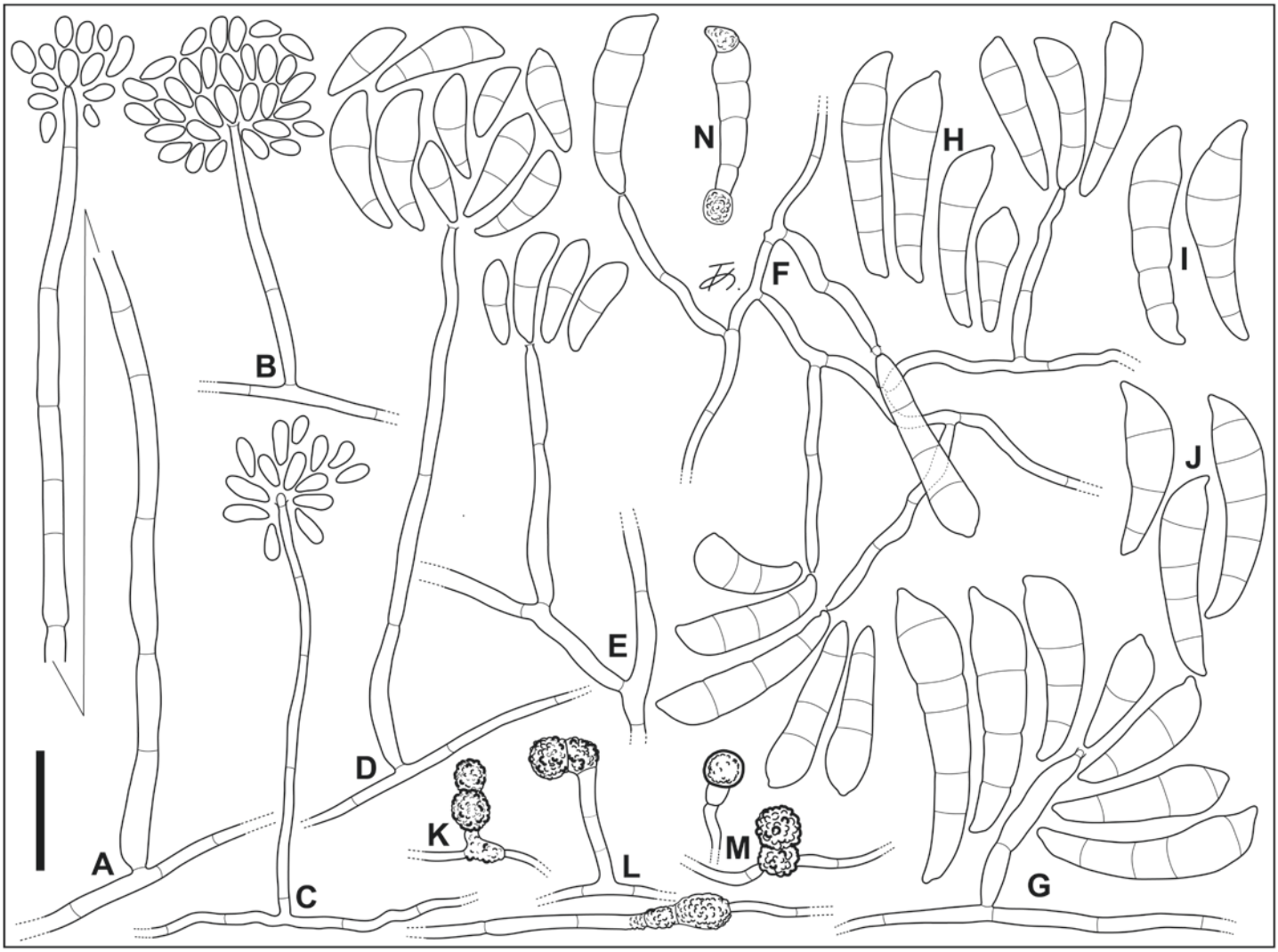

Fig. 6. *Fusarium ambrosium* cultured on SNA under black light. **A–C.** Tall and short aerial conidiophores forming 0-septate conidia. **D, E.** Short and tall aerial conidiophores forming septate conidia. **F, G.** Sporodochial conidiophores forming large clavate conidia. **H–J.** Clavate multiseptate septate conidia. **K–N.** Chlamydospores in hyphae (K–M) and conidium (N). A, E, F, L from NRRL 62605; B, D, H, K, N from NRRL 20438; C, G, M from NRRL 22346 (ex-epitype); I from NRRL 22345; J from NRRL 46583. Bar = 25 µm.

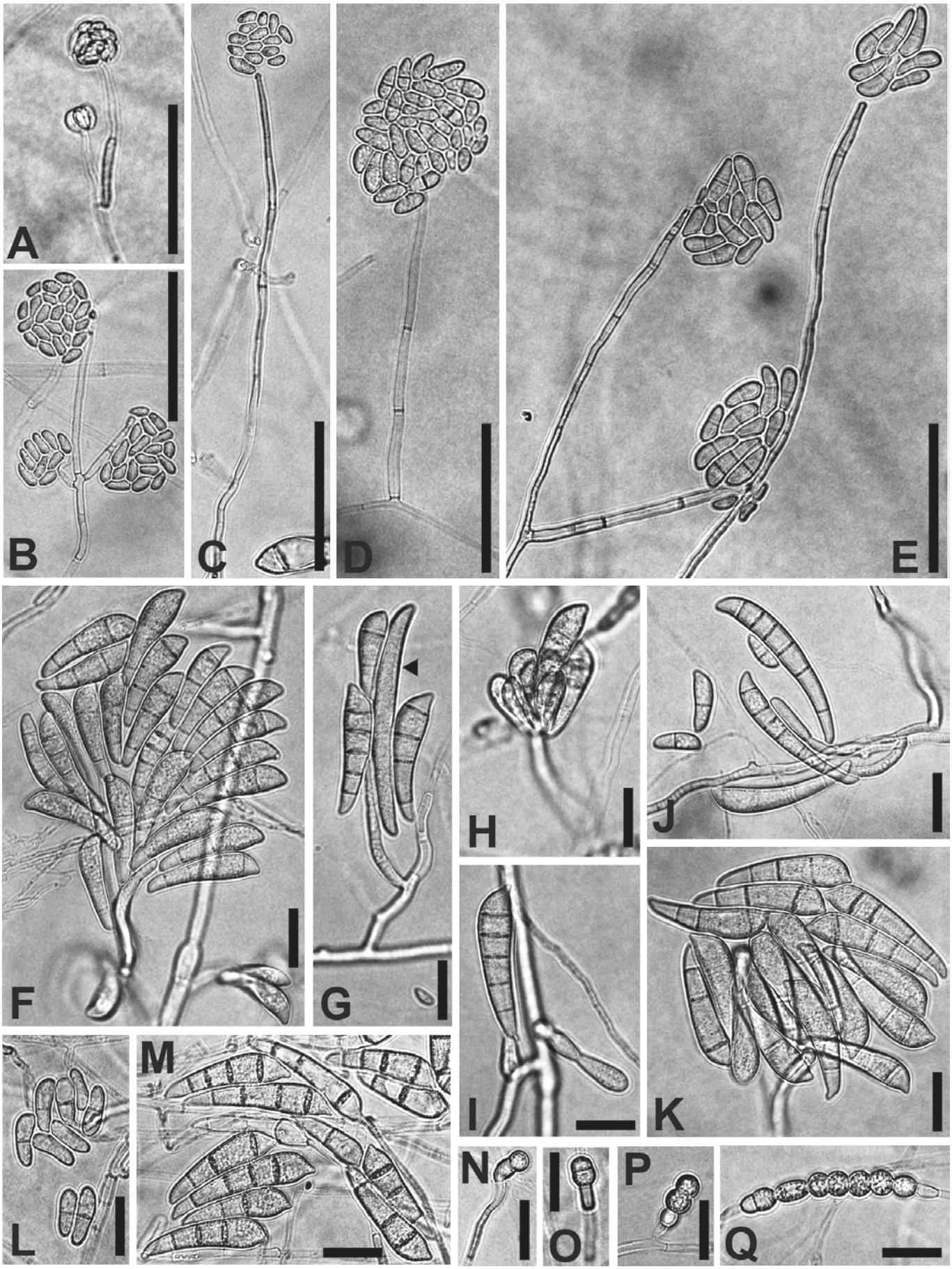

Fig. 7. *Fusarium ambrosium* cultured on SNA under black light. **A–E.** Aerial conidiophores forming 0–1(–2)-septate conidia. **F–K.** Sporodochial conidiophores forming mostly multiseptate clavate conidia, swollen apically with (2–)3–5(–6)-septa; (Arrowhead in G:) crescent-shaped conidium without septa. **L.** 0–1-septate conidia formed on aerial conidiophores. **M.** Clavate conidia formed on sporodochial conidiophores. **N–Q.** Chlamydospores formed in hyphae. A, L, M, O from NRRL 62605; B, C, E, I, K, N from NRRL 22346 (ex-epitype); D, F–H, J, P, Q from NRRL 20438. (A–E: Aerial view without a cover slip; F–Q: Mounted in water with a cover slip.) Bars: A–E = 50 µm, F–Q = 20 µm.

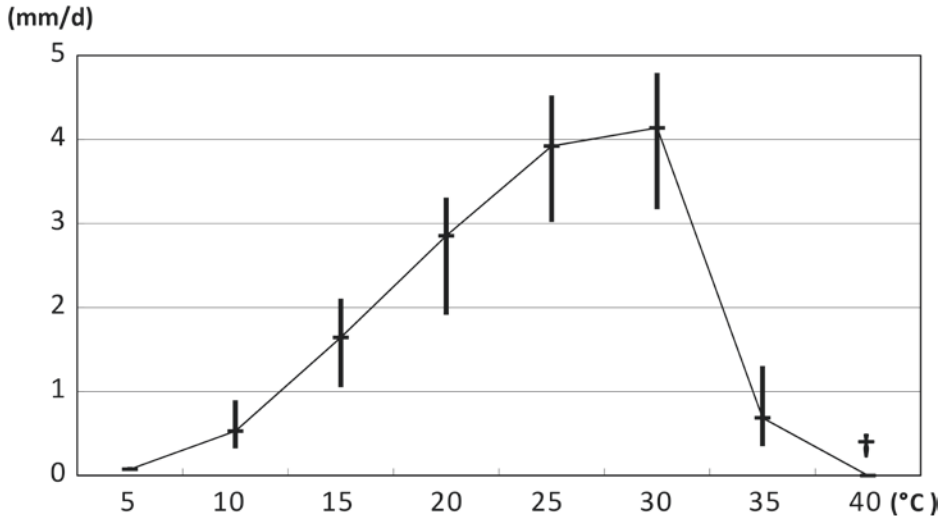

Fig. 8. Radial mycelial growth rate of *Fusarium ambrosium* per day on PDA cultured at eight different temperatures. Thick horizontal and vertical bars indicate means and total ranges, respectively, of the 6 isolates analyzed. All isolates failed to grow and died at 40 °C.

(BPI 910524 – epitype MBT 378232, designated in this study; NRRL 22346 = BBA 65390 = CBS 571.94 = MAFF 246287 – ex-epitype cultures). **Sri Lanka**, an illustration of a mature conidium of the fungus taken from a gallery of *Euwallacea fornicatus* infesting a tea tree, *Camellia sinensis*, C.H. Gadd & C.A. Loos (*Trans. Brit. Mycol. Soc.* **31** (1 & 2): 16, Text-fig. 5 (1987) – lectotype, MBT379562, designated in this study; the original description by Gadd & Loos (1947) did not designate type material of *M. ambrosium* and it was not found in the IMI, K, and BPI herbaria).

Additional strains examined: **India**, *Maharashtra*: Chinchona, isolated from a gallery of *E. fornicatus* infesting a *C. sinensis* tree, 17 Jul. 1985, (*unknown collector)* (NRRL 20438 = IMI 296597 = MAFF 246291, preserved as the ex-holotype strain of *F. bugnicourtii*); *(State name of India not recorded)*: Upari Tea Institute, isolated from a gallery of *E. fornicatus* infesting *C. sinensis* tree, 9 May 1990, *V. Agnihothrudu* (NRRL 22345 = BBA 65389 = MAFF 246288; NRRL 36510 = BBA 65390 = MAFF 246289 as a duplicate of NRRL 22346); *Tamil Nadu*: Coimbatore District, Anamallais, High Forest Estate, isolated from a gallery of *E. fornicatus* infesting stem of *C. sinensis* tree, 26 Mar. 1990, *(unknown collector)* (NRRL 46583 = IMI 339338 = MAFF 246290, preserved as *F. bugnicourtii*). **Sri Lanka**, *Talawakelle*, St. Coombs, Tea Research Institute of Sri Lanka, isolated from a gallery of *E. fornicatus* infesting *C. sinensis* tree, Mar. 2012, *S. Freeman* (NRRL 62605).

Description: *Colonies* on PDA showing radial mycelial growth rates of 1.9–3.3 mm/d at 20 °C and 3.0–4.5 mm/d at 25 °C in the dark. Colony color on PDA white (1A1) to yellowish-white (4A2) in the dark, white (1A1) to yellowish-white (3–4A2) or pale yellow (4A3) under black light. Aerial mycelium white (1A1) sparse to floccose in the dark, more abundantly formed and often covering entire surface of colonies under black light. Colony margin entire to undulate. Reverse pigmentation absent or yellowish-white (3–4A2) or pale yellow (4A3) in the dark and under black light. Olive (3E–F5–8) to olive-brown (4E–F5–8) spots in some strains when sporodochia formed on PDA. *Exudates* absent. *Odor* absent, or slightly moldy or sweet in some strains. *Hyphae* on SNA 1.5–9.5 µm wide. *Chlamydospores* present in hyphae and in septate sporodochial conidia, mostly subglobose to round ellipsoidal, intercalary or terminal, single or often in chains, hyaline or slightly pale yellow, smooth to often minutely rough-walled, 5–31.3 × 4.5–13 µm. *Sclerotia* absent. *Sporulation* on SNA and PDA generally rapid and abundant under black light, less abundant in some strains, delayed or sometimes less production in the dark; generally light-colored on SNA and PDA under black light or under daylight; sporodochia formed sparsely on SNA, and rarely on PDA under daylight; olive (3E–F5–8) to olive brown (4E–F5–8) when produced in mass. *Aerial conidiophores* formed abundantly on SNA under black light, erect, short or tall, mostly narrow but rarely thicker, mostly unbranched, rarely branched sparsely, up to 320 µm long, 2.5–7 µm wide at base, thin-walled, forming monophialides integrated in the apices. *Phialides* on aerial conidiophores simple, subcylindrical to subulate, tapering towards apex, often with a minute collarette at the tip, 15–66 × 2.5–4 µm. *Aerial conidia* typically (1) elliptical, oblong-elliptical, fusiform-elliptical to short clavate, occasionally reniform, some obovate, 0–1-septate; 0-septate on SNA in the dark: 3.5–14 × 1.5–7 µm in total range, 8.4–9.4 × 2.8–3.3 µm on average [ex epitype (NRRL 22346): 3.5–14 × 2–5.5 µm in total range, 9.4±2.4 × 3.2±0.6 µm on average ± S.D.]; 0-septate on SNA under black light: 3.5–22 × 2–7.5 µm in total range, 7.8–10.8 × 3.5–4.5 µm on average [ex epitype (NRRL 22346): 4.5–18.5 × 2–7.5 µm in total range, 10.2±2.4 × 4.1±1.1 µm on average ± S.D.]; 1-septate on SNA in the dark: 8–26 × 2.5–6.5 µm in total range, 13.6–16.3 × 3.7–4.9 µm on average [ex epitype: 12–19 × 3.5–5.5 µm in total range, 15.3±1.9 × 4.8±0.4 µm on average ± S.D.]; 1-septate on SNA under black light: 8.5–37 × 2.5–10.5 µm in total range, 15.3–20.1 × 5.4–6.6 µm on average [ex epitype: 10.5–36 × 2.5–10.5 µm in total range, 19.3±5.1 × 6.2±1.5 µm on average ± S.D.]; sometimes with (2) larger, falcate to clavate, or curved clavate, (1–)2(–3)-septate conidia, morphologically continuous with falcate sporodochial conidia. *Sporodochial conidiophores* generally short, unbranched or rarely sparsely branched, contorted, monophialides integrated apically, 20–61.3 × 3.5–5 µm. *Sporodochial phialides* simple, subulate, lanceolate or subcylindrical, often with a conspicuous collarette at the tip, 13–60 × 3–6 µm. *Sporodochial conidia* hyaline, mostly falcate to long clavate, sometimes curved cylindrical, mostly swollen in the upper part, tapering towards base, often with a round or papillate apical cell, and a distinct or indistinct foot-like, or rounded basal cell, swollen conidia often appear "dolphin-like" (Brayford 1987), (0–)2–5(–7)-septate, formed on SNA under black light, less frequently in the dark, sometimes formed on PDA under black light, rarely in the dark; 2-septate on SNA under black light: 15–61.5 × 3–12 µm in total range, 24.7–32.2 × 6.9–9.1 µm on average [ex-epitype: 15–61.5 × 4–10 µm in total range, 28.3±7.8 × 7.8±1.2 µm on average ± S.D.]; 3-septate on SNA under black light: 21–57.5 × 3.5–13 µm in total range, 34.1–40.4

× 8.3–10.0 µm on average [ex epitype: 30–57 × 7.5–12.5 µm in total range, 40.4±4.9 × 8.8±1.1 µm on average ± S.D.]; 4-septate on SNA under black light: 25.5–78.5 × 6–12.5 µm in total range, 40.7–45.6 × 8.8–10.4 µm on average [ex epitype: 33–78.5 × 7.5–11.5 µm in total range, 45.3±6.1 × 8.8±0.9 µm on average ± S.D.]; 5-septate on SNA under black light: 30–64 × 7–12.5 µm in total range, 42.9–52.1 × 8.8–10.3 µm on average [ex epitype: 37–51.5 × 7.5–12 µm in total range, 45.6±3.7 × 8.8±1.1 µm on average ± S.D.]. Together with multiseptate sporodochial conidia, often forming (0-)1(-2)-septate, oblong to naviculate or short-clavate, straight or curved conidia, with a rounded apex and a truncate base.

Notes: Strain NRRL 62605 did not form conidia on SNA or PDA in the dark. Therefore, the description of conidia in the dark was based on the three other strains examined in this study. All of the strains studied were isolated from galleries of *E. fornicatus* infesting *C. sinensis* trees in India and Sri Lanka. *Fusarium ambrosium* was originally isolated from *E.* (*Xyleborus*) *fornicatus* galleries in stems of Chinese tea and caster-oil trees in Sri Lanka, and was described as a new species of *Monacrosporium*, i.e., *M. ambrosium* by Gadd & Loos (1947). Forty years later, it was redescribed by Brayford (1987) as *F. bugnicourtii* based on collections from beetle galleries in Chinese tea in India and borer-damaged *Hevea brasiliensis* and *Theoborma cacao* in Malaysia. Brayford (1987) considered *F. bugnicourtii* to be conspecific with *F. tumidum* var. *coeruleum* (Bugnicourt 1939) isolated from *H. brasiliensis*, but distinct from *F. tumidum*. *Fusarium bugnicourtii* was recognized as conspecific with Gadd and Loos' species, *M. ambrosium* from the shot-hole borer on tea, and synonymized under the new combination, *F. ambrosium* based on its nomenclatural priority (Nirenberg 1990). Because type material of *M. ambrosium* (≡ *F. ambrosium*) was not designated in the original description by Gadd & Loos (1947) and not found in the IMI, K, and BPI herbaria, we selected a line-drawing (illustration) of a conidium from Gadd & Loos (1947) as the lectotype, according to Art. 9.2 and 9.3 of the ICNafp (McNeill et al. 2012). To supplement the lectotype, BPI 910524, a dried culture of NRRL 22346 (= BBA 65390 = CBS 571.94), isolated from a gallery of *E. fornicatus* infesting *C. sinensis* in India by V. Agnihothrudu, was selected as the epitype according to Art. 9.8 of the code. Because an authentic strain of *F. ambrosium*, IMI 296597 (= NRRL 20438), isolated from *E.* (*Xyleborus*) *fornicatus* galleries in tea tree from India in 1985, was designated as the holotype of *F. bugnicourtii* by Brayford (1987), this material was not selected for the epitype, per Art. 52.1 and 52.2 of the ICN (McNeill *et al.* 2012). Although NRRL 62605 was isolated from an *E. fornicatus* gallery in Sri Lanka from the same host and type locality, it was not selected as the epitype because it appears to be an interspecific hybrid that contains alleles from what appear to be two other AFC species (Kasson *et al.* 2013). The present epitypification was prepared to stabilize the taxonomy of this species.

Fusarium ambrosium is most similar morphologically to *F. euwallaceae* (Freeman *et al.* 2013, Kasson *et al.* 2013). *Fusarium ambrosium* and *F. euwallaceae* produce very similar falcate to clavate, septate sporodochial conidia that are swollen in their upper half, together with ovoid to ellipsoid, 0-septate aerial conidia. The conidial sizes and number of septa of these two fusaria are almost identical (Table 2, Figs 6, 7; Freeman *et al.* 2013). However, *F. ambrosium* and *F. euwallaceae* can be distinguished by the production of hyaline or olive to olive-brown conidia when old in the former and bluish to greenish conidia in the latter when produced in mass on PDA after 1 mo (Freeman *et al.* 2013). Production of sporodochial conidia in *F. euwallaceae* is easily observed on SNA and PDA in the dark and under black light, but in *F. ambrosium* it is often delayed or limited without black light illumination, even if cultured on SNA.

A preliminary morphological comparison of the sporodochial conidia for 10 of the 12 AFC species has been conducted (T. Aoki *et al.* unpubl.). Ten of the species produced clavate sporodochial conidia that were swollen apically, and two, including AF-6 associated with *Euwallacea* sp. #2 in avocado in the Miami-Dade area of southern Florida, USA and AF-9 from *Xyleborus ferrugineus* in Costa Rica and *Delonix regia* (royal poinciana) in southern Florida only produced curved fusiform, septate sporodochial conidia in culture, as commonly observed in typical members of the *F. solani* species complex. Three novel AFC species that were reported recently (O'Donnell *et al.* 2015, Na *et al*. 2018), including AF-10 from Singapore, AF-11 from Sri Lanka, and AF-12 (= *F. kuroshium*) from San Diego County, California, produced clavate sporodochial conidia in culture.

The available data suggests that most of the AFC species might possess diagnostic phenotypic characters. For example, AF-3 ex *Euwallacea interjectus* infesting *Acer negundo* in Gainesville, Florida produced sporodochial conidia that were variable in size and shape; AF-5 from Malaysia ex *Hevea brasiliensis* produced the shortest sporodochial conidia when comparing those with the same number of septa produced by the members of the AFC; AF-7 from *Euwallacea* sp. #3 ex *Persea americana* in Australia produced sporodochial conidia that were densely and/or obliquely septate; AF-8 from *Euwallacea* sp. #2 ex avocado in the Miami-Dade area of southern Florida appeared to be unique in that it is the only AFC species that produced swollen clavate and curved fusiform sporodochial conidia (Kasson *et al.* 2013); AF-10 from Singapore produced clavate sporodochial conidia that were narrower than conidia of other AFC species with the same number of septa; AF-11 ex *E. fornicatus* in Chinese tea from Sri Lanka produced sporodochial conidia that were frequently pointed and curved or hooked to one side; *F. kuroshium* AF-12 ex *Euwallacea* sp. #5 from *Platanus racemosa* (California sycamore) and several other woody hosts in San Diego County, California was distinguished by the production of clavate sporodochial conidia together with crescent- or comma-shaped conidia (T. Aoki *et al.* unpubl.). By way of contrast, AF-6 ex *Euwallacea* sp. #2 in avocado in the Miami-Dade area of Florida and AF-9 from Costa Rica and the Miami-Dade area of Florida only produced curved cylindrical multiseptate conidia and elliptical to oblong aerial conidia (Kasson *et al.* 2013). However, the sporodochial conidia formed by AF-9 are atypical of the *F. solani* species complex because they were pointed at both ends, terminating in a short apical beak and a conspicuous foot-like basal cell. Detailed studies will be required to assess whether AF-6 and AF-9 possess morphological characters that distinguish them from other members of the *F. solani* species complex.

DISCUSSION

The AFC symbiont cultivated by the ambrosia beetle *Euwallacea validus* in *Ailanthus altissima* in eastern U.S. is formally described herein as *Fusarium oligoseptatum*. Sampling across 7 eastern U.S. states and 17 tree hosts confirmed that *F. oligoseptatum* is the

primary symbiont of *E. validus* and dominant, regardless of plant host with few exceptions. This species can be distinguished from the 11 other known AFC species by producing significantly more 0–2-septate clavate sporodochial conidia that are swollen apically and via multilocus molecular phylogenetics where it was strongly supported as a genealogically exclusive species-level lineage in the analyses reported here and in previous studies (Kasson *et al*. 2013, O'Donnell *et al*. 2015, Na *et al*. 2018). *Fusarium oligoseptatum* was strongly supported as a reciprocally monophyletic sister to *F. euwallaceae* + *Fusarium* sp. (AF-3) in Kasson *et al*. (2013), but the sister group relationship of *F. oligoseptatum* was unresolved in analyses that included the closely related *F. kuroshium* (AF-12) from San Diego, California (O'Donnell *et al*. 2015, and present study). Efforts to develop a robust hypothesis of evolutionary relationships among these four AFC species, which are estimated to have shared a most recent common ancestor approximately 1.6 Mya (O'Donnell *et al*. 2015), might benefit from the comparative phylogenomic analyses that are currently underway (Stajich *et al*., pers. comm.).

Herein, an epitype of *F. ambrosium* was designated based on material originally isolated from a gallery of *E. fornicatus* infesting Chinese tea in India to provide nomenclatural stability for this species. AFC species have been collected in eight different countries, including Sri Lanka (*F. ambrosium* AF-1 and *Fusarium* sp. AF-11), India (*F. ambrosium* AF-1), Malaysia (*Fusarium* sp. AF-5), Singapore (*Fusarium* sp. AF-10), Australia (*Fusarium* sp. AF-7), Israel (*F. euwallaceae* AF-2), Costa Rica (*Fusarium* sp. AF-9) and the United States (*F. euwallaceae* AF-2, *Fusarium* sp. AF-3, *F. oligoseptatum* AF-4, *Fusarium* spp. AF-6, AF-8, AF-9 and *F. kuroshium* AF-12)(Brayford 1987, Nirenberg 1990, Freeman *et al.* 2013, Kasson *et al.* 2013, O'Donnell *et al.* 2015, Short *et al.* 2017, Na *et al*. 2018). To date only three species within the AFC have been described formally, i.e., *F. ambrosium* (AF-1; Gadd & Loos 1947, Nirenberg 1990), *F. euwallaceae* (AF-2; Freeman *et al.* 2013) and *F. oligoseptatum* (AF-4; in this study). Although nine of the AFC species are currently unnamed, the prospects for naming them are excellent because most of them appear to possess unique phenotypic/morphological features. Delimitations of such features may, in time, help to uncover the mechanisms underlying the production of clavate conidia, a posited adaptation for the *Euwallacea* – *Fusarium* symbiosis (Kasson *et al.* 2013). Indeed, analogous adaptations in agaricalean fungi (i.e., gongylidia) farmed by higher and occasionally lower attine ants (Schultz & Brady 2008, Masiulionis *et al.* 2014) also appear to exhibit variation among closely related lineages. However, quality of the substrate, pH, and temperature have also been shown to affect the growth and size of gongylidia in some higher attine ant cultivars when cultivated under lab conditions (Powell & Stradling 1986).

It remains unclear whether *F. ambrosium, F. euwallaceae*, or *F. oligoseptatum* are each farmed by a single *Euwallacea* species, including within their native range, where evidence of hybridization and co-cultivation with other closely related AFC members have been reported (O'Donnell *et al.* 2015). However, it has been shown that *F. euwallaceae* from avocado is obligately required for the survival and development of *Euwallacea* sp. #1 sensu O'Donnell *et al.* (2015) currently occurring in Israel, whereas *F. ambrosium* does not support development of this beetle species (Freeman *et al*. 2012). Likewise, specificity exists for *F. ambrosium* and its beetle host. Future studies focused on vector specificity could help clarify the threats these beetle-fungus consortia pose to our native ecosystems. This is especially important given that some AFC members such as *F. euwallaceae* have caused significant damage to orchard, landscape and forest trees and threaten avocado production worldwide (Mendel *et al.* 2012, Eskalen *et al.* 2013, Kasson *et al.* 2013), while other AFC members such as *F. oligoseptatum* appear to be quite innocuous when challenged against numerous plant species (Berger 2017).

The FSSC includes over 60 species (Zhang *et al.* 2006, O'Donnell *et al*. 2008, Short *et al.* 2013), a majority of which lack formal Latin binomials thus making it difficult to link specific plant diseases with specific phylogenetic species within the FSSC, including the AFC (Montecchio *et al*. 2015). The designation of formal Latin binomials for a majority of phylogenetic species within the FSSC coupled with recent abolishment of the dual system of fungal nomenclature will likely reduce confusion surrounding molecular identification of taxa within this large species complex. Nevertheless, the use of multilocus phylogenetic studies will remain the gold standard to discriminate closely related members in the FSSC.

Another avenue to further resolve these closely related phylogenetic species is to examine functional differences among closely related AFC. A recent study by Kasson *et al.* (2016) assessed the enzyme activity and wood degrading capacity of *F. oligoseptatum* and *R. subfusca*, the two known symbionts of *E. validus* in the eastern U.S. Polyphenol oxidase production was detected from *F. oligoseptatum* but not *R. subfusca*. An earlier study by Norris (1980) on AFC member *Fusarium* sp. AF-9 revealed this fungus was capable of degrading lignin. Further enzymatic studies among closely related AFC may compliment morphological and phylogenetic studies within the *Euwallacea* – *Fusarium* mutualism, revealing significant differences in enzyme activity. This is particularly important given recent studies by Aylward *et al.* (2015) that showed a diverse but consistent set of enzymes present in gongylidia, which are essential for initial degradation of plant substrates in the leaf-cutter ant-*Leucoagaricus* mutualism.

The results of this study suggest that many of the unnamed AFC species like *F. oligoseptatum* possess unique phenotypic/morphological features, which will facilitate formal description of these economically important pathogens. Phenotypic/morphological studies on the four additional AFC species from Tawain (Na *et al*. 2018) are, therefore, also fully expected. Our ongoing research is focused on advancing the systematics of the AFC to promote accurate communication within the global scientific community.

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DISCLAMER

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

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