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Three novel Ambrosia *Fusarium* Clade species producing multiseptate "dolphin-shaped" conidia, and an augmented description of *Fusarium kuroshium*

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ABSTRACT

The Ambrosia *Fusarium* Clade (AFC) is a monophyletic lineage within clade 3 of the *Fusarium solani* species complex (FSSC) that currently comprises 19 genealogically exclusive species. These fungi are known or predicted to be farmed by adult female *Euwallacea* ambrosia beetles as a nutritional mutualism (Coleoptera: Scolytinae; Xyleborini). To date, only eight of the 19 AFC species have been described formally with Latin binomials. We describe three AFC species, previously known as AF-8, AF-10, and AF-11, based on molecular phylogenetic analysis of multilocus DNA sequence data and comparative morphological/phenotypic studies. *Fusarium duplospermum* (AF-8) farmed by *E. perbrevis* on avocado in Florida, USA, is distinguished by forming two morphologically different types of multiseptate conidia and brownish orange colonies on potato dextrose agar (PDA). *Fusarium drepaniforme* (AF-10), isolated from an unknown woody host in Singapore and deposited as Herb IMI 351954 in the Royal Botanic Gardens, Kew, UK, under the name *F. bugnicourtii*, is diagnosed by frequent production of multiseptate sickle-shaped conidia. *Fusarium papillatum* (AF-11), isolated from mycangia of *E. perbrevis* infesting tea in Kandy, Sri Lanka, forms multiseptate clavate conidia that possess a papillate apical cell protruding toward the ventral side. Lastly, we prepared an augmented description of *F. kuroshium* (AF-12), previously isolated from the heads or galleries of *E. kuroshio* in a California sycamore tree, El Cajon, California, USA, and recently validated nomenclaturally as *Fusarium*. Conidia formed by *F. kuroshium* vary widely in size and shape, suggesting a close morphological relationship with *F. floridanum*, compared with all other AFC species. Maximum likelihood and maximum parsimony analyses of a multilocus data set resolve these three novel AFC species, and *F. kuroshium*, as phylogenetically distinct based on genealogical concordance. Given the promiscuous nature of several *Euwallacea* species, and the overlapping geographic range of several AFC species and *Euwallacea* ambrosia beetles, the potential for symbiont switching among sympatric species is discussed.

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INTRODUCTION

The Ambrosia *Fusarium* Clade is a monophyletic lineage within clade 3 of the *Fusarium solani* species complex (FSSC; Kasson et al. 2013; O'Donnell et al. 2015; Geiser et al. 2021). The AFC currently comprises 19 phylogenetically distinct species, most of which are known to be farmed by adult female *Euwallacea* ambrosia beetles (Coleoptera: Scolytinae) as a nutritional mutualism (Freeman et al. 2013; Kasson et al. 2013; O'Donnell et al. 2015; Aoki et al. 2018, 2019; Na et al. 2018; Carrillo et al. 2019; Lynn et al. 2020). Because *F. ambrosium* (Gadd & Loos) Agnihothr. & Nirenberg (Gadd and Loos 1947; Nirenberg 1990) was the only AFC species that possessed a Latin binomial when this clade was first discovered (Kasson et al. 2013), an informal ad hoc nomenclature was adopted that distinguishes the AFC species as AF-1 through AF-19. Currently, 19 AFC species are recognized but only eight have been formally described (Nirenberg 1990; Freeman et al. 2013; Aoki et al. 2018, 2019, 2020; Na et al. 2018; Lynn et al. 2020). Most of the AFC species produce apically swollen, multiseptate clavate conidia atypical of the

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FSSC, described as "dolphin-like" (Brayford 1987; Aoki et al. 2018) or clavate. This peculiar morphology was proposed to represent an adaptation for the mutualism with *Euwallacea* ambrosia beetles (Kasson et al. 2013).

Conidia of the AFC species are maintained and transported by adult female *Euwallacea* ambrosia beetles in their mycangia (Spahr et al. 2020). These fusaria are farmed in galleries in the xylem of woody hosts, where they serve as the primary source of nutrition for their developing larvae and adults (Six 2003, 2012; Freeman et al. 2012a, 2013; Mendel et al. 2012). Several *Fusarium*-farming *Euwallacea* beetles cause dieback and death of economically/agriculturally important host trees or shrubs in their native range in southern Asia, including Chinese tea (*Camellia sinensis* (L.) Kuntze), cacao (*Theobroma cacao* L.), citrus (*Citrus* spp.), rubber (*Hevea brasiliensis* Müll. Arg.), and wattle (*Acacia* spp.) (Brayford 1987; Freeman et al. 2012a, 2012b, 2013; Lynn et al. 2020). In addition, invasive *Euwallacea* beetles are responsible for dieback symptoms of avocado (*Persea americana* Mill.) in commercial groves, as well as many other woody hosts in urban areas and native forests in the United States, Israel, Australia, Mexico, and South Africa (Mendel et al. 2012; Eskalen et al. 2013; Freeman et al. 2013; O'Donnell et al. 2016; Paap et al. 2018; Aoki et al. 2019).

Here, we formally describe AFC species AF-8, AF-10, and AF-11, which form characteristic apically swollen, multiseptate clavate conidia. These include *F. duplospermum* (AF-8) farmed by an ambrosia beetle (*E. perbrevis* (Schedl) = *Euwallacea* sp. 2; Stouthamer et al. 2017; Smith et al. 2019); *F. drepaniforme* (AF-10) collected from an unknown host in Singapore; and *F. papillatum* (AF-11) isolated from tea infested with tea shot hole borer (TSHB) beetles (*E. perbrevis*; Stouthamer et al. 2017; Smith et al. 2019) in Kandy, Sri Lanka. In addition, because *F. kuroshium* (AF-12) was first validated nomenclaturally as *Neocosmospora kuroshio* F. Na, J.D. Carrillo & A. Eskalen ex Sand.-Denis & Crous (Sandoval-Denis et al. 2019), it was recombined as *Fusarium kuroshium* because *Neocosmospora* is nested within a monophyletic *Fusarium* (Aoki et al. 2020; Geiser et al. 2013, 2021; O'Donnell et al. 2020). An augmented description of *F. kuroshium* is provided here.

MATERIALS AND METHODS

Fungal isolates.— Collections of ambrosia beetles (*Euwallacea perbrevis*) were made in an avocado (*Persea americana*) grove in Homestead, Miami-Dade County, Florida, USA, in May and Jun 2012, and from tea (*Camellia sinensis*) bushes in Kandy, Central Province, Sri Lanka, in Feb 2014. In Sri Lanka, symptomatic tea bushes were showing typical dieback and canker symptoms. Live mature female adult beetles that possess oral mycangia (Kasson et al. 2013) were selected for fungal isolation described as follows. Female beetles were surface sterilized in 70% ethanol for 15 s, and their heads were removed with a sterile scalpel. The head of each beetle was macerated in 100 µL of sterile deionized water in a 1.5-mL Eppendorf tube. The homogenate was streaked on half-strength potato dextrose agar (PDA) amended with streptomycin and incubated at 25 C. Single colonies were subcultured on PDA amended with streptomycin. Once AFC isolates were identified using multilocus DNA sequence data, they were deposited in the Agriculture Research Culture Collection (NRRL), National Center for Agricultural Utilization Research, Peoria, Illinois, and the NARO Genebank Microorganisms Section (MAFF), Genetic Resources Center, National Agriculture Research Organization, Tsukuba, Ibaraki, Japan. Other strains examined were obtained from NRRL. The KOD and UCR strain designations refer to collections maintained by Kerry O'Donnell and the University of California, Riverside, respectively.

Molecular phylogenetic analyses.— Methods for preparing total genomic DNA, polymerase chain reaction (PCR) amplification of portions of four marker loci, and DNA sequencing were published previously (Kasson et al. 2013; O'Donnell et al. 2015; Aoki et al. 2019). The loci amplified and sequenced included translation elongation factor 1α (*TEF1*, 684 bp alignment), nuclear ribosomal internal transcribed spacer region (ITS rDNA, 445 bp alignment), and DNA-directed RNA polymerase II largest (*RPB1*, 1588 bp alignment) and second largest (*RPB2*, 1635 bp alignment) subunit. All of the sequences analyzed were downloaded from GenBank or TreeBASE (Kasson et al. 2013, TreeBASE accession no. S13974; Na et al. 2018, table 2; Aoki et al. 2019, TreeBASE accession no. S24402; Lynn et al. 2020, table 1). Sequences of *Fusarium* spp. AF-17, AF-18, and *F. rekanum* (AF-19) were not included in the analyses because their *TEF1* sequences were too short (i.e., 272–274 bp in length) to analyze with IQ-TREE and *RPB1* was missing. Sequences from two isolates of *F. neocosmosporiellum*, a related species within clade 3 of the FSSC (O'Donnell 2000), were used as the outgroup taxa. Maximum likelihood (ML) and maximum parsimony (MP) bootstrap (BS) analyses were conducted, respectively, with IQ-TREE 1.6.12 (Nguyen et al. 2015; http://www.iqtree.org/) and PAUP* 4.0a.168 (http://phylosolutions.com/paup-test/). ML-BS and MP-BS support was assessed by conducting 5000 pseudoreplicates of the data. A ML-BS analysis of the

combined data set was conducted after the best-fit model of molecular evolution was determined for each partition using ModelFinder (Kalyaanamoorthy et al. 2017) based on the Bayesian information criterion (BIC) scores (Chernomor et al. 2016).

Phenotypic characterization.— Phenotypic data, including colony color and morphology, growth rates, and morphological data of conidial structures, were collected following published methods (Freeman et al. 2013; Aoki et al. 2018, 2019). Strains originating as single-conidial isolates were cultured on potato dextrose agar (PDA; Difco, Detroit, Michigan) and synthetic low-nutrient agar (SNA; Nirenberg and 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. Cultures were incubated at 20 C in the dark on PDA in 9-cm Petri dishes, which were used to characterize colony color, odor, and morphology, unless stated otherwise. The Methuen Handbook of Colour (Kornerup and Wanscher 1978) was used as the color standard. Half-strength PDA was also used for documenting images of *F. duplospermum* colonies. PDA cultures inoculated with colony plugs ca. 5 × 5 mm in size were used for determining mycelial growth rates in the dark at eight different temperatures from 5 to 40 C at 5 C intervals (Aoki et al. 2013). Their growth at 36, 37, 38, and 39 C was also tested to assess their potential to infect humans. Cultures were examined at 1 and 4 d after inoculation, and colony margins were marked on the reverse side of the Petri dishes. Radial mycelial growth rates were calculated as arithmetic mean values per day by measuring the distance of 16 points around the colony radius and the agar plug. Measurements of growth rate at different temperatures were repeated twice, and the data were averaged for each strain. Microscopic characters were investigated on SNA at 20 C under continuous black light, unless otherwise stated, as described by Freeman et al. (2013) and Aoki et al. (2018, 2019). For *F. drepaniforme* (AF-10), morphological differences between cultures grown in the dark and under black light were also compared in the description. A Zeiss Stemi DRC stereo microscope (Zeiss, Jena, Germany) with a Nikon digital camera DS-Fi2 (Nikon, Tokyo, Japan) were used to record production of sporodochia in cultures. Conidia and conidiophores produced on SNA were examined and documented with a Zeiss Axioskop microscope using a Nikon digital camera DS-Fi2 after they were mounted in water and via direct observation of conidiogenous structures in aerial mycelia without a coverslip. Average and standard deviation (SD) for the size of individual conidial types for each strain were calculated from measurements of at least 50 conidia randomly chosen based on the number of septa. Measurements are indicated in the descriptions as follows: for the total range of a species: {minimum length–(minimum average length of a strain–maximum average length of a strain, among strains examined)–maximum length} × {minimum width–(minimum average width of a strain–maximum average width of a strain, among strains examined)–maximum width}; for the ex-type strain: {minimum length–(average length ± SD)–maximum length} × {minimum width–(average width ± SD)–maximum width}. Phenotypic characters were compared with previously published data for *F. ambrosium* (Aoki et al. 2018), *F. euwallaceae* (Freeman et al. 2013), *F. oligoseptatum* (Aoki et al. 2018), *F. kuroshium* (Na et al. 2018), *F. floridanum, F. tuaranense*, and *F. obliquiseptatum* (Aoki et al. 2019), and *F. rekanum* (Lynn et al. 2020). Swollen multiseptate aerial conidia were measured together with those formed on conidiophores on the substrate mycelium.

RESULTS

Molecular phylogenetic analyses.— The optimal model of molecular evolution found by ModelFinder (Kalyaanamoorthy et al. 2017) for each partition was as follows: *TEF1* = HKY+F+I, ITS rDNA = TIM2e+I, *RPB1* = TIMe+I+G4, *RPB2* = TNe+G4. The best tree found by a partitioned ML analysis of the combined data set with IQ-TREE (Nguyen et al. 2015) possessed a negative log-likelihood of −9356.275 (FIG. 1). Two AFC species (AF-8 + AF-9) in Clade A formed a well-supported sister group to Clade B, which contained the other AFC species. Considering phylogenetic relationships of the four AFC species that are the focus of the present study, a sister-group relationship of *F. duplospermum* (AF-8) + *Fusarium* sp. (AF-9) from Florida and Costa Rica and of *F. kuroshium* (AF-12) + *Fusarium* sp. (AF-13) from Taiwan received strong bootstrap support (i.e., ML-BS = 99%, MP-BS = 95–100%). However, a sister-group relationship of *F. drepaniforme* (AF-10) + *F. papilliatum* (AF-11) was not supported by ML and MP bootstrapping (FIG. 1). In addition, most nodes along the backbone of the phylogeny were not supported by ML and MP bootstrapping (FIG. 1).

TAXONOMY

Fusarium duplospermum T. Aoki, Konkol, R.C. Ploetz, J.A. Smith, Kasson, S. Freeman, Geiser & O'Donnell, sp. nov. FIGS. 2–4; SUPPLEMENTARY FIG. 1A, E, I–L
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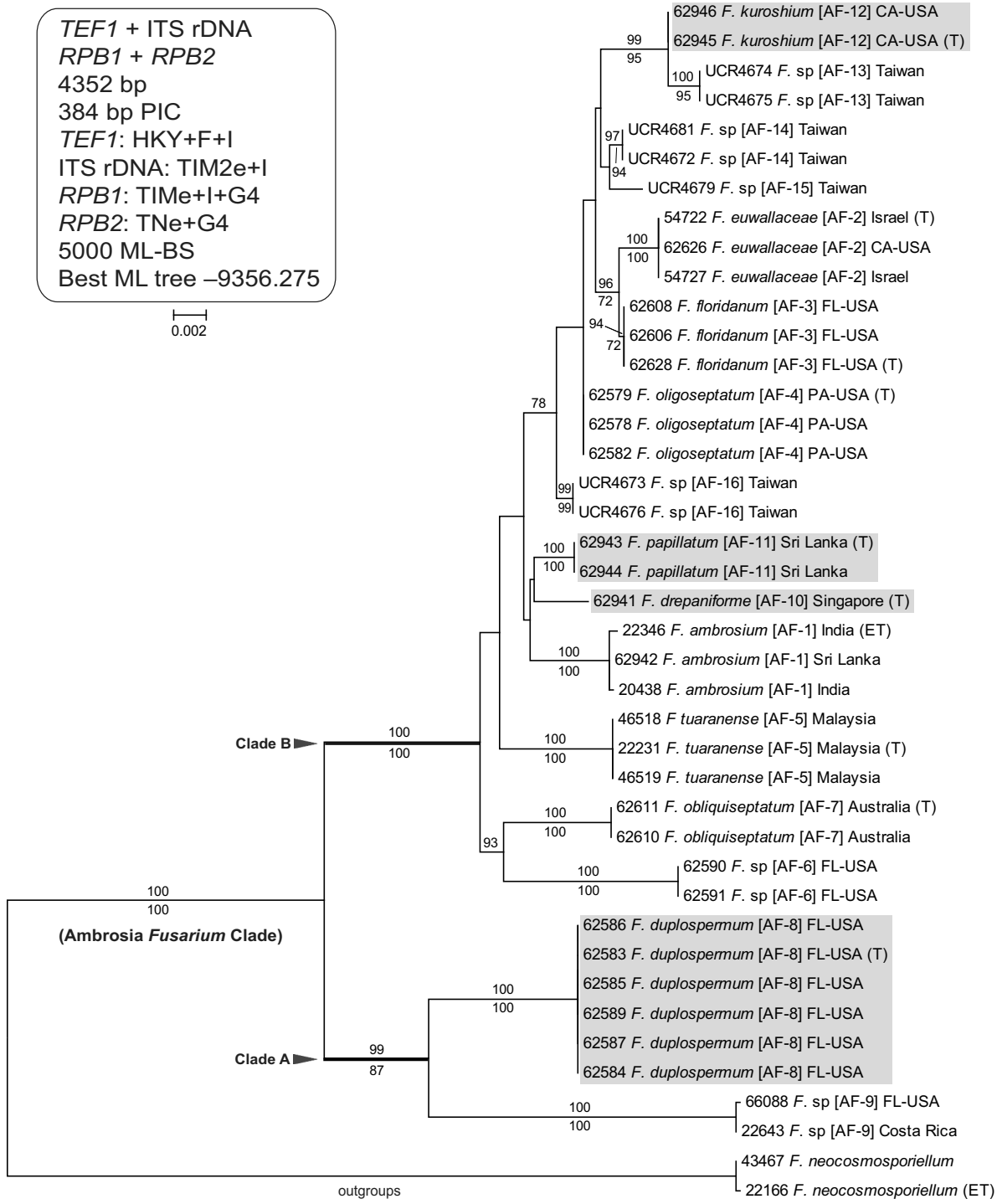

Figure 1. Maximum likelihood bootstrap (ML-BS) analysis of 16 AFC species, which are distinguished by an informal ad hoc nomenclature (i.e., AF-1 through AF-16). Eleven of the AFC species have been formally described, including four highlighted in gray reported herein. The combined 4.35-kb 4-locus data set was analyzed via maximum likelihood bootstrapping (ML-BS) with IQ-TREE (Nguyen et al. 2015) and maximum parsimony bootstrapping (MP-BS) with PAUP* 4.0a.168 (http://phylosolutions.com/paup-test/). Sequences of *F. neocosmosporiellum* were chosen to root the 39 ingroup AFC sequences based on more inclusive phylogenetic analyses (O'Donnell et al. 2013; Geiser et al. 2021). ML-BS and MP-BS support values ≥70% are reported above and below nodes, respectively, based on 5000 pseudoreplicates of the data. The 34 strains from the ARS Culture Collection are identified by a 5-digit accession number. Sequences of the seven University of California–Riverside (URC; Na et al. 2018) strains were downloaded from GenBank. PIC, parsimony-informative character.

Typification: USA. FLORIDA: Miami-Dade County, Homestead, originally isolated from the oral mycangium of an ambrosia beetle (*E. perbrevis* (Schedl)) trapped in an avocado (*Persea americana*) grove, 11 May 2012, *Joshua Konkol Amb1* (**holotype** BPI 923529, a dried specimen from a culture of NRRL 62583, Herbarium US National Fungus Collection, USA). Ex-holotype culture NRRL 62583 = MAFF 247220.

Etymology: *duplo-* (doubled) + *-spermus* (-spored), based on production of two morphologically distinct types of multiseptate conidia.

Diagnosis: *Fusarium duplospermum* can be distinguished from the other AFC species by forming two morphologically distinct types of multiseptate conidia, i.e., (i) long, slender, and falcate, or (ii) relatively short, apically swollen, curved and clavate. Conidia of the latter type are sometimes "dolphin-like" in appearance, as found in many AFC species, whereas the narrower falcate conidia resemble multiseptate conidia typically formed by most FSSC species that do not produce apically swollen "dolphin-like" conidia. Some isolates produce distinctly verticillate conidiophores. *Fusarium duplospermum* is characterized by forming brownish orange colonies on PDA, which differs from all other described AFC species, which typically produce whitish, yellowish, or grayish dull-colored mycelia on PDA.

Observation on PDA: Radial mycelial growth rates of colonies 1.8–2.3 mm per day at 20 C and 2.4–3.4 mm per day at 25 C; no growth at 38 C or higher, dead at 39 C. White (1A1), yellowish white (4A2), pale yellow (4A3), light yellow (4A4) to greyish yellow (4B3–5) initially, becoming pale orange (5A3) to greyish orange (5B3–5), or reddish white (7–8A2) to pale red (7–8A3), reddish grey (7–8B2) to greyish red (7–8B3) with age, often with a brownish orange (5–7C3–5) center when cultured in the dark. When cultured under black light, becoming more brownish centrally, or yellowish brown (5D–E4–6). Aerial mycelium white (1A1), sparsely formed, but sometimes floccose or funiculose. Colony margin typically entire, undulate in some strains. Reverse pigmentation absent or yellowish white (4A2) or pale yellow (4A3) to light yellow (4A4–5), some greyish orange (5B3–5), brownish orange (5C3–5) to yellowish brown (5D–E4–6) or brown (6D–E4–6), sometimes with yellowish pigments in the agar. Exudates absent. Odor absent or moldy. When cultured under black light, forming pale yellowish sporodochia as minute pustules with abundant conidial production after ca. 1 mo.

Microscopic characters on SNA: Hyphae 1–9 µm wide. Chlamydospores formed but delayed, intercalary in or on hyphae and conidia, terminally on lateral hyphal branches, or sometimes directly on hyphae and conidia, mostly subglobose to oblong-ellipsoidal, single or sometimes in chains of up to 3, hyaline to slightly pale yellow, smooth to often minutely rough-walled, 3.5–14 × 3–10.5 µm. Sclerotia absent. Sporulation generally starts within 2–3 d and abundant under black light, reduced in the dark in many strains; sporodochia formed sparsely and absent in some strains.

Aerial conidiophores ordinarily formed abundantly, erect, often tall and narrow, unbranched or branched sparsely to verticillately, up to 245 µm long, 2.5–6 µm wide at the base, often thin-walled, sometimes thick-walled, forming monophialides often integrated as apical segments of conidiophore branches, or sometimes as a proliferated phialide. Phialides on aerial conidiophores simple, subcylindrical to subulate, or integrated at the apex of a conidiophore, tapering toward the apex, often with a minute collarette at the tip, 9–56.5 × 1.5–5.5 µm. Aerial conidia hyaline, mostly (i) elliptical, oblong-elliptical, fusiform-elliptical to short-clavate, straight or sometimes curved, reniform or crescent-shaped, some obovate to comma-shaped, 0–1-septate; 0-septate: 3.5–(8.3–9.1)–21 × 1.5–(2.9–3.5)–6.5 µm [ex-type: 4.5–(8.3 ± 2.8)–21 × 1.5–(2.9 ± 0.6)–4.5 µm]; 1-septate: 10–(14.1–16.9)–34 × 2.5–(4.0–4.4)–7 µm [ex-type: 10.5–(14.1 ± 2.3)–20 × 2.5–(4.0 ± 0.6)–5.5 µm]; often also forming (ii) larger, falcate to clavate, sometimes curved cylindrical, (1–)3–4(–5)-septate conidia that are morphologically indistinguishable from multiseptate conidia formed on conidiophores from the substrate mycelium.

Conidiophores arising from the substrate mycelium or in sporodochia generally shorter than aerial conidiophores, sometimes tall and thick, unbranched or branched, up to 127.5 µm long, 2.5–6 µm wide at the base, straight or sometimes contorted or twisted, forming apical monophialides integrated at the apex of conidiophores, or reduced to a simple phialide on substrate mycelium. Phialides simple, subulate or subcylindrical, often with a conspicuous collarette at the tip, 8–60 × 2.5–6 µm. Conidia formed on conidiophores arising directly from the substrate mycelium or in sporodochia frequently under black light, less frequently in the dark, hyaline, of two distinct shapes: usually (i) not swollen apically, falcate, lanceolate to long clavate, or curved cylindrical, crescent-shaped, with a rounded or pointed apex, tapering gradually toward the base, with a distinct or indistinct foot-like basal cell, and sometimes (ii) straight or curved and swollen in their upper parts with a tapering apical cell, tapering toward the base with a distinct or indistinct foot-like basal cell, some "dolphin-like" (Brayford 1987), (1–)3–5(–7)-septate, 1-septate: 10–34 × 2.5–7 µm; 2-septate: 13–41.5 × 3–7 µm; 3-septate: 17.5–(28.1–41.6)–55 × 3.5–(4.9–5.6)–8

µm [ex-type: 17.5–(29.8 ± 5.7)–45.5 × 4–(5.2 ± 0.6)–8 µm]; 4-septate: 28–(37.0–49.9)–60 × 4–(5.1–6.1)–9 µm [ex-type: 28–(38.3 ± 5.8)–55 × 4–(5.7 ± 0.6)–7.5 µm]; 5-septate: 29.5–(43.3–55.2)–65 × 4–(5.5–6.4)–9 µm [ex-type: 34–(46.1 ± 5.5)–59 × 5–(6.4 ± 0.6)–9 µm]; 6-septate: 39.5–62.5 × 5–7.5 µm. Short-clavate to obovate or naviculate, straight or curved conidia, with a rounded apex and a truncate base, 0–1(–2)-septate, sometimes formed together with multiseptate conidia on shorter conidiophores arising from the substrate mycelium.

Additional isolates studied: (Only collected from the type locality.) USA. FLORIDA: Miami-Dade County, Homestead, isolated from the oral mycangium of an ambrosia beetle (*E. perbrevis*) trapped in an avocado grove, 11 May 2012, *Joshua Konkol Amb2* = NRRL 62584 = MAFF 247221; 11 May 2012, *AF4* = NRRL 62585 = MAFF 247222; 11 May 2012, *AF5* = NRRL 62586 = MAFF 247223; 1 Jun 2012, *AF6* = NRRL 62587 = MAFF 247224; 1 Jun 2012, *AF8* = NRRL 62589 = MAFF 247225.

Notes: This species forms two morphologically distinct multiseptate conidia: (i) narrow multiseptate falcate conidia (FIGS. 2H–L, 3L–N, Q–T) as observed in many FSSC species, and less frequently (ii) swollen "dolphin-like" multiseptate clavate conidia (FIGS. 2M–Q, 3O, P, U, V) as typically observed in many AFC species. Multiseptate conidia formed on conidiophores arising directly from the substrate mycelium or in sporodochia overlap morphologically with multiseptate aerial conidia (FIGS. 2G, 3H, I). This species forms chlamydospores in or on hyphae and conidia (FIGS. 2R, S, 3W–Y) that are hyaline or slightly pale yellow, smooth to often minutely rough-walled on SNA. Brownish orange colonies on PDA (FIG. 4) is a distinctive character of this species. *Fusarium duplospermum*'s fastest growth rate was at 30 C (FIG. 5), except for NRRL 62587, which grew fastest at 25 C. Except for two

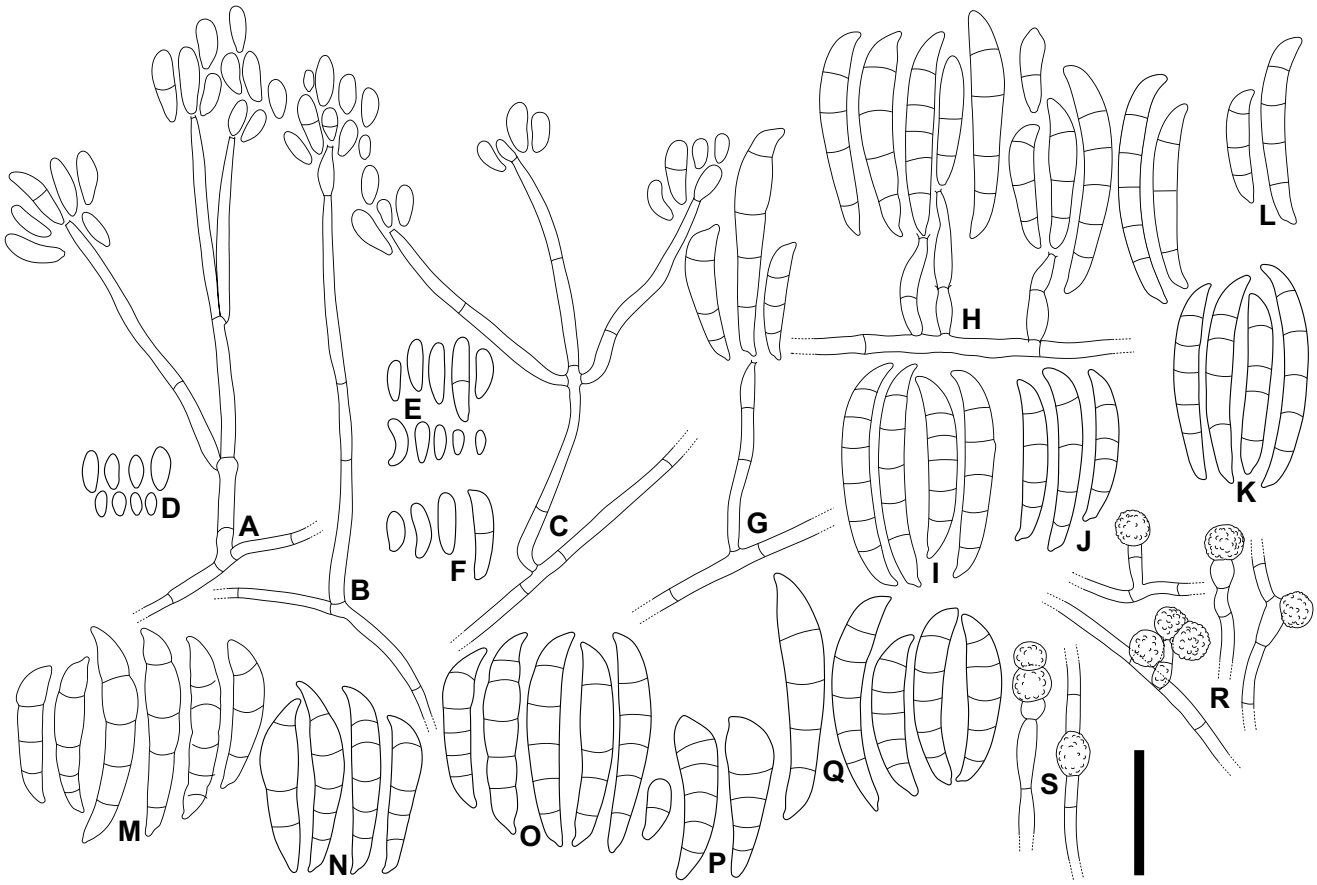

Figure 2. *Fusarium duplospermum*. A–C. Tall aerial conidiophores forming 0- and 1-septate conidia (B. Proliferated phialide formed through apex). D–F. 0- and 1-septate aerial conidia. G. Erect aerial conidiophore forming multiseptate conidia. H. Short conidiophores arising from substrate mycelium forming multiseptate falcate conidia, with some 1- and 2-septate shorter conidia. I–L. Falcate, curved cylindrical to crescent-shaped, multiseptate conidia, formed from short conidiophores on substrate mycelium. M–Q. Apically swollen, curved multiseptate clavate conidia, with a tapering or papillate apical cell, formed from conidiophores on substrate mycelium. R, S. Chlamydospores formed in hyphae. All from SNA cultures grown under black light; A, K, N, S from NRRL 62587; B, I, O from NRRL 62585; C, H, R from NRRL 62583 (ex-holotype); D, M from NRRL 62584; E, J, P from NRRL 62586; F, G, L, Q from NRRL 62589. Bar = 25 µm.

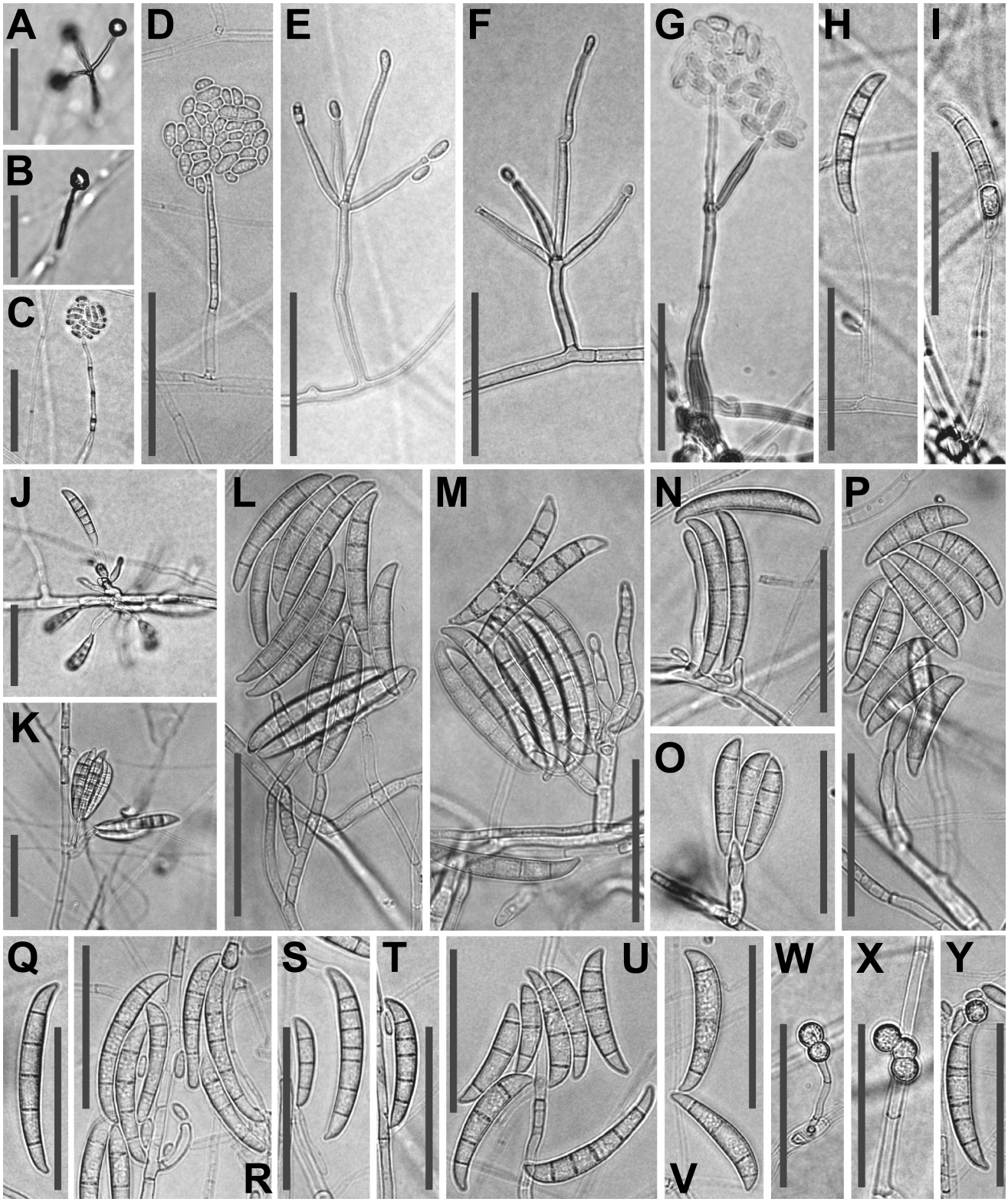

Figure 3. *Fusarium duplospermum*. A–G. Aerial conidiophores forming 0- and 1-septate conidia. H, I. Aerial conidiophores forming multiseptate falcate conidia. J–P. Short conidiophores arising from substrate mycelium forming multiseptate conidia that are falcate (L–N) or swollen in upper parts, curved clavate (O, P). Q–V. 3–5(–7)-septate multiseptate falcate conidia (Q–T) or ones swollen in upper parts and curved clavate (U, V). W–Y. Chlamydospores formed in hyphae (W, X) and on apex of conidium (Y). All from SNA cultures grown under black light; A, W from NRRL 62584; B, C, G, L, X from NRRL 62585; D, K, O, P, R, U, V from NRRL 62589; E, F, H, J, N, Q, S, T, Y from NRRL 62583 (ex-holotype); I, M from NRRL 62587. A–C, J, K. Direct observation without a coverslip. D–I, L–Y. Mounted in water with a coverslip. Bars = 50 µm.

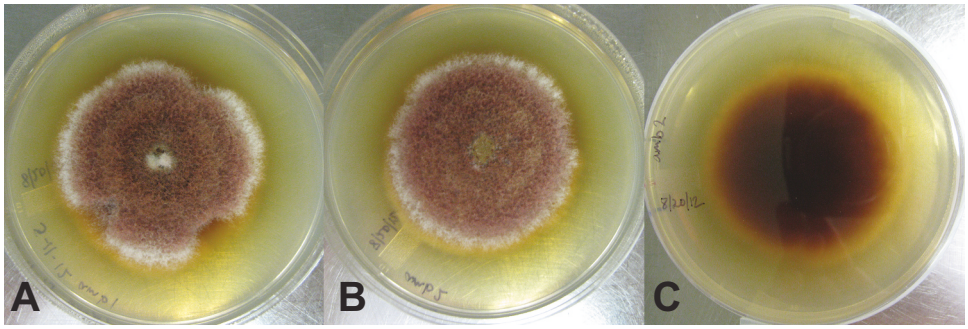

Figure 4. Colony morphology of *Fusarium duplospermum* on half-strength PDA in the dark. A, B. Colony surface. C. Colony undersurface. A. NRRL 62583 (ex-holotype). B, C. NRRL 62584. *Fusarium duplospermum* is distinguished by forming brownish orange colonies on PDA.

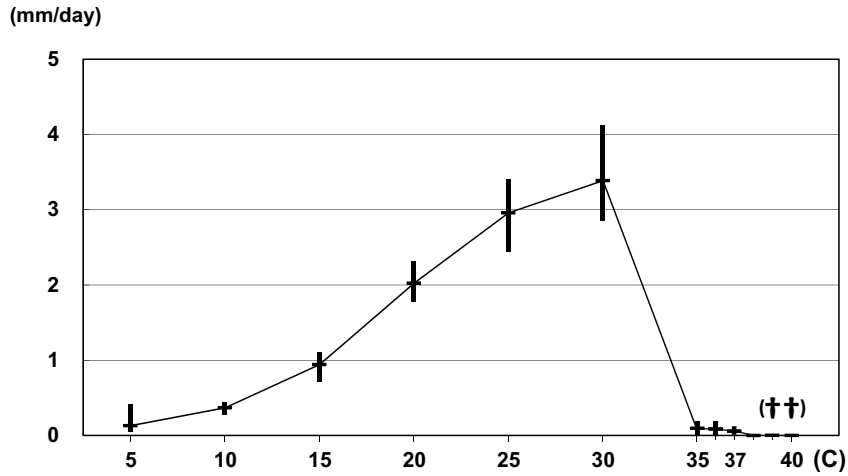

Figure 5. Daily radial mycelial growth rate of *Fusarium duplospermum* on PDA cultured at eight different temperatures. Thick horizontal and vertical bars indicate means and total ranges, respectively, of the six isolates analyzed. All isolates failed to grow and died at 40 C.

strains, this species grew very slowly, i.e., <0.1 mm per day at 37 C. The genome sequence of NRRL 62584 is available in Genbank, i.e., BioSample: SAMN07244161; BioProject: PRJNA389173.

Fusarium drepaniforme T. Aoki, Kasson, J.A. Smith, S. Freeman, Geiser & O'Donnell, sp. nov. FIGS. 6–8; SUPPLEMENTARY FIG. 1B, F, M–Q

Index Fungorum: IF558018

Typification: SINGAPORE. Isolated from unknown woody host, no further collection information found (**holotype** BPI 923530, a dried specimen from a culture of NRRL 62941, Herbarium of US National Fungus Collections, USA); **isotype** Herb IMI 351954, the IMI fungarium, Mycology Department, Royal Botanic Gardens, Kew, UK [as *F. bugnicourtii*]); ex-holotype culture NRRL 62941 (= KOD 147) = MAFF 247230.

Etymology: drepani- (sickle-) + *forme* (shaped), based on morphology of multiseptate conidia.

Diagnosis: The species can be distinguished from all other AFC species by frequent production of multiseptate sickle-shaped conidia.

Observations on PDA: Radial mycelial growth rates 3.5 mm per day at 20 C and 4.8 mm per day at 25 C; no growth at 36 C or higher, dead at 40 C. White (1A1), yellowish white (4A2) to pale yellow (4A3) initially, becoming pale orange (5A3), light orange (5A4–5) to greyish orange (5B3–5) with age. Aerial mycelium white (1A1), sparsely formed. Colony margin entire. Reverse pigmentation yellowish white (4A2) or pale yellow (4A3) to greyish yellow (4B3–4). Exudates absent. Odor absent or moldy. When cultured under black light, forming pale yellow (4A3) to light yellow (4A4–5) sporodochia with abundant conidial production after ca. 1 mo.

Microscopic characters on SNA: Hyphae on SNA 1.5–7.5 µm wide. Chlamydospores in or on hyphae and conidia, mostly subglobose to round ellipsoidal, intercalary or terminal, often single, sometimes in chains or

in mass of up to 4, hyaline or pale yellow, smooth to often minutely rough-walled, 8–11.5 × 3–7 µm. Sclerotia absent. Sporulation starts within 2–3 d and abundant under black light, less abundant in the dark; sporodochia formed sparsely.

Aerial conidiophores usually formed abundantly, erect, often tall and narrow, often unbranched, some sparsely branched, up to 219 µm long, 2.5–5 µm wide at the base, mostly thin-walled, sometimes thick-walled, forming monophialides integrated at the apices. Phialides on aerial conidiophores simple, subcylindrical to subulate, integrated, tapering toward the apex, often with a minute collarette at the tip, 10.5–40 × 2–4.5 µm. Aerial conidia hyaline, of two types: (i) elliptical, oblong-elliptical, fusiform-elliptical to clavate, straight or sometimes curved and reniform or crescent-shaped, some obovate to comma-shaped, 0–1(–3)-septate; 0-septate: 5–(9.9 ± 1.8)–14.5 × 2–(3.6 ± 0.7)–5 µm in the dark, 4.5–(9.4 ± 2.0)–14.5 × 1.5–(3.6 ± 0.7)–7 µm under black light; 1-septate: 7.5–(14.2 ± 2.7)–22.5 × 3–(4.3 ± 0.7)–5.5 µm in the dark, 10.5–(14.4 ± 2.1)–21 × 3–(4.4 ± 0.5)–5.5 µm under black light; often forming (ii) larger, clavate to falcate, sometimes curved cylindrical, (1–)3–4(–5)-septate swollen conidia, morphologically overlapping and indistinguishable from multiseptate conidia formed on conidiophores from the substrate mycelium.

Conidiophores arising from the substrate mycelium or in sporodochia generally shorter than aerial conidiophores, but sometimes relatively tall and thick, unbranched or branched, up to 87.5 µm long, 2.5–6.5 µm wide at the base, straight or sometimes contorted or twisted, forming apical integrated monophialides, or reduced to a simple phialide on substrate mycelium. Phialides simple, subulate or subcylindrical, rarely ampulliform, often with a minute collarette at the tip, 10–47.5 × 2.5–4.5 µm. Conidia formed on conidiophores arising directly from the substrate mycelium or in sporodochia, often clavate and straight in the dark, becoming falcate and curved under black light, with a tapering and papillate apical cell, gradually tapering toward the base, sickle-shaped, with a distinct or indistinct foot-like basal cell, often swollen in the upper parts, and sometimes "dolphin-like" (Brayford 1987), hyaline, (0–)3–7-septate, often becoming larger when cultured under black light than in the dark; 0-septate: 8–11.5 × 4.5–7.5 µm in the dark, 10–18 × 4–8 µm under black light; 1-septate: 11.5–22.5 × 3.5–7 µm in the dark, 11.5–29.5 × 4.5–8.5 µm under black light; 2-septate: 13–33 × 5–10 µm in the dark, 15.5–44.5 × 4.5–11.5 µm under black light; 3-septate: 19.5–(29.7 ± 5.0)–45.5 × 5.5–(7.5 ± 1.0)–10.5 µm in the dark, 19.5–(34.2 ± 6.2)–46 × 6.5–(9.1 ± 1.6)–12 µm under black light; 4-septate: 18–(35.9 ± 5.4)–48.5 × 6.5–(8.2 ± 0.8)–10.5 µm in the dark, 30–(42.8 ± 5.0)–54.5 × 7.5–(10.3 ± 1.2)–12.5 µm under black light; 5-septate: 25.5–(37.7 ± 4.0)–45.5 × 6.5–(8.1 ± 0.7)–10 µm in the dark, 26.5–(45.9 ± 7.6)–57.5 × 7–(10.0 ± 1.1)–12 µm under black light; 6-septate: 32.5–56 × 7–9.5 µm in the dark, 29.5–(51.2 ± 5.2)–63.5 × 7.5–(10.2 ± 0.8)–12.5 µm under black light; 7-septate: 42.5–59.5 × 8.5–11.5 µm formed only under black light. Short-clavate to obovate or naviculate, straight or slightly curved conidia, with a rounded apex and a truncate base, 0–1(–2)-septate, sometimes formed together with multiseptate conidia from shorter conidiophores on the substrate mycelium.

Notes: The single strain of this species, originally deposited in the HerbIMI, Kew, and the CABI Culture Collection as *F. bugnicourtii* Brayford IMI 351954, was accessioned in the NRRL and MAFF culture collections and deposited in BPI as the holotype. *Fusarium drepaniforme* frequently forms multiseptate sickle-shaped conidia (FIGS. 6F–I, 7N, O), especially under black light. These are larger in size than those formed in the dark, which distinguishes it from all other described AFC species. Some multiseptate sickle-shaped conidia become swollen apically and appear "dolphin-like" (Brayford 1987). When the ex-type strain was cultured on SNA in the dark, it produced narrower and straight multiseptate clavate conidia (FIG. 6E, H, J), whereas those produced under black light were thicker and longer, often falcate and curved (FIGS. 6F, G, I, K, 7N, O). *Fusarium drepaniforme* grew fastest at 25 C (FIG. 8).

Fusarium papillatum T. Aoki, Liyanage, Kasson, J. A. Smith, S. Freeman, Geiser & O'Donnell, sp. nov. FIGS. 9–11; SUPPLEMENTARY FIG. 1C, G, R–W

Index Fungorum: IF558019

Typification: SRI LANKA. CENTRAL PROVINCE: Kandy, originally isolated from the mycangium of a living female TSHB beetle (*E. perbrevis*) from a gallery in a branch of an infested tea (*C. sinensis*) bush, 16 Feb 2014, *Pradeepa N.H. Liyanage PL-KH1* (**holotype** BPI 923531, a dried specimen from a culture of NRRL 62943, Herbarium of US National Fungus Collection, USA). Ex-holotype culture NRRL 62943 (= KOD 796) = MAFF 247228.

Etymology: papillatus (papillate), the epithet is based on the papillate morphology of multiseptate conidia.

Diagnosis: This species forms multiseptate clavate conidia that possess a papillate apical cell that protrudes toward the ventral side.

Observations on PDA: Radial mycelial growth rates 3.4–3.7 mm per day at 20 C and 4.4–4.7 mm per day at 25 C; no growth at 38 C or higher, dead at 40 C. White (1A1), yellow white (4A2) to pale yellow (4A3), orange white (5A2) initially, becoming partly pale orange (5A3)

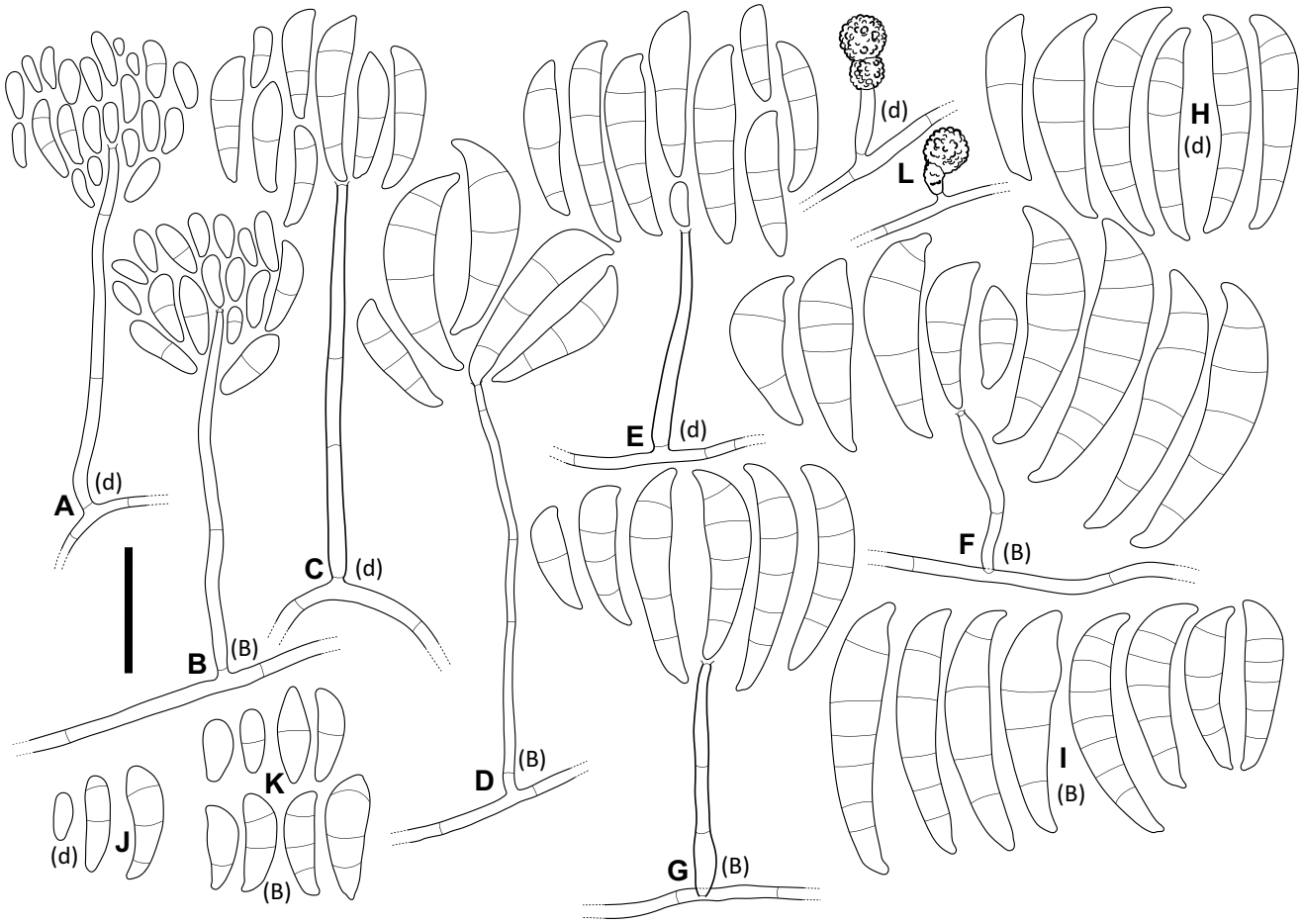

Figure 6. *Fusarium drepaniforme* NRRL 62941 ex-holotype. A, B. Aerial conidiophores forming 0- and 1-septate conidia. C, D. Aerial conidiophores forming multiseptate conidia. E–G. Short conidiophores arising from substrate mycelium forming apically swollen multiseptate conidia. H, I. Apically swollen, curved multiseptate clavate conidia, formed from short conidiophores on substrate mycelium. J, K. 0–3-septate conidia, formed from short conidiophores together with curved multiseptate conidia. L. Chlamydospores formed in hyphae. All from SNA cultures; A, C, E, H, J, L formed in the dark (d); B, D, F, G, I, K formed under the black light (B). Bar = 25 µm.

to greyish orange (5B3–4) with age. Aerial mycelium white (1A1), cottony to felty. Colony margin entire. Reverse pigmentation pale yellow (4A3) to light yellow (4A4–5), or greyish yellow (4B4–6). Exudates absent. Odor absent or moldy. When cultured under black light, forming orange (5–6A–B7–8), greyish yellow (2–4B–C3–6) to olive (2–3D–F3–7) sporodochia with abundant conidial production after ca. 1 mo.

Microscopic characters on SNA: Hyphae on SNA 2–9 µm wide. Chlamydospores present in hyphae and conidia, mostly subglobose to round ellipsoidal, intercalary, or terminal, often single or sometimes in chains of up to several, hyaline or slightly pale yellow, smooth to often minutely rough-walled, 5.5–11.5 × 4–10 µm. Sclerotia absent. Abundant sporulation starts within 2–3 d; sporodochia formed abundantly after ca. 1 mo. Aerial conidiophores formed abundantly, erect, often tall, and narrow, often unbranched but some sparsely branched, up to 400 µm long, 2.5–6.5 µm wide at the base, thin-walled, forming monophialides integrated at the apex. Phialides on aerial conidiophores simple, subcylindrical to subulate, integrated, tapering toward the apex, often with a minute collarette at the tip, 13.5–70 × 2.5–6.5 µm. Aerial conidia hyaline, variable in shape, oblong-elliptical, fusiform-elliptical to clavate, straight or crescent- or comma-shaped, also sometimes forming swollen clavate to falcate, straight or curved conidia, 0–1(–3)-septate; 0-septate: 5–(9.6)–15 × 2–(2.9–3.1)–5.5 µm [ex-type: 6.5–(9.6 ± 1.9)–15 × 2–(3.1 ± 0.6)–5.5 µm]; 1-septate: 10.50–(14.6–17.6)–33.5 × 2.5–(4.4–4.5)–7.5 µm [ex-type: 11–(14.6 ± 2.0)–20 × 2.5–(4.4 ± 0.9)–7.5 µm]; 2-septate: 15–23.5 × 4–7.5 µm.

Conidiophores arising from substrate mycelium or sporodochia generally shorter than aerial conidiophores,

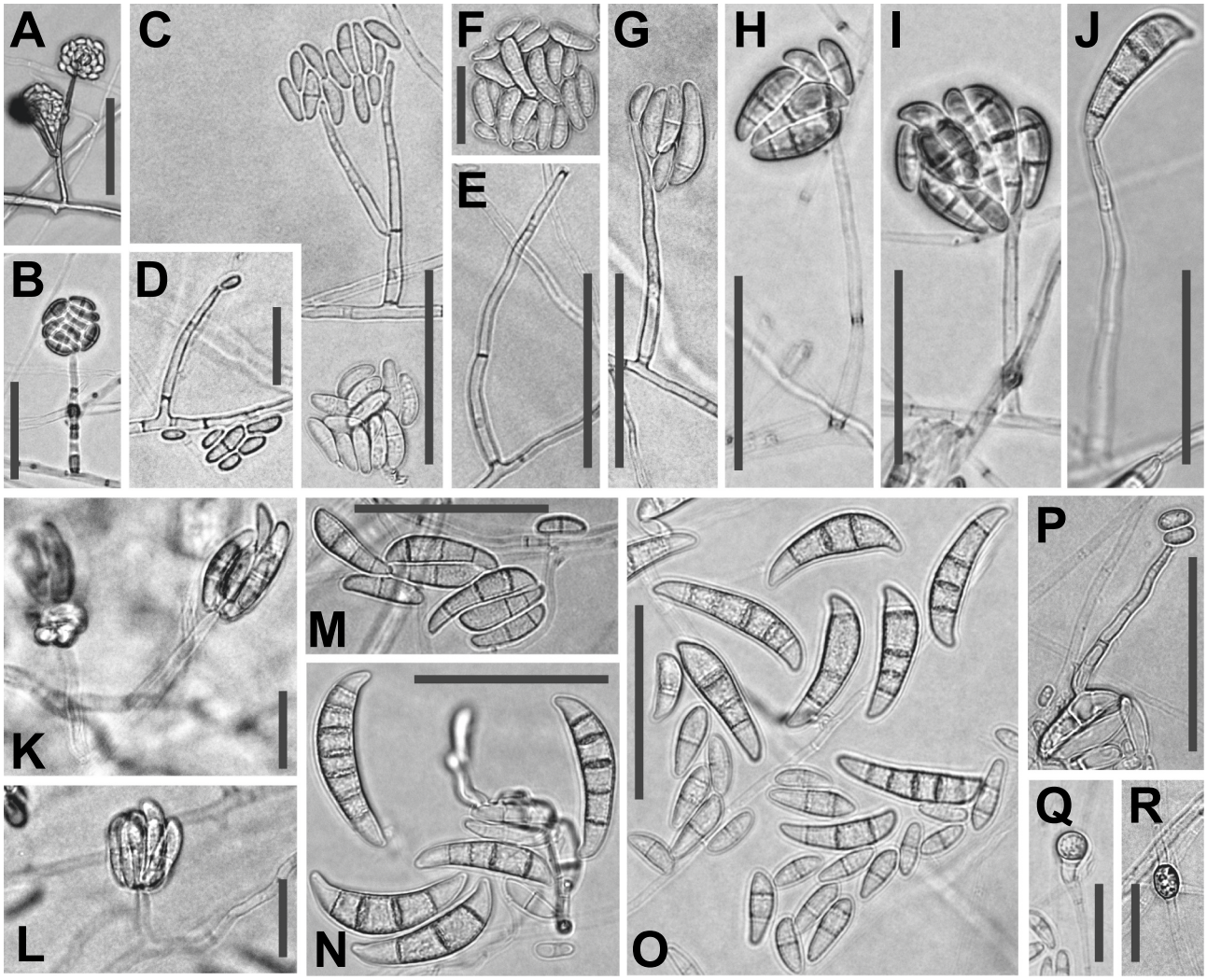

Figure 7. *Fusarium drepaniforme* NRRL 62941 ex-holotype. A–G. Aerial conidiophores forming 0- and 1(–2)-septate conidia (F. Aerial conidia formed from conidiophore in E). H–J. Aerial conidiophores forming multiseptate conidia. K, L. Short conidiophores arising from substrate mycelium forming multiseptate conidia. M–O. 1–5(–7)-septate clavate, falcate or apically swollen, and multiseptate sickle-shaped conidia. P. Microcycle conidiation with 0-septate conidia formed on phialide on septate conidia. Q, R. Chlamydospores formed in hyphae. All from SNA cultures grown under black light. A, B. Direct observation without a coverslip. C–R. Mounted in water with a coverslip. Bars: A–C, E, G–J, M–P = 50 µm; D, F, K, L, Q, R = 20 µm.

but sometimes relatively tall and thick, unbranched or branched, up to 87.5 µm long, 3.5–6.6 µm wide at the base, straight or sometimes contorted, forming apical integrated monophialides, or reduced to a simple phialide on substrate mycelium. Phialides simple, subulate or subcylindrical, sometimes ampulliform, often with a minute collarette at the tip, 9.5–48 × 2.5–6.5 µm. Conidia formed on conidiophores arising directly from the substrate mycelium or sporodochia, clavate to falcate, often gently curved, sometimes crescent-shaped, often swollen in their upper parts with a papillate apical cell resembling a protruded beak toward the ventral side, gradually tapering toward the base, with a distinct or indistinct foot-like basal cell, some "dolphin-like" (Brayford 1987), (0–)3–7(–8)-septate, formed frequently and abundant under black light, less abundant and smaller in the dark; 1-septate: 15.5–41 × 4.5–10 µm; 2-septate: 15–42.5 × 5–9.5 µm; 3-septate: 18.5–(37.5–39.9)–60 × 5.5–(9.6–10.1)–12.5 µm [ex-type: 18.5–(37.5 ± 6.6)–48.5 × 5.5–(10.1 ± 1.4)–12.5 µm]; 4-septate: 28.5–(42.4–46.5)–61.5 × 7–(10.3–10.7)–12.5 µm [ex-type: 28.5–(42.4 ± 4.0)–49.5 × 8–(10.7 ± 0.8)–12.5 µm]; 5-septate: 35.5–(44.8–48.7)–58.5 × 8.5–(10.7–11.0)–13.5 µm [ex-type: 35.5–(44.8 ± 4.0)–58.5 × 9.5–(11.0 ± 0.6)–12.5 µm]; 6-septate: 38–(47.1–52.0)–77.5 × 8–(10.6–11.1)–13 µm [ex-type: 38–(47.1 ± 4.1)–57 × 9.5–(11.1 ± 0.6)–12.5 µm]; 7-septate: 29.5–(50.4–53.1)–64 × 9.5–(10.6–11.1)–13.5 µm [ex-type: 43.5–(50.4 ± 3.7)–64 × 10–(11.1 ± 0.8)–13.5 µm]. Short-clavate to obovate or naviculate, straight or curved conidia, with a rounded apex and

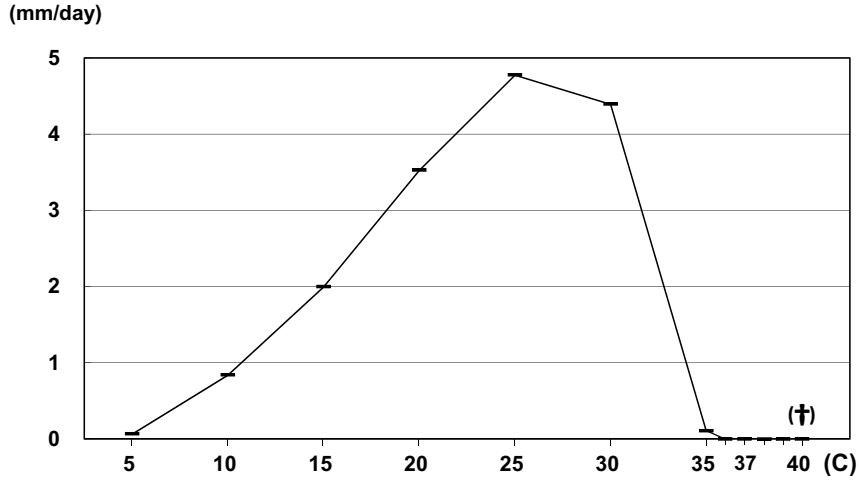

Figure 8. Daily radial mycelial growth rate of *Fusarium drepaniforme* on PDA cultured at eight different temperatures. Thick horizontal and vertical bars indicate means and total ranges, respectively, of the single isolate (NRRL 62941 ex-holotype) analyzed. The isolate failed to grow and died at 40 C.

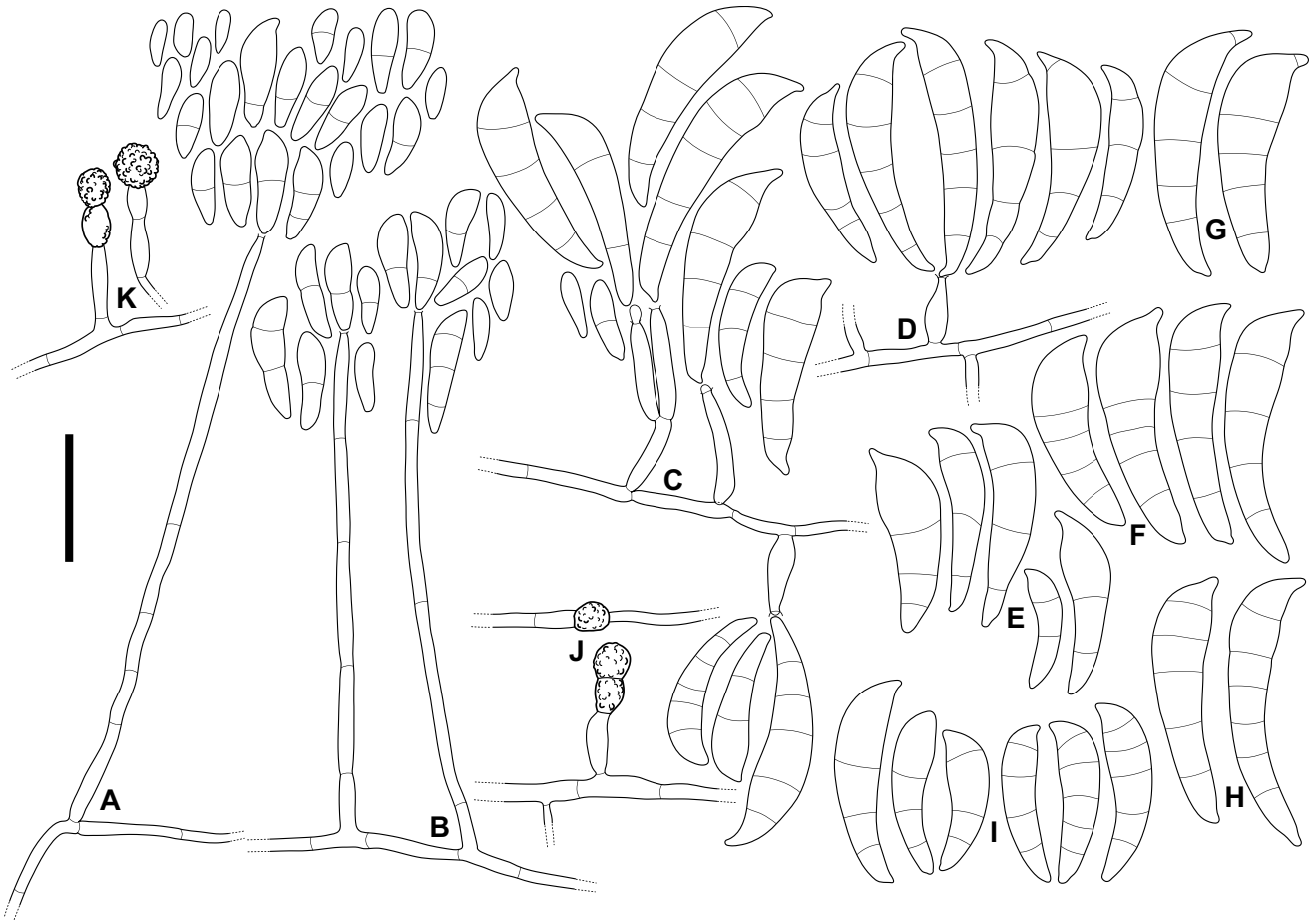

Figure 9. *Fusarium papillatum*. A, B. Tall aerial conidiophores forming 0- and 1(–3)-septate conidia. C, D. Short conidiophores arising from substrate mycelium forming large multiseptate clavate conidia, with some 0- and 1-septate small conidia. E–H. Multiseptate clavate conidia with a papillate apical cell formed under black light. I. Smaller multiseptate conidia formed in dark. J, K. Chlamydospores formed in hyphae. All from SNA cultures. A–H, J, K formed under black light, I in the dark; A, C, E–H, J from NRRL 62943 (ex-holotype); B, D, I, K from NRRL 62944. Bar = 25 µm.

a truncate base, 0–1(–3)-septate, sometimes formed together with multiseptate conidia from conidiophores on substrate mycelium or sporodochia.

Additional isolate studied: (Only collected from the type locality.) SRI LANKA. CENTRAL PROVINCE: Kandy, isolated from the mycangium of a living female TSHB beetle from a gallery in a branch of a infested tea bush, located approx. 10 m from the holotype collection site, 16 Feb 2014, *Pradeepa N.H. Liyanage PL-KH2* = NRRL 62944 (= KOD 797) = MAFF 247229.

Notes: This species frequently forms multiseptate clavate conidia with papillate apical cells that protrude ventrally, especially under black light (FIGS. 9C–H, 10K–Q), which distinguishes it from all other described AFC species. *Fusarium papillatum* is similar morphologically to *F. drepaniforme* in that it forms curved multiseptate conidia of similar size and number of septa. However, multiseptate conidia of *F. papillatum* often possess a papillum protruding ventrally from the apical cells, and their ultimate and penultimate apical cells are often swollen so that they are widest in the terminal half. By contrast, multiseptate conidia of *F. drepaniforme* are often widest at the second to fourth cells from their apices. Some multiseptate conidia of *F. papillatum* are "dolphin-like" (Brayford 1987). This species grew very slowly, i.e., <0.2 mm per day at 37 C (FIG. 11).

Fusarium kuroshium (F. Na, J.D. Carrillo & A. Eskalen ex Sand.-Denis & Crous) O'Donnell, Geiser, Kasson & T. Aoki, Index Fungorum 440:2. 2020. [IF557669] FIGS. 12–14; SUPPLEMENTARY FIG. 1D, H, X, Y

≡ *Neocosmospora kuroshio* F. Na, J.D. Carrillo & A. Eskalen ex Sand.-Den. & Crous, Persoonia 43:137. 2019. [MB831184]

≡ *Fusarium kuroshium* F. Na, J.D. Carrillo & A. Eskalen, Plant Disease 102:1159. 2018. Nom. inval., Art. 40.7 of ICNafp (Turland et al. 2018). [MB821907]

Type: USA. CALIFORNIA: San Diego County, El Cajon, originally isolated from the surface of a Kuroshio shot hole borer (KSHB; *Euwallacea kuroshio* Gomez and Hulcr) gallery in an infested California sycamore tree (*Platanus racemosa* Nutt.), 14 Dec 2013, *Akif Eskalen* (**holotype** BPI 910340, **isotype** UCR 3641). Ex-holotype culture CBS 142642 = NRRL 62945 = MAFF 247226 = UCR3641.

Etymology: Derived from the ambrosia beetle vector, the Kuroshio shot hole borer (Na et al. 2018; Sandoval-Denis et al. 2019).

Diagnosis: This species can be differentiated from all other described AFC species by the morphology of its conidia that vary widely in size, shape, and number of septa, i.e., (0–)1–5(–8)-septate. The straight or curved multiseptate conidia of this species are swollen apically and taper toward the apex, which is often rounded and not distinctly papillate. These morphological features distinguish *F. kuroshium* from the other described AFC species.

Observations on PDA: Radial mycelial growth rates 2.8–3.3 mm per day at 20 C and 3.5–4.4 mm per day at 25 C; no growth at 35 C or higher, dead at 39 C. White (1A1) initially, becoming yellow white (4A2) to pale yellow (4A3), light yellow (4A4) to partly greyish yellow (4B4–5) when aged. Aerial mycelium white (1A1), cottony to felty, moderate to abundant. Colony margin entire. Reverse pigmentation pale yellow (4A3) to greyish yellow (4B4–5). Exudates absent. Odor absent or moldy.

Microscopic characters on SNA: Hyphae 1–7.5 µm wide. Chlamydospores present in or on hyphae and conidia, mostly subglobose to round ellipsoidal, intercalary or terminal, often single or sometimes in chains or in masses of up to 4, hyaline or slightly pale yellow, smooth to minutely rough-walled, 5.5–11 × 3.5–9 µm. Sclerotia absent. Abundant sporulation starts within 2–3 d under black light, less abundant in the dark in some strains; sporodochia formed abundantly under black light, less abundant in the dark in ca. 1 mo. Aerial conidiophores formed abundantly, erect, often tall and narrow, often unbranched or some sparsely branched, up to 113 µm long, 2.5–6.5 µm wide at the base, thin-walled or sometimes slightly thick-walled, forming monophialides integrated at the apex. Phialides on aerial conidiophores simple, subcylindrical to subulate, some curved, integrated, tapering toward the apex, often with a minute collarette at the tip, 10.5–52.5 × 2.5–5 µm, sometimes formed as a percurrent-proliferated phialide. Aerial conidia hyaline, variable in shape: (i) obovate to clavate, elliptical, oblong-elliptical, fusiform-elliptical, fusiform, straight or sometimes crescent- or comma-shaped, 0–1(–3)-septate; 0-septate: 4–(7.4–9.6)–17.5 × 2–(3.8–5.5)–8.5 µm; 1-septate: 10.5–(13.5)–18 × 3.5–(4.2–4.8)–6 µm; often also forming (ii) larger, falcate to clavate, sometimes curved cylindrical, (1–)3–5-septate conidia, morphologically overlapping and indistinguishable from multiseptate conidia formed on conidiophores from the substrate mycelium.

Conidiophores arising from the substrate mycelium or in sporodochia generally shorter than aerial conidiophores, but sometimes tall, unbranched or branched, up to 85 µm long, 2.5–5.5 µm wide at the base, straight or sometimes contorted, forming apical integrated monophialides, or reduced to a simple phialide on substrate mycelium. Phialides simple, subulate or subcylindrical, sometimes ampulliform, often with a minute collarette at the tip, 9.5–52.5 × 2.5–5.5 µm, or sometimes percurrently or sympodially proliferating from previous phialides. Conidia formed on conidiophores arising directly from substrate mycelium or in sporodochia hyaline,

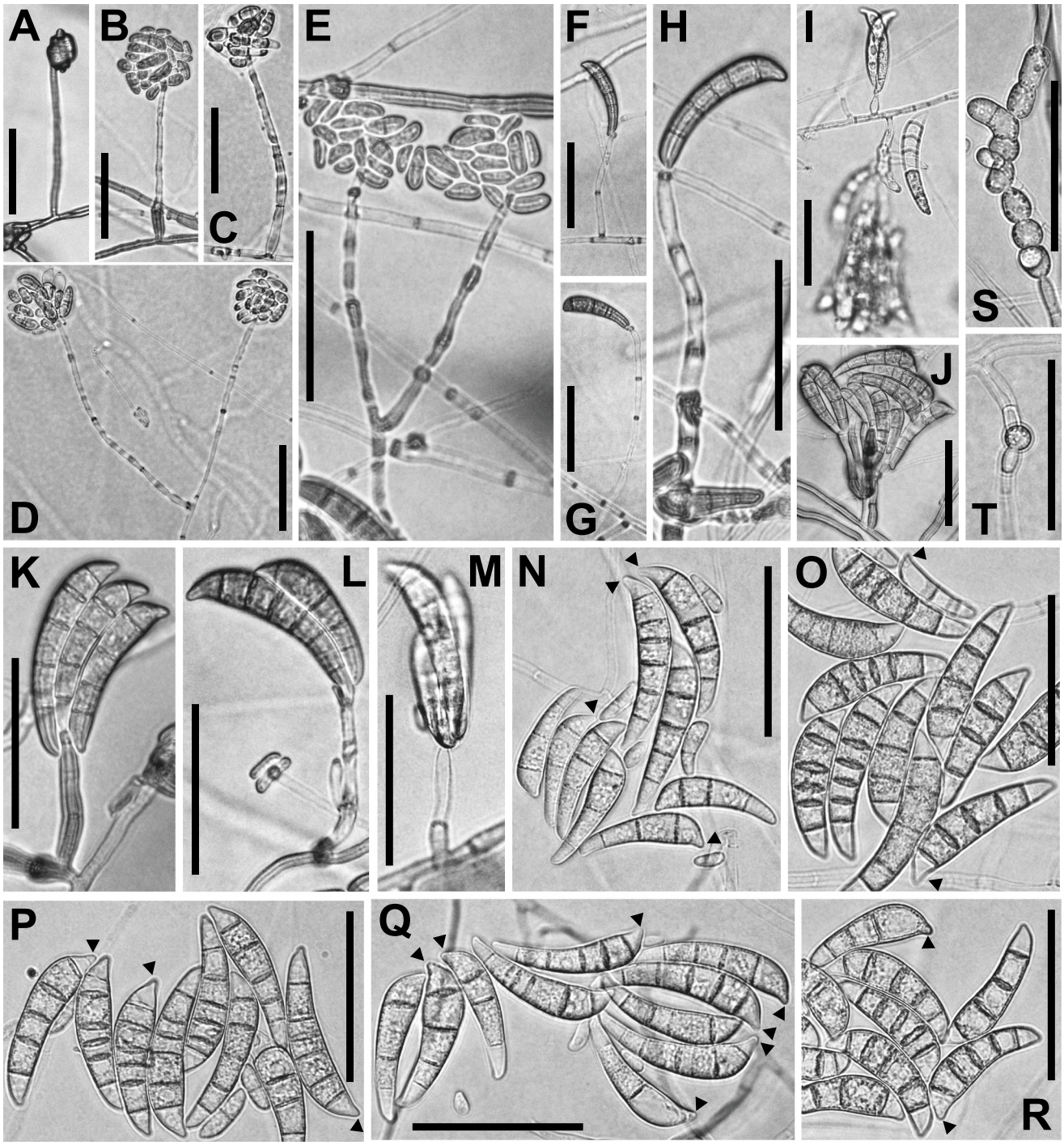

Figure 10. *Fusarium papillatum*. A–E. Aerial conidiophores forming 0- and 1(–2)-septate conidia. F–H. Aerial conidiophores forming multiseptate clavate conidia. I–M. Short conidiophores arising from substrate mycelium forming multiseptate clavate conidia. N–R. Multiseptate clavate conidia swollen apically and papillate with (1–)3–7 septa (arrowheads: papillate apical protrusion on ventral side of conidia). S, T. Chlamydospores formed in hyphae. All from SNA cultures. A–Q, S, T formed under black light, R in the dark; A–H, J–N, Q–S from NRRL 62943 (ex-holotype); I, O, P, T from NRRL 62944. A–D, F, G. Direct observation without a coverslip. E, H–T. Mounted in water with a coverslip. Bars = 50 µm.

variable in size, clavate to falcate, straight or curved, sometimes crescent-shaped, often swollen in their upper parts and with an apical cell tapering toward an often rounded apex or sometimes slightly protruded like a beak, but not distinctly papillate, tapering gradually toward the base, with or without a distinct foot-like basal cell under black light, some "dolphin-like" (Brayford 1987), (0–)1–5(–8)-septate, formed frequently under

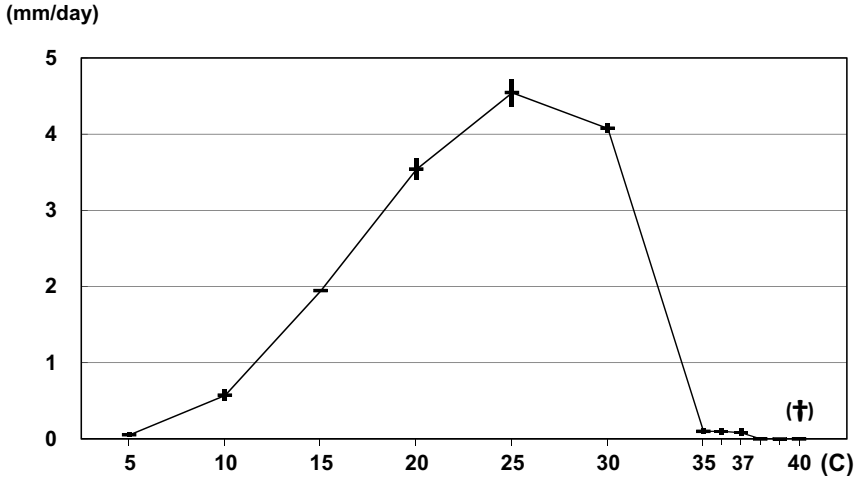

Figure 11. Daily radial mycelial growth rate of *Fusarium papillatum* cultured on PDA at eight different temperatures. Thick horizontal and vertical bars indicate means and total ranges, respectively, of the two isolates analyzed. Both isolates failed to grow and died at 40 C.

black light, less frequently in the dark; 1-septate: 9.5–(16.8–18.1)–30.5 × 4–(6.1–6.2)–11.5 µm; 2-septate: 15–(23.5–24.7)–37.5 × 4–(6.4–6.9)–9.5 µm; 3-septate: 20–(30.4–34.9)–54.5 × 4.5–(7.0–7.6)–11 µm; 4-septate: 23–(37.6–42.8)–53 × 5–(8.2–8.9)–12 µm; 5-septate: 28–(46.6–47.5)–64.5 × 4–(8.7–9.1)–12 µm.

When cultured in the dark, multiseptate conidia less abundant and shorter in size. Obovate to short-clavate or naviculate, or reniform to crescent or comma-shaped, straight or curved conidia, with a rounded apex and a truncate base, 0–1(–2)-septate, often formed together with swollen multiseptate conidia from conidiophores arising directly from the substrate mycelium.

Original description and illustration: Na et al. (2018).

Isolates studied: USA. CALIFORNIA: San Diego County, El Cajon, originally isolated from the surface of a KSHB beetle (*E. kuroshio*) gallery in an infested California sycamore tree, 14 Dec 2013, *Akif Eskalen AE-FD420-G47*, ex-holotype culture NRRL 62945 (= KOD 793) = MAFF 247226 = UCR 3641; ibid., 2014, *Francis Na AE-FD422-G69*, NRRL 62946 (= KOD 795) = MAFF 247227 = UCR 3644.

Notes: The description was prepared based on NRRL 62945 and NRRL 62946 on PDA and SNA. This species was first described by Na et al. (2018) with pictures of the colony together with conidial and chlamydospore morphology. However, because the holotype indicated two specimens in the description, it was invalid nomenclaturally (Sandval-Denis et al. 2019). Based on the species description given by Na et al. (2018), and by designating one of the specimens as the holotype (BPI 910340), Sandval-Denis et al. (2019) established a new species, *N. kuroshio*, validating it under the generic name *Neocosmospora*. However, because comparative phylogenomic data support inclusion of the FSSC in a monophyletic circumscription of *Fusarium* (Geiser et al. 2013, 2021; O'Donnell et al. 2020), *N. kuroshio* (as the basionym) was recombined in *Fusarium* as *F. kuroshium* (Aoki et al. 2020). Based on study of two NRRL strains, including the ex-holotype, *F. kuroshium* frequently forms apically swollen multiseptate conidia, especially on SNA under black light (FIGS. 12E–H, 13H–T), with a "dolphin-like" morphology (Brayford 1987). In our study, multiseptate conidia of this species were variable in size, shape, and number of septa, together with smaller rounded or short-clavate, 0–1(–2)-septate conidia, which distinguishes it from all other AFC species. Although this morphology is similar to the diverse types of conidia produced by *F. floridanum* (Aoki et al. 2019), the multiseptate clavate conidia of *F. floridanum* possess a papillate apical cell. By contrast, *F. kuroshium* produces multiseptate conidia with a tapering but often rounded and less papillate apical cell. *Fusarium kuroshium* grew fastest at 25 C (FIG. 14; Na et al. 2018).

DISCUSSION

The objective of this study was to use multilocus molecular phylogenetic and phenotypic data to formally describe three unnamed species within the AFC that produce multiseptate "dolphin-like" conidia (Brayford 1987). These include *F. duplospermum, F. drepaniforme*, and *F. papillatum*. Based on the present study, 11 of the 19 AFC species have been formally described (Gadd and Loos 1947; Nirenberg 1990; Freeman et al. 2013; Aoki et al. 2018, 2019, 2020; Na et al. 2018; Sandoval-Denis et al. 2019; Lynn et al. 2020). The three newly described

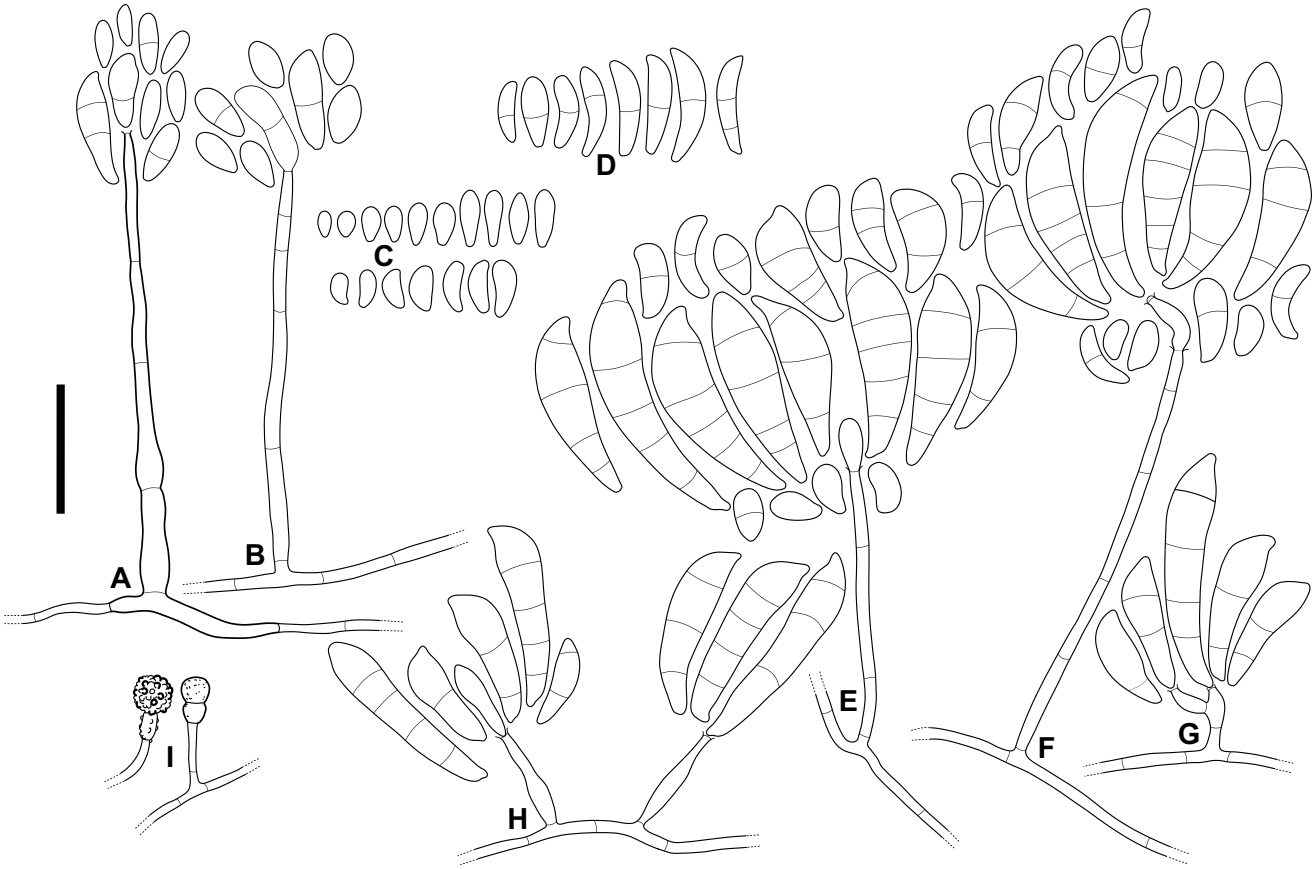

Figure 12. *Fusarium kuroshium*. A, B. Tall aerial conidiophores forming 0- and 1(–2)-septate conidia. C–D. Aerial conidia. C. 0-septate conidia. D. 1- and 2-septate conidia. E, F. Aerial conidiophores forming 0- to multiseptate, conidia of various size, shape, and septation. G, H. Short conidiophores arising from substrate mycelium forming multiseptate apically swollen, clavate conidia. I. Chlamydospores formed in hyphae. All from SNA cultures grown under black light; A, B, E, F, I from NRRL 62945 (ex-holotype); C, D, G, H from NRRL 62946. Bar = 25 µm.

species can be differentiated from all other AFC species phylogenetically and can be diagnosed phenotypically. Two different types of multiseptate conidia are produced by *F. duplospermum*, multiseptate conidia of *F. drepaniforme* are sickle-shaped and those produced by *F. papillatum* frequently possess a papillate apical cell that protrudes ventrally. Although multiseptate conidia with a papillate apical cell were also found in *F. floridanum* and *F. obliquisetatum*, the apical cell produced by the latter two species mostly protruded parallel to the curvature of the conidia. The apical cell of conidia in *F. papillatum*, by contrast, was often hooked and the papillum projected ventrally. In contrast to *F. papillatum*, multiseptate conidia in *F. floridanum* are highly variable in shape and size and those of *F. obliquisetatum* often possess oblique or thicker septa (Aoki et al. 2019). We discovered that conidia produced by *F. kuroshium* vary widely in size, shape, and number of septa, i.e., (0–)1–5(–8)-septate (FIGS. 12A–H, 13A–T), a feature shared with *F. floridanum*. However, multiseptate clavate conidia produced by *F. kuroshium* possess a tapering apical cell that is often rounded and less papillate, whereas conidia of *F. floridanum* typically possess a papillate apical cell. Conidia of *F. kuroshium* are diagnostic when compared with all other AFC species.

Among the three novel species described in this study, *F. duplospermum* from Florida grew fastest at 30 C (FIG. 5). By contrast, *F. drepaniforme* from Singapore (FIG. 8), *F. papillatum* from Sri Lanka (FIG. 11), and *F. kuroshium* from southern California grew fastest at 25 C (FIG. 14; Na et al. 2018). Several other AFC species exhibited their highest growth rate at 30 C, i.e., *F. ambrosium* from India (Aoki et al. 2018), *F. tuaranense* from Malaysia, *F. obliquiseptartum* from Australia (Aoki et al. 2019), and the undescribed species AF-6 and AF-9 from Florida that only produce slender, multiseptate falcate conidia (T. Aoki, unpublished data). Two of the six strains of *F. floridanum* from Florida, USA, also grew fastest at 30 C (Aoki et al. 2019). Because most FSSC and other fusaria grow fastest at 25 C, this is an additional attribute observed among the AFC species.

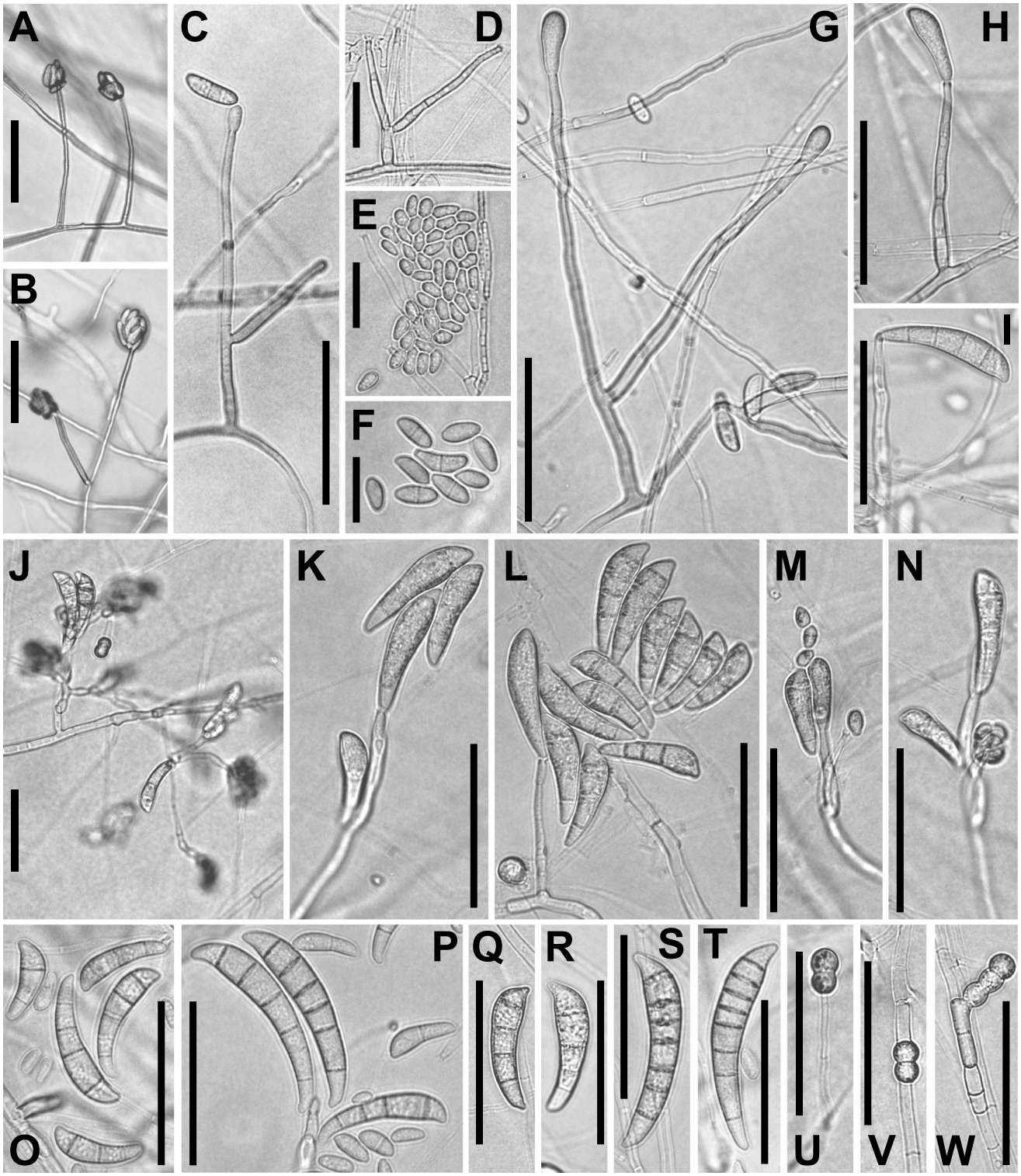

Figure 13. *Fusarium kuroshium* cultured on SNA under black light. A–G. Aerial conidiophores forming 0- and 1-septate conidia. H, I. Aerial conidiophores forming multiseptate clavate conidia. J–N. Short conidiophores arising from substrate mycelium forming multiseptate clavate conidia, sometimes together with 0-septate conidia (M, N). O–T. Multiseptate clavate conidia swollen in upper half and tapering toward an often rounded apex with 1–8 septa. U–W. Chlamydospores formed in hyphae. All from SNA cultures grown under black light; A, F, J, Q–S, V, W from NRRL 62946; B–E, G–I, K–P, T, U from NRRL 62945 (ex-holotype). A, B, J. Direct observation without a coverslip. C–I, K–W. Mounted in water with a coverslip. Bars: A–C, G–W = 50 µm; D–F = 20 µm.

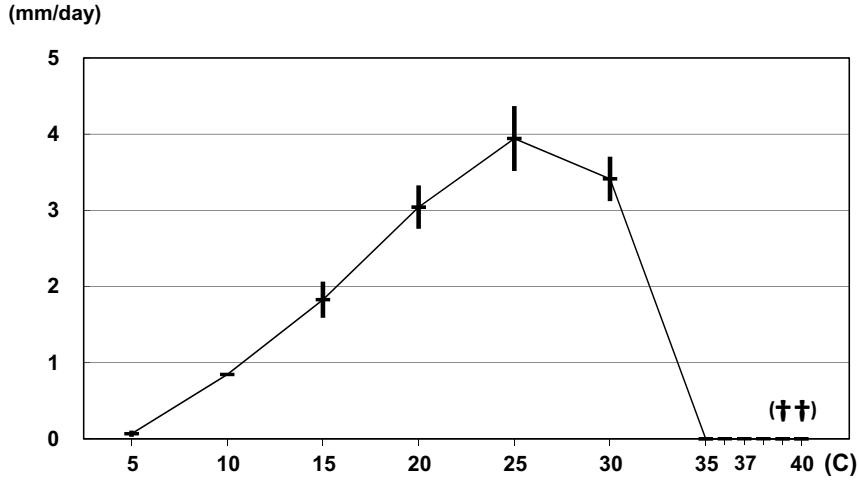

Figure 14. Daily radial mycelial growth rate of *Fusarium kuroshium* on PDA cultured at eight different temperatures. Thick horizontal and vertical bars indicate means and total ranges, respectively, of the two isolates analyzed. Both isolates failed to grow at 35 C but died at 40 C.

Two new AFC species, *F. duplospermum* and *F. papillatum*, were able to grow at 37 C, but very slowly, less than 0.2 mm per day (FIGS. 5, 11).

Most AFC species produce apically swollen multiseptate "dolphin-like" conidia, which distinguishes them from all other fusaria. Another AFC species, *F. rekanum* Lynn & Marinc., was recently described from Indonesia as a symbiont of *E. perbrevis* (as TSHBa), an important pest in *Acacia* plantations (Lynn et al. 2020). *Fusarium rekanum* also forms curved clavate, (0–)2–6-septate conidia that are swollen in the upper part. According to Lynn et al. (2020), swollen conidia of this species formed on SNA are slightly curved but sometimes also straight and relatively slender (Lynn et al. 2020, Fig. 4d–f, i), which distinguishes it from the three AFC species described in the current study. Two undescribed AFC species isolated in Florida, USA, i.e., AF-6 isolated from *E. perbrevis* on avocado and AF-9 from royal poinciana (*Delonix regia* (Boj. ex Hook.) Raf.), only produced slender, multiseptate falcate conidia and no apically swollen "dolphin-like" conidia (T. Aoki, unpublished data). Therefore, comparative morphological studies with other groups within the FSSC will be required in order to formally describe them and identify their diagnostic features.

The AFC represents one of the 13 known independent evolutionary origins of fungal genera farmed by ambrosia beetles (Jordal and Cognato 2012; Li et al. 2015; Hulcr and Stelinski 2017), including some *Geosmithia* species (Kolařik and Kirkendall 2010; Kolařik et al. 2015) within the Hypocreales. AFC mutualists are carried by female *Euwallacea* beetles in their mycangia (Spahr et al. 2020) and are farmed in galleries in the xylem of diverse woody hosts (Hulcr and Stelinski 2017) as a source of nutrition for adult beetles and their larvae (Freeman et al. 2012a). The relationship between AFC species and *Euwallacea* ambrosia beetles that farm them have long been considered an obligate mutualistic association (Freeman et al. 2012a; Aoki et al. 2018). However, several *Euwallacea* species are promiscuous in that they farm more than one AFC species (O'Donnell et al. 2015; Carrillo et al. 2019, 2020).

Based on prior reports (Gadd and Loos 1947; Brayford 1987; Kasson et al. 2013; O'Donnell et al. 2015; Aoki et al. 2018, 2019; Lynn et al. 2020) and the current study, the geographic range of several AFC species and *Euwallacea* ambrosia beetles overlap (Cognato et al. 2015; Short et al. 2017; Carrillo et al. 2020), e.g., (i) *F. ambrosium* and *F. papillatum* are farmed by the TSHB beetle *E. perbrevis* (Stouthamer et al. 2017; Smith et al. 2019) in tea trees in Sri Lanka (current study); (ii) undescribed AFC species AF-6 and *F. duplospermum* are both farmed by the TSHB beetle *E. perbrevis* on avocado, and *F. floridanum* is farmed by *E. interjectus* on box elder (*Acer negundo*) in Florida, USA; and (iii) *F. kuroshium* is farmed by *E. kuroshio* (= *Euwallacea* sp. 5) and *F. euwallaceae* by *E. fornicatus* on avocado in California, USA. Undescribed AFC species AF-9 has also been isolated from royal poinciana in Florida, USA, but it remains to be determined whether this species is farmed by *Euwallacea*. Several unnamed AFC species have also been isolated in Taiwan (Na et al. 2018; Lynn et al. 2020). In addition, *E. interjectus* is sympatric with *E. validus* at the southernmost limits of the latter species' known range in northern Georgia and western South Carolina, USA (Cognato et al. 2015).

By experimental switching the primary (*F. kuroshium* or *F. euwallaceae*) and/or auxiliary (*Graphium*

kuroshium, G. euwallaceae, or *Paracremonium pembeum*) fungal symbionts, Carrillo et al. (2020) found that brood size was highest when *Euwallacea* was grown with its primary *Fusarium* symbiont or its congener within the AFC. However, because host shifts have helped drive speciation within the AFC (O'Donnell et al. 2015), symbiont switching among sympatric AFC species in natural populations has the potential to bring together more damaging *Fusarium-Euwallacea* mutualists.

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LITERATURE CITED

Aoki T, Geiser DM, Kasson MT, O'Donnell K. 2020. Nomenclatural novelties. Index Fungorum 440:1–5.

Aoki T, Kasson MT, Berger MC, Freeman S, Geiser DM, O'Donnell K. 2018. *Fusarium oligoseptatum* sp. nov., a mycosymbiont of the ambrosia beetle *Euwallacea validus* in the Eastern U.S. and typification of *F. ambrosium*. Fungal Systematics and Evolution 1:23–39.

Aoki T, Smith JA, Kasson MT, Freeman S, Geiser DM, Geering ADW, O'Donnell K. 2019. Three novel Ambrosia *Fusarium* Clade species producing clavate macroconidia known (*F. floridanum* and *F. obliquiseptatum*) or predicted (*F. tuaranense*) to be farmed by *Euwallacea* spp. (Coleoptera: Scolytinae) on woody hosts. Mycologia 111:919–935.

Aoki T, Smith JA, Mount LL, Geiser DM, O'Donnell K. 2013. *Fusarium torreyae* sp. nov., a pathogen causing canker disease of Florida torreya (*Torrea taxifolia)*, a critically endangered conifer restricted to northern Florida and southwestern Georgia. Mycologia 105:312–319.

Brayford D. 1987. *Fusarium bungicourtii* sp. nov., and its relationship to *F. tumidum* and *F. tumidum* var. *coeruleum*. Transactions of the British Mycological Society 89:347–351.

Carrillo JD, Dodge C, Stouthamer R, Eskalen A. 2020. Fungal symbionts of the polyphagous and Kuroshio shot hole borers (Coleoptera: Scolytinae, *Euwallacea* spp.) in California can support both ambrosia beetle systems on artificial media. Symbiosis 80:155–168.

Carrillo JD, Rugman-Jones PF, Husein D, Stajich JE, Kasson MT, Carrillo D, Stouthamer R, Eskalen A. 2019. Members of the *Euwallacea fornicatus* species complex exhibit promiscuous mutualism with ambrosia fungi in Taiwan. Fungal Genetics and Biology 133:103269.

Chernomor O, von Haeseler A, Minh BQ. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. Systematic Biology 65:997–1008.

Cognato AI, Hoebeke ER, Kajimura H, Smith SM. 2015. History of the exotic ambrosia beetles *Euwallacea interjectus* and *Euwallacea validus* (Coleoptera: Curculionidae: Xyleborini) in the United States. Journal of Economic Entomology 108:1129–1135.

Eskalen A, Stouthamer R, Lynch SC, Twizeyimana M, Gonzalez A, Thibault T. 2013. Host range of Fusarium dieback and its ambrosia beetle (Coleoptera: Scolytinae) vector in southern California. Plant Disease 97:938–951.

Freeman S, Protasov A, Sharon M, Mohotti K, Eliyahu M, Okon-Levy N, Maymon M, Mendel Z. 2012a. Obligate feed requirement of *Fusarium* sp. nov., an avocado wilting agent, by the ambrosia beetle *Euwallacea* aff. *fornicata*. Symbiosis 58:245–251.

Freeman S, Protasov A, Wysoki M, Ben Yehuda S, Noi M, Rabaglia R, O'Donnell K, Sharon M, Okon-Levy N, Zveibil A, Eliyhu M, Mendel Z. 2012b. A pernicious agent affecting avocado in Israel: a novel symbiotic *Fusarium* sp. associated with the ambrosia beetle *Euwallacea fornicatus*. Phytoparasitica 40: 258.

Freeman S, Sharon M, Maymon M, Mendel Z, Protasov A, Aoki T, Eskalen A, O'Donnell K. 2013. *Fusarium euwallaceae* sp. nov.—a symbiotic fungus of *Euwallacea* sp., an invasive ambrosia beetle in Israel and California. Mycologia 105:1595–1606.

Gadd CH, Loos CA. 1947. The ambrosia fungus of *Xyleborus fornicatus* Eich. Transactions of the British Mycological Society 31:13–18.

Geiser DM, Al-Hatmi AMS, Aoki T, Arie T, Balmas V, Barnes I, Bergstrom GC, Bhattacharyya MK, Blomquist CL, Bowden RL, Brankovics B, Brown DW, Burgess LW, Bushley K, Busman M, Cano-Lira JF, Carillo JD, Chang H-X, Chen C-Y, Chen W, Chivers M, Chulze S, Coleman JJ, Cuomo CA, de Beer ZW, de Hoog GS, Del Castillo-Múnera J, Del Ponte EM, Diéguez-Uribeondo J, Di Pietro A, Edel-Hermann V, Elmer WH, Epstein L, Eskalen A, Esposto MC, Everts KL, Fernández-Pavía SP, Ferreira da Silva G, Foroud NA, Fourie G, Frandsen RJN, Freeman S, Freitag M, Frenkel O, Fuller KK, Gagkaeva T, Gardiner DM, Glenn AE, Gold SE, Gordon TR, Gregory NF, Gryzenhout M, Guarro J, Gugino BK, Gutiérrez S, Hammond-Kosack KE, Harris LJ, Homa M, Hong C-F, Hornok L, Huang J-W, Ilkit M, Jacobs A, Jacobs K, Jiang C, Jiménez-Gasco MM, Kang S, Kasson MT, Kazan K, Kennell JC, Kim H-S, Kistler HC, Kuldau GA, Kulik T, Kurzai O, Laraba I, Laurence MH, Lee T, Lee Y-W, Lee Y-H, Leslie JF, Liew ECY,

Lofton LW, Logrieco AF, López-Berges MS, Luque AG, Lysøe E, Ma L-J, Marra RE, Martin FN, May SR, McCormick SP, McGee C, Meis JF, Migheli Q, Mohamed Nor NMI, Monod M, Moretti A, Mostert D, Mulè G, Munaut F, Munkvold GP, Nicholson P, Nucci M, O'Donnell K, Pasquali M, Pfenning LH, Prigitano A, Proctor RH, Ranque S, Rehner SA, Rep M, Rodríguez-Alvarado G, Rose LJ, Roth MG, Ruiz-Roldán C, Saleh AA, Salleh B, Sang H, Scandiani MM, Scauflaire J, Schmale DG III, Short DPG, Šišić A, Smith JA, Smyth CW, Spahr E, Stajich JE, Steenkamp E, Steinberg C, Subramaniam R, Suga H, Summerell BA, Susca A, Swett CL, Toomajian C, Torres-Cruz TJ, Tortorano AM, Urban M, Vaillancourt LJ, Vallad GE, Van der Lee TAJ, Vanderpool D, Van Diepeningen AD, Vaughan MM, Venter E, Vermeulen M, Verweij PE, Viljoen A, Waalwijk C, Wallace EC, Walther G, Wang J, Ward TJ, Wickes BL, Wiederhold NP, Wingfield MJ, Wood AKM, Xu J-R, Yang X-B, Yli-Mattila T, Yun S-H, Zakaria L, Zhang H, Zhang N, Zhang SX, Zhang X. 2021. Phylogenomic analysis of a 55.1 kb 19-gene dataset resolves a monophyletic *Fusarium* that includes the *Fusarium solani* species complex. Phytopathology [in press].

Geiser DM, Aoki T, Bacon CW, Baker SE, Bhattacharyya MK, Brandt ME, Brown DW, Burgess LW, Chulze S, Coleman JJ, Correll JC, Covert SF, Crous PW, Cuomo CA, De Hoog GS, Di Pietro A, Elmer WH, Epstein L, Frandsen RJN, Freeman S, Gagkaeva T, Glenn AE, Gordon TR, Gregory NF, Hammond-Kosack KE, Hanson LE, del Mar Jímenez-Gasco M, Kang S, Kistler HC, Kuldau GA, Leslie JF, Logrieco A, Lu G, Lysøe E, Ma L-J, McCormick SP, Migheli Q, Moretti A, Munaut F, O'Donnell K, Pfenning L, Ploetz RC, Proctor RH, Rehner SA, Robert VARG, Rooney AP, Bin Salleh B, Scandiani MM, Scauflaire J, Short DPG, Steenkamp E, Suga H, Summerell BA, Sutton DA, Thrane U, Trail F, Van Diepeningen A, Van Etten HD, Viljoen A, Waalwijk C, Ward TJ, Wingfield MJ, Xu J-R, Yang X-B, Yli-Mattila T, Zhang N. 2013. One fungus, one name: defining the genus *Fusarium* in a scientifically robust way that preserves longstanding use. Phytopathology 103:400–408.

Hulcr J, Stelinski LL. 2017. The ambrosia symbiosis: from evolutionary ecology to practical management. Annual Review of Entomology 62:285–303.

Jordal BH, Cognato AI. 2012. Molecular phylogeny of bark and ambrosia beetles reveals multiple origins of fungus farming during periods of global warming. BMC Evolutionary Biology 12:133.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14:587–589.

Kasson MT, O'Donnell K, Rooney AP, Sink S, Ploetz RC, Ploetz JN, Konkol JL, Carrillo D, Freeman S, Mendel Z, Smith JA, Black AW, Hulcr J, Bateman C, Stefkova K, Campbell PR, Geering ADW, Dann EK, Eskalen A, Mohotti K, Short DPG, Aoki T, Fenstermacher KA, Davis DD, Geiser DM. 2013. An inordinate fondness for *Fusarium*: phylogenetic diversity of fusaria cultivated by ambrosia beetles in the genus *Euwallacea* on avocado and other plant hosts. Fungal Genetics and Biology 56:147–157.

Kolařik M, Hulcr J, Kirkendall L. 2015. New species of *Geosmithia* and *Graphium* associated with the ambrosia beetles in Costa Rica. Czech Mycology 67:29–35.

Kolařik M, Kirkendall L. 2010. Evidence for a new lineage of primary ambrosia fungi in *Geosmithia* Pitt (Ascomycota: Hypocreales). Fungal Biology 114:676–689.

Kornerup A, Wanscher JH. 1978. Methuen handbook of colour. London, UK: Eyre Methuen. 252 p.

Li Y, Simmons DR, Bateman CC, Short DPG, Kasson MT, Rabaglia RJ, Hulcr J. 2015. New fungus-insect symbiosis: culturing, molecular, and histological methods determine saprophytic polyporales mutualists of *Ambrosiodmus* ambrosia beetles. PLoS ONE 10:e0137689.

Lynn KMT, Wingfield MJ, Durán A, Marincowitz S, Oliveira LSS, de Beer ZW, Barnes I. 2020. *Euwallacea perbrevis* (Coleoptera: Curculionidae: Scolytinae), a confirmed pest on *Acacia crassicarpa* in Riau, Indonesia, and a new fungal symbiont; *Fusarium rekanum* sp. nov. Antonie van Leeuwenhoek 113:803–823.

Mendel Z, Protasov A, Sharon M, Zveibil A, Ben Yehuda S, O'Donnell K, Rabaglia R, Wysoki M, Freeman S. 2012. An Asian ambrosia beetle *Euwallacea fornicatus* and its novel symbiotic fungus *Fusarium* sp. pose a serious threat to the Israeli avocado industry. Phytoparasitica 40:235–238.

Na F, Carrillo JD, Mayorquin JS, Ndinga-Muniania C, Stajich JE, Stouthamer R, Huang Y-T, Lin Y-T, Chen C-Y, Eskalen A. 2018. Two novel fungal symbionts *Fusarium kuroshium* sp. nov. and *Graphium kuroshium* sp. nov. of Kuroshio shot hole borer (*Euwallacea* sp. nr. *fornicatus*) cause Fusarium dieback on woody host species in California. Plant Disease 102:1154–1164.

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Molecular Biology and Evolution 32:268–274, doi:10.1093/molbev/msu300

Nirenberg HI. 1990. Recent advances in the taxonomy of *Fusarium*. Studies in Mycology 32:91–101.

Nirenberg HI, O'Donnell K. 1998. New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. Mycologia 90:434–458.

O'Donnell K. 2000. Molecular phylogeny of the *Nectria haematococca-Fusarium solani* species complex. Mycologia 92:919–938.

O'Donnell K, Al-Hatmi AMS, Aoki T, Brankovics B, Cano-Lira JF, Coleman JJ, de Hoog GS, Di Pietro A, Frandsen RJN, Geiser DM, Gibas CFC, Guarro J, Kim H-S, Kistler HC, Laraba I, Leslie JF, López-Berges MS, Lysøe E, Meis JF, Monod M, Proctor RH, Rep M, Ruiz-Roldán C, Šišić A, Stajich JE, Steenkamp ET, Summerell BA, Van der Lee TAJ, Van Diepeningen AD, Verweij PE, Waalwijk C, Ward TJ, Wickes BL, Wiederhold NP, Wingfield MJ, Zhang N, Zhang SX. 2020. No to *Neocosmospora*: phylogenomic and practical reasons for continued inclusion of the *Fusarium solani* species complex in the genus *Fusarium*. mSphere 5: e00810–20, doi:10.1128/mSphere.00810-20

O'Donnell K, Libeskind-Hadas R, Hulcr J, Bateman C, Kasson MT, Ploetz RC, Konkol JL, Ploetz JN, Carrillo D, Campbell A, Duncan RE, Liyanage PNH, Eskalen A, Lynch SC, Geiser DM, Freeman S, Mendel Z, Sharon M, Aoki T, Cossé AA, Rooney AP. 2016. Invasive Asian *Fusarium–Euwallacea* ambrosia beetle mutualists pose a serious threat to forests, urban landscapes and the avocado industry. Phytoparasitica 44:435–442.

O'Donnell K, Rooney AP, Proctor RH, Brown DW, McCormick SP, Ward TJ, Frandsen RJN, Lysøe E, Rehner SA, Aoki T, Robert VARG, Crous PW, Groenewald JZ, Kang S, Geiser DM. 2013. Phylogenetic analyses of *RPB1* and *RPB2* support a middle Cretaceous origin for a clade comprising all agriculturally and medically important fusaria. Fungal Genetics and Biology 52:20–31.

O'Donnell K, Sink S, Libeskind-Hadas R, Hulcr J, Kasson MT, Ploetz RC, Konkol JL, Ploetz JN, Carrillo D, Campbell A, Duncan RE, Liyanage NH, Eskalen A, Na F, Geiser DM, Bateman C, Freeman S, Mendel Z, Sharon M, Aoki T, Cossé AA, Rooney AP. 2015. Discordant phylogenies suggest repeated host shifts in the *Fusarium–Euwallacea* ambrosia beetle mutualism. Fungal Genetics and Biology 82:277–290.

Paap T, de Beer ZW, Migliorini D, Nel WJ, Wingfield MJ. 2018. The polyphagous shot hole borer (PSHB) and its fungal symbiont *Fusarium euwallaceae*: a new invasion in South Africa. Australasian Plant Pathology 47:231–237.

Sandoval-Denis M, Lombard L, Crous PW. 2019. Back to the roots: a reappraisal of *Neocosmospora*. Persoonia 43:90–185.

Short DP, O'Donnell K, Stajich JE, Hulcr J, Kijimoto T, Berger MC, Macias AM, Spahr EJ, Bateman CC, Eskalen A, Lynch SC. 2017. PCR multiplexes discriminate *Fusarium* symbionts of invasive *Euwallacea* ambrosia beetles that inflict damage on numerous tree species throughout the United States. Plant Disease 101:233–240.

Six DL. 2003. Bark beetle-fungus symbioses. In: Bourtzis K, Miller TA, eds. Insect symbiosis. Boca Raton, Florida: CRC Press. p. 97–114.

Six DL. 2012. Ecological and evolutionary determinants of bark beetle—fungus symbioses. Insects 3:339–366.

Smith SM, Gomez DF, Beaver RA, Hulcr J, Cognato AI. 2019. Reassessment of the species in the *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) complex after the rediscovery of the "lost" type specimen. Insects 10:1–11.

Spahr E, Kasson MT, Kijimoto T. 2020. Micro-computed tomography permits enhanced visualization of mycangia across development and between sexes in *Euwallacea* ambrosia beetles. PLoS ONE 15:e0236653, doi:10.1371/journal.pone.0236653

Stouthamer R, Rugman-Jones P, Thu PQ, Eskalen A, Thibault T, Hulcr J, Wang L-J, Jordal BH, Chen C-Y, Cooperband M, Lin C-S, Kamata N, Lu S-S, Masuya H, Mendel Z, Rabaglia R, Sanguansub S, Shih H-H, Sittichaya W, Zong S. 2017. Tracing the origin of a cryptic invader: phylogeography of the *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) species complex. Agricultural and Forest Entomology 19:366–375.

Turland NJ, Wiersema JH, Barrie FR, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Kusber W-H, Li D-Z, Marhold K, May TW, McNeill J, Monro AM, Prado J, Price MJ, Smith GF. (eds.). 2018. International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books, doi:10.12705/Code.2018