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## 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

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### ABSTRACT

The Ambrosia *Fusarium* Clade (AFC) comprises at least 16 genealogically exclusive species-level lineages within clade 3 of the *Fusarium solani* species complex (FSSC). These fungi are either known or predicted to be farmed by Asian *Euwallacea* ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) in the tribe Xyleborini as a source of nutrition. To date, only 4 of the 16 AFC lineages have been described formally. In the absence of Latin binomials, an ad hoc nomenclature was developed to distinguish the 16 species lineages as AF-1 to AF-16. Herein, *Fusarium* species AF-3, AF-5, and AF-7 were formally described as *F. floridanum*, *F. tuaranense*, and *F. obliquiseptatum*, respectively. *Fusarium floridanum* farmed by *E. interjectus* on box elder (*Acer negundo*) in Gainesville, Florida, was distinguished morphologically by the production of sporodochial conidia that were highly variable in size and shape together with greenish-pigmented chlamydospores. *Fusarium tuaranense* was isolated from a beetle-damaged Paía rubber tree (*Hevea brasiliense*) in North Borneo, Malaysia, and was diagnosed by production of the smallest sporodochial conidia of any species within the AFC. Lastly, *F. obliquiseptatum* was farmed by an unnamed ambrosia beetle designated *Euwallacea* sp. 3 (*E. fornicatus* species complex) on avocado (*Persea americana*) in Queensland, Australia. It uniquely produces some clavate sporodochial conidia with oblique septa. Maximum likelihood analysis of a multilocus data set resolved these three novel AFC taxa as phylogenetically distinct species based on genealogical concordance. Particularly where introduced into exotic environments, these exotic mutualists pose a serious threat to the avocado industry, native forests, and urban landscapes in diverse regions throughout the world.

### ARTICLE HISTORY

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## INTRODUCTION

The Ambrosia *Fusarium* Clade (AFC) was named for a recently discovered monophyletic lineage within the *F. solani* species complex (FSSC) that includes 16 phylogenetically distinct species (Kasson et al. 2013; O'Donnell et al. 2015; Na et al. 2018). Because only one of the species possessed a Latin binomial when the AFC was first discovered, an ad hoc nomenclature was adopted in which the phylogenetically distinct species were distinguished by the prefix AF followed by a unique Arabic number (i.e., AF-1 through AF-16). These fusaria are known or predicted to be farmed by female *Euwallacea* ambrosia beetles (Coleoptera:

Scolytinae) in an obligate mutualistic association. The AFC is unique and represents the Hypocreales as one of the 12 independent evolutionary origins of fungus-farming by ambrosia beetles (Jordal and Cognato 2012; Li et al. 2015), although there are some additional ambrosia fungal species within the genus *Geosmithia* of the same order (Kolařík and Kirkendall 2010; Kolařík et al. 2015). Diversification time estimates place the origin of the *Euwallacea*-*Fusarium* mutualism in the early Miocene, approximately 21 million years ago (Mya; Kasson et al. 2013). Robust species-level multilocus molecular systematic data suggest that at least seven *Euwallacea* species and 16 AFC species are engaged in the obligate mutualism. Cophylogenetic

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analyses strongly suggest that host-shift speciation, rather than diversifying coevolution, has played a central role in shaping the evolution of this unique mutualism (O'Donnell et al. 2015).

AFC mutualists are carried in specialized cavities called mycangia by female *Euwallacea* beetles, which farm them in galleries they construct in the xylem of their woody hosts and use them as a source of nutrition for themselves and their larvae (Hulcr and Stelinski 2017). This mutualism has attracted considerable attention by agricultural scientists because several xyleborine *Fusarium*-farming *Euwallacea* beetles cause dieback and death of economically important hosts, including Chinese tea (*Camellia sinensis*), cacao (*Theobroma cacao*), rubber tree (*Hevea brasiliensis*), citrus (*Citrus* spp.) (Brayford 1987), and avocado (Mendel et al. 2012).

The discovery of invasive Asian *Euwallacea* farming *Fusarium* associated with wilt and dieback of avocado (*Persea americana*), as well as diverse woody hosts in managed urban landscapes and naïve forests in the United States, Israel, Australia, Mexico, and South Africa, during the past decade has spurred intensive research efforts focused on understanding the genetic diversity of these exotic mutualists (Mendel et al. 2012; Freeman et al. 2013; O'Donnell et al. 2016; Paap et al. 2018). The present work identified phenotypic characters that can be used to distinguish the AFC species (Aoki et al. 2018) and elucidate their host range and pathogenic potential (Eskalen et al. 2013), providing a basis for developing management, monitoring, and eradication strategies. To date, only 4 of the 16 AF species have been described formally, and these include *F. ambrosium* (Gadd and Loos 1947; Brayford 1987; Nirenberg 1990) farmed by the shot hole borer of tea (*Euwallacea perbrevis*) in Sri Lanka and India; *F. euwallaceae* (Freeman et al. 2013) farmed by the polyphagous shot hole borer of avocado (*E. fornicatus*; O'Donnell et al. 2015; Smith et al. 2019) and numerous other woody hosts in Los Angeles and surrounding counties in California, Israel (Freeman et al. 2013), and South Africa (Paap et al. 2018); *F. kuroshium* (Na et al. 2018) farmed by the kuroshio shot hole borer (*Euwallacea kuroshio*; Gomez et al. 2018) in San Diego (California) and Mexico (García-Avila et al. 2016); and *F. oligoseptatum* (Aoki et al. 2018) farmed by *E. validus* on the invasive Asian endemic tree of heaven (*Ailanthus altissima*) in eastern North America (Kasson et al. 2013).

The present study reports on multilocus molecular phylogenetic analyses and detailed phenotypic data that were used to distinguish three unnamed AFC species analyzed by Kasson et al. (2013) that form clavate and

apically swollen multiseptate conidia described as “dolphin-like” (Brayford 1987; Aoki et al. 2018). Herein, these species are formally described as *Fusarium floridanum* (AF-3) farmed by *E. interjectus* on boxelder (*Acer negundo*) in Gainesville, Florida; *Fusarium tuar-anense* (AF-5) predicted to be farmed by an unknown *Euwallacea* species on Pará rubber trees (*Hevea brasiliensis*) in Malaysia; and *Fusarium obliquiseptatum* (AF-7) farmed by *Euwallacea* sp. 3, an unnamed member of the *E. fornicatus* clade, on avocado in Queensland, Australia (O'Donnell et al. 2015). Of these, *Fusarium floridanum* and *F. obliquiseptatum* are known to cause limited cankers associated with beetle attacks on boxelder and avocado, respectively (FIG. 1). These symbiotic fusaria are unique in that they all produce apically swollen, clavate, multiseptate macroconidia that represent a potential adaptation for the symbiosis (Kasson et al. 2013).

## MATERIALS AND METHODS

**Phenotypic characterization.**—Strains were grown on potato dextrose agar (PDA; Difco, Detroit, Michigan) and synthetic low-nutrient agar (SNA; Nirenberg 1990; 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 incubated at 20 C in the dark on PDA in 9-cm Petri dishes were used to characterize colony color, odor, and morphology. Kornerup and Wanscher (1978) was used as the color standard. PDA cultures were also employed for determining mycelial growth rates in the dark at eight temperatures (5–40 C) at 5 C intervals (Aoki et al. 2013). Cultures were examined at 1 and 4 d after inoculation, and radial growth rates were calculated as arithmetic mean values per day by measuring 16 radii around the colonies. Measurements of growth rate at different temperatures were repeated twice, and the data were averaged for each strain. Cultures on SNA were examined for microscopic characters as described by Freeman et al. (2013) and Aoki et al. (2018). Conidia and conidiophores produced on SNA under continuous black light were mounted in water and then examined and photographed with a Zeiss Axioskop microscope (Zeiss, Jena) using a Nikon DS-Fi2 digital camera (Nikon, Tokyo). Phenotypic characters were compared with published data on related AFC species, i.e., *F. ambrosium* (as *Monacrosporium ambrosium*; Gadd and Loos 1947), *F. euwallaceae* (Freeman et al. 2013), and *F. oligoseptatum* (Aoki et al. 2018).



**Figure 1.** Abundant entry holes and frass associated with *Euwallacea interjectus* attacks on *Acer negundo* (left) and *Fusarium* cankers caused by *Fusarium floridanum* on debarked portion of stem surrounding individual beetle entry holes (right). White arrow denotes female *E. interjectus* emerging from gallery. Infested *A. negundo* trees were sampled in Loblolly Woods Nature Park, Gainesville, Florida, USA.

**Molecular phylogenetic analyses.**—The 29 isolates from the FSSC included in the present study were cultured in yeast-malt broth on a rotary shaker set at 150 rpm for 3–4 d at 25 C. Once the cultures had grown, mycelium was collected on a Whatman No. 1 filter paper disc (Sigma-Aldrich, St. Louis, Missouri) on a Büchner funnel, freeze-dried overnight, and then genomic DNA was extracted using a cetyltrimethylammonium bromide (CTAB; Sigma-Aldrich) protocol (Gardes and Bruns 1993). Portions of four phylogenetically informative loci (translation elongation factor 1 $\alpha$  [*TEF1*], internal transcribed spacer [ITS] rDNA, and DNA-directed RNA polymerase II largest [*RPB1*] and second largest [*RPB2*] subunit) were obtained via Sanger sequencing with an ABI 3730 genetic analyzer (ABI, Emeryville, California). Sequencher 5.2.4 (Gene Codes, Ann Arbor, Michigan) was used to edit ABI chromatograms, after which they were exported as FASTA files. Sequences in the four individual partitions, including those of eight strains from Na et al. (2018) downloaded from GenBank, were aligned with MUSCLE (Edgar 2004), and

then ModelFinder (Kalyaanamoorthy et al. 2017) was used to identify the optimal model of molecular evolution for the four partitions based on the Bayesian information criterion scores. ML analyses of the combined four-locus data set were implemented in IQ-TREE (Nguyen et al. 2015; <http://www.iqtree.org/>) using separate models for each partition (Chernomor et al. 2016). Clade support was assessed by 5000 maximum likelihood (ML) bootstrap pseudoreplicates of the combined data set. Sequences analyzed in the present study were deposited in GenBank as part of several prior studies (Kasson et al. 2013; O'Donnell et al. 2015; Na et al. 2018). The four-locus data set and the ML-bootstrapped tree were deposited in TreeBASE (accession no. S24402 and tree no. Tr117309).

## RESULTS

**Phylogenetic analyses.**—The aligned four-locus data set consisted of the following gene regions, with total sites/parsimony-informative sites indicated in

parentheses: *TEF1* (684/50), ITS rDNA (445/11), *RPB1* (1588/166), and *RPB2* (1635/157). The optimal model of molecular evolution found by ModelFinder (Kalyaanamoorthy et al. 2017) was HKY+F+I for *TEF1*, TIM2e+I for ITS rDNA, TNe+G4 for *RPB1*, and TNe+I for *RPB2*. The negative log-likelihood ( $-\ln L$ ) of the best ML tree found by IQ-TREE (Nguyen et al. 2015) was  $-9270.603$  (FIG. 2). The ML-bootstrapped phylogram was rooted on sequences of *Fusarium neocosmosporiellum* NRRL 22166 and NRRL 43467 based on more inclusive analyses (O'Donnell et al. 2013). ML bootstrapping failed to support most of the nodes along the backbone of the phylogeny, but the species lineages represented by two or more isolates were all strongly supported as genealogically exclusive (FIG. 2), including the AFC species described herein. In addition, *F. floridanum* (AF-3) + *F. euwallaceae* (AF-2) and *F. obliquiseptatum* (AF-7) + *Fusarium* sp. (AF-6) were strongly supported as sisters. However, sister-

group relationships of *F. tuaranense* (AF-5) from Malaysia were unresolved. Separate trees for each partition were provided as SUPPLEMENTARY FIG. 1.

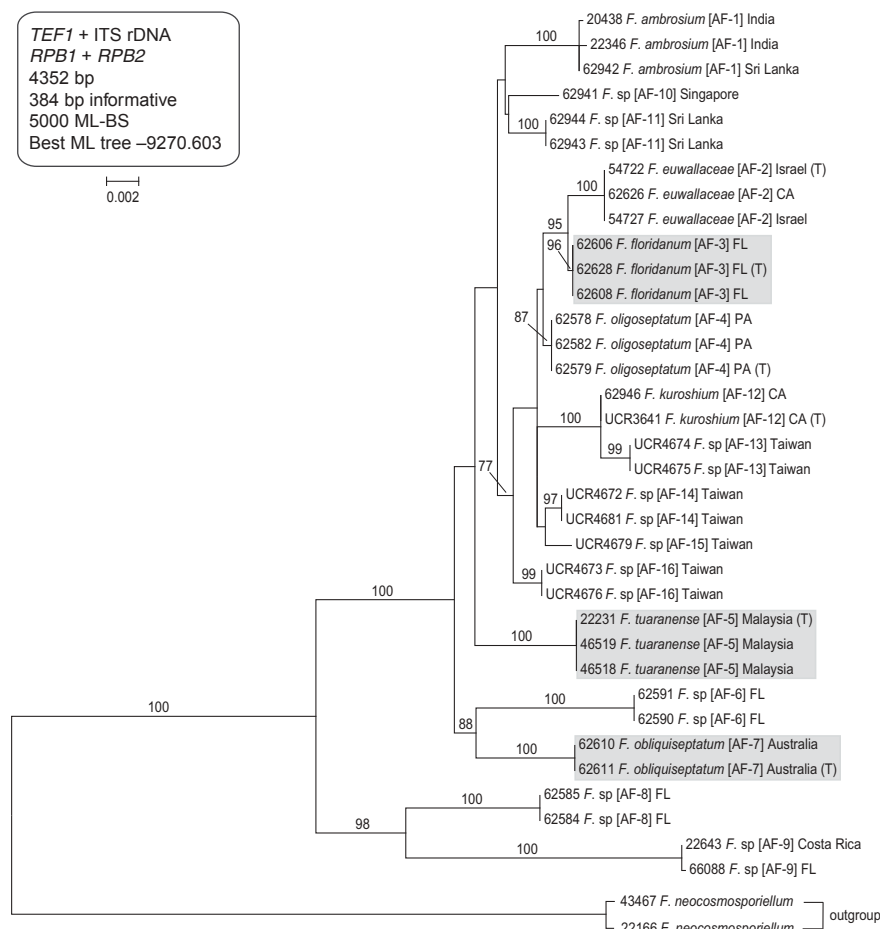
## TAXONOMY

Based on multilocus phylogenetic analyses and a detailed comparison of phenotypic/morphological characters, we formally describe three novel AFC species within the FSSC.

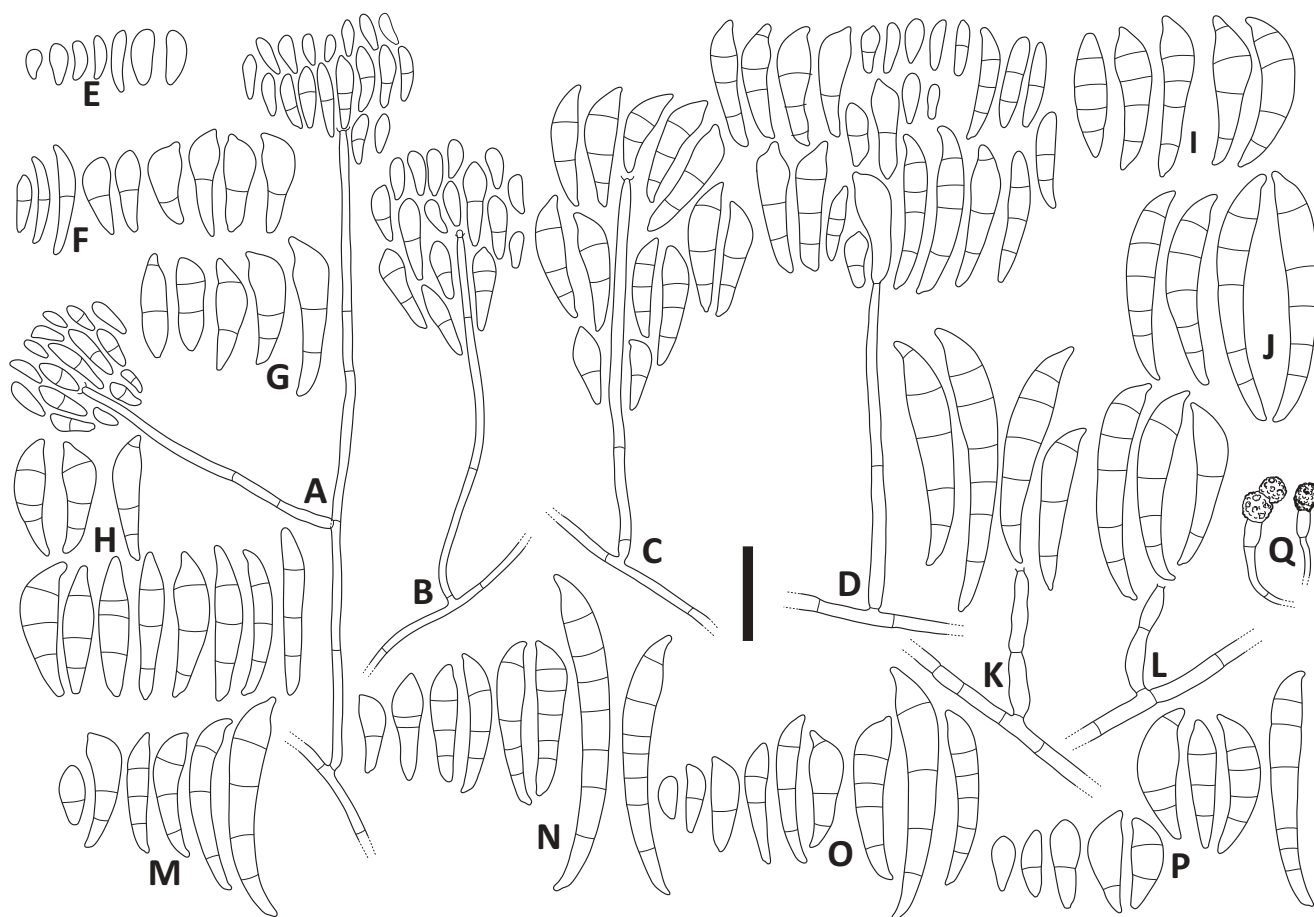
***Fusarium floridanum*** T. Aoki, J.A. Smith, Kasson, S. Freeman, Geiser & O'Donnell, sp. nov. FIGS. 3–5; SUPPLEMENTARY FIG. 2A–D, I

MycoBank MB830011

**Typification:** USA. FLORIDA: Gainesville, originally isolated from the mycangium of an ambrosia beetle (*Euwallacea interjectus*) infesting a box elder tree (*Acer negundo*), 18 Oct 2012, Jason A. Smith No. 1190



**Figure 2.** Partitioned maximum likelihood bootstrap (ML-BS) analysis of 16 ingroup Ambrosia *Fusarium* Clade (AFC) species distinguished by an informal ad hoc nomenclature (AF-1 through AF-16). Seven of the ambrosia fusaria possess Latin binomials, including three species formally described herein identified by gray highlight. The 4.35 kb, four-locus data set included portions of *TEF1*, ITS rDNA, *RPB1*, and *RPB2*. The ingroup was rooted on sequences of *F. neocosmosporiellum* based on more inclusive analyses (O'Donnell et al. 2013). Numbers above nodes represent ML-BS support  $\geq 70\%$  based on 5000 pseudoreplicates of the data implemented in IQ-TREE (Nguyen et al. 2015). Twenty-nine of the strains are identified by the 5-digit ARS Culture Collection number (NRRL); sequences of eight UCR isolates were downloaded from GenBank (Na et al. 2018).



**Figure 3.** *Fusarium floridanum* cultured on SNA under black light. A–D. Tall aerial conidiophores forming 0- to multiseptate conidia. E–J. Shape and septation of conidia formed on tall aerial conidiophores. E. 0-Septate conidia. F. 1-Septate conidia. G. 2-Septate conidia. H. 3-Septate conidia. I. 4-Septate conidia. J. 5-Septate conidia. K, L. Sporodochial conidiophores forming clavate multiseptate conidia. M–P. Conidia formed by different strains. Q. Chlamydospores formed in hyphae. A–L, Q from NRRL 62628 (ex-holotype); M from NRRL 62608; N from NRRL 62606; O from NRRL 62607; P from NRRL 62629. Bar = 25 µm.

(holotype BPI 910972, a dried culture of NRRL 62628, Herbarium of US National Fungus Collections, designated in this study). Ex-holotype culture NRRL 62628 = MAFF 246849.

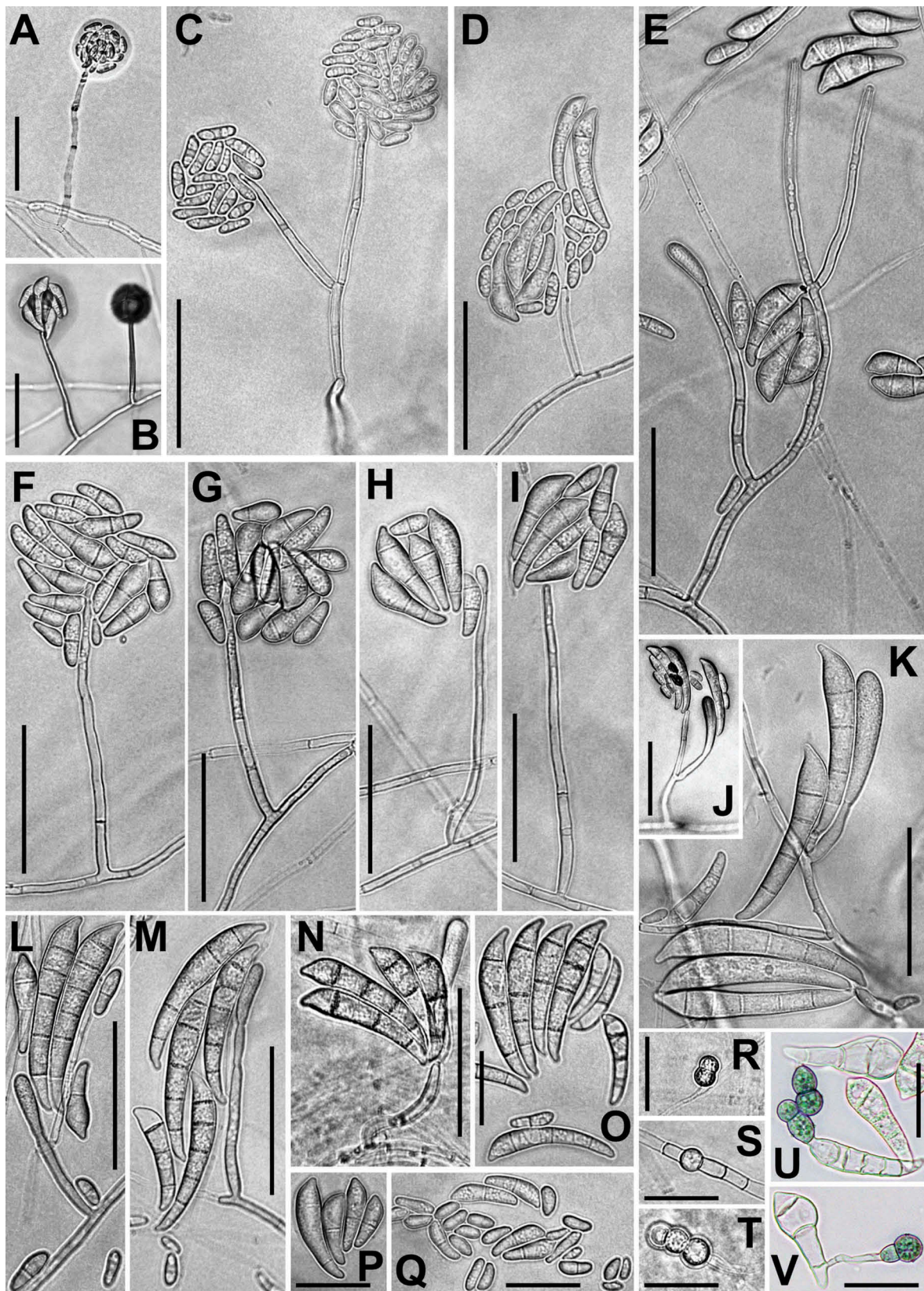
**Etymology:** *floridanum*, referring to the type locality in Florida.

**Diagnosis:** This species can be diagnosed by the highly variable size and shape of the sporodochial conidia. They are clavate, multiseptate, and swollen, “dolphin-like” in appearance, and narrower falcate septate conidia that varied in shape, including ones that were comma-shaped and 1-septate. Production of greenish-pigmented chlamydospores on PDA is an additional diagnostic feature.

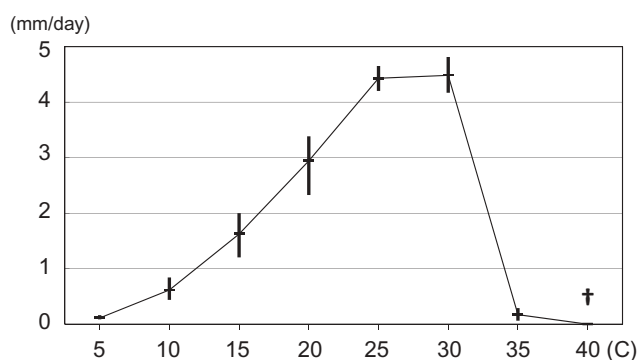
**Observations on PDA:** Colonies showing radial mycelial growth rates of 2.3–3.4 mm per day at 20 C and 4.2–4.7 mm per day at 25 C in the dark. Colony color white (1A1), yellowish-white (3–4A2) to yellowish-gray (4B2) or pale yellow (4A3), or orange white (5A2) to orange gray (5B2), grayish-orange (5B3) to brownish-

orange (5C3), some light brown (5–7D4–5) to grayish-brown (6–8D–E3) in the dark, white (1A1), yellowish-white (4A2) to pale yellow (4A3) or grayish-yellow (4B3–4) under black light. Aerial mycelium white (1A1), generally sparse, but sometimes floccose or funiculate in the dark, produced more abundantly under black light. Colony margin entire to undulate. Reverse pigmentation absent or yellowish-white (4A2) or pale yellow (4A3), pale orange (5A3) to grayish-orange (5B3–5) or brownish-orange (5C2–5), some light brown (5–7D4–5) to grayish-brown (6–8D–E3) in the dark and yellowish-white (4A2) to pale yellow (4A3) or grayish-yellow (4B3–4) under black light. Exudates absent. Odor moldy.

**Microscopic characters:** Hyphae on SNA 1–7 µm wide. Chlamydospores present but formation delayed in or on hyphae and conidia, mostly subglobose to round ellipsoidal, intercalary or terminal, single or sometimes in catenate chains up to 4 cells in length, typically hyaline to very slightly pale yellow, surface



**Figure 4.** *Fusarium floridanum* cultured on SNA under black light (except for U and V on PDA). A–I. Aerial conidiophores forming 0–4-septate conidia. J–N. Sporodochial conidiophores forming mostly clavate multiseptate conidia, swollen apically with 1–6 septa, and some oblong to short-clavate conidia (J, L, M). O. 0- to 4-septate sporodochial conidia. P, Q. 0- to 2-septate aerial conidia. R–T. Chlamydospores formed in hyphae. U, V. Greenish-pigmented chlamydospores formed on conidia cultured on PDA for more than 1 mo under ambient light conditions. A, E–I, L, S from NRRL 67607; B, M, O, T from NRRL 67608; C, D, J, K, P, Q from NRRL 62629; N, U, V from NRRL 62628, R from NRRL 62606 (A, B: aerial view without a cover slip; C–V: mounted in water with a cover slip; A–T: on SNA in black and white; U, V: on PDA in color). Bars: A–N = 50  $\mu$ m; O–V = 25  $\mu$ m.



**Figure 5.** Daily radial mycelial growth rate of *Fusarium florida-num* 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.

smooth to often minutely roughened,  $4.5\text{--}15.5 \times 4\text{--}11.5$   $\mu\text{m}$ ; on PDA chlamydospores formed in conidia sometimes becoming bluish to greenish after more than 1 mo. Sclerotia absent. Sporulation on SNA or on PDA generally rapid and abundant under black light, conidiation developing slowly and greatly reduced in the dark. Some strains produce fewer conidia on PDA in the dark; sporodochia formed sparsely on SNA and PDA. Chlamydospores in clusters of sporodochial conidia appear greenish-gray in some strains incubated for more than 1 mo under ambient light conditions.

Aerial conidiophores ordinarily formed abundantly on SNA under black light, erect, short or tall and narrow, unbranched or sometimes branched sparsely, up to 223  $\mu\text{m}$  long, 2–6  $\mu\text{m}$  wide at the base, thin-walled, forming monophialides integrated in the apices. Phialides on aerial conidiophores simple, subcylindrical to subulate, tapering toward apex, often with a minute collarette at the tip,  $11\text{--}60 \times 1.5\text{--}4$   $\mu\text{m}$ . Aerial conidia mostly (1) elliptical, oblong-elliptical, fusiform-elliptical to short-clavate, straight or often slightly curved, occasionally reniform, some obovate to comma-shaped, 0–1 (–2)-septate; 0-septate on SNA under black light:  $4\text{--}17.5 \times 2\text{--}7$   $\mu\text{m}$  in total range,  $7.1\text{--}11.6 \times 3.4\text{--}3.5$   $\mu\text{m}$  on average (ex-type:  $6\text{--}17.5 \times 2.5\text{--}5$   $\mu\text{m}$  in total range,  $11.6 \pm 2.5 \times 3.5 \pm 0.7$   $\mu\text{m}$  on average  $\pm$  SD); 1-septate on SNA under black light:  $8\text{--}28.5 \times 2.5\text{--}8$   $\mu\text{m}$  in total range,  $15.7\text{--}18.4 \times 4.7\text{--}4.9$   $\mu\text{m}$  on average (ex-type:  $9.5\text{--}26 \times 2.5\text{--}8$   $\mu\text{m}$  in total range,  $18.4 \pm 3.9 \times 4.9 \pm 1.2$   $\mu\text{m}$  on average  $\pm$  SD); but often forming (2) larger, falcate to clavate, sometimes curved cylindrical, (1–)2–3(–4)-septate conidia, morphologically similar to falcate to clavate sporodochial conidia. Sporodochial conidiophores generally shorter than aerial conidiophores, but some are relatively tall and thick,

unbranched or rarely sparsely branched, up to 138  $\mu\text{m}$  long, 1.5–5  $\mu\text{m}$  wide at the base, mostly straight, sometimes contorted, forming apical integrated monophialides, or reduced to a simple phialide on substrate mycelium. Sporodochial phialides simple, subulate or subcylindrical, often with a conspicuous collarette at the tip,  $13\text{--}64 \times 2\text{--}5$   $\mu\text{m}$ . Sporodochial conidia hyaline, highly variable in shape and size, falcate, lanceolate to long clavate, or curved cylindrical, crescent-shaped, often swollen in their upper parts with a papillate apical cell, tapering abruptly toward the base, with a distinct or indistinct foot-like basal cell, or simply rounded at base, swollen conidia sometimes becoming “dolphin-like” in appearance, or rarely comma-shaped when 1-septate and without an apical papillum, but narrower (0–)1–5(–7)-septate conidia formed frequently under black light, less frequently in the dark on SNA, sometimes formed on PDA under black light; 0-septate on SNA under black light:  $6.5\text{--}22.5 \times 3\text{--}10$   $\mu\text{m}$  in total range,  $11.5\text{--}13.9 \times 4.9\text{--}5.2$   $\mu\text{m}$  on average (ex-type:  $7.5\text{--}17.5 \times 3\text{--}7.5$   $\mu\text{m}$  in total range,  $11.5 \pm 2.3 \times 4.9 \pm 0.8$   $\mu\text{m}$  on average  $\pm$  SD); 1-septate on SNA under black light:  $10.5\text{--}38 \times 3.5\text{--}12$   $\mu\text{m}$  in total range,  $19.1\text{--}20.9 \times 6.2\text{--}6.8$   $\mu\text{m}$  on average (ex-type:  $10.5\text{--}29.5 \times 3.5\text{--}10$   $\mu\text{m}$  in total range,  $20.2 \pm 4.4 \times 6.8 \pm 1.4$   $\mu\text{m}$  on average  $\pm$  SD); 2-septate on SNA under black light:  $15\text{--}61 \times 5\text{--}11$   $\mu\text{m}$  in total range,  $26.0\text{--}28.3 \times 7.0\text{--}7.6$   $\mu\text{m}$  on average (ex-type:  $22\text{--}61 \times 5\text{--}9.5$   $\mu\text{m}$  in total range,  $28.0 \pm 5.7 \times 7.4 \pm 1.0$   $\mu\text{m}$  on average  $\pm$  SD); 3-septate on SNA under black light:  $21\text{--}62 \times 5\text{--}11.5$   $\mu\text{m}$  in total range,  $33.2\text{--}37.1 \times 7.5\text{--}8.8$   $\mu\text{m}$  on average (ex-type:  $23\text{--}44.5 \times 5\text{--}10$   $\mu\text{m}$  in total range,  $33.8 \pm 5.0 \times 7.6 \pm 1.0$   $\mu\text{m}$  on average  $\pm$  SD); 4-septate on SNA under black light:  $28\text{--}65 \times 5\text{--}12$   $\mu\text{m}$  in total range,  $38.6\text{--}51.1 \times 8.1\text{--}9.4$   $\mu\text{m}$  on average (ex-type:  $30.5\text{--}54 \times 5\text{--}10$   $\mu\text{m}$  in total range,  $38.6 \pm 4.8 \times 8.1 \pm 0.9$   $\mu\text{m}$  on average  $\pm$  SD); 5-septate on SNA under black light:  $26.5\text{--}74.5 \times 6\text{--}12.5$   $\mu\text{m}$  in total range,  $44.6\text{--}58.0 \times 8.9\text{--}9.6$   $\mu\text{m}$  on average (ex-type:  $33\text{--}67 \times 6.5\text{--}12.5$   $\mu\text{m}$  in total range,  $44.6 \pm 6.5 \times 8.9 \pm 0.9$   $\mu\text{m}$  on average  $\pm$  SD); 6-septate on SNA under black light:  $37.5\text{--}72.5 \times 8\text{--}11$   $\mu\text{m}$  in total range. Short-clavate to obovate or naviculate, straight or curved conidia, with a rounded apex and a truncate base, 0–1(–2)-septate, often formed together with multiseptate conidia borne on thick sporodochial conidiophores.

*Additional isolates studied:* NRRL 62606 = MAFF 246846 isolated from gallery of ambrosia beetles (*Euwallacea interjectus*) infesting *Acer negundo*, Gainesville, Florida, USA, 18 Oct 2012, by Jason A. Smith (No. PL1499); NRRL 62607 = MAFF 246847 isolated from gallery of ambrosia beetles (*Euwallacea interjectus*) infesting *Acer negundo*, Gainesville, Florida, USA, 18 Oct 2012, by Jason A. Smith (No. PL1500);

NRRL 62608 = MAFF 246848 isolated from red-stained sapwood of *Acer negundo*, Gainesville, Florida, USA, 18 Oct 2012, by Jason A. Smith (No. PL1501); NRRL 62629 = MAFF 246850 isolated from mycangium of an ambrosia beetle (*Euwallacea interjectus*) infesting *Acer negundo*, Gainesville, Florida, USA, 18 Oct 2012, by Jason A. Smith (No. 1191) (a dried culture of NRRL 62629 was also deposited as BPI 910976 for an additional specimen of the species).

**Notes:** This member of the AFC was reported previously as *Fusarium* sp. (AF-3) (Kasson et al. 2013; O'Donnell et al. 2015; Short et al. 2017; Aoki et al. 2018). It produces diverse types of aerial conidia on tall conidiophores. The conidia are variable in shape and size, i.e., 0- to multiseptate, obovoid to oblong, elliptical to short-clavate, falcate, naviculate to apically swollen long clavate with or without apical papilla (FIGS. 3A–J, 4A–I), or even comma-shaped 0- to 1-septate (FIGS. 3B–F, 4E–I). Sporodochial conidia are often long clavate, and some are swollen apically; when swollen, sometimes appearing “dolphin-like” (FIGS. 3K–P, 4K–O). Aerial and sporodochial conidia are morphologically similar in that they both form comma-shape 1-septate conidia with or without apical papilla, and obovoid to oblong 0-septate conidia (FIG. 4J, L, O). The sizes of aerial and sporodochial conidia are also similar (FIGS. 3A–P, 4A–Q). This species forms chlamydospores in or on hyphae and conidia (FIG. 3Q, 4R–V), but their formation is often delayed. Chlamydospores are typically hyaline to slightly pale yellow, and smooth- to often minutely rough-walled on SNA; sometimes becoming bluish- to greenish-pigmented when cultured for more than 1 mo on PDA (FIG. 4U, V). *Fusarium floridanum* grew fastest at 30 C (FIG. 5). Colony morphology of NRRL 62628 (ex-holotype) and NRRL 62628 on PDA at 25 C is provided in SUPPLEMENTARY FIG. 2A–D. A whole-genome shotgun (WGS) sequence of *F. floridanum* was deposited at GenBank for isolate NRRL 62606 = MAFF 246846 under accession NKCL00000000.1 and Bioproject PRJNA389173.

***Fusarium tuaranense*** T. Aoki, Kasson, S. Freeman, Geiser & O'Donnell, sp. nov. **FIGS. 6–8;**  
SUPPLEMENTARY FIG. 2E, F, J  
Mycobank MB830012

**Typification:** MALAYSIA. SABAH STATE (NORTH BORNEO): Tuaran, originally isolated from a Pará rubber tree (*Hevea brasiliensis*) damaged by an unknown ambrosia beetle, Agriculture Research Centre, Tuaran, Sabah, 19 Nov 1964, by unknown collector (**holotype** BPI 910971, a dried culture of NRRL 22231, designated in this study; **isotype** IMI 110107, Herbarium IMI, the

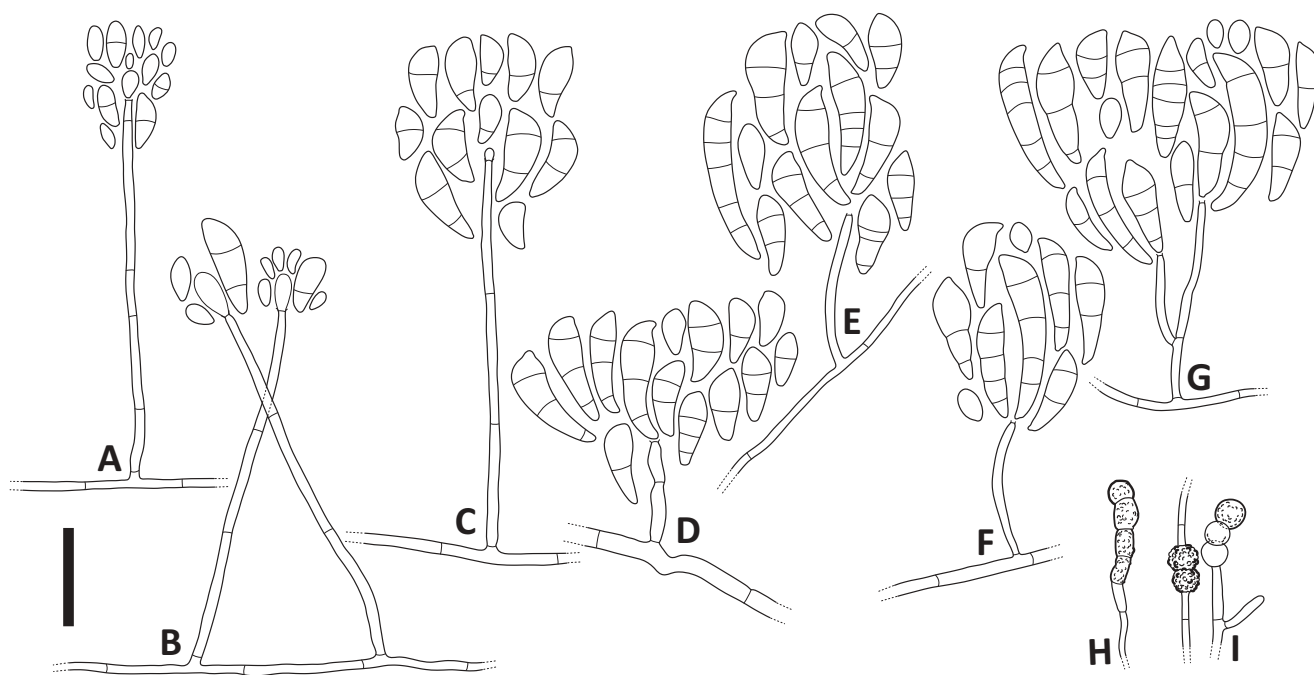
Mycology Department, the Royal Botanic Gardens, Kew, UK [as *Fusarium bugnicourtii*]). Ex-holotype culture NRRL 22231 = ATCC 16563 = MAFF 246842.

**Etymology:** *tuaranense*, referring to the type locality of Tuaran in the west costal region of Sabah State, Malaysia.

**Diagnosis:** This species can be diagnosed by its smaller sporodochial conidia. They are septate and clavate, appearing “dolphin-like,” and they are often shorter compared with other species within the AFC.

**Observations on PDA:** Colonies showing radial mycelial growth rates of 3.9–4.0 mm per day at 20 C and 4.9–5.1 mm per day at 25 C in the dark. Colony color on PDA yellowish-white (4A2) to pale yellow (4A3), or dull yellow (4B3), orange white (5A2), orange gray (5B2) to grayish-orange (5B3) in the dark; yellowish-white (4A2) to pale yellow (4A3) or light yellow (4A4) under black light. Aerial mycelium white (1A1), absent or sparsely formed in the dark and under black light. Colony margin entire to undulate. Reverse pigmentation absent or yellowish-white (4A2), pale yellow (4A3) to light yellow (4A4–5) in the dark and under black light. Exudates absent. Slight or strong moldy odor.

**Microscopic characters:** Hyphae on SNA 1–6.5  $\mu$ m wide. Chlamydospores present but formation delayed in or on hyphae and conidia, mostly subglobose to round ellipsoidal to cylindrical, intercalary or terminal, single or in catenate chains of up to 5 cells in length, ordinarily hyaline to slightly pale yellow, smooth- to often minutely rough-walled, 5–17.5  $\times$  4.5–11.5  $\mu$ m. Sclerotia absent. Sporulation on SNA or on PDA generally rapid and abundant under black light, delayed and reduced in the dark; sporodochia formed sparsely on SNA and PDA. Aerial conidiophores formed abundantly on SNA under black light, erect, short or tall and narrow, unbranched or sometimes branched sparsely, up to 363  $\mu$ m long, 2–5  $\mu$ m wide at the base, thin-walled, forming monophialides integrated in the apices. Phialides on aerial conidiophores simple, subcylindrical to subulate, tapering toward apex, often with a minute collarette at the tip, 28.5–66.5  $\times$  2.5–5  $\mu$ m. Aerial conidia (1) elliptical, oblong-elliptical, fusiform-elliptical to short-clavate, straight or often slightly curved, occasionally reniform, some obovate to comma-shaped, 0–1(–2)-septate; 0-septate on SNA under black light: 4.5–16  $\times$  2–6.5  $\mu$ m in total range, 7.8–8.4  $\times$  3.8–4.1  $\mu$ m on average (ex-type: 4.5–12.5  $\times$  2–6.5  $\mu$ m in total range, 7.8  $\pm$  1.8  $\times$  3.8  $\pm$  0.9  $\mu$ m on average  $\pm$  SD); 1-septate on SNA under black light: 5–34.5  $\times$  2.5–9  $\mu$ m in total range, 11.4–13.2  $\times$  4.9–5.5  $\mu$ m on average (ex-type: 8.5–17.5  $\times$  3–8.5  $\mu$ m in total range, 13.2  $\pm$  2.1  $\times$  5.5  $\pm$  0.9  $\mu$ m on average  $\pm$  SD); but often together with (2)



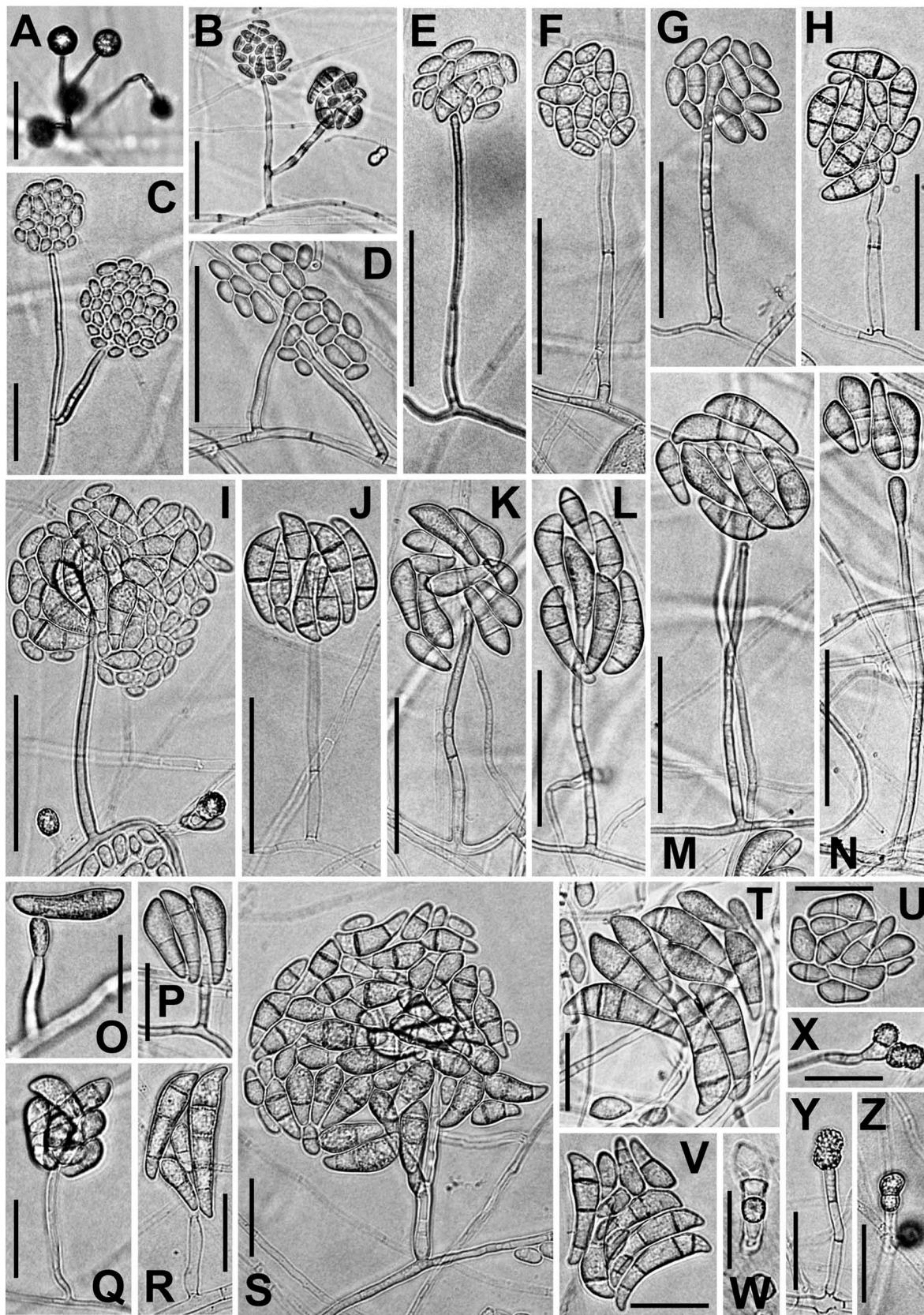
**Figure 6.** *Fusarium tuaranense* cultured on SNA under black light. A–C. Tall aerial conidiophores forming 0- to 2-septate conidia. D–G. Sporodochial conidiophores forming 0- to multiseptate conidia. H, I. Chlamydospores formed in hyphae. A–E, H from NRRL 22231 (ex-holotype); F, G, I from NRRL 46518. Bar = 25 µm.

larger, falcate to clavate, sometimes curved cylindrical, 1–2(–5)-septate conidia, morphologically similar to the falcate to clavate sporodochial conidia. Sporodochial conidiophores generally much shorter than aerial conidiophores, but sometimes tall or thicker, unbranched or rarely sparsely branched, up to 144 µm long, 2–4.5 µm wide at the base, mostly straight, forming apical integrated monophialides, or often reduced to be a simple phialide on substrate mycelium. Sporodochial phialides simple, subulate or subcylindrical, often with a conspicuous collarette at the tip, 12.5–50 × 2–4.5 µm. Sporodochial conidia hyaline, smaller in size, falcate, clavate, or cylindrical, often curved, swollen in upper parts, often with a pointed or slightly protruding apical cell, tapering gradually toward the base, with a distinct or indistinct foot-like basal cell, or simply rounded at base, swollen and longer conidia often appear “dolphin-like,” 0–4(–5)-septate, formed frequently under black light, less frequently in the dark on SNA, also formed on PDA under black light; 0-septate on SNA under black light: 7.5–30 × 3.5–9.5 µm in total range, 11.5–14.8 × 5.7–6.5 µm on average (ex-type: 7.5–21.5 × 3.5–9.5 µm in total range, 11.5 ± 2.6 × 5.7 ± 1.2 µm on average ± SD); 1-septate on SNA under black light: 11–26 × 5–10 µm in total range, 16.2–16.8 × 6.8–7.1 µm on average (ex-type: 11–24.5 × 5–10 µm in total range, 16.8 ± 2.9 × 6.8 ± 1.2 µm on average ± SD); 2-septate on SNA under black light: 13–31 × 5–12.5 µm in total

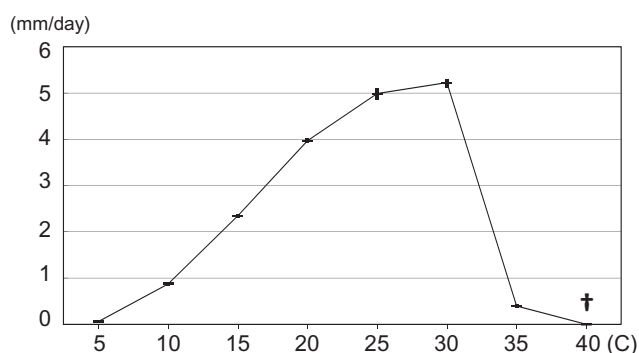
range, 22.0–22.7 × 7.8–8.3 µm on average (ex-type: 13–30.5 × 5–11 µm in total range, 22.7 ± 3.3 × 7.8 ± 1.0 µm on average ± SD); 3-septate on SNA under black light: 16.5–38 × 6–11.5 µm in total range, 26.8–27.1 × 7.8–8.8 µm on average (ex-type: 17.5–35.5 × 6–9.5 µm in total range, 26.8 ± 3.9 × 7.8 ± 0.7 µm on average ± SD); 4-septate on SNA under black light: 22.5–43.5 × 7.5–12 µm in total range, 32.2–33.9 × 8.8–9.1 µm on average (ex-type: 25–43.5 × 7.5–10 µm in total range, 33.9 ± 4.2 × 8.8 ± 0.8 µm on average ± SD); 5-septate on SNA under black light: 25.5–47 × 7.5–10 µm in total range. Short-clavate to obovate, or elliptical to naviculate, straight or curved conidia, with a rounded apex and a truncate base, (0–)1(–2)-septate, often formed together with multiseptate conidia from sporodochial conidiophore, resulting in aerial and sporodochial conidial structures that are morphologically similar.

*Additional isolate studied:* NRRL 46518 = FRC S-2075 = BBA 67605 = MAFF 246843 isolated from a Pará rubber tree (*Hevea brasiliensis*) in Malaysia, Mar 1993, collector unknown (a dried culture of NRRL 46518 was also deposited as BPI 910977 for an additional specimen of the species).

*Notes:* This species was reported previously as *Fusarium* sp. (AF-5), which is nested within the AFC (Kasson et al. 2013; O'Donnell et al. 2015; Aoki et al. 2018). *Fusarium tuaranense* forms conidia that are typically smaller compared with other species within the AFC.



**Figure 7.** *Fusarium tuaranense* cultured on SNA under black light. A–N. Aerial conidiophores forming 0- to 5-septate conidia. O–T. Sporodochial conidiophores forming 0- to multiseptate conidia, often swollen apically with 1–5 septa, and some oblong to short-clavate with 0–2 septa. U. 0- to 2-septate aerial conidia. V. 1- to 4-septate sporodochial conidia. W–Z. Chlamydospores formed in conidia and in hyphae. A–C, E, F, H–J, O, Q–S, V, Y from NRRL 22231; D, G, K–N, P, T, U, W, X, Z from NRRL 46518 (A, B: aerial view without a cover slip; C–Z: mounted in water with a cover slip; all on SNA). Bars: A–N = 50 µm; O–Z = 25 µm.



**Figure 8.** Daily radial mycelial growth rate of *Fusarium tuara-nense* 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 and died at 40 C.

Aerial and sporodochial conidiophores form septate conidia, but these are often shorter than other AFC species when conidia with the same number of septa are compared (FIGS. 6A–G, 7E–U). Aerial and sporodochial conidiophores produce morphologically similar 0- to multiseptate conidia that are obovate to short-clavate, elliptical to naviculate, or falcate to curved clavate with swollen upper parts (FIGS. 6A–G, 7A–V). The optimal temperature for growth was 30 C (FIG. 8). Colony morphology of NRRL 22231 (ex-holotype) on PDA at 25 C is provided in SUPPLEMENTARY FIG. 2E, F.

***Fusarium obliquiseptatum*** T. Aoki, Geering, Kasson, S. Freeman, Geiser & O'Donnell, sp. nov. FIGS. 9–11; SUPPLEMENTARY FIG. 2G, H, K, L  
MycoBank MB830013

**Typification:** AUSTRALIA. QUEENSLAND: Sunshine Coast, Beerwah, originally isolated from a gallery of an ambrosia beetle (*Euwallacea* sp. 3) infesting an avocado tree (*Persea americana*), Nov 2012, Andrew D.W. Geering & Paul R. Campbell No. QLD 2 (holotype BPI 910970, a dried culture of NRRL 62611, designated in this study). Ex-holotype culture NRRL 62611 = MAFF 246845.

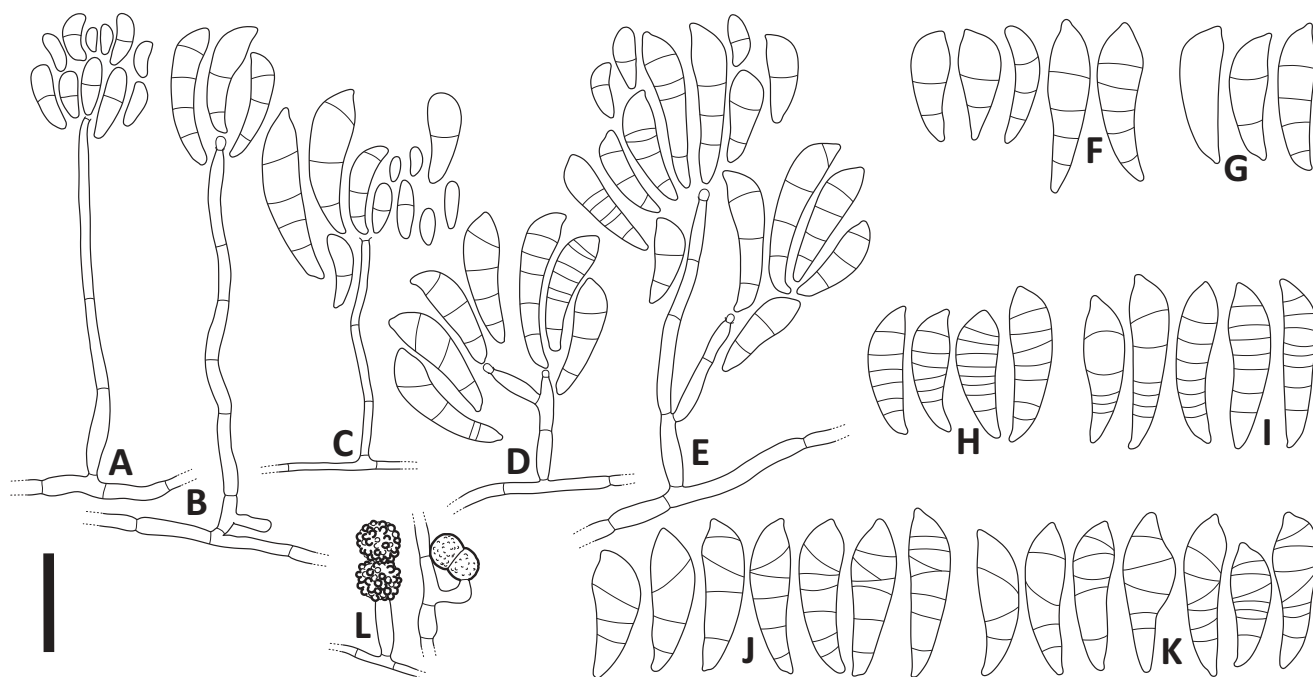
**Etymology:** *obliqui* + *septatum* (Latin), based on the occasional production of obliquely septate conidia.

**Diagnosis:** The species can be diagnosed by the dense septation and occasional oblique septa in swollen clavate conidia.

**Observations on PDA:** Colonies on PDA showing radial mycelial growth rates of 3.9–4.1 mm per day at 20 C and 4.8–4.9 mm per day at 25 C in the dark. Colony color on PDA white (1A1), yellowish-white (4A2) to pale yellow (4A3), grayish-yellow (4B–C3), or orange white (5A2) to grayish-orange (5B3), sometimes with greenish or bluish mycelial mass in the dark, yellowish-white (4A2) to pale yellow (4A3) or grayish-yellow (4B–C4–6) under black light. Aerial mycelium

white (1A1), absent or sparse in the dark and under black light. Colony margin entire to undulate. Reverse pigmentation absent or orange white (5A2) to pale orange (5A3), light orange (5A4–5) to grayish-orange (5B3–5) or brownish-orange (5C3–5), or grayish-brown (5D3) to light brown (5D4) in the dark and yellowish-white (4A2) to pale yellow (4A3) or grayish-yellow (4B–C4–6) under black light. Exudates absent. Odor moldy.

**Microscopic characters:** Hyphae on SNA 1.5–10 µm wide. Chlamydospores present but formation delayed in or on hyphae and conidia, mostly subglobose to round ellipsoidal, intercalary or terminal, single or sometimes in catenate chains up to 5 cells in length, typically hyaline to very slightly pale yellow, smooth- to often minutely rough-walled, 5–12 × 4.5–11.5 µm. Sclerotia absent. Sporulation on SNA or on PDA generally rapid and abundant under black light, delayed but often formed abundantly in the dark; sporodochia initially formed sparsely, but later formed abundantly on SNA and PDA. Aerial conidiophores formed abundantly on SNA under black light, erect or sometimes prostrate, short or very tall and narrow, unbranched or sometimes branched sparsely, up to 645 µm long, 2–4 µm wide at the base, thin-walled, forming monophialides integrated in the apices. Phialides on aerial conidiophores simple, subcylindrical to subulate, tapering toward apex, often with a minute collarette at the tip, 13.5–57.5 × 1.5–5 µm. Aerial conidia mostly (1) obovate, elliptical, oblong-elliptical, fusiform-elliptical to short-clavate, straight or often slightly curved, occasionally reniform, 0–1(–2)-septate; 0-septate on SNA under black light: 3–14.5 × 2–6 µm in total range, 6.7–8.1 × 3.4–3.7 µm on average (ex-type: 3–14.5 × 2.5–4.5 µm in total range, 8.1 ± 2.6 × 3.4 ± 0.5 µm on average ± SD); 1-septate on SNA under black light: 10.5–24.5 × 3–7 µm in total range, 13.4–18.2 × 5.2–5.3 µm on average (ex-type: 13–24.5 × 3–7 µm in total range, 18.2 ± 2.9 × 5.2 ± 0.8 µm on average ± SD); often formed together with (2) larger, falcate to clavate, sometimes curved cylindrical, (1–)2–3(–4)-septate conidia, morphologically similar to the falcate to clavate sporodochial conidia. Sporodochial conidiophores, often formed in a sporodochial mass, generally shorter than aerial conidiophores, but relatively tall and thick, unbranched or sparsely to densely branched, up to 218 µm long, 2–4 µm wide at the base, straight or sometimes contorted, forming apical integrated monophialides, or reduced into a simple phialide on substrate mycelium. Sporodochial phialides simple, subulate or subcylindrical, often with a conspicuous collarette at the tip, 15–31 × 2.5–4.5 µm. Sporodochial conidia hyaline, falcate, lanceolate to clavate, or curved cylindrical,



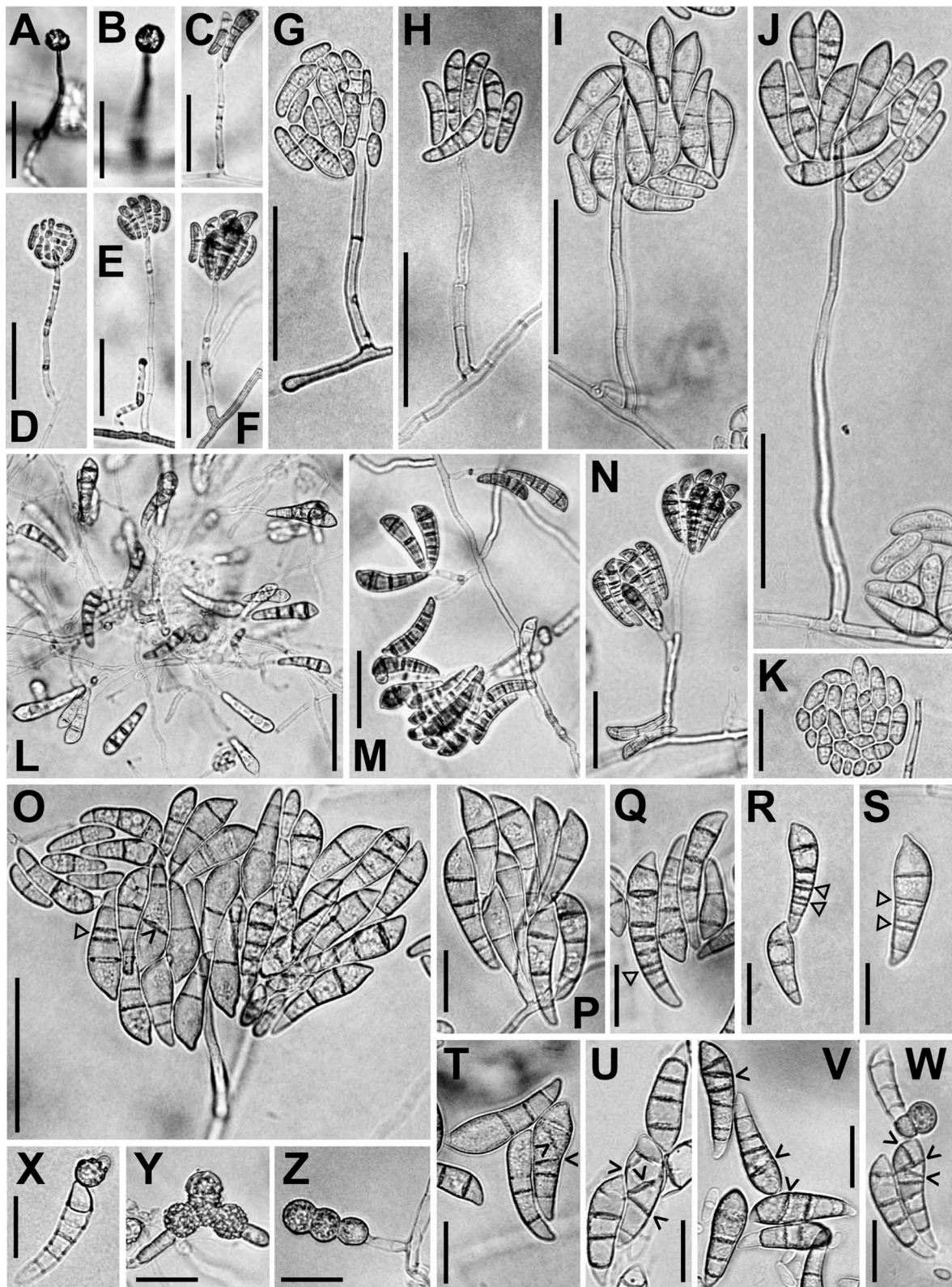
**Figure 9.** *Fusarium obliquiseptatum* cultured on SNA under black light. A–C. Aerial conidiophores forming 0- to 4-septate conidia. D, E. Sporodochial conidiophores forming 1- to multiseptate conidia, some of which possess dense or oblique septa. F–K. Sporodochial conidia. F, G. Apically swollen clavate conidia with transverse septa. H, I. Apically swollen clavate conidia with dense transverse septa. J, K. Apically swollen clavate conidia with oblique septa. L. Chlamydospores formed in hyphae. A–E, G, I, K, L from NRRL 62611 (ex-holotype); F, H, J from NRRL 62610. Bar = 25  $\mu$ m.

crescent-shaped, often swollen in their upper parts and with a papillate apical cell, gradually tapering toward the base, often with an indistinct, but sometimes distinct, foot-like basal cell, or simply rounded at base, swollen conidia often become “dolphin-like” in appearance, (0–)2–5(–7)-septate, mostly transversely septate, but often densely or sometimes obliquely septate, formed abundantly under black light and in the dark on SNA, also sometimes formed abundantly on PDA under black light; 0-septate on SNA under black light: 22.5–37.5  $\times$  5.5–10  $\mu$ m in total range; 1-septate on SNA under black light: 16.5–46.5  $\times$  5–12  $\mu$ m in total range, 22.1–24.9  $\times$  7.4–7.6  $\mu$ m on average (ex-type: 12.5–46.5  $\times$  4.5–12  $\mu$ m in total range, 20.4  $\pm$  4.7  $\times$  6.6  $\pm$  1.3  $\mu$ m on average  $\pm$  SD); 2-septate on SNA under black light: 16–43.5  $\times$  3.5–11.5  $\mu$ m in total range, 24.7–29.5  $\times$  7.0–8.7  $\mu$ m on average (ex-type: 16–43.5  $\times$  3.5–10  $\mu$ m in total range, 24.7  $\pm$  4.6  $\times$  7.0  $\pm$  1.1  $\mu$ m on average  $\pm$  SD); 3-septate on SNA under black light: 18.5–45  $\times$  4.5–12.5  $\mu$ m in total range, 27.5–33.9  $\times$  7.5–9.2  $\mu$ m on average (ex-type: 18.5–44.5  $\times$  4.5–10.5  $\mu$ m in total range, 27.5  $\pm$  5.7  $\times$  7.5  $\pm$  1.1  $\mu$ m on average  $\pm$  SD); 4-septate on SNA under black light: 18.5–53.5  $\times$  6–12  $\mu$ m in total range, 35.4–37.3  $\times$  8.7–9.0  $\mu$ m on average (ex-type: 18.5–53.5  $\times$  6–11.5  $\mu$ m in total range, 35.4  $\pm$  8.2  $\times$  8.7  $\pm$  1.1  $\mu$ m on average  $\pm$  SD); 5-septate on SNA under black light: 23.5–58  $\times$  6.5–12.5  $\mu$ m in total range,

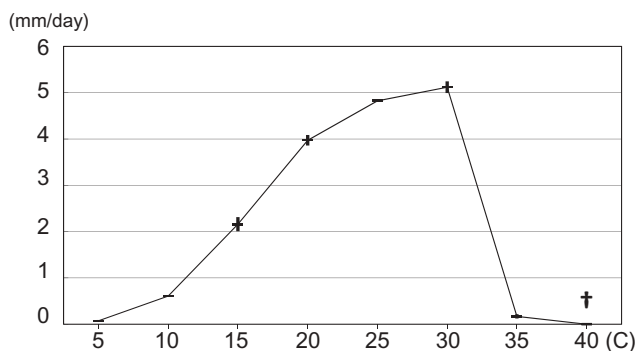
38.7–43.7  $\times$  9.2–9.4  $\mu$ m on average (ex-type: 23.5–58  $\times$  6.5–12  $\mu$ m in total range, 43.7  $\pm$  6.4  $\times$  9.4  $\pm$  1.1  $\mu$ m on average  $\pm$  SD); 6-septate on SNA under black light: 27.5–58  $\times$  7–12  $\mu$ m in total range, 41.1–43.7  $\times$  9.4–9.8  $\mu$ m on average (ex-type: 27.5–58  $\times$  7–12  $\mu$ m in total range, 41.1  $\pm$  6.3  $\times$  9.8  $\pm$  1.0  $\mu$ m on average  $\pm$  SD). 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 from sporodochial conidiophores.

**Additional isolate studied:** NRRL 62610 = MAFF 246844, a single conidial isolate from the crushed head of an ambrosia beetle (*Euwallacea* sp. 3) infesting avocado tree (*Persea americana*), at an avocado orchard, Glasshouse Mountains, Sunshine Coast, Queensland, Australia, Nov 2012, by Andrew D.W. Geering and Paul R. Campbell (No. QLD 1), University of Queensland (a dried culture of NRRL 62610 was also deposited as BPI 910975 for an additional specimen of the species).

**Notes:** This species was reported previously as *Fusarium* sp. (AF-7) within the AFC (Kasson et al. 2013; O'Donnell et al. 2015; Aoki et al. 2018). The most striking morphological feature of this species is the production of clavate septate sporodochial conidia that are swollen apically with a terminal protrusion,



**Figure 10.** *Fusarium obliquiseptatum* cultured on SNA under black light. A–J. Aerial conidiophores forming 0- to 4-septate conidia. K. 0- to 1-septate aerial conidia. L. Sporodochium formed on agar surface. M–O. Sporodochial conidiophores forming 1- to multiseptate conidia, often swollen apically with 1–5 septa, and some oblong to short-clavate conidia with 1–2 septa. P–W. Sporodochial conidia with transverse, oblique, or longitudinal septa (open triangles: dense septation; arrowheads: oblique, sometimes longitudinal septation). W–Z. Chlamydospores formed in conidia and in hyphae. A–F, H, M, N, Q, R, T, U, X–Z from NRRL 62610; G, I–L, O, P, S, V, W from NRRL 62611 (A–F: aerial view without a cover slip; G–Z: mounted in water with a cover slip). Bars: A–J, L–O = 50 µm; K, P–Z = 20 µm.



**Figure 11.** Daily radial mycelial growth rate of *Fusarium obliquiseptatum* 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 and died at 40 C.

frequent dense transverse septa (FIGS. 9D, E, H, I, 10O–S), and occasional oblique septa when cultured on SNA (FIGS. 9D, J, K, 10O, T–W). Oblique septa are often formed in the swollen upper part of conidia, which may appear zigzag-like (FIGS. 9J, K, 10T, V) or cruciate (FIGS. 9J, 10U, W). The optimal temperature for growth was 30 C (FIG. 11). Colony morphology of NRRL 62611 (ex-holotype) on PDA at 25 C is provided in SUPPLEMENTARY FIG. 2G, H, together with that of sporodochia formed in culture (SUPPLEMENTARY FIG. 2K, L).

## DISCUSSION

The primary objective of the present study was to use multilocus molecular phylogenetic and phenotypic data to formally describe three phylogenetic species within the AFC that were previously reported as *Fusarium* species AF-3, AF-5, and AF-7 (Kasson et al. 2013; O'Donnell et al. 2015; Na et al. 2018). Partitioned maximum likelihood (ML)-bootstrapped phylogenetic analyses of a four-gene, 4.3 kb data set strongly supported the recognition of these three phylopecies as *F. floridanum* (AF-3), *F. tuaranense* (AF-5), and *F. obliquiseptatum* (AF-7) based on their genealogical exclusivity (Taylor et al. 2000). These three fusaria are known or predicted to be farmed by exotic Asian *Euwallacea* ambrosia beetles as a source of nutrition. *Fusarium floridanum* was isolated from the mycangia of *Euwallacea interjectus* infesting a boxelder tree (*Acer negundo*) in Gainesville, Florida; *F. tuaranense* was recovered from a Pará rubber tree (*Hevea brasiliensis*) damaged by an unknown ambrosia beetle (presumably *Euwallacea*) in North Borneo, Malaysia (Brayford 1987); and *F. obliquiseptatum* was isolated from a gallery of *Euwallacea* sp. 3 (*E. fornicatus* species

complex) infesting an avocado tree (*Persea americana*) in Queensland, Australia (O'Donnell et al. 2015).

The present study provides the strongest bootstrap support to date for a sister-group relationship of *F. floridanum* and *F. euwallaceae* from Los Angeles and surrounding counties, Israel (Freeman et al. 2013), and South Africa (Paap et al. 2018; Van den Berg et al. 2019). These fusaria are farmed by *E. interjectus* and the polyphagous shot hole borer, *E. fornicatus* (Smith et al. 2019), respectively. The present study also obtained much stronger support for the sister-group status of *F. obliquiseptatum* and *Fusarium* sp. (AF-6) than previously reported (O'Donnell et al. 2015). The former species was isolated from galleries of *Euwallacea* sp. 2 (*E. fornicatus* species complex) in avocado trees in Miami-Dade County, Florida. However, phylogenetic relationships of *F. tuaranense* from Malaysia were unresolved, as were most of the nodes along the backbone of the phylogeny.

In addition to the results presented here, previous multilocus molecular phylogenetic studies have consistently reported strong monophyly bootstrap support for the AFC (Kasson et al. 2013; O'Donnell et al. 2015; Na et al. 2018), which is nested within clade 3 of the *F. solani* species complex (FSSC; O'Donnell 2000). Recently Guarnaccia et al. (2018) erroneously reported that the *Euwallacea*-*Fusarium* mutualism represents “one of the best examples of host-fungus coevolution,” but this baseless conclusion is strongly contradicted by the evolutionary analyses presented in O'Donnell et al. (2015), which indicate that the incongruent phylogenies of these mutualists is consistent with host-shift speciation sensu de Vienne et al. (2013) rather than diversifying coevolution. Furthermore, contrary to the report published by Sandoval-Denis and Crous (2018), in which they falsely claim that the circumscription of *Fusarium* by Geiser et al. (2013; i.e., including the FSSC) is polyphyletic, every comprehensive phylogenetic analysis of this genus published to date has resolved the FSSC as part of a monophyletic *Fusarium* (Gräfenhan et al. 2011; Schroers et al. 2011; Geiser et al. 2013; O'Donnell et al. 2013; Lombard et al. 2015). Because the monophyletic circumscription of *Fusarium* presented in Geiser et al. (2013) preserves long-standing use by avoiding unnecessary name changes of agriculturally and medically important fusaria (de Hoog et al. 2015), it has received near unanimous support from the global *Fusarium* community. Although we anticipate that the three AFC species described herein, and others within the FSSC, will be recombined in *Neocosmospora* (see

Lombard et al. 2015), we urge end users to continue to use the *Fusarium* names and reject the de facto dual nomenclature promoted by Lombard et al. (2015) and Sandoval-Denis and Crous (2018). Because the taxonomic reclassification of members of the FSSC in *Neocosmospora* is based on the faulty premise that *Fusarium* sensu Geiser et al. (2013) is polyphyletic, these taxa will be recombined in *Fusarium* in a forthcoming paper on the circumscription of *Fusarium* supported by comparative phylogenomic data (Stajich et al., in prep.).

Phenotypic/morphological characters were discovered that distinguish the three newly described species from the other members of the AFC (Gadd and Loos 1947; Freeman et al. 2013; Aoki et al. 2018; Na et al. 2018). These include production of sporodochial conidia that are highly variable in size and shape in *F. floridanum*, the smallest sporodochial conidia of any member of the AFC in *F. tuaranense*, and swollen clavate sporodochial conidia with dense and occasional oblique septa in *F. obliquiseptatum*. These three species together with the other species within clade B of the AFC that we have studied produce “dolphin-like” apically swollen, clavate and septate conidia exclusively, or a mixture of clavate and fusiform multiseptate sporodochial conidia (Kasson et al. 2013; Aoki et al. 2018). Even though strains of the four putatively novel AFC species (i.e., AF-13 to AF-16) from Taiwan were not available for study (Na et al. 2018), we predict that they all produce apically swollen “dolphin-like” sporodochial conidia (Aoki et al. 2018) because this character appears to be fixed in the more derived species lineages within the AFC. The transition from production of only fusiform (i.e., *Fusarium* species AF-6 and AF-9) to apically swollen clavate conidia is thought to represent an adaptation for the symbiosis (Kasson et al. 2013). Although an optimal temperature of 30 C distinguishes the three AFC species described herein from fastest growth at 25 C in *F. oligoseptatum* from Pennsylvania and *F. euwallaceae* from the central coastal region of Israel (Freeman et al. 2013), this character appears to be of limited diagnostic value because it is strain dependent in *F. ambrosium* (Aoki et al. 2018).

Prior to 2013, *Fusarium ambrosium* was the only species in this genus known to be farmed by an ambrosia beetle, the tea shot hole borer *E. perbrevis* (classified formerly as *E. fornicatus*; Smith et al. 2019) in Sri Lanka and India (Gadd and Loos 1947; Nirenberg 1990). Due to the significant threat the invasive *Euwallacea-Fusarium* mutualists pose to urban landscapes, naïve forests, and the avocado industry worldwide, extensive pathogen surveys and multilocus molecular phylogenetic analyses conducted over the past 6 years led to the surprising discovery of 15 additional phylopecies within the AFC (Freeman et al. 2013;

O'Donnell et al. 2015; Aoki et al. 2018; Na et al. 2018). Similarly, analyses of a six-gene data set revealed that fusaria are farmed by at least eight different *Euwallacea* species, including six cryptic species within the *E. fornicatus* clade (O'Donnell et al. 2015). Preliminary results show that three of the beetles in this clade produce species-specific mixtures of two volatile ketones (i.e., 2-heneicosanone and 2-tricosanone) that appear to function as pheromones (Cooperband et al. 2017). Further support of the beetle's species status is based on cophylogenetic analyses that revealed the seven *Euwallacea* species available for study all farm different AFC species (O'Donnell et al. 2015). Although recent cytochrome oxidase (*COI*)-based phylogenetic analyses of extensive *Euwallacea* spp. collections from Asia revealed considerably more phylogenetic diversity than reported in O'Donnell et al. (2015), the authors suggested that the *E. fornicatus* clade only comprised three or four species (Stouthamer et al. 2017; Gomez et al. 2018), which we deem overly conservative based on the deep divergences in the *COI* gene trees. The fusarial symbionts of the *Euwallacea* spp. from Asia were not reported in these studies; however, we predict that multiple novel AFC species will be discovered when they are analyzed phylogenetically.

Although the available data suggest that the 12 AFC species reported in O'Donnell et al. (2015) can be distinguished using morphological characters (Aoki, unpubl.), the discovery of additional members of the AFC (Na et al. 2018) and *Euwallacea* farming fusaria in Asia (Stouthamer et al. 2017; Gomez et al. 2018) strongly suggests that DNA data will be required to accurately identify these mutualists. Species-level studies of *Euwallacea* could benefit significantly from more informative marker loci because most of the phylogenetic signal in the six-gene data set came from *COI* and the nuclear large subunit (LSU) rDNA (O'Donnell et al. 2015). The same is true for the AFC because sequence from two of the four loci (i.e., LSU rDNA and *RPB1*) failed to distinguish all of the fusaria that are farmed by *Euwallacea* (Kasson et al. 2013). In addition to facilitating identification of more informative loci, whole-genome sequence data provide a means for developing high-throughput molecular diagnostic assays for the detection, identification, and active surveillance of these invasive and economically destructive mutualists (Short et al. 2017). Although the majority of the fungus-farming *Euwallacea* are not known to cause widespread damage (Hulcr and Stelinski 2017), the likelihood that they might switch symbionts (O'Donnell et al. 2015) offers the real potential of creating more aggressive and economically destructive *Euwallacea-Fusarium* pest-pathogen associations. Therefore, detailed knowledge of their genetic diversity

and pathogenic potential is urgently needed to assist quarantine efforts at international ports of entry to prevent the introduction of additional exotic *Fusarium*-farming *Euwallacea* into the United States and other non-native regions worldwide.

There are nine unnamed species within the AFC clade, including four newly discovered phylopecies from Taiwan. We plan to describe them based on detailed morphological/phylogenetic analyses. In addition, the mating type loci will be analyzed to assess the potential of these fusaria to reproduce sexually.

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