



## **Identification, characterisation, and localisation of hyaline spore-forming endophytic fungi in tissues of *Echinochloa glabrescens* Munro ex Hook. f.**

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### **Abstract**

The information on the identities, characteristics, and tissue-specificity of dark spore-forming endophytic fungi in tissues of *E. glabrescens* had already been known. However, less information is available when it comes to hyaline spore-forming (HSF) ones. Therefore, seeds, leaves, leaf sheaths, and roots of healthy and mature *E. glabrescens* were sampled to determine and describe the identities, characteristics, and tissue locations of HSF endophytic fungi. Of the 147 isolates recovered in the dry season, 60 belonged to *Fusarium*; 55 to *Trichoconiella*; 11 to EF-ds163; eight to EF-ds571; four to EF-ds290; two each to *Aspergillus*, *Hansfordia*, and *Peyronallaea*; and one each to *Colletotrichum*, *Geotrichum*, and *Mucor*. Of the 415 isolates recovered in the wet season, 196 belonging to *Trichoconiella*, 115 to *Hansfordia*, 67 to *Colletotrichum*, 20 to *Fusarium*, six to *Penicillium*, five to *Paecilomyces*, three to *Aspergillus*, and one each to *Geotrichum*, *Humicola*, and *Mucor*. *Fusarium* sp. ds529 and *Hansfordia* sp. ds143 were the most dominant in the seeds. *Trichoconiella* sp. ds333, *Hansfordia* sp. ds143, and *Colletotrichum* sp. ds361 are dominant in the upper leaves; *Trichoconiella* sp. ds333 and *Hansfordia* sp. ds143 in the lower leaves; and *Trichoconiella* sp. ds333 in the leaf sheaths and roots. This study confirmed that HSF endophytic fungi also reside in different tissues of *E. glabrescens*.

**Keywords** – barnyard grass – fungal diversity – summed-dominance ratio

### **Introduction**

Endophytic fungi are organisms that are capable of symptomless occupation in healthy plant tissues, although some of them, after incubation, may cause disease in their hosts (Petrini 1991, Stone et al. 2004). Numerous works have already shown their potential uses, particularly in the agriculture and medical sectors. Hence, explorations about their ecological role, beneficial uses for human and animal health, growth promotions, drought tolerance, and protection of crops against pests are widely increasing around the world (Arnold et al. 2003, Evans 2003, Tian et al. 2004, Kuldau & Bacon 2008, Ting et al. 2008, Ahmad et al. 2010, Bungihan et al. 2013, Hipol 2012, Zainudin et al. 2021).

*Echinochloa glabrescens* Munro ex Hook. f. is an annual C<sub>4</sub> plant that belongs to the family Poaceae (Graminae). It is an erect plant with a stout stand, erect to decumbent stem, acuminate leaves and glabrous leaf sheaths, narrow pyramidal panicles with numerous green spikes, and 1 to 3 cm long awned spikelets (Moody et al. 2014). It is differentiated from *E. colona* (L.) Link. based on the shapes of lemmas and glumes and the presence of awns; *Echinochloa crus-galli* (L.) Beauv. and *E. hispidula* (Retz.) Honda, on the other hand, differed based on the shapes and types of inflorescences (Pancho & Obien 1995).

*Echinochloa glabrescens* is one of the common weeds of irrigated lowland rice in the Philippines (Donayre et al. 2018). It possesses competitive characteristics which, if not properly controlled, could drastically reduce the yield of rice. For example, allowing its younger seedlings to grow and compete at a 100% level of infestation after planting rice resulted in a 94% grain yield reduction on wet direct-seeded rice, and at 40-100% level of infestations resulted in 73–100% grain yield reductions on transplanted rice (Rao & Moody 1992). In another study, *E. glabrescens* reduced 29% of the grain yield of transplanted rice at a 29% level of infestation over the total number of hills per square meter of the crop (Rao & Moody 1987). It is also an alternative host for different rice pests like the green leafhopper [*Nephotettix virescens* (Distant)], rice black bug [*Scotinophara latiuscula* (Breddin)], invasive apple snail (*Pomacea canaliculata* Lam.), and diseases such as ragged stunt caused by rice ragged stunt virus and tungro by rice tungro bacilliform virus (RTBV) and rice tungro spherical virus as the causal organisms (Salamat et al. 1987, Khan et al. 1991, Joshi et al. 2006, Litsinger 2007).

Despite the undesirable impacts on rice production, it has also been reported to harbor beneficial organisms like endophytic fungi (Donayre et al. 2014). Previous reports showed that tissues of *E. glabrescens* also harbored dark-spore-forming endophytic fungi, mostly belonging to Family Dematiaceae and formerly to the Class Deuteromycetes (Donayre & Dalisay 2016). However, only a few literature reported the hyaline spore-forming (HSF) endophytic fungi from tissues of *E. glabrescens*. Thus, this paper aimed to determine and describe the identities, characteristics, and locations of HSF endophytic fungi in tissues of *E. glabrescens*.

## Materials & Methods

### Collection of the weed

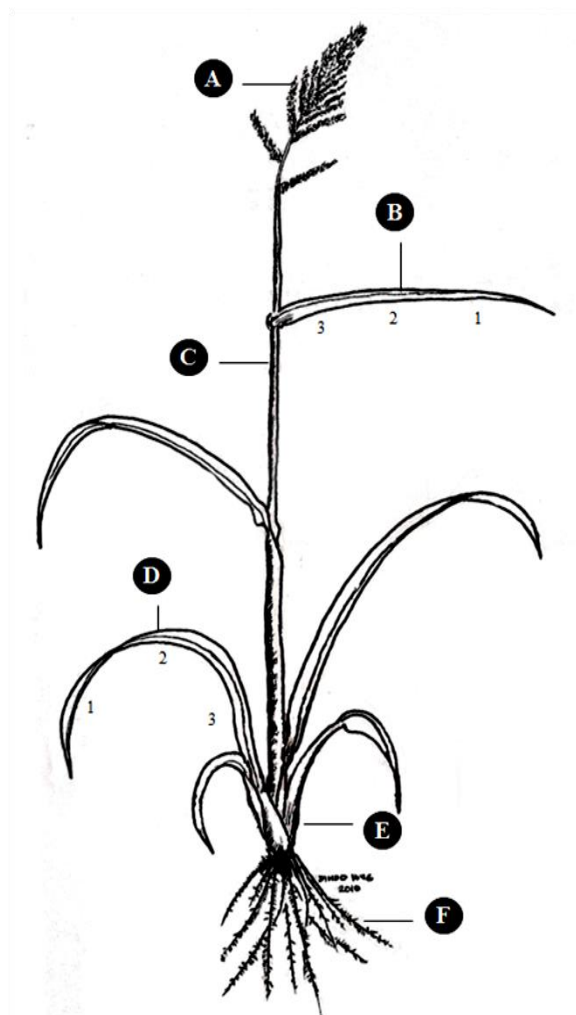
The hyaline spore-forming (HSF) endophytic fungi described in this study were obtained from the previous work of Donayre et al. (2014) with the isolation technique presented in the works by Donayre & Dalisay (2015, 2016). Healthy and mature *E. glabrescens* were collected in lowland rice fields in 16 municipalities of Nueva Ecija, Philippines. The first collection was conducted in March 2010 (coinciding with the dry season), while the second was in October (the wet season). The entire month of March had 2 mm total rainfall, 23.1 °C mean temperature (min = 28.8 °C, max = 34.5 °C), and 87% relative humidity, while October had 297.4 mm total rainfall, 24 °C mean temperature (min = 28.2 °C, max = 32.3 °C), and 79% relative humidity. In each rice field, three plants were carefully pulled out equidistantly in a diagonal pattern, placed separately inside plastic bags, and brought into the Plant Pathology Laboratory of the Crop Protection Division, Philippine Rice Research Institute Central Experiment Station. The coordinates of the collection sites where *E. glabrescens* was collected were marked using a GPS tool (Garmin: Etrex Summit) in order to identify the same ricefield for the second collection. The sampling point of each field was >5 km and <80 km apart by radius.

### Preparation of media

Potato dextrose agar (PDA) and malt extract agar (MEA) were prepared by mixing 39 g and 33 g, respectively, in a liter of distilled water. Each culture medium was transferred separately to storage bottles (500 ml capacity), heated in the microwave for 15 min, and sterilized inside the autoclave for 15 min at 121 °C.

### Isolation of endophytic fungi

Fresh and symptomless seeds, leaves, leaf sheaths, and roots of *E. glabrescens* were used for the isolation of HSF endophytic fungi (Fig. 1). Leaves and leaf sheaths were divided as upper part (younger, first order from the apex of each plant) and lower part (older, 2<sup>nd</sup> order from the base of each plant). The top, middle, and bottom leaf blades, as well as the midribs of each leaf, were also sampled for isolation. Portions of each sample tissue were cut into 2 mm × 2 mm sections in a “Z” pattern (Gamboa et al. 2002). For roots, 2-mm segments were randomly cut from each plant sample. All cut sections were surface sterilized by immersing in 95% ethanol for 30 sec, 10% NaOCl for 5 min, and 95% ethanol for 30 sec (Schultz et al. 1993, Stone et al. 2004). Cut sections were then rinsed three times in sterile distilled water and blotted-dry inside a folded, clean, sterilized tissue paper. Five sections of each sample tissue were immediately planted inside a petri plate with MEA. The plates were incubated inverted for 5 to 10 days at 25°C room temperature.



**Fig. 1** – Line drawing of *Echinochloa glabrescens* showing its different parts that were sampled for isolation of hyaline spore-forming endophytic fungi. A Seeds. B Upper leaf including the leaf blade and leaf midrib. C Upper leaf sheath. D Lower leaf including the leaf blade and leaf midrib. E Lower leaf sheath. F Roots. 1 Top. 2 Middle. 3. Bottom.

### Identities and morphological characteristics

Mycelial growth in MEA was excised using a sterile 5-mm cork borer. One disc of each isolate was transferred and plated on another petri plate with PDA. Petri plates were then incubated for 7 to 30 days at room temperature. In each plate, the mycelial growth of the respective isolate was observed, measured, and recorded daily. To identify and further characterize the assemblage, the slide mount technique through the agar block was done for each isolate (Dhingra & Sinclair

1995). When fruiting structures were ready for microscopic examination, 7 to 50 conidia and conidiophores were randomly selected for capturing and measuring using a camera-aided microscope (Olympus DP72-BSW). Captured images of each isolate were then compared to images reported in previous works. Each isolate was identified up to the genus level only through morphological characteristics captured in the camera-aided microscope.

### Tissue specificity

The abundance of HSF endophytic fungi was determined by calculating the absolute number of times a species was isolated in each tissue of the three sampled *E. glabrescens* plants. Their tissue specificities and dominances were determined by calculating the summed-dominance ratio (SDR) following the formula of Moody (1995), as shown below:

$$\text{SDR of a species} = \frac{\text{Relative density} + \text{Relative frequency} + \text{Relative dominance}}{3} [1]$$

where:

$$\text{Relative density of a species} = \frac{\text{Absolute number of a species in all sampled plant}}{\text{Total absolute number of all species in all sampled plant}}$$

$$\text{Relative frequency of a species} = \frac{\text{Absolute frequency of a species}}{\text{Total of all absolute frequencies of all species}}$$

$$\text{Frequency abundance of a species} = \text{Absolute frequency} \times \text{Average abundance}$$

## Results

### Identities and morphological characteristics

A total of 147 and 415 hyaline-spore-forming (HSF) endophytic fungal isolates were recovered in different tissues of *E. glabrescens* during the dry and wet seasons, respectively. Of the 147 isolates in the dry season, 60 of these belonged to *Fusarium*; 55 to *Trichoconiella*; 11 to EF-ds163; eight to EF-ds571; four to EF-ds290; two each to *Aspergillus*, *Hansfordia*, and *Peyronallaea*; and one each to *Colletotrichum*, *Geotrichum*, and *Mucor*. Of the 415 isolates in the wet season, on the other hand, 196 belonged to *Trichoconiella*; 115 to *Hansfordia*; 67 to *Colletotrichum*; 20 to *Fusarium*; six each to *Penicillium* and *Mucor*; three to *Aspergillus*; and one each to *Geotrichum*, *Humicola*, and *Mucor*. Based on morphological and cultural characteristics, the genus *Fusarium* had eight, *Aspergillus* had four, and *Penicillium* had two morpho species. The identities, characteristics, and assemblages of all HSF endophytic fungi of *E. glabrescens* were described and shown below:

#### *Aspergillus* sp. ds153

Descriptions: conidiophores pale green, geniculate, occasionally straight, simple; conidia pale green, globose, smooth, catenulate, a chain composed of 50–60 conidia; phialides pale green, uniseriate; conidial heads dark green, clavate; vesicles pale green (Fig. 2A). Dimensions: conidiophores 75.23–151.23 × 2.06–4.38 μm ( $\bar{x}$  = 100.23 × 3.21 μm, n = 50), conidia 3.0–3.96 × 2.22–3.92 μm ( $\bar{x}$  = 3.48 × 2.94 μm, n = 50), phialides 6.23 × 9.98 μm tall ( $\bar{x}$  = 3.21 μm, n = 20), conidial heads 42.33–88.48 × 9.35–18.88 μm ( $\bar{x}$  = 60.39 × 12.76 μm, n = 20), vesicles 1.56–3.13 × 1.79–3.71 μm ( $\bar{x}$  = 2.53 × 2.45 μm, n = 20). References used for morphological identification: Rayner (1970), Pitt & Hocking (2009), Watanabe (2010), Santiago et al. (2011).

Cultural characteristics in PDA: colony growth 80mm (diameter) after nine days of incubation, irregular and spreading, thin, pale mouse to mouse grey (obverse and reverse).

Source of tissue and plant material: the top part of the leaf blade, upper leaf; Poblacion, Rizal, Nueva Ecija, Philippines (N 15° 42' 03.8", E 121° 05' 27.5").

### ***Aspergillus* sp. ws840**

Descriptions: conidiophores pale brown, erect, simple, bearing radiate conidial heads; conidia brown, globose, echinulate, catenulate, 1 chain composed of 20-25 conidia; phialides uniseriate; conidial heads hyaline; and vesicles sub-globose (Fig. 2B). Dimensions: conidiophores  $6.4\text{--}30.94 \times 0.36\text{--}1.45 \mu\text{m}$  ( $\bar{x} = 18.87 \times 0.91 \mu\text{m}$ ,  $n = 27$ ); conidia  $0.48\text{--}1.92 \times 1.11\text{--}1.79 \mu\text{m}$  ( $\bar{x} = 1.42 \times 1.52 \mu\text{m}$ ,  $n = 50$ ); phialides  $2.5 \times 3.17 \mu\text{m}$  tall ( $\bar{x} = 2.82 \mu\text{m}$ ,  $n = 5$ ); conidial heads  $4.98\text{--}11.01 \times 5.35\text{--}10.17 \mu\text{m}$  ( $\bar{x} = 7.72 \times 6.96 \mu\text{m}$ ,  $n = 12$ ); vesicles  $0.81\text{--}2.28 \times 0.66\text{--}1.50 \mu\text{m}$  ( $\bar{x} = 1.57 \times 1.05 \mu\text{m}$ ,  $n = 12$ ). References used for morphological identification: Rayner (1970), Pitt & Hocking (2009), Watanabe (2010), Santiago et al. (2011).

Cultural characteristics in PDA: colony growth 85mm (diameter) after four days of incubation, medium dense, irregular, powdery in texture, dense, without zonation, greyish septa in color (obverse and reverse).

Source of tissue and plant material: middle part of the leaf blade, upper leaf; Carmen, Zaragosa, Nueva Ecija, Philippines (N  $15^{\circ} 27' 13.4''$ , E  $120^{\circ} 49' 49.3''$ ).

### ***Aspergillus* sp. ds397**

Descriptions: conidiophores green, bent, bearing nearly radiating conidial heads; conidia pale green, smooth, sub-globose, catenate, 1 chain composed of 20-40 conidia; conidial heads nearly radiating; and vesicles sub-globose (Fig. 2C). Dimensions: conidiophores  $14.36\text{--}46.20 \times 0.7\text{--}1.35 \mu\text{m}$  ( $\bar{x} = 26.54 \times 0.94 \mu\text{m}$ ,  $n = 25$ ); conidia pale green  $0.55\text{--}1.30 \times 0.46\text{--}1.00 \mu\text{m}$  ( $\bar{x} = 0.88 \times 0.76 \mu\text{m}$ ,  $n = 50$ ); conidial heads  $8.72\text{--}12.66 \times 3.37\text{--}13.53 \mu\text{m}$  ( $\bar{x} = 11.15 \times 9.34 \mu\text{m}$ ,  $n = 25$ ); vesicles  $2.08\text{--}4.48 \times 2.01\text{--}4.00 \mu\text{m}$  ( $\bar{x} = 2.91 \times 3.21 \mu\text{m}$ ,  $n = 25$ ). References used for morphological identification: Rayner (1970), Pitt & Hocking (2009), Watanabe (2010), Santiago et al. (2011).

Cultural characteristics in PDA: colony growth was 47.5mm (diameter) after nine days of incubation; dense, irregular, raised margin, concentric, glaucous blue-green in the center with white margin (obverse); entirely white in reverse position.

Source of tissue and plant material: seed; Sanggalang, Jaen, Nueva Ecija, Philippines (N  $15^{\circ} 23' 06.9''$ , E  $120^{\circ} 50' 29.3''$ ).

### ***Aspergillus* sp. ws960**

Descriptions: conidiophores hyaline, erect, occasionally bent, bearing sub-globose conidial; conidia pale green, sub-globose, catenate (1 chain composed of 15-20 conidia); conidial heads and vesicles sub-globose (Fig. 2D). Dimensions: conidiophores  $13.01\text{--}17.48 \times 0.59\text{--}1.06 \mu\text{m}$  ( $\bar{x} = 20.02 \times 0.81 \mu\text{m}$ ,  $n = 25$ ); conidia  $0.51\text{--}1.06 \times 0.58\text{--}0.92 \mu\text{m}$  ( $\bar{x} = 0.76 \times 0.71 \mu\text{m}$ ,  $n = 50$ ); conidial heads; and vesicles  $2.76\text{--}5.07 \times 3.05\text{--}5.44 \mu\text{m}$  ( $\bar{x} = 4.21 \times 4.20 \mu\text{m}$ ,  $n = 25$ ). References used for morphological identification: Rayner (1970), Pitt & Hocking (2009), Watanabe (2010), Santiago et al. (2011).

Cultural characteristics in PDA: colony growth 29mm (diameter) after seven days of incubation, irregular, primrose zonation color from the center with white margin (obverse); and ochreous (reverse).

Source of tissue and plant material: the top part of the leaf blade, upper leaf; Sta Rosa, Nueva Ecija (N  $15^{\circ} 26' 03.7''$  E  $120^{\circ} 53' 54.6''$ ).

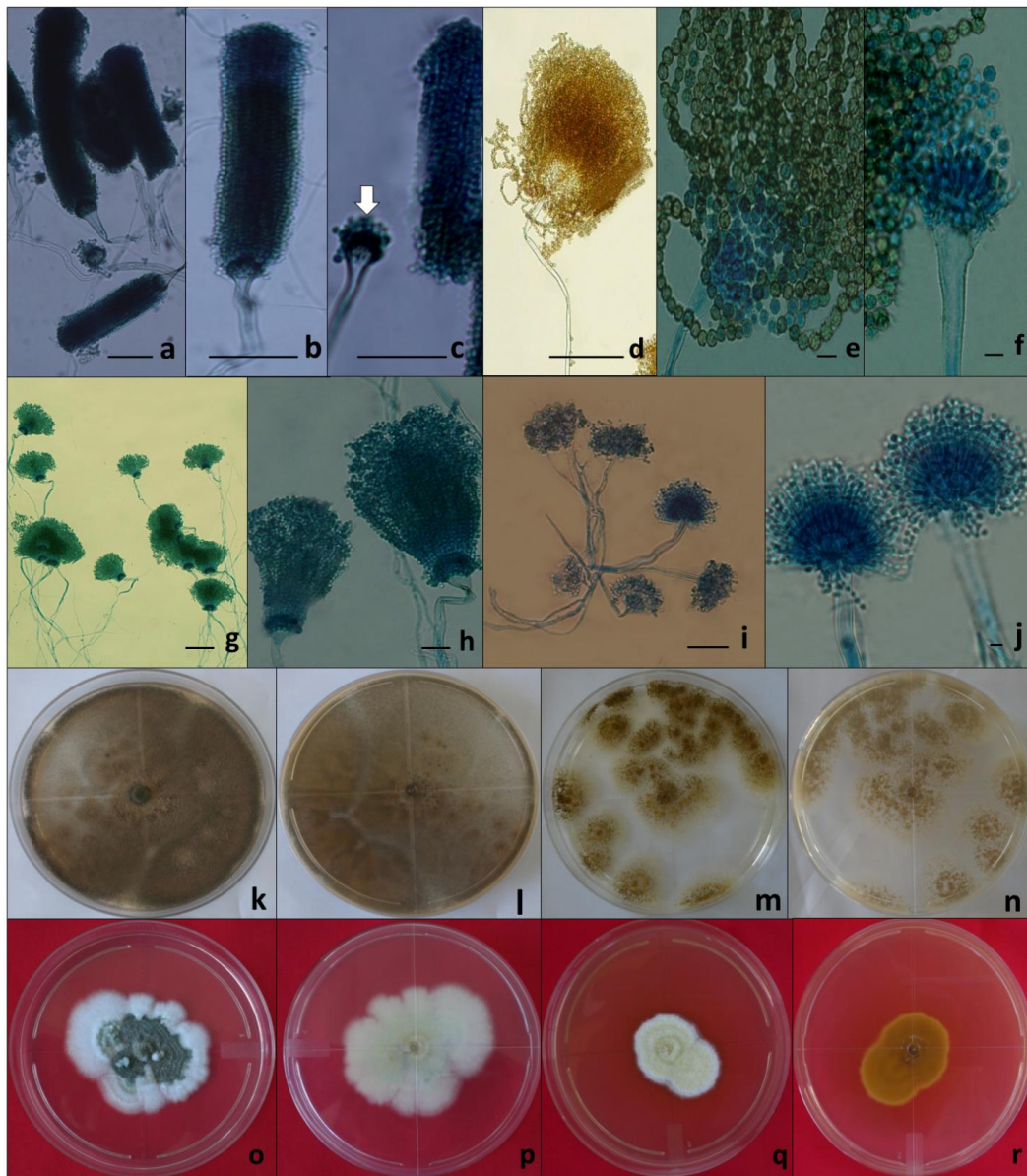
### ***Colletotrichum* sp. ds361**

Descriptions: conidiophores hyaline, simple, erect, bearing a conidium; conidia hyaline, 1-celled, cylindrical; and setae brown, tapering toward apexes (Fig. 3). Dimensions: conidiophores  $0.95\text{--}5.47 \times 0.29\text{--}1.13 \mu\text{m}$  ( $\bar{x} = 2.69\text{--}0.68 \mu\text{m}$ ;  $n = 20$ ), conidia  $33.40\text{--}46.13 \times 8.40\text{--}13.02 \mu\text{m}$  ( $\bar{x} = 38.57 \times 10.72 \mu\text{m}$ ;  $n = 50$ ), setae brown  $8.55\text{--}18.96 \times 0.24\text{--}0.55 \mu\text{m}$  ( $\bar{x} = 13.10 \times 0.44 \mu\text{m}$ ;  $n = 8$ ). References used for morphological identification: Watanabe (2010), Rayner (1970).

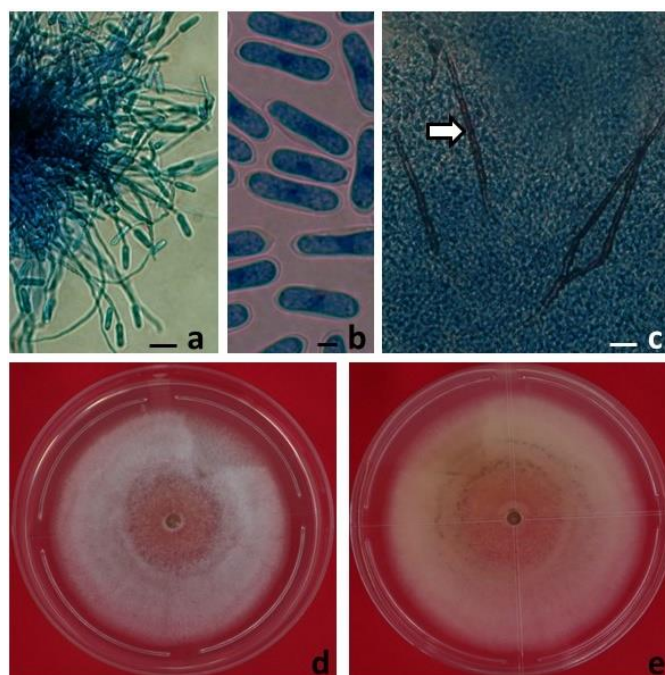


Cultural characteristics in PDA: colony growth 72 mm (diameter) after six days of incubation, dense, smooth, round, raised, white to lavender grey (obverse and reverse).

Source of tissue and plant material: top part of the leaf midrib, lower leaf; Bertici, Quezon, Nueva Ecija, Philippines (N 15° 34' 26.1", E 120° 49' 24.2").



**Fig. 2** – Assemblages of *Aspergillus* species. *Aspergillus* sp. ds153. a–b Conidiophores bearing long chains of conidia. c Dislodge spores exposing the vesicle. k–l Front and reverse view of the colony after nine days in PDA; *Aspergillus* sp. ws840. d–e Conidiophores bearing brush-like masses of conidia, f Vesicle bearing phialides and conidia, m–n Front and reverse view of the colony after four days in PDA; *Aspergillus* sp. ds397. g–h Conidiophores bearing long chains of conidia. o–p Front and reverse view of the colony after nine days in PDA; *Aspergillus* sp. ws960. i Cluster of conidiophores. j Close-up of conidiophores with phialides and conidia. q–r Front and reverse view of the colony after nine days in PDA. Scale bars: a–c = 1  $\mu$ m, d, h, i = 10  $\mu$ m, e–f = 3  $\mu$ m, g = 50  $\mu$ m, j = 2  $\mu$ m.



**Fig. 3** – Assemblages of *Colletotrichum* sp. ds361. a Conidiophores bearing conidia. b Mature conidia. c Dark brown setae. d–e Front and reverse view of the colony after six days in PDA. Scale bars: a = 30  $\mu$ m, b = 10  $\mu$ m, c = 5  $\mu$ m.

#### ***Fusarium* sp. ds529**

Descriptions: conidiophores hyaline, simple, erect, bearing one conidium; conidia lunate, more curved dorsally (upper) than ventrally (lower) (Fig. 4A). Dimensions: conidiophores 19.33–198.78  $\times$  4.62–5.89  $\mu$ m ( $\bar{x}$  = 121.75  $\times$  5.36  $\mu$ m; n = 50); conidia 41.02–79.44  $\times$  3.65–8.59  $\mu$ m ( $\bar{x}$  = 58.40  $\times$  5.76  $\mu$ m; n = 50). References used for morphological identification: Rayner (1970), Leslie & Summerell (2006).

Cultural characteristics in PDA: 59.5 mm (diameter) after 12 days of incubation, dense, wavy, white with concentric saffron (obverse and reverse).

Source of tissue and plant material: the top part of the leaf midrib, lower leaf; Sta Rosa, Nueva Ecija (N 15<sup>o</sup> 26' 03.7" E 120<sup>o</sup> 53' 54.6").

#### ***Fusarium* sp. ds498**

Descriptions: conidiophores hyaline, short; conidia lunate, short, 2–4-celled, dorsal more curved, apically hooked-shaped, basally barely notched shaped (Fig. 4B). Dimensions: conidiophores 23.69–67.32  $\times$  7.08–8.89  $\mu$ m ( $\bar{x}$  = 47.22  $\times$  7.76  $\mu$ m; n = 50); conidia 33.64–69.37  $\times$  4.16–9.16  $\mu$ m ( $\bar{x}$  = 49.76  $\times$  6.59  $\mu$ m; n = 50). References used for morphological identification: Rayner (1970), Leslie & Summerell (2006).

Cultural characteristics in PDA: 77 mm (diameter) after six days of incubation, medium dense, smooth, concentric, raised, white (obverse and reverse).

Source of tissue and plant material: seed; Sta Rosa, Nueva Ecija (N 15<sup>o</sup> 26' 03.7" E 120<sup>o</sup> 53' 54.6").

#### ***Fusarium* sp. ds111**

Descriptions: conidia lunate, elongated, 2–4-celled, dorsally curved, apically tapering, basally foot-shaped (Fig. 4C). Dimensions: conidiophores 64.34–222.08  $\times$  6.42–10.33  $\mu$ m ( $\bar{x}$  = 117.79  $\times$  7.78  $\mu$ m; n = 50); conidia 26.03–52.98  $\times$  3.87–8.40  $\mu$ m ( $\bar{x}$  = 41.16  $\times$  6.11  $\mu$ m; n = 50). References used for morphological identification: Rayner (1970), Leslie & Summerell (2006).

Cultural characteristics in PDA: 48 mm (diameter) after 13 days of incubation, medium dense, smooth, round with raised margin, umbonate, white to salmon with lavender grey margin (obverse), fuscous black at the center with salmon to lavender grey margin (reverse).

Source of tissue and plant material: the top part of the leaf blade, upper leaf; Sanggalang, Jaen, Nueva Ecija, Philippines (N 15° 23' 06.9", E 120° 50' 29.3").

#### ***Fusarium* sp. ds556**

Descriptions: microconidia straight, 1-celled, ovoid; macroconidia filiform, 2–4-celled, straight, occasionally curved, in between cells separated by thick septa, apically blunt, basal barely notched (Fig. 4D). Dimensions: microconidia 13.74–21.98 × 2.06–6.35 μm ( $\bar{x}$  = 15.84 × 3.73 μm; n = 50); macroconidia 43.09–80.90 × 3.61–7.67 μm ( $\bar{x}$  = 53.91 × 5.37 μm; n = 10). References used for morphological identification: Rayner (1970), Leslie & Summerell (2006).

Cultural characteristics in PDA: 43.5 mm (diameter) after six days of incubation, thin, irregular, flat, orange with luteous margin (obverse and reverse).

Source of tissue and plant material: middle part of the leaf blade, lower leaf; Sta Rosa, Nueva Ecija (N 15° 26' 03.7" E 120° 53' 54.6").

#### ***Fusarium* sp. ds519**

Descriptions: conidiophore branched, smooth, bearing 2–6 conidia; macroconidia lunate, short, slightly curved dorsally, 2–4-celled, apically hooked shaped, basally barely notched (Fig. 4E). Dimensions: conidiophore 34.40–204.55 × 5.0–7.24 μm ( $\bar{x}$  = 101.23 × 6.06 μm; n = 50); macroconidia 18.90–69.69 × 3.11–10.11 μm ( $\bar{x}$  = 39.54 × 5.21 μm; n = 50). References used for morphological identification: Rayner (1970), Leslie & Summerell (2006).

Cultural characteristics in PDA: 44.5 mm (diameter) after three days of incubation, medium dense, smooth, irregular, raised, pale greenish grey (obverse), dark brick (reverse).

Source of tissue and plant material: the bottom part of the leaf blade, upper leaf; Sanggalang, Jaen, Nueva Ecija, Philippines (N 15° 23' 06.9", E 120° 50' 29.3").

#### ***Fusarium* sp. ds516**

Descriptions: macroconidia falcate, largely elongated, dorsal more curved than the ventral, apically tapering, basally foot shaped (Fig. 4F). Dimensions: macroconidia 33.63–103.29 × 4.13–10.18 μm ( $\bar{x}$  = 72.65 × 6.29 μm; n = 50). References used for morphological identification: Rayner (1970), Leslie & Summerell (2006).

Cultural characteristics in PDA: dense, wavy, round with radiating margin, raised, white (obverse and reverse).

Source of tissue and plant material: middle part of the midrib, upper leaf; Sta Rosa, Nueva Ecija (N 15° 26' 03.7" E 120° 53' 54.6").

#### ***Fusarium* sp. ds172**

Descriptions: conidiophore branched, smooth, 2–4 conidia; macroconidia lunate, dorsally curved, 2–4-celled, apically tapering, basally foot-shaped (Fig. 4G). Dimensions: conidiophore 19.71–262.07 × 5.11–7.98 μm ( $\bar{x}$  = 116.24 × 6.27 μm; n = 10); macroconidia 30.02–106.95 × 2.58–9.60 μm ( $\bar{x}$  = 73.41 × 6.82 μm; n = 50). References used for morphological identification: Rayner (1970), Leslie & Summerell (2006).

Cultural characteristics in PDA: 51 mm (diameter) after three days of incubation, dense, irregular, white with concentric saffron (obverse and reverse).

Source of tissue and plant material: lower leaf sheath; Poblacion, Rizal, Nueva Ecija, Philippines (N 15° 42' 03.8", E 121° 05' 27.5").

#### ***Fusarium* sp. ds142**

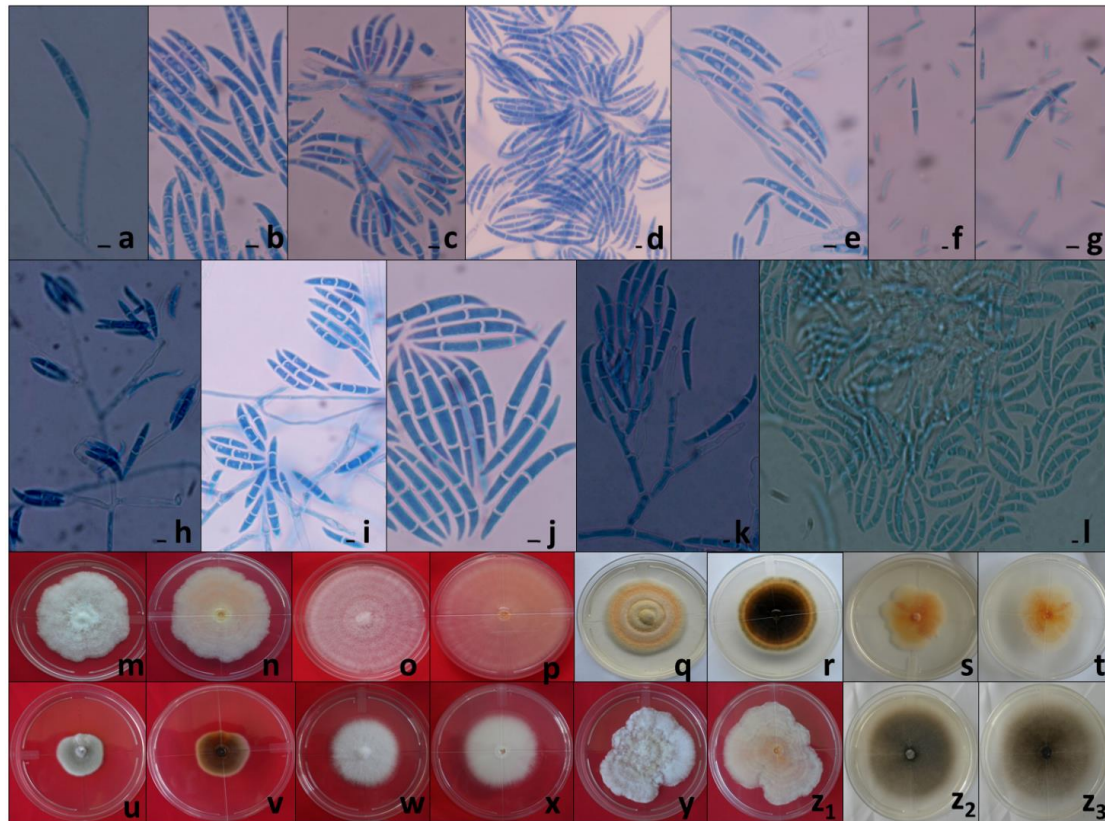
Descriptions: macroconidia lunate, short, dorsal and ventral equally curved, 2–4-celled, apically hooked shaped, basally foot shaped (Fig. 4H). Dimensions: macroconidia 21.42–58.68 ×



4.38–10.64  $\mu\text{m}$  ( $\bar{x} = 44.81 \times 7.24 \mu\text{m}$ ;  $n = 50$ ). References used for morphological identification: Rayner (1970), Leslie & Summerell (2006).

Cultural characteristics in PDA: 61.5 mm (diameter) after nine days of incubation, medium dense, round with radiating margin, flat, mouse grey (obverse), pale mouse grey (reverse).

Source of tissue and plant material: root; Pinili, San Jose, Nueva Ecija (N 15° 45' 22.3" E 121° 01' 10.1").



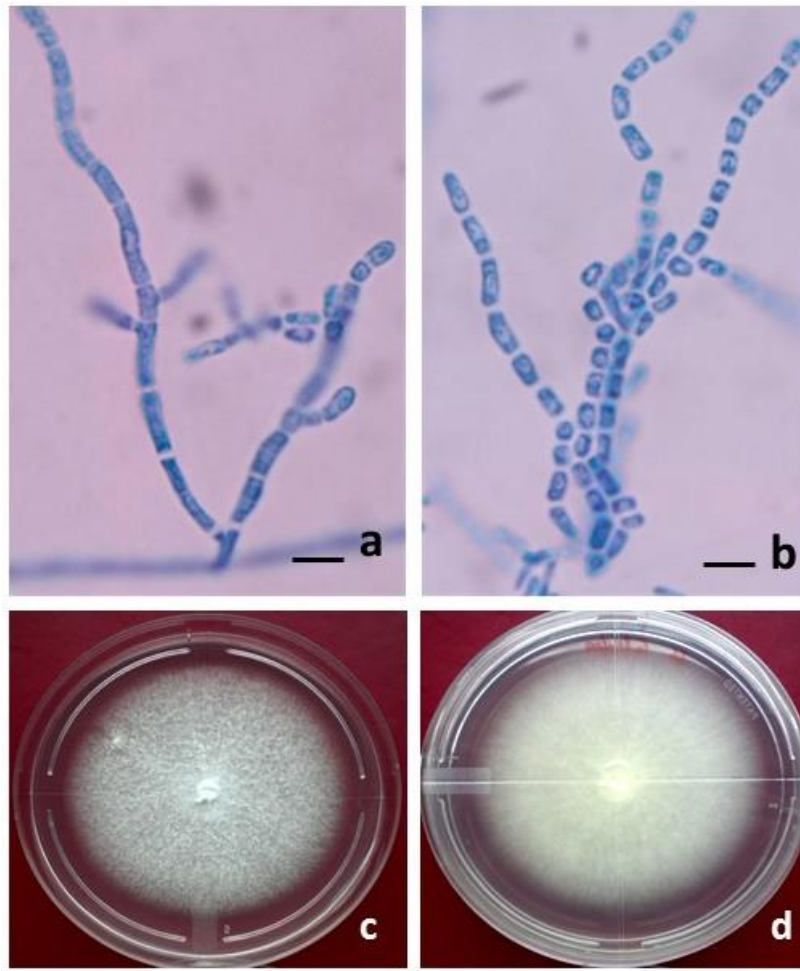
**Fig. 4** – Assemblages of *Fusarium* species. *Fusarium* sp. ds529. a–b Conidiophores and conidia. m–n Front and reverse view of the colony after 12 days in PDA; *Fusarium* sp. ds498. c Conidiophore and conidia. o–p Front and reverse view of the colony after 12 days in PDA; *Fusarium* sp. ds111. d–e Multiseptate and apically-curved conidia. q–r Front and reverse view of the colony after 13 days in PDA; *Fusarium* sp. ds556. f–g Macro and micro-conidia. s–t Front and reverse view of the colony after six days in PDA; *Fusarium* sp. ds519. h–i Branched and long phialides bearing conidia; u–v Front and reverse view of the colony after six days in PDA; *Fusarium* sp. ds516. j Multiseptate conidia with curved tips. w–x Front and reverse view of the colony after nine days in PDA; *Fusarium* sp. ds172. k Phialides tapering towards the tip. y–z<sub>1</sub> Front and reverse view of the colony after nine days in PDA; *Fusarium* sp. ds142: l Multiseptate and fusoid conidia, z<sub>2</sub>–z<sub>3</sub> Front and reverse view of the colony after nine days in PDA. Scale bars: a–b, g, l = 5  $\mu\text{m}$ , c, e, f, h–i, j = 10  $\mu\text{m}$ , d, k = 20  $\mu\text{m}$ .

#### ***Geotrichum* sp. ds104-16**

Descriptions: conidiophores and chlamydoconidia absent; arthroconidia hyaline, barrel-shaped, catenate (Fig. 5). Dimensions: arthroconidia 0.48–1.99  $\times$  0.39–1.0  $\mu\text{m}$  ( $\bar{x} = 1.12 \times 0.62 \mu\text{m}$ ,  $n = 50$ ). References used for morphological identification: Rayner (1970), Pitt & Hocking (2009), Watanabe (2010).

Cultural characteristics in PDA: colony growth 69 mm (diameter) after three days of incubation; medium dense, smooth, L-form, flat, white (obverse and reverse).

Source of tissue and plant material: the bottom part of the leaf blade, upper leaf; Campus, Talavera, Nueva Ecija (N 15° 36' 41.5" E 120° 55' 25.9").



**Fig. 5** – Assemblages of *Geotrichum* sp. ds104-16. a–b Thalloconidia. c–d Front and reverse view of the colony after three days in PDA. Scale bars: a–b = 2  $\mu$ m.

***Hansfordia* sp. ds143**

Descriptions: conidiophores hyaline, erect, rugose, surface, occasionally smooth, branched apically, bearing 6 to 10 conidia terminally and laterally at the apical parts of the branches; conidia hyaline, 1-celled, ovate to cylindrical (Fig. 6). Dimensions: conidiophores 1.48–9.86  $\times$  0.30–1.10  $\mu$ m ( $\bar{x}$  = 3.99  $\times$  0.71  $\mu$ m, n = 28), conidia 0.61–1.57  $\times$  0.31–0.94  $\mu$ m ( $\bar{x}$  = 1.12  $\times$  0.61  $\mu$ m, n = 50). References used for morphological identification: Barron (1968), Rayner (1970), Gene et al. (2000), Pitt & Hocking (2009), Watanabe (2010), Cheng et al. (2011).

Cultural characteristics in PDA: colony growth 41 mm (diameter) after three days of incubation; medium dense, wavy, convex, white to lavender grey (obverse and reverse).

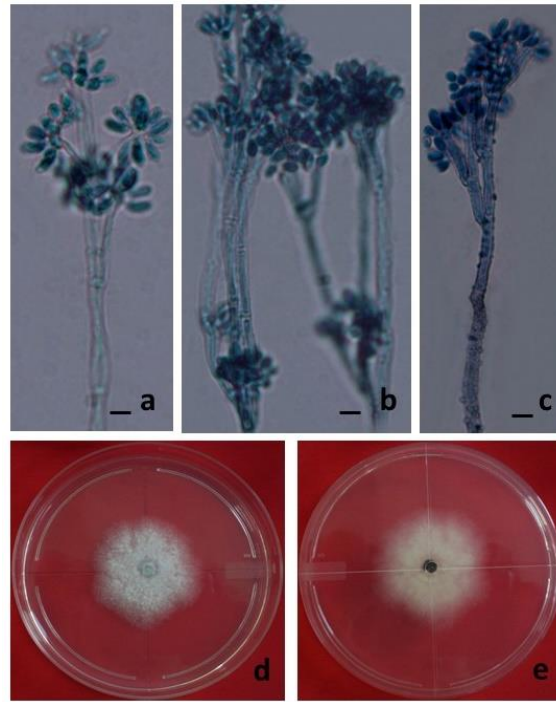
Source of tissue and plant material: the top part of the midrib, upper leaf; Sanggalang, Jaen, Nueva Ecija, Philippine (N 15<sup>0</sup> 23' 06.9", E 120<sup>0</sup> 50' 29.3").

***Humicola* sp. ws891**

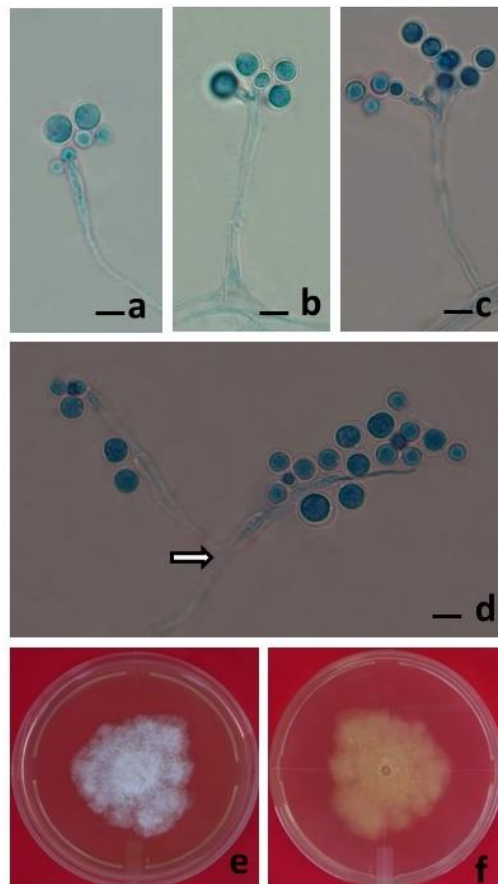
Descriptions: conidiophores hyaline, branched, smooth bearing 4 to 17 conidia apically and laterally; conidia hyaline, spherical, thick-walled (Fig. 7). Dimensions: conidia 0.91–2.52  $\times$  0.45–2.24  $\mu$ m ( $\bar{x}$  = 1.64  $\times$  1.57  $\mu$ m, n = 50). References used for morphological identification: Pitt & Hocking (2009), Watanabe (2010).

Cultural characteristics in PDA: colony growth 45.5mm (diameter) after seven days of incubation; dense, irregular, convex, white (obverse); pale luteos (reverse).

Source of tissue and plant material: root; Carmen, Zaragosa, Nueva Ecija (N 15<sup>0</sup> 27' 13.4" E 120<sup>0</sup> 49' 49.3").



**Fig. 6** – Assemblages of *Hansfordia sp. ds143*. a–b Branched conidiophores bearing whorls of conidia - scale bars = 2  $\mu$ m. c Conidiophore having roughened surface. d–e Front and reverse view of the colony after three days in PDA. Scale bars: a–b = 2  $\mu$ m.



**Fig. 7** – Assemblages of *Humicola sp. ws891*. a–c Unbranched conidiophore. d Branched conidiophore. e–f Front and reverse view of the colony after seven days in PDA. Scale bars: a–d = 2  $\mu$ m.

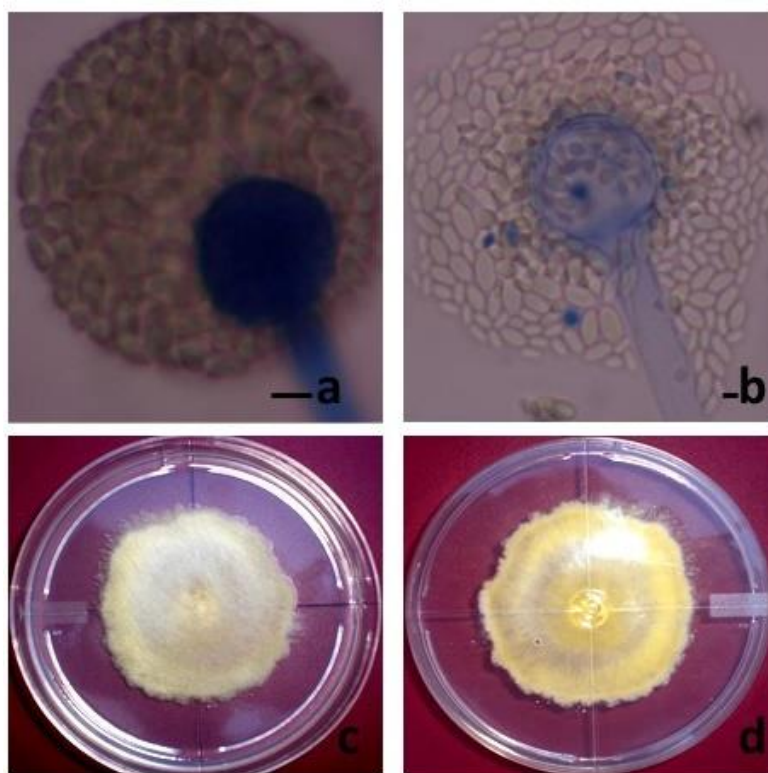


### ***Mucor* sp. ds153-2**

Descriptions: sporangiophores hyaline, erected, unbranched, bearing sporangia terminally and dehiscent columella; sporangia yellow and terminal; columella globose and apophysate; sporangiospores ellipsoid, reniform and yellow in color (Fig. 8). Dimensions: sporangiophores  $7.83\text{--}29.62 \times 1.05\text{--}1.59 \mu\text{m}$  ( $\bar{x} = 14.04 \times 1.29 \mu\text{m}$ ,  $n = 25$ ), columella  $2.33\text{--}5.08 \times 4.17\text{--}7.17 \mu\text{m}$  ( $\bar{x} = 3.98 \times 5.98 \mu\text{m}$ ,  $n = 11$ ), and sporangiospores  $0.84\text{--}2.18 \times 0.46\text{--}0.71 \mu\text{m}$  ( $\bar{x} = 1.62 \times 0.73 \mu\text{m}$ ,  $n = 50$ ). References used for morphological identification: Watanabe (2010), Pitt & Hocking (2009).

Cultural characteristics in potato dextrose agar (PDA): colony growth 49 mm diameter at four days of incubation; dense, irregular, erose, spreading, convex, and yellow to pure yellow color in the top and reverse views.

Source of tissue and plant material: seed; Sangalang, Jaen, Nueva Ecija, Philippines (N  $15^{\circ} 23' 06.9''$ , E  $120^{\circ} 50' 29.3''$ ).



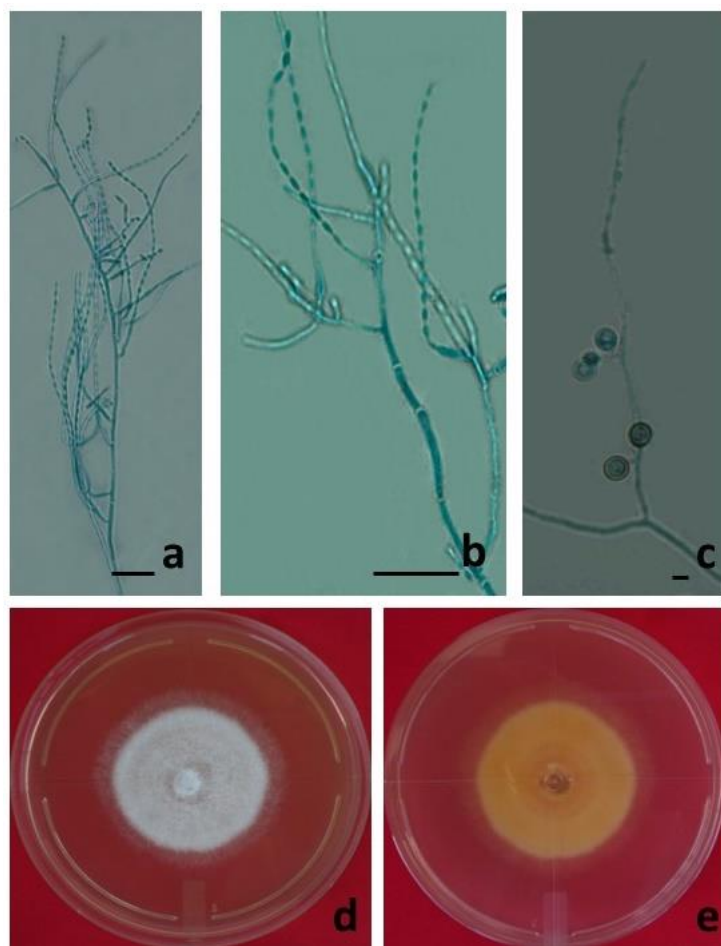
**Fig. 8** – Assemblages of *Mucor* sp. ds153-2. a–b Sporangiophores bearing collumellate sporangia and lemon-shaped, hyaline conidia. c–d Front and reverse view of the colony after four days in PDA. Scale bars: a =  $2 \mu\text{m}$ , b =  $1 \mu\text{m}$ .

### ***Paecilomyces* sp. ws158**

Descriptions: conidiophores hyaline, thin, branched, curved; conidia, hyaline, ellipsoidal, catenate, in a chain (1 chain = 12–15 conidia); chlamydospores hyaline, occasionally pale brown, globose, thick-walled (Fig. 9). Dimensions: conidiophores  $1.68\text{--}11.90 \times 0.19\text{--}0.73 \mu\text{m}$  ( $\bar{x} = 3.88 \times 0.39 \mu\text{m}$ ,  $n = 25$ ); conidia  $0.32\text{--}1.11 \times 0.18\text{--}0.57 \mu\text{m}$  ( $\bar{x} = 0.70 \times 0.43 \mu\text{m}$ ,  $n = 25$ ); chlamydospores  $1.27\text{--}1.99 \times 0.91\text{--}1.89 \mu\text{m}$  ( $\bar{x} = 1.60 \times 1.53 \mu\text{m}$ ,  $n = 25$ ). References used for morphological identification: Rayner (1970), Watanabe (2010).

Cultural characteristics in PDA: colony growth 48.5 mm (diameter) after seven days of incubation in PDA, dense, round with radiating margin, raised, entirely white (obverse); and salmon (reverse).

Source of tissue and plant material: the bottom part of the leaf blade, upper leaf; Burgos, Sto. Domingo, Nueva Ecija, Philippines (N  $15^{\circ} 37' 04.3''$ , E  $120^{\circ} 52' 37.8''$ ).



**Fig. 9** – Assemblages of *Paecilomyces* sp. ws158. a Fruiting structure along hyphae. b Branched conidiophore with catenate conidia. c Chlamydozoospores along the hypha. d-e Front and reverse view of the colony after seven days PDA. Scale bars: a, b = 5  $\mu$ m, c = 1  $\mu$ m.

#### ***Penicillium* sp. ws356**

Descriptions: conidiophores hyaline, short, erect, bi-verticillate; phialides tapering toward the tips; conidia hyaline, 1-celled, ellipsoidal, catenate (1 chain = 50–60 conidia) (Fig. 10A). Dimensions: conidiophores 6.66–17.45  $\times$  0.26–0.53  $\mu$ m ( $\bar{x}$  = 11.17  $\times$  0.37  $\mu$ m, n = 20); metulae 2.14–6.15  $\times$  0.45–1.06  $\mu$ m ( $\bar{x}$  = 3.72  $\times$  0.72  $\mu$ m, n = 20), phialides 2.47–4.11  $\times$  0.23–0.69  $\mu$ m ( $\bar{x}$  = 3.24  $\times$  0.41  $\mu$ m, n = 20); conidia 0.80–1.51  $\times$  0.41–0.89  $\mu$ m ( $\bar{x}$  = 1.11  $\times$  0.62  $\mu$ m, n = 50). References used for morphological identification: Rayner (1970), Quimio (1983), Watanabe (2010), Dugan (2004).

Cultural characteristics in PDA: colony growth 61 mm (diameter) after four days of incubation; medium dense, smooth, irregular, raised, white with grey olivaceous concentric rings (obverse and reverse).

Source of tissue and plant material: middle part of the leaf blade, upper leaf; Poblacion, Rizal, Nueva Ecija, Philippines (N 15<sup>o</sup> 42' 03.8", E 121<sup>o</sup> 05' 27.5").

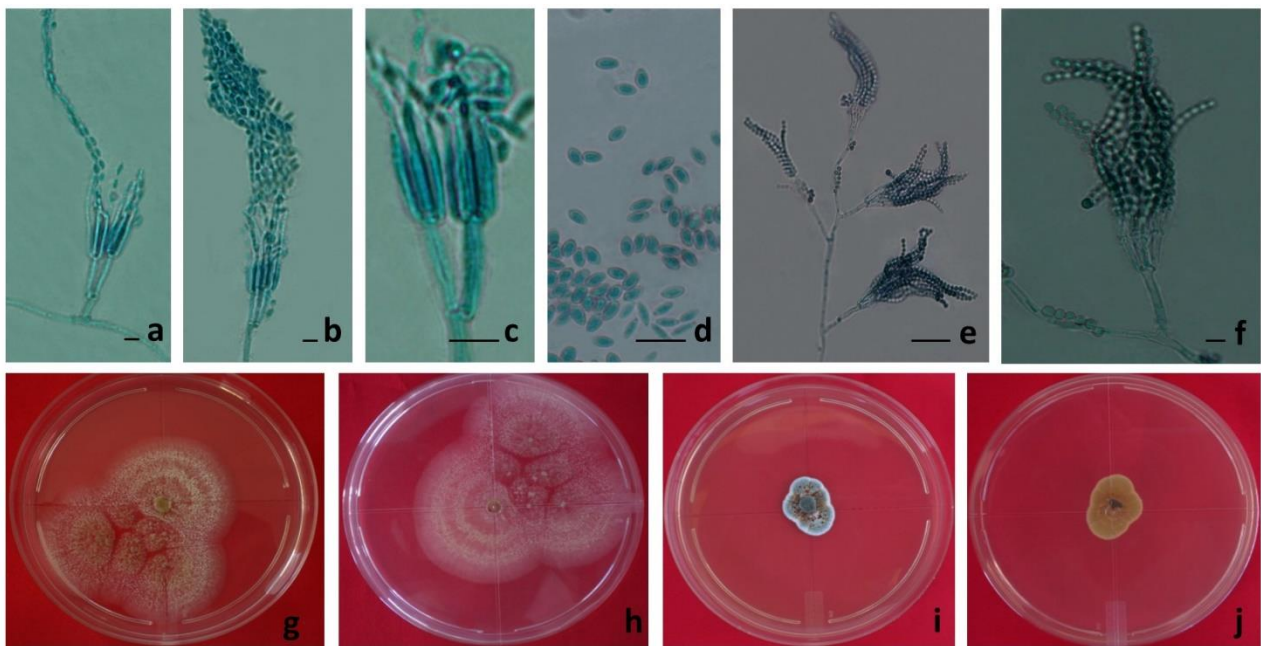
#### ***Penicillium* sp. ws587**

Descriptions: conidiophores pale green, short, erect, monoverticillate; phialides inflated toward the tips; conidia green, 1-celled, globose, catenate (1 chain = 20–45 conidia) (Fig. 10B). Dimensions: conidiophores 2.13–10.22  $\times$  0.23–0.66  $\mu$ m ( $\bar{x}$  = 5.11  $\times$  0.39  $\mu$ m, n = 25); phialides 2.08–4.17  $\times$  0.47–1.44  $\mu$ m ( $\bar{x}$  = 3.08  $\times$  0.81  $\mu$ m, n = 15); conidia 0.43–1.05  $\times$  0.56–0.99  $\mu$ m ( $\bar{x}$  = 0.74  $\times$  0.74  $\mu$ m, n = 50). References used for morphological identification: Rayner (1970), Quimio (1983), Watanabe (2010), Dugan (2004).



Cultural characteristics in PDA: colony growth 19 mm (diameter) after seven days of incubation; thin, irregular, raised, glaucous blue-green (obverse), primrose (reverse).

Source of tissue and plant material: middle part of the midrib, upper leaf; Ibabao Bana, Cabanatuan, Nueva Ecija, Philippines (N 15° 29' 11.4", E 120° 55' 31.4").



**Fig. 10** – Assemblages of *Penicillium* species. *Penicillium* sp. ws356. a Fruiting structure showing short conidiophore. b Long chain of conidia on phialides. c–d Biverticillate phialides and ellipsoidal conidia. g–h Front and reverse view of the colony after four days PDA; *Penicillium* sp. ws587. e Fruiting structures. f Monoverticillate phialides bearing globose conidia. i–j Front and reverse view of the colony after four days in PDA. Scale bars: a, b = 3  $\mu$ m, c–d = 5  $\mu$ m, e = 15  $\mu$ m, f = 2  $\mu$ m.

#### ***Peyronallaea* sp. EF-ds517**

Descriptions: conidiophores undetermined; conidia hyaline, unicellular, globose with double ring-like structures (Fig. 11). Dimensions: conidia 0.85–2.21  $\times$  0.71–1.69  $\mu$ m ( $\bar{x}$  = 1.47  $\times$  1.30  $\mu$ m, n = 50). References used for morphological identification: Rayner (1970).

Cultural characteristics in PDA: colony growth 33 mm (diameter) at three days of incubation; dense, smooth, round, raised, entirely white (obverse and reverse).

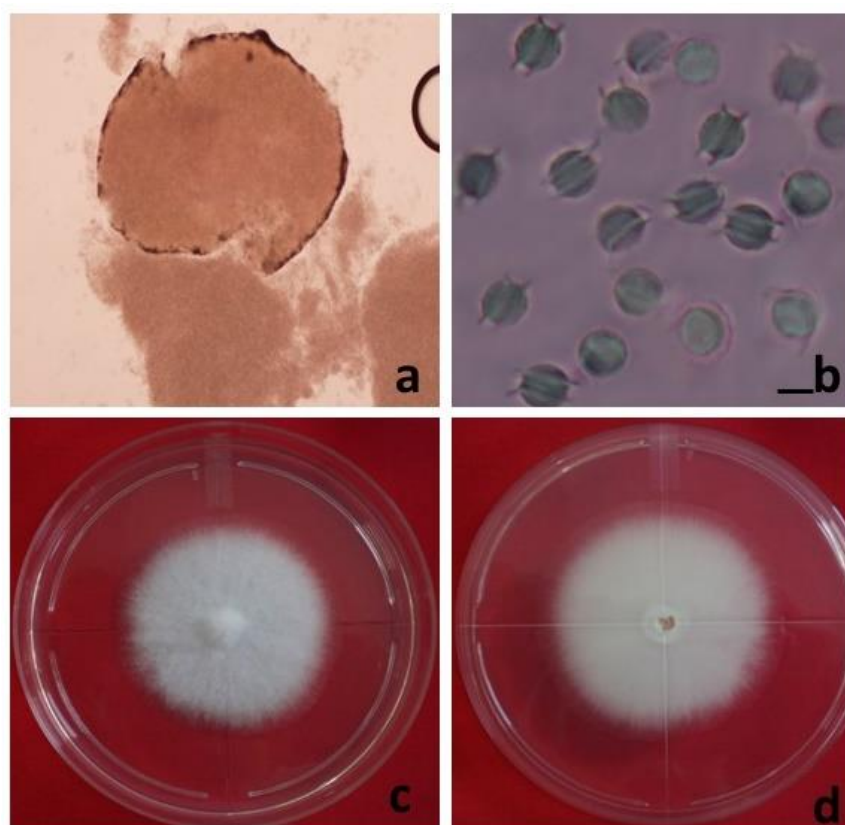
Source of tissue and plant material: the bottom part of the midrib, upper leaf; Ibabao Bana, Cabanatuan, Nueva Ecija, Philippines (N 15° 29' 11.4", E 120° 55' 31.4").

#### ***Trichoconiella* sp. ds143**

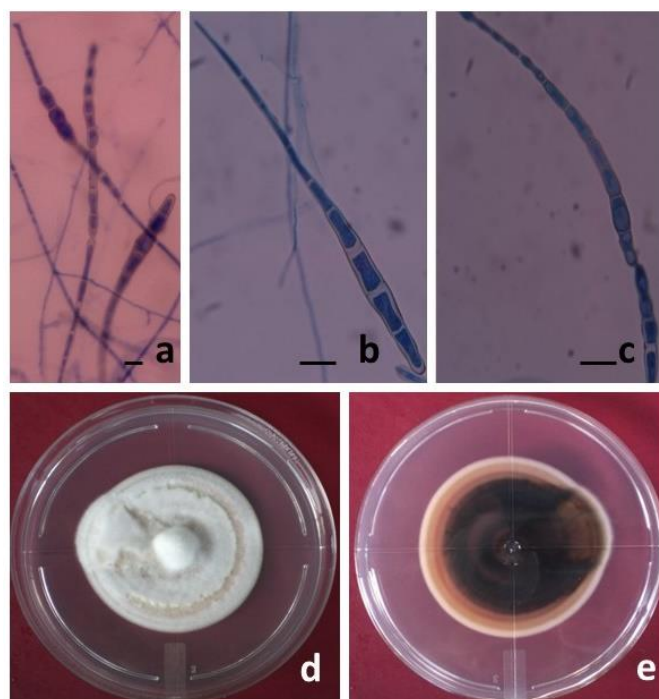
Descriptions: conidiophores hyaline, smooth, bearing one conidium apically; conidia hyaline, long-ellipsoidal tapering towards apex, with 8–12 cells (Fig. 12). Dimensions: conidiophores 7.69–108.66  $\times$  0.41–0.65  $\mu$ m ( $\bar{x}$  = 40.91  $\times$  0.56  $\mu$ m, n = 5); conidia 3.24–4.64  $\times$  0.76–1.24  $\mu$ m ( $\bar{x}$  = 4.02  $\times$  1.04  $\mu$ m, n = 5). References used for morphological identification: Rayner (1970), Mew & Gonzales (2002).

Cultural characteristics in PDA: colony growth 54.5 mm (diameter) after four days of incubation; dense, smooth, round with scalloped margin, white to saffron (obverse); olivaceous with saffron and white margin (reverse).

Source of tissue and plant material: the top part of the leaf blade, lower leaf; Bertici, Quezon, Nueva Ecija, Philippines (N 15° 34' 26.1", E 120° 49' 24.2").



**Fig. 11** – Assemblages of *Peyronallaea* sp. ds517. a Compact mass of conidia. b Unicellular, hyaline conidia with double ring-like structure. c–d Front and reverse view of the colony after three days in PDA. Scale bars: a, b = 2  $\mu$ m.



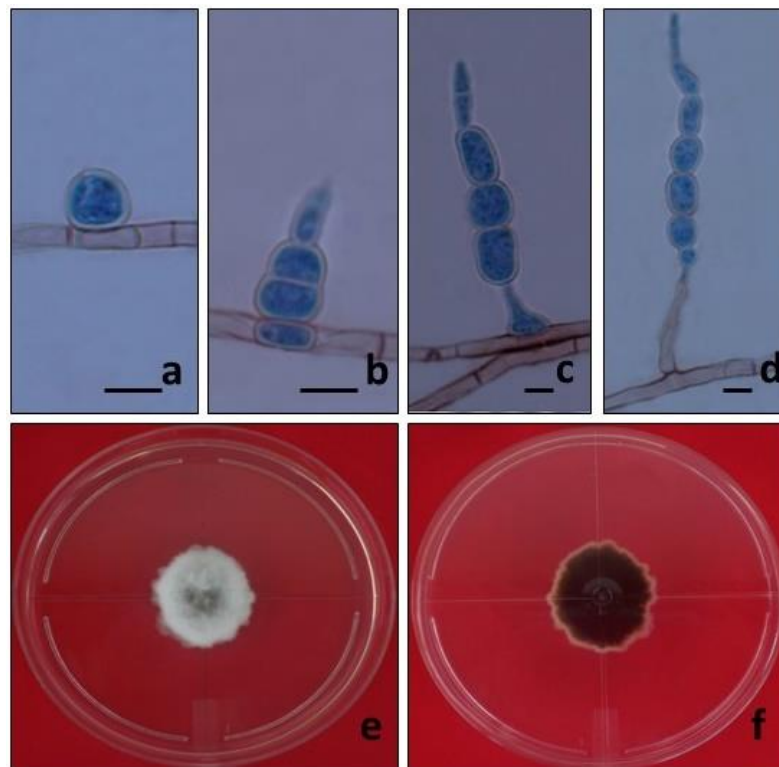
**Fig. 12** – Assemblages of *Trichoconiella* sp. ds333. a Solitary produced conidium. b–c Long multi-septate conidia. d–e Front and reverse view of the colony after ten days in PDA. Scale bars: a–c = 1  $\mu$ m.

### EF-ds163

Descriptions: conidiophore brown, short when immature; conidia hyaline, oval to ovoid, catenate (1 chain = 2–7 conidia) (Fig. 13). Dimensions: conidiophore  $15.73\text{--}19.57 \times 19.16\text{--}37.75 \mu\text{m}$  ( $\bar{x} = 22.54 \times 24.60 \mu\text{m}$ ,  $n = 38$ ); conidia  $5.73\text{--}22.86 \times 5.73\text{--}15.10 \mu\text{m}$  ( $\bar{x} = 10.79 \times 9.14 \mu\text{m}$ ,  $n = 50$ ).

Cultural characteristics in PDA: colony growth 27.5 mm (diameter) after six days of incubation; thin, wavy, round, raised, lavender grey (obverse); fuscous black (reverse).

Source of tissue and plant material: middle part of the leaf blade, lower leaf; Poblacion, Rizal, Nueva Ecija, Philippines (N  $15^{\circ} 42' 03.8''$ , E  $121^{\circ} 05' 27.5''$ ).



**Fig. 13** – Assemblages of EF-ds163. a Conidium initial. b Basipetal formation of conidia in a chain. c Formation of the stalk. d Mature fruiting structure. e–f Front and reverse colony growth after six days in PDA. Scale bars: a–d =  $15 \mu\text{m}$ .

### EF-ds571

Descriptions: conidiophores smooth, branched; conidia hyaline 1-celled, ovoid (Fig. 14). Dimensions: conidiophores  $4.75\text{--}29.14 \times 0.27\text{--}0.84 \mu\text{m}$  ( $\bar{x} = 12.07 \times 0.55 \mu\text{m}$ ,  $n = 20$ ); conidia  $0.69\text{--}2.34 \times 0.2\text{--}0.78 \mu\text{m}$  ( $\bar{x} = 1.5 \times 0.5 \mu\text{m}$ ,  $n = 50$ ).

Cultural characteristics in PDA: colony growth 50.5 mm (diameter) after ten days of incubation; dense, wavy, round with scalloped margin, umbonate, lavender grey (obverse); rosy buff with shades of vinaceous purple at the center (reverse).

Source of tissue and plant material: root; Sta Rosa, Nueva Ecija (N  $15^{\circ} 26' 03.7''$  E  $120^{\circ} 53' 54.6''$ ).

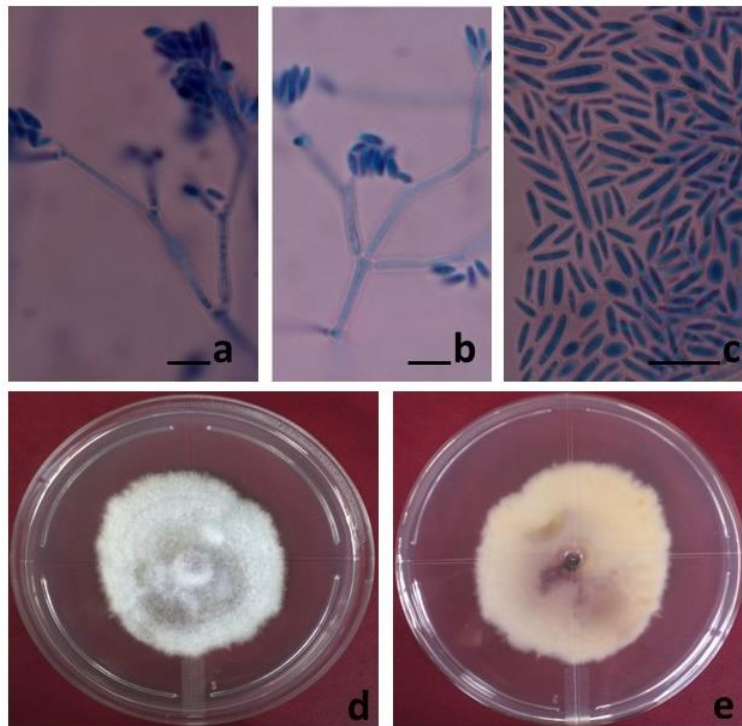
### EF-ds290

Descriptions: conidiophores undetermined; conidia hyaline, unicellular, and sub-globose (Fig. 15). Dimensions: conidia  $0.72\text{--}1.34 \times 0.61\text{--}1.30 \mu\text{m}$  ( $\bar{x} = 1.06 \times 0.96 \mu\text{m}$ ,  $n = 50$ ).

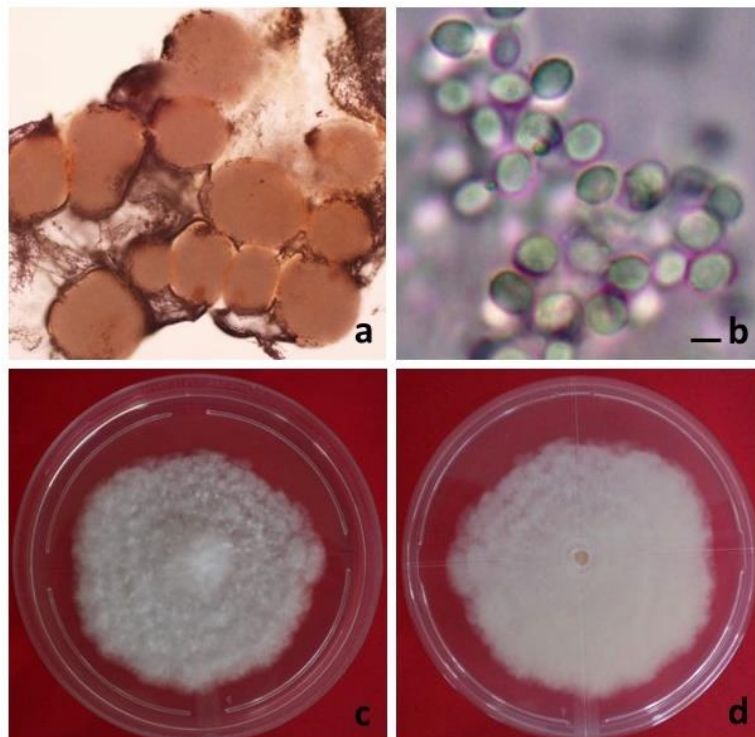
Cultural characteristics in PDA: colony growth 66.5 mm (diameter) at three days of incubation; dense, wavy, irregular, convex, entirely white (obverse and reverse).



Source of tissue and plant material: root; San Casimiro, Licab, Nueva Ecija (N 15° 33' 0.07" E 120° 46' 11.5").



**Fig. 14** – Assemblages of EF-ds571. a–b Branched conidiophores bearing conidia. c Close-up of conidia in various shapes. d–e Front and reverse view of the colony after six days in PDA. Scale bars: a–b = 0.5  $\mu\text{m}$ , c = 1  $\mu\text{m}$ .



**Fig. 15** – Assemblages of EF ds290. a Masses of conidiomata. b Unicellular, hyaline conidia. c–d Front and reverse view of the colony after five days in PDA. Scale bar: b = 1  $\mu\text{m}$ .

## Tissue specificity

Five HSF endophytic fungi were recovered in seeds of *E. glabrescens*, as shown in Table 1. Of these, *Fusarium* sp. ds529 was the most dominant in the dry season, *Hansfordia* sp. ds143, followed by *Trichoconiella* sp. ds143, was dominant in the wet season. In the upper leaves, on the other hand, sixteen HSF endophytic fungi were recovered in the upper part; ten in the lower part (Table 2). In the dry season, *T. padwickii* was the most dominant in the top, middle, and bottom of the leaf blade as well as in the top and middle parts of the leaf midrib. *Fusarium* sp. ds111 and *Fusarium* sp. ds519 dominated the bottom part of the leaf midrib. In the wet season, *T. padwickii*, *H. biophila*, and *Colletotrichum* sp. ds361 were the most dominant in the upper and lower parts of the leaf blade and leaf midrib.

**Table 1** Hyaline spore-forming endophytic fungi residing in seeds of *Echinochloa glabrescens*.

Endophytic Fungi	Summed-Dominance Ratio (%)	
	Dry Season	Wet Season
<i>Aspergillus</i> sp. ds397	6.7	0
<i>Fusarium</i> sp. ds111	6.7	9.5
<i>Fusarium</i> sp. ds519	0	11.8
<i>Fusarium</i> sp. ds529	39.8	0
<i>Hansfordia</i> sp. ds143	0	26.4
<i>Mucor</i> sp. ds158-2	6.7	0
<i>Penicillium</i> sp. ws587	0	4.2
<i>Trichoconiella</i> sp. ds333	0	18.3
EF-ds571	8.7	0

**Table 2** Hyaline spore-forming endophytic fungi residing in the upper leaves of *Echinochloa glabrescens*.

EF	Summed-Dominance Ratio (%)											
	Dry Season						Wet Season					
	Leaf Blade			Leaf Midrib			Leaf Blade			Leaf Midrib		
	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
Asp1	0	0	9.0	0	0	0	0	0	0	0	0	0
Asp2	0	0	0	0	0	0	0	0	0	0	4.3	0
Asp4	0	0	0	0	0	0	5.4	0	0	0	4.3	0
Col	0	0	0	0	0	0	5.4	14.2	14.1	24.2	18.8	9.5
Fus3	25.1	9.6	0	33.4	0	15.1	0	0	0	0	0	0
Fus5	0	9.6	0	0	11.7	19.2	0	0	0	0	4.3	0
Fus6	0	0	0	0	6.4	8.2	0	0	0	0	0	0
Geo	0	0	9.0	0	0	0	0	0	0	0	0	6.8
Han	0	0	0	0	9.1	0	21.3	28.0	19.1	22.3	14.3	28.6
Pae	0	0	0	0	0	0	0	0	7.6	0	0	9.5
Pen1	0	0	0	0	0	0	0	8.1	0	0	0	0
Pen2	0	0	0	0	0	0	3.7	0	0	5.6	0	0
Per	0	0	0	0	6.4	8.2	0	0	0	0	0	0
Tri	42.0	48.9	27.1	33.4	15.4	8.2	33.2	18.4	28.6	16.7	23.3	13.7
EF1	0	0	22.4	0	12.8	8.2	0	0	0	0	0	0
EF2	0	0	0	0	6.4	0	0	0	0	0	0	0

Asp1 - *Aspergillus* sp. ds153; Asp2 - *Aspergillus* sp. ws840; Asp4 - *Aspergillus* sp. ws960; Col - *Colletotrichum* sp. ds361; Fus3 - *Fusarium* sp. ds111; Fus5 - *Fusarium* sp. ds519; Fus6 - *Fusarium* sp. ds516; Geo - *Geotrichum* sp. ds104-16; Han - *Hansfordia* sp. ds143; Pae - *Paecilomyces* sp. ws158; Pen1 - *Penicillium* sp. ws356; Pen2 - *Penicillium* sp. ws587; Per - *Peronallaea* sp. ds517; Tri - *Trichoconiella* sp. ds333; EF1 - EF-ds163; and EF2 - EF-ds571

In the lower leaves, ten HSF endophytic fungi were recovered (Table 3). In the dry season, *T. padwickii* was the most dominant in all parts of the tissues except in the top and bottom parts of the leaf midrib. *Colletotrichum* sp. ds361 and *Fusarium* sp. ds529, on the other hand, were the



dominants in the top portion of the leaf midrib; *Fusarium* sp. ds556 in the bottom of the leaf midrib. In the wet season, *T. padwickii* was still the most dominant in all parts of the tissues except in the middle part of the leaf midrib; *H. biophila* was the second, particularly in the top and middle of the leaf blade, and middle and bottom of leaf midrib. *Colletotrichum* sp. ds361 dominated *H. biophila* in the bottom of the leaf blade and top of the leaf midrib.

**Table 3** Hyaline spore-forming endophytic fungi residing in the lower leaves of *Echinochloa glabrescens*.

EF	Summed-Dominance Ratio (%)											
	Dry Season						Wet Season					
	Leaf Blade			Leaf Midrib			Leaf Blade			Leaf Midrib		
	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
Col	0	0	0	33.4	0	0	16.2	11.3	22.3	16.7	24.9	10.8
Fus1	0	0	0	33.4	0	0	0	0	0	0	0	5.9
Fus3	0	0	10.9	0	19.8	12.3	0	0	0	0	0	0
Fus4	0	0	0	0	8.4	37.0	0	0	0	0	0	0
Fus5	0	19.9	10.9	0	17.9	0	0	5.2	0	0	0	0
Fus7	0	0	0	0	0		0	4.3	0	0	0	0
Han	0	0	0	0	0	0	22.1	17.4	16.6	14.9	28.5	19.3
Pen2	0	0	0	0	0	0	0	0	0	0	0	7.1
Tri	68.0	34.0	45.7	0	22.7	18.0	33.3	33.1	33.3	37.2	15.4	26.4
EF1	0	14.1	0	0	0	0	0	0	0	0	0	0

Col – *Colletotrichum* sp. ds361; Fus1 – *Fusarium* sp. ds529; Fus3 – *Fusarium* sp. ds111; Fus 4- *Fusarium* sp. ds556; Fus5 – *Fusarium* sp. ds519; Fus7 – *Fusarium* sp. ds412; Han - *Hansfordia* ds143; Pen2 - *Penicillium* sp. ws587; Tri - *Trichoconiella* sp. ds333; and EF1 - EF-ds163

In the leaf sheaths, seven HSF endophytic fungi were recovered in the dry season; six in the wet season (Table 4). In the dry season, *T. padwickii* was the most dominant over *Fusarium* sp. ds111 in the upper part, with equal dominance of EF-ds163 and EF-ds571 over *Fusarium* sp. 519 and *Fusarium* sp. 516 in the lower part. In the wet season, *T. padwickii* was still dominant, followed by *H. biophila* and *Fusarium* sp. ds529 in the upper and lower leaf sheaths, respectively. In roots, EF-ds290 was the most dominant in the dry season, and *T. padwickii* in the wet season (Table 5).

**Table 4** Hyaline spore-forming endophytic fungi residing in upper and lower leaf sheath of *Echinochloa glabrescens*.

Endophytic Fungi	Summed-Dominance Ratio (%)			
	Dry Season		Wet Season	
	Upper leaf sheath	Lower leaf sheath	Upper leaf sheath	Lower leaf sheath
<i>Colletotrichum</i> sp. ds361	0	0	5.7	5.3
<i>Fusarium</i> sp. ds529	0	0	0	15.5
<i>Fusarium</i> sp. ds111	17.9	0	0	0
<i>Fusarium</i> sp. ds498	0	0	5.7	0
<i>Fusarium</i> sp. ds519	0	12.9	0	0
<i>Fusarium</i> sp. ds516	0	12.9	0	0
<i>Fusarium</i> sp. ds172	0	9.8	0	0
<i>Hansfordia</i> sp. ds143	0	0	21.5	5.3
<i>Mucor</i> sp. ds158-2	0	0	0	5.3
<i>Trichoconiella</i> sp. ds333	49.3	0	36.5	32.3
EF-ds163	0	16.1	0	0
EF-ds571	0	16.1	0	0

**Table 5** Hyaline spore-forming endophytic fungi residing in roots of *Echinochloa glabrescens*.

Endophytic Fungi	Summed-Dominance Ratio (%)	
	Dry Season	Wet Season
<i>Aspergillus</i> sp. ws840	0	0
<i>Colletotrichum</i> sp. ds361	0	9.3
<i>Fusarium</i> sp. ds516	9.9	0
<i>Fusarium</i> sp. ds142	19.9	0
<i>Humicola</i> ws891	0	9.3
<i>Trichoconiella</i> sp. ds333	0	49.4
EF-ds163	4.2	0
EF-ds290	24.1	0
EF-ds571	9.9	0

## Discussion

Numerous HSF isolates had been recovered in the wet season than in the dry season. The abundance of HSF endophytic fungi in the wet season could be attributed to plentiful moisture. In fact, previous studies also have shown that the diversity and abundance of many endophytic fungi are influenced by too much moisture. For example, Rather et al. (2018) reported that the overall colonization and isolation rates of 38 fungal species were higher in wet periods (September–November) in Irula Tribe Women’s Welfare Society, Thandarai, Chennai, India for both *Asparagus racemosus* (Willd.) (92.22% and 95.11%) and *Hemidesmus indicus* (Linn.) (82% and 77.11%). Naik et al. (2009) also reported that the colonization rate of endophytic fungi in rice (*Oryza sativa* L.) in two sites of Southern India was higher in the winter season (33.08%) than in the dry season (14.41%). Lumyong et al. (2009) also had similar results on *Calamus kerrianus* Becc. and *Wallichia caryotoides* Roxb. in two sites in Chang Mai, Thailand. They reported that the colonization rate of endophytic fungi was higher in the wet season (94%) compared to the hotter season (52%). In Oregon, USA, Wilson & Carroll (1994) also reported that the infection rate of endophytic fungi *Discula quercina* (Westd.) von Arx increased as rainfall increased in the growing season of *Quercus garyanna* Dougl.

Hyaline spore-forming endophytic fungi were abundant in the lower leaves compared to other tissues. In the study of Rodriguez (1994), they also recovered more endophytic fungi in mature and expanded leaves (midrib and inter-midrib of leaf blades) of a mature tree of *Euterpe oleracea* Mart. than the unopened (young) leaves both in dry and wet seasons. According to Carroll and Carroll (1978), high colonization of endophytic fungi in older leaves is influenced by maturity accompanied by prolonged exposure to different environmental conditions and fungal species. Another attribution could be due to the entrapment of numerous endophytic fungi as a function of the wider surface area of older leaves compared to the developing young leaves. Bayman (2006), on the other hand, proposed that the tissue specificity of endophytic fungi may be affected by environmental differences among tissues (e.g., parts of the leaf may differ in relative humidity, O<sub>2</sub>, and CO<sub>2</sub> concentrations, carbohydrate and protein availability, and temperature), or by differences among microorganisms on how they enter into the leaf or vice versa, response of the host plant in the presence of invading microorganisms.

Previous studies also showed that different tissues of plants have been occupied by HSF endophytic fungi. For example, in 2,400 segments of rice (*Oryza sativa* L.) made by Naik et al. (2009), the overall colonization rates of the endophyte population from surface-sterilized tissues were 40.3 and 25.8% in roots and leaves during the winter season; 20.1 and 8.7% in the same order during the summer season. Of the 19 fungal taxa that were identified, *Penicillium chrysogenum* was one of the most dominant endophytic fungi. In the study of Larran et al. (2007) on endophytic fungi of wheat (*Triticum aestivum* L.), they also identified 30 genera of endophytic fungi out of 722 isolates that were isolated from 1,750 plant segments (leaves, stems, glumes, and grains). The researchers added that *Alternaria* sp., *Rhodotolura rubra*, *Penicillium* sp., and *Fusarium*

*graminearum* were the most dominant genera in terms of colonization frequency in all types of organs in the wheat plant. Among the organs, however, leaves harbored the most. Tian et al. (2004), in the study on the communities of endophytic fungi on rice in Guandong Province, South China, also reported that endophytic fungi were more diverse on leaves than in the roots of rice plants. Of the 72 endophytic fungi in site 1, species of *Fusarium*, *Pyricularia* and unidentified yeasts were found to be the most dominant genera, while out of 366 isolates in site 2, *Fusarium*, *Penicillium*, *Aspergillus*, *Paecilomyces*, two dark-spore-forming endophytic fungi, and species of yeasts were the most dominant ones. In healthy tissues of *Melia azedarach* L., a total of 59 endophytic fungi were also recovered by Geris dos Santos et al. (2003) from the root cortex, root xylem, stem cortex, leaves, and fruits of the plant. The identified endophytic fungi were *Balansia* sp.; *Pestalotiopsis versicolor*, *Aspergillus aculeatus* Lizuka, *A. carbonarius* Bain, *A. flavus* Link, *A. japonicus* Saito, *A. niger*, van Tieghem, *A. pulvurentus* Mc Alpine, *Aspergillus* sp., *F. nivale* (Fr.) Ces, *Fusarium moniliforme* Sheldon, *Gilmaniella* sp., *Nigrospora* sp., *Penicillium citrinum*, *P. herquei* Bainier and Sartory, *P. janthinellum*, *P. rubrum* Stoll, *P. rugulosum* Thom., *P. simplicissimum*, *P. implicatum* Biorgue, *Penicillium* sp., *Trichoderma koningii* Ouderm, *T. nivale*, *Trichoderma* sp., and 16 other unidentified endophytic fungi. Among the genera mentioned, the genus *Aspergillus* was found to be very common and dominant in the plant. Moreover, about 160 isolates of endophytic fungi were recovered by Cao et al. (2002) from 200 leaf samples of the *Musa acuminata* plant, mostly belonging to *Gloeosporium musae*, *Myxosporium* sp., *Deightoniella torulosa*, *Alternaria tenuis*, *Sphaceloma* sp., *Aureobasidium* sp., *Melida* sp., *Uncinula* sp., *Penicillium* sp., *Aspergillus* sp., *Sarcinella* sp., *Cladosporium* sp., *Cephalosporium* sp. and sterile mycelium. Meanwhile, 68 endophytic fungal cultures were isolated from 100 root samples of *Musa acuminata*, mostly belonging to *Aspergillus* sp., *Paecilomyces* sp., *Penicillium* sp., *Fusarium* sp., *Gloeosporium musae*, yeast, *Deightoniella torulosa*, *Spicaria* sp., *Cephalosporium* sp., *Meliola* sp. and sterile mycelium. Among the fungal cultures, *Gloeosporium musae*, *Myxosporium* sp., were the most dominant in leaves while *Aspergillus* sp. and *Penicillium* sp. in roots.

It is remarkable to note that the genera *Trichoconiella* and *Hansfordia* were the most common and dominant among the HSF endophytic fungi in all tissues of *E. glabrescens*. The reason behind their dominance in tissues of the weed could be due to their conidia, which are not born inside specialized structures like the ascus, perithecium, and basidium. This distinct characteristic makes them easily exposed and vulnerable to the actions of dispersing agents like the wind, rain splashes or even humans and animals. On the other hand, *Trichoconiella* was also reported as seed-borne fungal pathogens causing foliage and grain diseases of rice (Mew & Gonzales 2002). When disseminated, the spores of these endophytic fungi must have landed, penetrated, and resided in tissues of *E. glabrescens* without causing any infections and symptoms. Meanwhile, little information is known about the ecology of *Hansfordia* (Gene et al. 2010, Cheng et al. 2011). For example, a *Hansfordia* sp. parasitizing the black rust pathogen of papaya [*Asperisporium caricae* (Speg.) Maubl.], leaf mold pathogen of tomato [*Fulvia fulva* (Cooke.) Ciferri.] in Puerto Rico and Korea (Hepperly 1986, Park et al. 2010), and leaf mold pathogen of olive (*Cercospora tandojamensis* Kamal.) in India (Rathaiiah & Pavgi 1971).

## Conclusion

*Aspergillus*, *Colletotrichum*, *Fusarium*, *Geotrichum*, *Hansfordia*, *Humicola*, *Mucor*, *Paecilomyces*, *Penicillium*, *Trichoconiella*, and three unidentified isolates were the hyaline-spore forming endophytic fungi isolated and identified from different tissues of *E. glabrescens*. *Fusarium*, *Aspergillus*, and *Penicillium* had the most number of morpho species. All sampled tissues were occupied by different hyaline spore-forming endophytic fungi. However, the genera of *Trichoconiella* and *Hansfordia* were the most common and dominant in the seeds, leaves, leaf sheaths, and roots of *E. glabrescens*. This study confirmed that hyaline spore-forming endophytic fungi also reside in different tissues of *E. glabrescens*. To further explore the ecology of hyaline-spore forming endophytic fungi, it was recommended to determine their a) presence in other weeds of rice, b) potentials as biological control agents against major disease-causing pathogens and

insect pests of rice, and c) potentials as pathogens (particularly the *Aspergillus*, *Fusarium*, and *Penicillium*) to cause grain diseases during the soft dough to maturity stages as well as during the storage process of rice.

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