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Biodiversity of *Fusarium* species in Mexico associated with ear rot in maize, and their identification using a phylogenetic approach

Irma Morales-Rodríguez[1], María de J. Yañez-Morales[2], Hilda V. Silva-Rojas[1], Gabino García-de-los-Santos[1] & Doralinda A. Guzmán-de-Peña[3]
[1]*Colegio de Postgraduados (CP), Campus Montecillo, Seed Production Program, Montecillo, Edo. de Mexico, CP 56230, Mexico;* [2]*CP-Institute of Phytopathology, Montecillo, CP 56230, Edo. De Mexico, Mexico;* [3]*Department of Biotechnology and Biochemistry, CINVESTAV-Unidad Irapuato, km 9.6 Carr, Irapuato-León, Guanajuato, CP 36500, Mexico*

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Abstract

Fusarium proliferatum, *F. subglutinans*, and *F. verticillioides* are known causes of ear and kernel rot in maize worldwide. In Mexico, only *F. verticillioides* and *F. subglutinans*, have been reported previously as causal agents of this disease. However, *Fusarium* isolates with different morphological characteristics to the species that are known to cause this disease were obtained in the Highland-Valley region of this country from symptomatic and symptomless ears of native and commercial maize genotypes. Moreover, while the morphological studies were not sufficient to identify the correct taxonomic position at the species level, analyses based in the Internal Transcribed Spacer region and the Nuclear Large Subunit Ribosomal partial sequences allowed for the identification of *F. subglutinans*, *F. solani*, and *F. verticillioides*, as well as four species (*F. chlamydosporum*, *F. napiforme*, *F. poae*, and *F. pseudonygamai*) that had not previously been reported to be associated with ear rot. In addition, *F. napiforme* and *F. solani* were absent from symptomless kernels. Phylogenetic analysis showed genetic changes in *F. napiforme*, and *F. pseudonygamai* isolates because they were not true clones, and probably constitute separate sibling species. The results of this study suggest that the biodiversity of *Fusarium* species involved in ear rot in Mexico is greater than that reported previously in other places in the world. This new knowledge will permit a better understanding of the relationship between all the species involved in ear rot disease and their relationship with maize.

Key words: fungus, ITS, nLSU, seed, *Zea mays*

Introduction

Maize (*Zea mays* L.) is one of the four basic food staples of the world population [1] and at the same time is the crop that has the first place in production volume in the world. In developed countries most of the maize produced is used for animal feed and industrial uses other than human food [2]. In Mexico maize is the main human food source and *per capita* consumption is estimated to be 328 g day^{-1} [3].

The majority of the reported maize diseases, affecting roots, stalks, ears, and kernels, are caused by fungi [4]. Among these diseases, ear rot is one of the most important in all the countries where this cereal is grown. In addition to reduced crop yield, ear rot adverasely affects the physical, physiological, and phytosanitary qualities of the seed [5, 6]. The causal agents reported worldwide as responsible for ear rot are *Fusarium proliferatum*, *F. subglutinans*, and *F. verticillioides* [4]. These pathogens survive in the soil, in infected plant debris, and inside apparently healthy seed and can affect the embryo and pericarp without visible symptoms. Infection can be seedborne and systemic in the crop from seedling to harvest, or starting during the pollination where the silks are

infected by the airborne conidia. During harvest, ear rot appears as individual rotted kernels or as randomly scattered groups of rotted kernels [4, 7]. In addition, depending on the specific fungus responsible for the ear rot, the production of mycotoxins can be an important source of contamination.

The three *Fusarium* species involved in ear rot disease are included in the *Gibberella fujikuroi* complex [7, 8]. Morphological differences between the three species are an important step in the classification, however, currently there is no consensus definition among research groups that will allow definitive identification based on morphology alone. Revisions in recent years have asserted that the first criteria to define a *Fusarium* species [7], was the use of the biological species concept, but it has some limitations. Some researchers have adopted the concept of phylogenetic species which considers the use of one or more conserved genes or sequences to define a species of this genus [9], such as the Internal Transcribed Spacer (ITS) region of the ribosomal genes 18S-5.8S-28S [10].

In Mexico, the species that have been found to cause ear rot are *F. verticillioides* [11] and *F. subglutinans* [12, 13], and since this disease is the most important in the Highland Valley, based on reduced yield and grain quality [8], the aim of this research was to determine the biodiversity of the *Fusarium* species associated with ear rot in this geographical area of the country utilizing both a morphological and phylogenetic approach.

Materials and methods

During the fall-winter of 2002, at the Colegio de Postgraduados' plots, located in Montecillo-Highland-Valley (elevation 2250 m), in the central part of Mexico, 10 symptomatic and two symptomless ears from each one of 28 native maize genotypes were collected (Table 1).

Table 1. Relation of 28 native maize genotypes and their origin in the Highland of Mexico[a]

Genotypes	Origin
ASV11	Sierra Purepecha, Michoacan
ASV34	Sierra Purepecha, Michoacan
ASV36	Sierra Purepecha, Michoacan
ASV45	Sierra Purepecha, Michoacan
ASV49	Sierra Purepecha, Michoacan
ASV64	Sierra Purepecha, Michoacan
ASV71	Sierra Purepecha, Michoacan
ASV76	Sierra Purepecha, Michoacan
ASV84	Sierra Purepecha, Michoacan
ASV86	Sierra Purepecha, Michoacan
ASV87	Sierra Purepecha, Michoacan
ASV102	Sierra Purepecha, Michoacan
ASV111	Sierra Purepecha, Michoacan
ASV112	Sierra Purepecha, Michoacan
Qro-21 (Chalqueño Qro)	Sierra Purepecha, Michoacan
Zac-66 (Chalqueño Dgo-Zac)	Sierra Purepecha, Michoacan
Hgo-88 (Chalqueño Hgo)	Sierra Purepecha, Michoacan
Pedro Cruz (7a CSM) (Chalco Crema)	Sierra Purepecha, Michoacan
Santos Altamirano 6a CSM (Chalco palomo)	Valle de Chalco, Edo. de Mexico
Comp. Familias (Chalqueño Cajetes)	Valle de Chalco, Edo. de Mexico
Manuel Montes de Oca (Ciclo 8)	Valle de Chalco, Edo. de Mexico
David Rivera Enrrique (1a CSM)	Valle de Chalco, Edo. de Mexico
Pedro Cruz Linares (8a CSM)	Valle de Chalco, Edo. de Mexico
Ignácio Rosas (7a CSM)	Valle de Chalco, Edo. de Mexico
David Rivera Reyes (2a CSM)	Valle de Chalco, Edo. de Mexico
Pedro Hernandez (Ciclo 8)	Valle de Chalco, Edo. de Mexico
Santos Altamirano (7a CSM)	Valle de Chalco, Edo. de Mexico
Oaxaca 492	Oaxaca

[a]The native maize genotypes are grown from 1900 to 2700 m elevation.

Symptomatic and symptomless ear isolates

The ears of all the genotypes with visible rot symptoms were grouped according to the mycelium colour. Of each group, a fungal sample grown on the top of the kernels was taken to obtain monoconidial cultures. To isolate the fungi that were within the symptomless kernels, hundred of seeds were randomly chosen from the apparently healthy ears and mixed. The seeds were disinfested using a 3:1 solution of sodium hypochlorite 1.5 %, and ethanol 25% for 2 min. After that, they were rinsed three times with sterile distilled water. Internal fungi were obtained through the Blotter method [14]. The colonies that developed over the seed were examined microscopically. *Fusarium*

colonies were selected according to the mycelium colour.

Monoconidial cultures

The mycelium of each group selected from symptomatic and symptomless kernels was placed into 10 ml test tubes with 5 ml of sterile distilled water, and shaken in a Mini Vortex (VWR, USA). The content of individual tubes was poured into Petri dishes with water-agar (WA) (18 g of agar/l) and allowed to stay on the plates for 10 s and then the excess was removed. The Petri dishes were maintained at room temperature (22–24°C) for 24 h. The germinated conidia were transferred to new Petri dishes containing Potato Carrot Agar medium (PCA) (20 g of potato, 20 g of carrot, and 15 g of agar/l) and kept at room temperature with constant white light for seven days. The conidia were transferred into sterile glycerol 25%, and stored in Eppendorf tubes at −84°C.

Morphological characterization

To identify the fungi at the genus level, the Manual of Barnett and Hunter [15] was used and the determination of the species was according to Booth [16], Nelson et al. [17], and Burgess et al. [18]. Also the descriptions of Marasas et al. [19], Pascoe [20], and Nirenberg and O'Donnell [21] were referred to when necessary.

Colour of the colony

The *Fusarium* cultures were placed into slant tubes containing Potato Dextrose Agar medium (PDA) (250 g of potato, 20 g of dextrose, and 20 g of agar/l) amended with a half-cup of potato pulp. To promote sporulation and colouring, the tubes were maintained at 20°C with continuous white light for 10 days [17]. Finally, the colour of the mycelia was evaluated on both sides, the tubes were grouped according to the colour, and only one isolate was selected from each group for further morphological and phylogenetic analysis.

Structure formation

To set up microcultures in humidity chambers, Petri dishes were used [22]. Aluminium foil, a microscope slide, and a PDA square of 5 mm^3 were placed in the plate, in this order one over the other, and a portion of mycelia was taken and placed on each PDA square. A cover slide was placed over the inoculated PDA squares. The Petri dishes were sealed and maintained at ± 24°C under white (12 h) and black light (12 h) (365 nm-General Electric 40 W F40SL, Mexico) for seven days. After the mycelia colonized the cover slide it was removed with sterile forceps and permanently mounted on a microscope slide with a drop of acidified glycerol. The size, shape and other key characteristics of each structure were recorded and photomicrographs were taken with a digital camera (Nikon COOLPIX 5000, Japan).

Obtaining of macroconidia and napiforme microconidia

The isolates were placed on Carnation-Leaf-Agar medium (CLA) (18 g of agar/l), and 10 carnation leaves were amended to the medium before the agar solidified [23], the leaves were irradiated previously with gamma rays at the Nuclear Centre of Mexico. The Petri dishes were kept at room temperature (22–24°C) with white light for 25 days, and then permanently mounted for microscopic evaluation.

Formation of microconidia into chains

To stimulate this structure isolates were grown in KCl medium (8 g of KCl, and 14 g of agar/l) amended with 10 carnation leaves. The Petri dishes were kept at room temperature (22–24°C) under black light for 10 days, and the aerial mycelium was microscopically examined.

Formation of chlamydospores

This conserved structure was induced in PDA medium, and the Petri dishes were kept at room temperature (22–24°C) with white light for 14 days.

Phylogenetic analysis

The DNA of 21 monosporic cultures was obtained with the protocol of Ahrens and Seemüller [24], and quality was verified by electrophoresis on a 1% agarose gel (Ultrapure, Gibco, USA) using TBE buffer (Tris–Borate–EDTA pH 8.0). The

gel was stained with ethidium bromide and the amplicons were visualized in a transilluminator (Gel Doc 2000, BIO RAD®, USA). The DNA concentration was quantified in a Perkin-Elmer spectrophotometer (Lambda BIO 10, USA).

To amplify the ITS region ITS5 (5′-GGAAG-TAAAAGTCGTAACAAGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [10], primers were used, and to amplify a partial sequence of 28S rRNA gene, ITS5 and NL4 (5′-GGTCCGTGTT-TCAAGACGG-3′) primers were used [10, 11]. PCR master mix was prepared in a final volume of 25 μl containing 1× of *Taq* DNA buffer, 0.13 mM of $MgCl_2$, 16 μM of dNTPs, 20 ρmole of each primer, 0.4 U of *Taq*-DNA polymerase (University Biotecnologies®, Mexico), and 80 ng of DNA. PCR was carried out in a Perkin-Elmer thermocycler (CT 2400 ICA, USA) with an initial denaturing at 95°C for 2 min; 30 cycles of denaturing at 95°C for 1 min, annealing at 50°C for 30 s, and extension at 72°C for 2 min; afterwards at 72°C for 10 min as single final extension cycle. The PCR product was purified using a QIAquick kit (Qiagen, USA) according to the instructions given by the manufacturer. The fragment amplified was verified on a 0.8% agarose gel, and the gel was stained as above. The remaining PCR-products were sequenced in both directions in an ABI PRISM 3700 (Applied Biosystems, USA). The sequences were edited with Lasergene 2001, V.5 Software (DNASTAR Inc., USA), and the most related sequence was obtained using GenBank-Blast (NCBI-National Centre for Biotechnology Information). DNA sequences were aligned with the profile mode of Clustal W, and an evolutionary distances matrix was generated with Kimura 2 parameter substitution model. The evolutionary tree for the datasets was constructed with the neighbour-joining algorithm and the confidence of the tree was assessed by bootstrap analysis based on 5000 boot strap replications using MEGA 3.1 software [25].

Results and discussion

Isolates obtained

Of the symptomatic and symptomless kernels, eight and 13 isolates were obtained, respectively, on the basis of colour in PDA slant tubes (Table 2).

Morphological characterization

Typical *Fusarium* structures were observed in the 21 isolates selected. In general, we observed non-coloured septate mycelium, globular to fusiform microconidia with zero to three septate; falcate to cylindric macroconidia with seven septate according to the species; and the mesoconidia (the third type of *Fusarium* conidia) with zero to three septate [21], some species had brown coloured chlamydospores (Figure 1).

The species identified morphologically were *F. chlamydosporum* (9.52%), *F. napiforme* (14.29%), *F. poae* (14.29%), *F. pseudonygamai* (23.8%), *F. solani* (14.29%), *F. subglutinans* (14.29%), and *F. verticillioides* (9.52%) (Table 2).

Four species of *Fusarium*; *F. napiforme* (37.5%), *F. solani* (37.5%), *F. subglutinans* (12.5%), and *F. verticillioides* (12.5%), were obtained from symptomatic kernels, however from apparently healthy kernels five species were isolated out of which only two were present in both symptomatic and symptomless kernels; *F. subglutinans* (15.4%) and *F. verticillioides* (7.7%). The remaining *Fusarium* colonies belonged to three different species; *F. chlamydosporum* (15.4%), *F. poae* (23%), and *F. pseudonygamai* (38.5%). These results indicate that these species of *Fusarium* are latent inside the seed as reported by White and Munkvold and Desjardins [4, 5], and when the phytopathogenic species have appropriate environmental conditions they grow systemically and affect the crop, thus, becoming sources of dissemination of these pathogens to other areas.

The seven species found are associated with other maize diseases, and were found throughout the plants. *F. proliferatum*, *F. subgutinans*, and *F verticillioides* have been isolated in leaf axil and other maize tissues during ear fill, while *F. solani* was only reported associated with maize in India [26, 27]. *F. poae*, and *F. solani*, which cause head blight and root rot in maize, are transmitted by seed [16, 28]. *F. chlamydosporum*, *F. poae*, and *F. pseudonygamai* have been associated with other gramineous species such as *Pennisetum typhoides* and *Sorghum caffrorum* [19].

Five of the species identified produce mycotoxins such as fusaric acid and/or fumonisin (*F. napiforme*, *F. subglutinans*, and *F. verticillioides*); fusarin (*F. poae*, and *F. verticillioides*), moniliformin

Table 2. Morphological comparison of the structures of seven *Fusarium* species associated with ear rot of maize obtained from symptomatic and symptomless ears during fall to winter 2002 in Montecillo, Mexico

Morphological characteristics	*Gibberella fujikuroi* complex					*F. poae**,**	*F. solani***
	*F. napiforme***	*F. pseudonygamai**,**	*F. subglutinans**,**	*F. verticillioides**,**	*F. chlamydosporum**,**		
Microconidia (length × width in μm)	9.8 × 5.0[a] , 10.2 × 2.4[b], 19.25 × 3.9[c]	11.1 × 2.42, 5.0–13.8 × 1.9–4.0	11.32 × 3.1, 8–12 × 2.5–3	11.8 × 2.4, 5.0–12.0 × 1.5–2.5	9.7 × 3.3	11.46 × 7.0, 8–12 x 7–10 [d]	9.5 × 3, 8–16 × 2–4
Microconidia in chains	+	+ (short)	–	+	–	–	–
False heads microconidia	+	+	+	+	–	–	+
Mesoconidia	–	–	1–3 septate	–	0–3 septate.0	–	–
Shape and size (length × width in μm)			17.0 × 4.5		11.0 × 4		
Macroconidia (length × width in μm)	43.5 × 3.8	39.0 × 4.0	37.4 × 4.2	31.0 × 3.7	40.4 × 3.5	22.1 × 3.6	50.3 × 5.0
Conidiophore	Branched	Single or branched	Branched	Single or branched	Branched	Branched	Branched
Phialides (length × width in μm)	Monophialide	Monophialide	Monophialide	Monophialide	Monophialide Polyphialide	Monophialide	Monophialide
	27.0 × 3.0	26.0 × 2.43 Polyphialide	23.5 × 2.7 Polyphialide	22.0 × 3.5	26.7 × 2.7 Polyphialide	14.0 × 6.0	45.0–73.0 × 2.4
Chlamydospores	+	–	–	–	+	–	+
Colour[e]	White/purple	White/orange, gray	White purple /pink-purple	Purple gray /pink-purple	brown/brown	White pink /orange	Light cream /light cream
Isolates	2, 4, 6	10B, 13A, 13B, 18, 19	9, 11, 14	5, 10A	16, 17	12, 15A, 15B	3, 7, 8

*Isolates obtained from 100 symptomless kernels.
** isolates obtained from symptomatic kernels.
[a]Napiform conidia with 0–1 septate.
[b]Ovoid conidia with 0–1 septate.
[c]Ovoid conidia with 1–3 septate.
[d]Ampuliform conidia
[e]Slant tubes containing PDA with amended potato pulp.

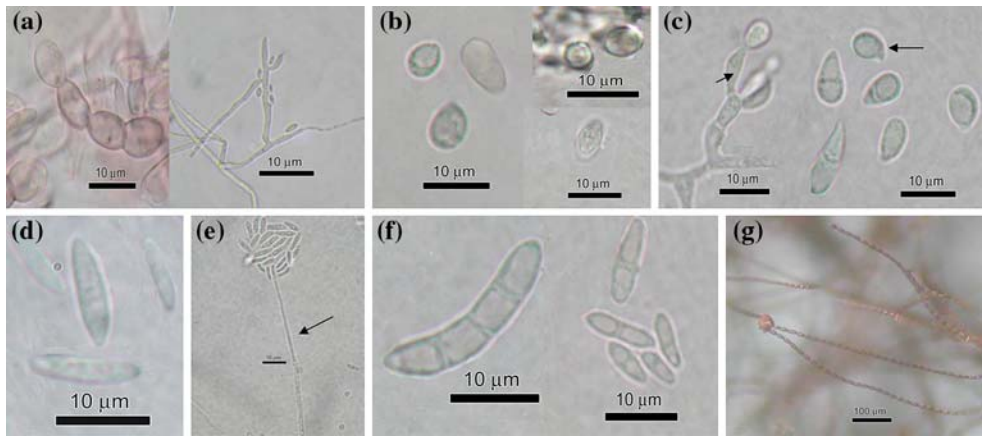


Figure 1. Morphological differential features of *Fusarium* species. (a) *F. chlamydosporum*: brown chlamydospores in chains and polyphialide, (b) *F. napiforme*: napiform microconidia (globose with a small protuberance), (c) *F. poae*: ampuliform microconidia with papilla, 0–1 septate and monophialide, (d). *F. pseudonygamai*: large microconidia aseptate, (e) *F. solani*: false heads microconidia, and large monophialide, (f) *F. subglutinans*: large mesoconidia, fusiform, straight 1–3 septate, and G. *F. verticillioides:* microconidia in large chains observed on Petri dish under the low power of the microscope 10×.

(*F. chlamydosporum*, *F. napiforme*, *F. subglutinans*, and *F. verticillioides*); trichothecenes (*F. poae*) and naphthazarin (*F. verticillioides*) in maize kernels [29]. *F. pseudonygamai*, and *F. solani* have not been reported to produce mycotoxins [21, 29, 30].

Phylogenetic analysis

Analysis of ITS and nLSU regions

PCR was successfully performed and bands ((550 bp) were obtained. Each sequence was compared to sequences of the same species deposited at GenBank. Similarities ranged from 99.6% to 100%. Total nucleotides amplified were 470–533 bp and corresponded to the ITS1 and ITS2 complete regions; the 3′ portion of the 18S gene, 5.8S complete sequence, and the 5′ end of the 28S gene. PCR-products corresponding to nLSU gene were approximately 600 bp. With respect to ITS sequences, the isolate identified morphologically as *F. chlamydosporum* did not have any ITS sequence corresponding to the species deposited at GenBank database, so its closest relative was *Fusarium* spp. (AF158314) with a percentage of similarity of 98.6% (Table 3).

The 28S rRNA gene partial sequences showed the same results as the ITS region. However, in the case of the isolate identified morphologically as *F. chlamydosporum* it was 98.6% equal to *F. chlamydosporum* (accession number AY213706). This result could be explained considering that for some species of *Gibberella fujikuroi* complex, the 28S gene gives a better resolution than ITS region. The ITS and nLSU sequences were deposited at Genbank NCBI (Table 3). Also, all isolates were placed in the Fungal Collection of Colegio de Postgraduados (registration on World Data Centre for Microorganisms in process).

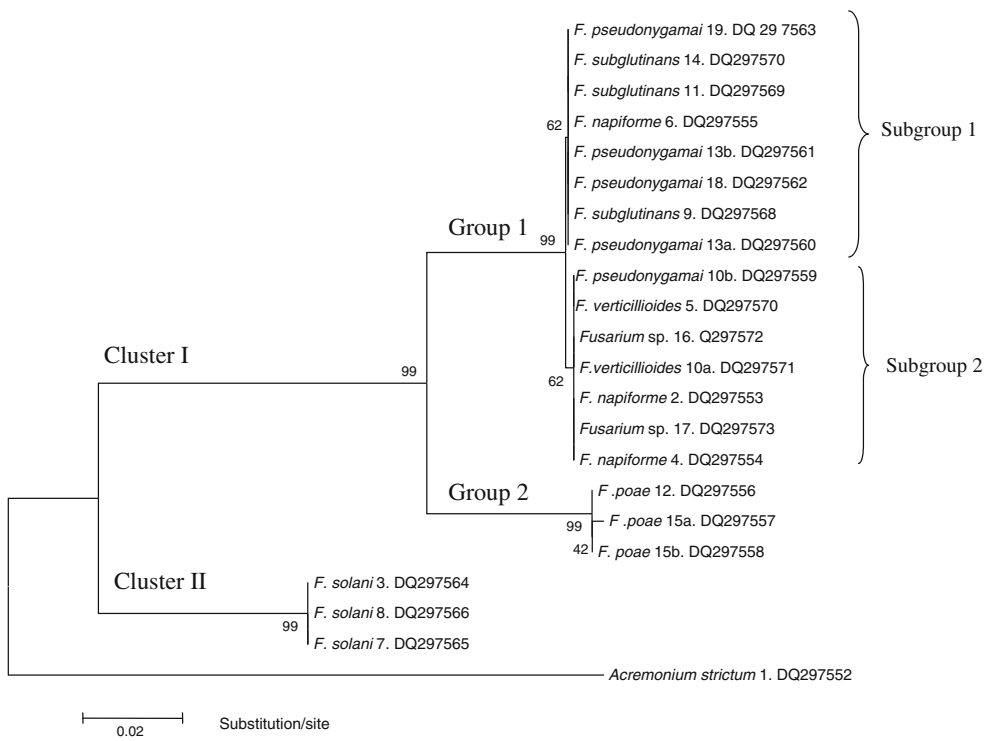
The species formed two clusters (Cluster I and II) (Figure 2). Cluster I had two groups (Group 1 and 2). Group 1 corresponded to two of the 16 sections as proposed by Wollenweber and Reinking [17] and both sections were in the *G. fujikuroi* complex [21]; Liseola section with *F. pseudonygamai*, *F. subglutinans*, and *F. verticillioides*, and Dlaminia section with *F. napiforme*. Group 2 also had two subgroups as evidenced by nucleotidic differences in the sequences of the isolates (Figure 3). *F. pseudonygamai* (DQ297559) had a cytosine at base 99 of ITS1, and not a thiamine as the other isolates of this species and *F. napiforme* (DQ297555) had a thiamine at base 99 of ITS1, and not a cytosine as in the isolate DQ297553. The isolate DQ 297554 of this species had a guanine at base 29, which corresponded to the beginning of the 28S gene. It also did

Figure 2. Phylogenetic tree based on ITS region of 21 Mexican isolates of *Fusarium* species obtained from ear rot (symptomatic and symptomless kernels) using Kimura 2 parameter substitution method. The evolutionary scheme was constructed with the neighbor-joining algorithm using MEGA 3.1 software. The confidence of the tree was assessed by bootstrap analysis based on 5000 replications.

Table 3. Molecular characterization of 21 *Fusarium* isolates from symptomatic and symptomless ear rot of maize using ITS region in Mexico

Isolates	Morphological identification	Accession most related (Blast-GenBank)	Value of A[a]	Similarity index[b]	Difference in nucleotides	Proceeding
16	*F. chlamydosporum* DQ297572	*Fusarium* sp. AY213706	1001	100	0	USA
17	*F. chlamydosporum* DQ297573	*Fusarium sp*. AY213706	1001	100	0	USA
2	*F. napiforme* DQ297553	*F. napiforme* X94175	1005	99.8	1	Netherlands
4	*F. napiforme* DQ297554	*F. napiforme* X94175	1021	99.6	2	Netherlands
6	*F. napiforme* DQ297555	*F. napiforme* X94175	1021	100	0	Netherlands
12	*F. poae* DQ297556	*F. poae* AY053440	963	100	0	Norway
15A	*F. poae* DQ297557	*F. poae* AY053440	942	99.8	0	Norway
15B	*F. poae* DQ297558	*F. poae* AY053440	942	100	0	Norway
10B	*F. pseudonygamai* DQ297559	*F. pseudonygamai* U34563	985	99.8	1	USA
13A	*F. pseudonygamai* DQ297560	*F. pseudonygamai* U34563	1005	100	0	USA
13B	*F .pseudonygamai* DQ297561	*F. pseudonygamai* U34563	1005	100	0	USA
18	*F. pseudonygamai* DQ297562	*F. pseudonygamai* U34563	985	100	0	USA
19	*F. pseudonygamai* DQ297563	*F. pseudonygamai* U34563	985	100	0	USA
3	*F. solani* DQ297564	*F. solani* AY755617	977	99.6	2	Mexico
7	*F. solani* DQ297565	*F. solani* AY755617	989	99.6	2	Mexico
8	*F. solani* DQ297566	*F. solani* AY755617	1019	99.6	2	Mexico
9	*F. subglutinans* DQ297567	*F. subglutinans* X94167	993	99.8	1	Netherlands
11	*F. subglutinans* DQ297568	*F. subglutinans* X94167	999	99.8	1	Netherlands
14	*F. subglutinans* DQ297569	*F. subglutinans* X94167	1019	99.8	1	Netherlands
5	*F. verticillioides* DQ297570	*F. verticillioides* AY533376	1043	99.8	1	Austria
10A	*F. verticillioides* DQ297571	*F. verticillioides* AY533376	999	99.8	1	Austria

[a]Alignment.
[b]Alignment done with Lasergene 2001 V.5 software (DNASTAR, Inc. Madison, USA).



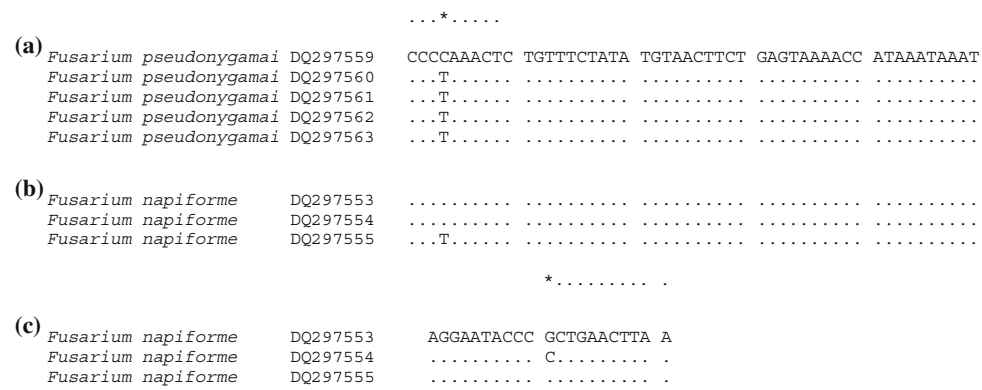


Figure 3. Alignment showing differences in nucleotides: (A) ITS1 of *Fusarium pseudonygamai*, base 99 (asterisk). (B) ITS1 of *F. napiforme*, base 99 (asterisk). (C) 28S gene of *F. napiforme*, base 29 (asterisk).

not have a cytosine as in the other two isolates analysed in this same region (Figure 3). These results clearly show that these two species, as well as *F. subglutinans*, are separated into reproductively isolated populations that probably constitute separate sibling species [7], and in the future they may form different monophyletic species.

Group 2 included three isolates of *F. poae*, of the Sporotrichiella section. Isolate DQ297557 had a thiamine at the 438 base of ITS2, and not an adenine as in the other two isolates.

In the cluster II there were the three *F. solani* isolates that have been grouped by Snyder and Hansen [18] in the Martiella y Ventricosum section of the *F. solani* complex [31]. *Acremonium strictum* was considered as and outgroup (Figure 3).

The position of species in the Liseola and Dlaminia sections in the same group can be explained by the fact that both had *Gibberella* as the teleomorph, are part of the same complex, and produce moniliformin. The isolates identified as *F. chlamydosporum*, according to its morphology and considered as *Fusarium* spp. by ITS Blast search, included in the same group was unexpected because its teleomorph is unknown. However, it produces moniliformin [29], and this fact might reflect its relation with the other species.

Through morphological and phylogenetic analysis, seven species of *Fusarium* identified as *F. chlamydosporum*, *F. napiforme*, *F. poae*, *F. pseudonygamai*, *F. solani*, *F. subglutinans*, and *F. verticillioides*, were found to be associated with ear rot disease, and *F. chlamydosporum*, *F. poae*, *F. pseudonygamai*, *F. subglutinans*, and *F. verticillioides* were found within asymptomatic kernels as well. Four of the identified species have not been reported previously to be associated with ear rot in Mexico. For this reason the biodiversity of *Fusarium* species involved in ear rot in Mexico appears to be greater than that reported previously in other places in the world. This new knowledge will permit a better understanding of the relationship between all the species involved in ear rot disease and their relationship with maize.

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Address for correspondence: I. Morales-Rodríguez, Colegio de Postgraduados, Campus Montecillo, Seed Production Program, km 36.5 Carr. Mexico-Texcoco, Montecillo, Edo. de Mexico CP 56230, Mexico
Phone: + 52-55-58045963; Fax: + 52-55-58045962
E-mail: imorales@colpos.mx