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TAXONOMY OF *FUSARIUM* GENUS, A CONTINUOUS FIGHT BETWEEN LUMPERS AND SPLITTERS

ABSTRACT: The genus *Fusarium* comprises a high number of fungal species that can be plant-pathogenic, causing diseases in several agriculturally important crops including cereals, and also can be harmful for humans and animals since many of them are toxigenic.

The identification of mycotoxigenic *Fusarium* species still remains a most critical issue, given that the number of species recognized in the genus has been constantly changing in the last century in accordance with the different taxonomic systems. Together with the morphological identification, current criteria for *Fusarium* species identification are also based on biological and phylogenetic species recognition. However these criteria rarely agree to each other. Therefore, it is still a charming scientific challenge to ascertain the taxonomic status of *Fusarium* species, which in the years have been continuously „splitted" and „lumpered" by scientists. The major cases of the taxonomic debates amongst the *Fusarium* community will be here discussed.

KEY WORDS: *Gibberella*, morhology, biological identification, phylogeny.

INTRODUCTION

The genus *Fusarium* comprises a high number of fungal species that can be plant-pathogenic, causing diseases in several agriculturally important crops, including cereals, and can also be harmful for humans and animals. Many of them produce a wide range of biologically active secondary metabolites (e. g. mycotoxins) with extraordinary chemical diversity. The biological activity of *Fusarium* mycotoxins can be detrimental to plants, and it is associated with cancer and other diseases in humans and domesticated animals. The combined effect of *Fusarium* species infecting several crops and producing mycotoxins in the field is the contamination of cereal grains and other plant-based foods. With many pathogenic and opportunistic species of the genus colonizing plants as a part of the complex of *Fusarium* species, it provides an interesting example of biodiversity, as well as the consequences of different environmental conditions that exist in the various agro-ecosystems in which crops are

cultivated. These conditions can also influence the fungal-plant interactions of the single species and their capability to produce mycotoxins. Moreover, the ability of various *Fusarium* species within the complexes to produce different classes of secondary metabolites combined with their ability to coexist in the same host or/and occur in quick succession have allowed these complexes to become „invincible armadas" against many plants.[1] Plant infections by *Fusarium* can occur at all developmental stages, from germinating seeds to mature vegetative tissues, depending on the host plant and *Fusarium* species involved. Therefore, since most *Fusarium* species have specific mycotoxin profiles, early and accurate identification of the *Fusarium* species occurring in the plants, at every step of their growth, is critical to predict the potential toxicological risk to which plants are exposed and to prevent toxins entering the food chain. However, the unambiguous identification of mycotoxigenic *Fusarium* species still remains a most critical issue, given that the number of species (which stands now over 80)[2] recognised in the genus was constantly changing during the last century in accordance with the different taxonomic systems. Furthermore, this genus is provided of few morphological characters useful for species discrimination based only on traditional technique, although, fortunately, some of the most important toxigenic and pathogenic *Fusarium* species can be diagnosed, with some experience, by using only their morphological traits. Considering that the current criteria for *Fusarium* species identification (e. g. morphological (MSR),[3, 4] biological (BSR)[5] and phylogenetic species recognition (PSR)[6]) rarely concur, and that, out of 101 most economically important plants, 81 have at least one plant associated with *Fusarium* disease,[2] along with the fact that each *Fusarium* species keeps its own toxicological profile,[7] it is a challenge to ascertain the taxonomic status of *Fusarium* species on their phenotypical characteristics (including pathogenicity and toxigenicity) alone (www.apsnet.org/online/common/search.asp).

41.1.1. Classification and morphology of *Fusarium*

The genus *Fusarium* belongs to the *Ascomycota* phylum, *Ascomycetes* class, *Hypocreales* order,[8] while the teleomorphs of *Fusarium* species are mostly classified in the genus *Gibberella*, and for a smaller number of species, *Hemanectria* and *Albonectria* genera. For a complete review of the main taxonomic systems that have contributed to the defining of the modern taxonomy of *Fusarium*, see the excellent work of Leslie and Summerell,[2] which contains an updated description, not only morphological, of 70 species within the genus.

The main approach for the *Fusarium* classification is still morphology, and the primary trait for species to be placed in *Fusarium* genus is the occurrence of the asexual spores, the distinctive banana-shaped macroconidia, firstly diagnosed by Link.[9] *Fusarium* species produce three types of spores: macroconidia, microconidia and chlamydospores.[2] Septated macroconidia can be produced on monophialides and polyphialides in the aerial mycelium, but also on short monophialides in specialized structures called sporodochia.[10] A mono-

phialide is a conidiation cell with a unique pore from which the endoconidia are released; a polyphialide can possess several such openings. Microconidia can vary in shape and size, and are produced in the aerial mycelium in clumps or chains, both on monophialides and polyphialides. Finally, chlamydospores are resistance structures with thickened walls and high lipid content; in the case of their presence, they can form in the middle of the hyphae or at their termini. The different shape of macroconidia remains the most important feature for distinguishing the species. Moreover, other traits, such as the presence/absence of microconidia and their shape, the presence/absence of chlamydospores, and the characteristics of the micro- and macro-conidiogenous cells, contribute to distinguishing species in *Fusarium*. In order to identify the species, all taxonomists suggest the use of strain cultures derived from single--spore isolation, and growing the strains on special media under standard incubation conditions. All taxonomic systems developed so far are based on a seminal work by Wollenweber and Reinking[11] with various modifications.[4, 12] This publication organized the genus in 16 Sections, including 65 species, 55 varieties and 22 forms. The main discriminating criteria among the Sections were based on morphology; in particular, on the presence and shape of microconidia, on the presence and position of chlamydospores in the hyphae, on the shape of macroconidia and their basal cells. The taxonomic system described by Gerlach and Nirenberg[3] kept the number of the Sections as Wollenweber and Reinking, while Nelson et al.[4] proposed a simpler classification method that divided the genus in 12 Sections. Although the main taxonomic systems have organized the Sections with species sharing common morphological characteristics, thus, supposedly genetically related, not all researchers accept this Section concept since some of the used morphological characteristics are now considered of poor reliability from an evolutionary point of view, according to the recent molecular investigations. On the other hand, the classification of *Fusarium* species has still a number of open question marks that need to be solved and should require the use of all species recognition methods in an integrated approach.

   The *G. fujikuroi* species complex. *Gibberella fujikuroi* (Sawada) Ito in Ito e K. Kimura has long been considered the teleomorph of several *Fusarium* species, morphologically placed by Nelson et al. in the *Liseola* section.[4] Within this complex, Nelson et al.[4] comprised 4 anamorphic species, including the maize pathogens, *F. moniliforme*, *F. proliferatum*, and *F. subglutinans*, and a minor species, *F. anthophilum*. On the other hand, Gerlach and Nirenberg[3] identified 10 species in Section *Liseola* and adopted the name of *F. verticillioides* (Sacc.) Nirenberg (teleomorph *Gibberella moniliformis*), instead of *F. moniliforme*, as generally accepted by the research community of *Fusarium*.[13] As for many other fungal phyla, taxonomic results based on MSR, BSR and PSR (see Introduction) have recently started being compared also for *G. fujikuroi* species complex. According to BSR, many reports have now clarified that *G. fujikuroi* species complex includes at least 11 different biological species or Mating Populations (MPs): MP-A (*G. moniliformis*, anamorph *F. verticillioides*), MP-B (*G. sacchari* anamorph *F. sacchari*), MP-C (*G. fujikuroi* anamorph *F. fujikuroi*), MP-D (*G. intermedia* anamorph *F. proliferatum*), MP-E

(*G. subglutinans* anamorph *F. subglutinans*), MP-F (*G. thapsina* anamorph *F. thapsinum*), MP-G (*G. nygamai* anamorph *F. nygamai*), MP-H (*G. circinata* anamorph *F. circinatum*), MP-I (*G. konza* anamorph *F. konzum*), MP-J (*G. gaditjirrii* anamorph *F. gaditjirrii*), and MP-K (*G. xylarioides* anamorph *F. xylarioides*).[2] The results of sexual crosses, integrated with morphological observations and molecular data by using RAPD, AFLP, RFLP and DNA sequencing,[6, 14—17] have shown that the results of the three classification methods (biological, morphological, phylogenetic) are largely congruent. However, phylogenetic analyses carried out by O'Donnell et al.[14] using several genes, among which b-tubulin and calmodulin, revealed 46 species in the *G. fujikuroi* complex, of which 23 are new to science. Among the 46 species, the 11 species identified by using biological species concept have been reported identical to the phylogenetic species, which indicates that phylogenetic approach can provide the same information as biological approach and that other MPs still need to be identified.

The *F. graminearum* species complex. The recent re-classification of *F. graminearum* (teleomorph, *G. zeae*), a worldwide pathogen of wheat and maize, is controversial. *Fusarium graminearum* produces several mycotoxins, mainly trichothecenes, which are tricyclic sesquiterpenes, that have been strongly associated with chronic and fatal toxicoses of humans and animals, and zearalenones, which have estrogenic activity.[7] *Fusarium graminearum* produces multiple trichothecene analogues, in particular deoxynivalenol (DON) and nivalenol (NIV), and their acetylated derivatives, 3-acetil-DON (3A-DON) and 15-acetil-DON (15A-DON). Within this species, strains differ in their trichothecene production profiles; some strain produce DON, some produce NIV, and others produce DON and NIV. Such chemotype diversity within *F. graminearum* is a result of loss of gene function. DNA sequence-based phylogenetic analysis of *F. graminearum* field isolates from six continents delineated eight phylogenetically distinct lineages that were considered biogeographically structured.[18] Among the lineages, lineage 7 was considered as the most geographically widespread, predominating on wheat and maize in North and South America, and in Europe, producing primarily DON.[18] The lineages were considered genetically isolated because each was reciprocally monophyletic within genealogies when the six nuclear genes were analyzed both individually and together. Further studies based on DNA sequence polymorphisms from eleven nuclear genes and three intergenic regions led O'Donnell et al.[19] to describe nine lineages within *F. graminearum* clade and to elevate these lineages to the rank of species. Finally, Starkey et al.[20] described two novel species within the *F. graminearum* species complex based on phylogenetic analyses of multi-locus DNA sequence data of 13 genes. However, not all *Fusarium* researchers agree with the division of *F. graminearum* into multiple species. Some authorities considered the lineages to be subspecies rather than species.[2] This opinion was supported by the finding that in general, isolates from partially inter-fertile phylogenetic species tend to have AFLP band identities in the range of 40—65%, and that *F. asiaticum* and *F. graminearum* have AFLP band identities of 50%. Moreover, only three of the nine lineages show conidial morphology traits useful for differentiating them, and there is no correlation between

lineages and specific mycotoxin profile.[18, 19, 21] Finally, sequencing a portion of *tri101* gene of 400 strains of the *F. graminearum* species complex, Leslie et al.[22] generated both a phylogenetic tree and a genetic network that led the authors to a conclusion that „there is only a single species within *F. graminearum/G. zeae*". Due to these apparently contradictory data, additional studies are necessary to determine whether the different lineages of *F. graminearum* represent distinct phylogenetic species or subspecies lineages. Such studies should also provide insight into the practical implications dividing *F. graminearum* into multiple species with respect to disease management, quarantine regulations and plant breeding strategies, and to understand the ecology, epidemiology, and population dynamics of *F. graminearum* species complex.

The third case, *Fusarium oxysporum*: a species complex? *Fusarium oxysporum* is a plant pathogen causing a wide range of plant diseases mainly related to vascular wilts. However, within the species, many populations isolated mainly from soil have been shown as non pathogenic and they are used as bio-control agents against several diseases also caused by *Fusarium* species.[23] Morphologically, these strains cannot be differentiated from pathogenic strains, although a wide genetic diversity of the population originating from soils has been reported.[23] On the other hand, the majority of the isolates causing vascular wilts are specific for a certain host plant. From taxonomic point of view, these strains have differentiated from each other on the basis of pathogenicity as *formae speciales*. Therefore, the identification of these strains traditionally involves tests of pathogenicity with the appropriate hosts, which are time consuming and can require several months for some *formae speciales*. Moreover, since pathogenicity is not an ancestral character, taxonomic distinctions of strains based only on this are not reliable from an evolutionary point of view, and *formae speciales* should not be considered monophyletic in origin. On the other hand, the basis for *formae speciales* names need not be grounded in traits that are monophyletic in origin [24,25] in order to avoid mistakes in breeding for resistance, and to set up inappropriate quarantine measures.[2]

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ТАКСОНОМИЈА РОДА *FUSARIUM*, СТАЛНА БОРБА МЕЂУ ТАКСОНОМИСТИМА

Антонио Н. Морети

Резиме

Род *Fusarium* обухвата велик број гљивичних врста које могу бити патогене за биљке и које изазивају обољења код неколико важних пољопривредних култура, укључујући и житарице; а како је већина њих токсигена, могу исто тако бити штетне за људе и животиње.

Идентификација микотоксигених *Fusarium* врста је још увек проблематична, с обзиром на то да се број познатих врста овог рода стално мењао у току прошлог века, а у складу са различитим таксономским системима. Уз морфолошку идентификацију, тренутни критеријуми идентификације *Fusarium* врста се базирају на идентификацији биолошких и филогенетичких врста. Међутим, ови критеријуми се ретко међусобно подударају. Из тог разлога је утврђивање таксономског статуса *Fusarium* врсте прави изазов, јер их научници све до данас разврставају по различитим системима који или истичу сличности или истичу разлике између врста. У раду ће бити размотрене неке од најважнијих научних дебата у вези са *Fusarium* заједницом.