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Research Article

Unique Phylogenetic Lineage Found in the *Fusarium*-like Clade after Re-examining BCCM/IHEM Fungal Culture Collection Material

David Triest[1,*], Koen De Cremer[2], Denis Piérard[3] and Marijke Hendrickx[1]

[1]*BCCM/IHEM Collection of Biomedical Fungi, Service of Mycology & Aerobiology, Scientific Institute of Public Health, 1050 Brussels, Belgium*
[2]*Service of Health & Environment, Scientific Institute of Public Health, 1050 Brussels, Belgium*
[3]*Department of Microbiology & Infection Control, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, 1090 Brussels, Belgium*

Abstract Recently, the *Fusarium* genus has been narrowed based upon phylogenetic analyses and a *Fusarium*-like clade was adopted. The few species of the *Fusarium*-like clade were moved to new, re-installed or existing genera or provisionally retained as "*Fusarium*." Only a limited number of reference strains and DNA marker sequences are available for this clade and not much is known about its actual species diversity. Here, we report six strains, preserved by the Belgian fungal culture collection BCCM/IHEM as a *Fusarium* species, that belong to the *Fusarium*-like clade. They showed a slow growth and produced pionnotes, typical morphological characteristics of many *Fusarium*-like species. Multilocus sequencing with comparative sequence analyses in GenBank and phylogenetic analyses, using reference sequences of type material, confirmed that they were indeed member of the *Fusarium*-like clade. One strain was identified as "*Fusarium*" *ciliatum* whereas another strain was identified as *Fusicolla merismoides*. The four remaining strains were shown to represent a unique phylogenetic lineage in the *Fusarium*-like clade and were also found morphologically distinct from other members of the *Fusarium*-like clade. Based upon phylogenetic considerations, a new genus, *Pseudofusicolla* gen. nov., and a new species, *Pseudofusicolla belgica* sp. nov., were installed for this lineage. A formal description is provided in this study. Additional sampling will be required to gather isolates other than the historical strains presented in the present study as well as to further reveal the actual species diversity in the *Fusarium*-like clade.

Keywords *Fusarium*, *Fusicolla*, *Microcera*, *Nectriaceae*, Phylogeny, *Pseudofusicolla*

In 2011, a phylogenetic study from Gräfenhan *et al.* [1] showed that the *Fusarium* genus (*Hypocreales*, *Nectriaceae*) was not monophyletic. They found that the genus was divided into two groups in their phylogenic tree of *Nectriaceae*, separated by a large number of species classified in genera such as *Neonectria* and *Volutella* [1]. These groups were referred to as the "basal *Fusarium* clade" and the "terminal *Fusarium* clade." In order to retain the monophyly of *Fusarium*, Gräfenhan *et al.* [1] narrowed the generic concept of *Fusarium* and the basal *Fusarium* clade was no longer considered as *Fusarium* sensu stricto. The few species and lineages represented in this *Fusarium*-like clade were moved to new, re-installed or existing genera (i.e., *Atractium*, *Microcera*, *Macroconia*, *Fusicolla*, *Dialonectria*, and *Stylonectria*), or provisionally retained as "*Fusarium*" or as one of its teleomorphs (i.e., *Nectria* and *Cosmospora*), based upon phylogenetic and morphological analyses [1, 2].

Recently, Lombard *et al.* [3] phylogenetically re-evaluated the generic concepts in the *Nectriaceae* and confirmed the monophyly of each of these *Fusarium*-like genera. Lombard *et al.* [3] also further narrowed *Fusarium* sensu Gräfenhan *et al.* [1] according to its internal phylogenetic structure and thereby applied the "one fungus, one name" concept adopted in the International Code of Nomenclature for Algae, Fungi and Plants as of January 2013 in order to abolish the dual naming system (cfr. teleomorphic name is preferred). They installed a new genus *Bisifusarium* and applied several teleomorphic names for some important groups of *Fusarium* anamorphs. In doing so, Lombard *et al.* [3] rejected the

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*Corresponding author
E-mail: David.Triest@wiv-isp.be

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"*Fusarium*-first" proposal made by the *Fusarium* working community [2] to conserve the name *Fusarium* above all linked teleomorphic names for the sake of nomenclatural stability as well as rejected their broad phylogenetic definition of *Fusarium*, which was based upon *Fusarium* sensu Gräfenhan *et al.* [1] and the refinements made by O'Donnell *et al.* [4].

Species of the *Fusarium*-like clade are mostly slow growing and produce a characteristic orange, conidial slime, known as pionnotes, rather than an aerial mycelium [1]. Species identification based upon morphology is difficult in the *Fusarium*-like clade. They often occur as saprophytes in the soil, on trees or other fungi, and in aquatic environments [1, 2]. In contrast to the well-studied species diversity of *Fusarium* sensu Gräfenhan *et al.* [1], they have not been reported as plant pathogens or opportunistic human pathogens and only a few have been described to produce mycotoxins [2, 5]. Members of the *Fusarium*-like clade have therefore been largely neglected for a long period of time. As a consequence, not much is known about the actual phylogenetic species diversity in this clade. It was only after the taxonomical revision of Gräfenhan *et al.* [1] that some DNA marker sequences became available.

Among the *Fusarium* strains preserved by the Belgian culture collection of biomedical fungi BCCM/IHEM, six appeared to belong to the *Fusarium*-like clade based upon their morphological appearance during re-identification [6]. All six strains were slow growing and produced pionnotes, i.e., typical morphological characteristics of only some *Fusarium*, but many *Fusarium*-like species. The objective of the present study was to determine whether these strains indeed belong to the *Fusarium*-like clade in the phylogeny of the *Nectriaceae* as well as to confirm their identity up to the genus and species level. This was achieved by performing multilocus sequencing and phylogenetic analyses using reference sequences of type material. Also morphological analyses and mating experiments were performed.

MATERIALS AND METHODS

Strains. The six strains, used in this study, were collected and preserved by the BCCM/IHEM fungal culture collection after their isolation from different substrates (Table 1). All were identified based upon their morphology and identified as a *Fusarium* species at the time of their deposit in the collection, more than 20 years ago. They were recently subjected to re-identification, together with the other *Fusarium* strains in the BCCM/IHEM collection [6].

Morphological re-identification. The six strains were re-analyzed morphologically, according to the procedure previously applied by Hosoya and Tubaki [7] for their description of *Fusicolla matuoi*, a member of the *Fusarium*-like clade. This involved cultivation on nutrient-rich agar, i.e., potato dextrose agar (PDA), as well as nutrient-poor agar, i.e., synthetic nutrient agar (SNA) with or without fragments of carnation leaf, at 23°C for 30 days under alternating cycles of light (i.e., day) and dark (i.e., night) conditions. Growth rates were measured from the PDA plates. Colony morphology, pigmentation, conidiogenesis, conidial characteristics, and presence of chlamydospores or other features were described from both the PDA and SNA plates. Conidial measurements were taken from 20 randomly selected conidia. A Nikon eclipse E600 microscope (Nikon, Tokyo, Japan) was used and pictures were recorded with the NIS-Elements BR 4.0 imaging software. The strains were described according to the terminology applied in "The *Fusarium* laboratory manual" of Leslie *et al.* [8].

Re-identification by multilocus sequencing. Multilocus sequencing was applied on the six strains and comparative sequence analyses were performed in GenBank. The internal transcribed spacer (ITS) region and part of the ribosomal large subunit (LSU) were amplified as well as partial fragments of the commonly used *Fusarium* DNA marker genes: translation elongation factor 1-alpha (TEF1α), beta-tubulin (BT), and the second largest subunit of RNA polymerase II (RPB2). DNA extraction, PCR amplification, and sequencing were achieved according to the protocol applied by Beguin *et al.* [9]. Primers were described previously: for ITS by White *et al.* [10], for LSU by Hopple and Vilgalys [11], for TEF1α by Carbone and Kohn [12], for BT by Glass and Donaldson [13], and for RPB2 by Van Hove *et al.* [14].

Phylogenetic analyses. In order to determine whether our six strains belong to the *Fusarium*-like clade in the phylogeny of the *Nectriaceae*, a Bayesian phylogenetic analysis was conducted. Therefore, we used the ITS and RPB2 reference sequences of type material published by Gräfenhan *et al.* [1] and included our strains. The combined sequence dataset was first aligned by the ClustalW algorithm in MEGA4 [15] and edited manually. Four ambiguously aligned regions (two in the ITS and two in the RPB2 gene marker) were excluded from the analysis. Subsequently, Bayesian inference analysis was executed with MrBayes3.2 [16], applying the GTR + I + Γ model of evolution for both loci and estimating parameters separately for each locus. The Monte Carlo Markov chain method was used with runs of one million generations and sampling a tree every 100 generations. The first 25% of sampled trees were discarded (i.e., burn-in) and the consensus tree, with posterior probabilities, was assessed from the remaining trees. The *Acremonium* sp. A104 strain from Grum-Grzhimaylo *et al.* [17] was chosen as outgroup. Tracer v1.5 [18] was used to check the convergence of the likelihood scores and the effective sample sizes for the different parameters.

In order to determine the phylogenetic position of our six strains, taking into account all currently known phylogenetic species diversity of the *Fusarium*-like clade, three other Bayesian phylogenetic trees were constructed according to the same methodology. A first by using the RPB2 reference

Table 1. Strains of the Belgian fungal culture collection BCCM/IHEM used in this study

Strain reference No.	Identity according to multilocus phylogeny	Substrate, geographical origin and time period of isolation	GenBank accession numbers of the amplified DNA marker sequences[a]					Morphological characteristics[b]							
			ITS	RPB2	LSU	BT	TEF1α	Diameter (mm)	Macroconidia: presence, shape, septation	Macroconidial length and width (μm)	Macroconidial apical cell morphology	Macroconidial basal cell morphology	Microconidia: presence, shape, septation	Microconidial length and width (μm)	Chlamydospores: presence and presentation
IHEM 2040	*Fusicolla merismoides*	Humidifier water from air-conditioning, Brussels (Belgium), 1984	KJ125529	KP835471	KJ126417	KJ125825	KJ126121	24	Abundantly produced, slightly falcate, smooth-walled, 3-septate	21~42 × 2~4	Blunt or hooked	Poorly developed	Absent	-	Absent
IHEM 2989	"*Fusarium*" *ciliatum*	Leaf of *Fagus sylvatica* (beech), Pommeroeul (Belgium), 1984	KJ125591	KP835472	KJ126479	KJ125887	KJ126183	25	Abundantly produced, slightly falcate, smooth-walled, 3-septate	25~51 × 2~4	Blunt or hooked	Foot-shaped or poorly developed	Absent	-	Absent
IHEM 5322	Unique lineage in *Fusarium*-like clade	Recycled humidifier water from air-conditioning, Antwerp (Belgium), 1990	KJ125590	KP835473	KJ126478	KJ125886	KJ126182	22	Abundantly produced, strongly falcate, smooth-walled, 0~3 septate (mostly 1-septate, seldom 3-septate)	17~31 × 2~3.5	Blunt or hooked	Poorly developed	Abundantly produced, strongly falcate, 0~1 septate	6~16 × 2~3.5	Abundantly produced, singly and in chains (intercalary and lateral)
IHEM 2105	Unique lineage in *Fusarium*-like clade	Recycled humidifier water from air-conditioning, Brussels (Belgium), 1983	KP835478	KP835476	KP835480	KP835482	KP835484	23	Abundantly produced, strongly falcate, smooth-walled, 0~3 septate (mostly 1-septate, seldom 3-septate)	17~30 × 2~3.5	Blunt or hooked	Poorly developed	Abundantly produced, strongly falcate, 0~1 septate	5~16 × 2~3.5	Abundantly produced, singly and in chains (intercalary and lateral)
IHEM 2413	Unique lineage in *Fusarium*-like clade	Recycled water from spray humidifier, Brussels (Belgium), 1983	KJ125588	KP835474	KJ126476	KJ125884	KJ126180	22	Abundantly produced, strongly falcate, smooth-walled, 0~3 septate (mostly 1-septate, seldom 3-septate)	17~32 × 2~3.5	Blunt or hooked	Poorly developed	Abundantly produced, strongly falcate, 0~1 septate	5~16 × 2~3.5	Abundantly produced, singly and in chains (intercalary and lateral)
IHEM 2440	Unique lineage in *Fusarium*-like clade	Humidifier water from air-conditioning, Belgium, 1984	KJ125589	KP835475	KJ126477	KJ125885	KJ126181	22	Abundantly produced, strongly falcate, smooth-walled, 0~3 septate (mostly 1-septate, seldom 3-septate)	17~32 × 2~3.5	Blunt or hooked	Poorly developed	Abundantly produced, strongly falcate, 0~1 septate	6~15 × 2~3.5	Abundantly produced, singly and in chains (intercalary and lateral)

[a]DNA markers that were sequenced include the internal transcribed spacer (ITS), the ribosomal large subunit (LSU), the translation elongation factor 1-alpha (TEF1α), the beta-tubulin (BT) and the second largest subunit of RNA polymerase II (RPB2).

[b]Diameter after 14 days of growth on potato dextrose agar at 23°C under alternating cycles of light (i.e., day) and dark (i.e., night) conditions. Other morphological characteristics were assessed from the synthetic nutrient agar plates after 30 days of growth at 23°C under alternating cycles of light (i.e., day) and dark (i.e., night) conditions.

sequences of type material from the *Fusarium*-like clade published by Gräfenhan *et al.* [1]. In doing so, more type material from the *Fusarium*-like clade could be included compared to the ITS-RPB2 combined tree, since all reference strains from Gräfenhan *et al.* [1] had been sequenced for RPB2, but not all of them had been sequenced for ITS. A second tree was constructed by using type material published by Bills *et al.* [5] from the "*Fusarium*" *larvarum* complex and the "*Fusarium*" *merismoides* complex, of which the members were respectively moved to *Microcera* and *Fusicolla* by Gräfenhan *et al.* [1]. The ITS, LSU, and BT reference sequences were used, excluding one ambiguously aligned region for the BT gene marker and using the *Viridispora alata* CBS 421.88 strain from Bills *et al.* [5] as outgroup. A third Bayesian phylogenetic tree was constructed by using the ITS reference sequences of the *Fusarium*-like type material from both the studies of Gräfenhan *et al.* [1] and Bills *et al.* [5]. Also for this tree, we used the *Viridispora alata* CBS 421.88 strain from Bills *et al.* [5] as outgroup.

Mating experiments. Sexual crossing experiments were carried out between IHEM 2105, IHEM 2413, IHEM 2440, and IHEM 5322 by co-incubating each time two strains on V8 Juice Agar (VJA) at 23°C for 30 days under alternating cycles of light (i.e., day) and dark (i.e., night) conditions.

RESULTS

Morphological re-identification. The six strains showed a colony diameter between 22~25 mm after 14 days of growth on PDA. All six strains produced a dense sterile, white to (pale) orange, aerial mycelium on PDA (reverse cream colored) and pionnotes on SNA with or without fragments of carnation leaf. Orange pigmentation was

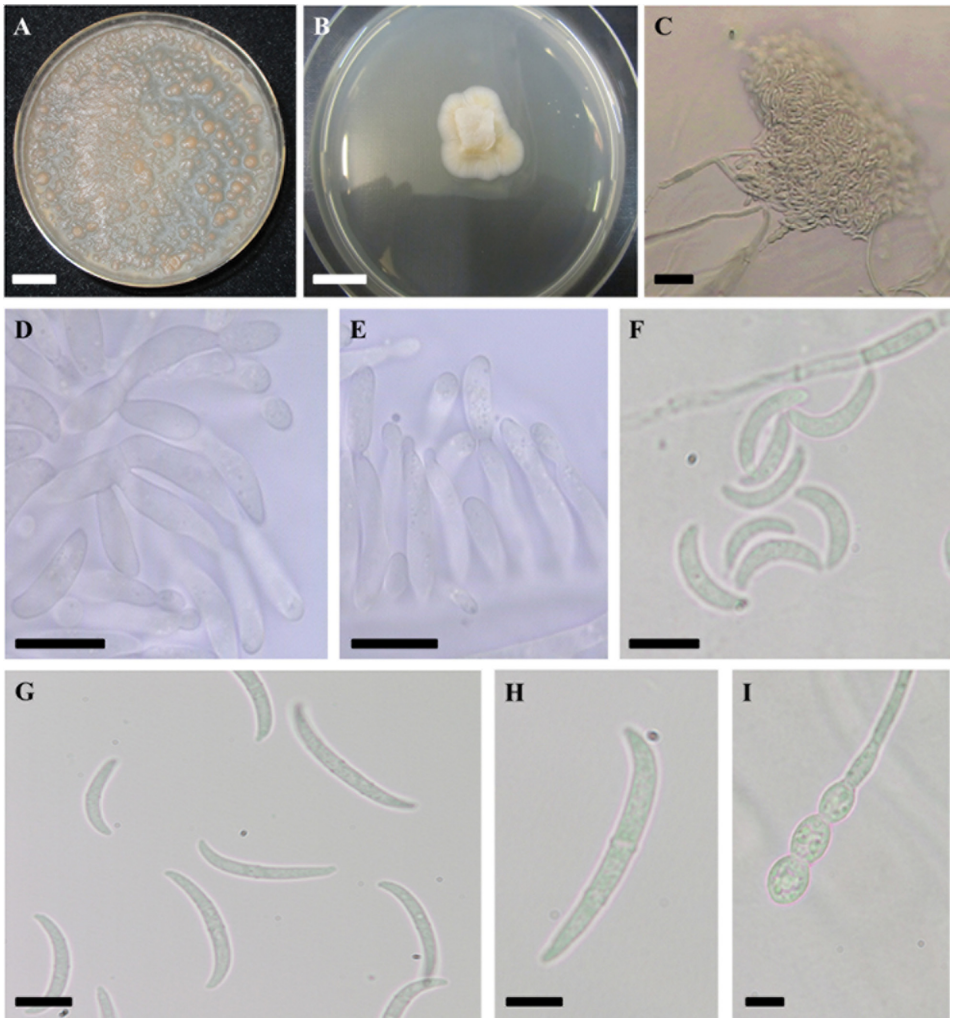

Fig. 1. Morphological characterization of *Pseudofusicolla* gen. nov. and *Pseudofusicolla belgica* sp. nov. (type strain IHEM 2413). A, Pionnotal growth after 30 days on synthetic nutrient agar (SNA); B, Sterile hyphal growth after 7 days on potato dextrose agar; C~I, Characteristics observed on SNA; C, Mass of conidia; D, E, Conidiogenesis; F, Microconidia; G, Macroconidia; H, Characteristic one-septate macroconidium; I, Chlamydospores (scale bars: A, B = 1 cm, C = 20 µm, D, E, H, I = 5 µm, F, G = 10 µm).

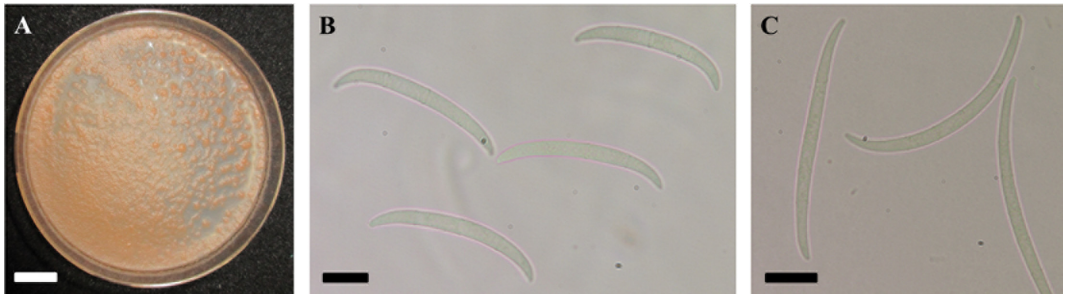

Fig. 2. Macroscopic and microscopic observations made for *Fusicolla merismoides* IHEM 2040 (A, B) and "*Fusarium*" *ciliatum* IHEM 2989 (C). A, Pionnotal growth after 30 days on synthetic nutrient agar (SNA); B, C, Macroconidia on SNA (scale bars: A = 1 cm, B, C = 10 μm).

more pronounced for IHEM 2040 than for the other strains. No aerial mycelium was observed on SNA for any of the strains and conidiogenesis started after a few days from phialides, consisting of branched conidiogenous cells or conidiogenous cells scattered along branched hyphae. Growth rates and conidial measurements as well as information about other morphological characteristics of the strains are enlisted in Table 1 and pictures from the macroscopic/microscopic observations are shown in Fig. 1A~1I and Fig. 2A~2C.

Re-identification by multilocus sequencing. All sequences were deposited in GenBank with the accession numbers given in Table 1. By performing comparative sequence analyses in GenBank, using the amplified sequences for the different loci, we could provisionally identify two of our strains according to their RPB2 sequence. IHEM 2040 and IHEM 2989 were identified as *Fusicolla epistroma* (with 99% sequence similarity) and "*Fusarium*" *ciliatum* (with 100% sequence similarity), respectively, both members of the *Fusarium*-like clade. The identification of IHEM 2989 based upon its RPB2 sequence was confirmed by a BLAST query with its ITS sequence (99% sequence similarity), but not by a BLAST query with its BT sequence (only 94% similarity with a "*Fusarium*" *merismoides* strain). Also the RPB2 identification of IHEM 2040 could not be confirmed by a BLAST query with its ITS sequence (99% similarity with uncultured fungal strains) nor by a BLAST query with its BT sequence, which lead to a *Fusicolla merismoides* identification (99% sequence similarity).

No identification could be obtained for IHEM 2105, IHEM 2413, IHEM 2440, and IHEM 5322, of which the sequences were 100% identical for all five amplified DNA markers. Nevertheless, the highest similarity scores, using the RPB2 and BT sequences of these strains, were, although low (i.e., 85% and 92%, respectively), associated with members of the *Fusarium*-like clade (i.e., *Fusicolla aquaeductuum* and *Microcera larvarum*, respectively). Moreover, comparative sequence analyses performed with the ITS sequences resulted in highest similarity scores (i.e., 99%) associated with sequences of uncultured fungal strains from aquatic habitats or from soils. These strains could, as such, not be allocated to any described taxon.

For all six strains, screening of the LSU sequences resulted in highest similarities (i.e., 99%) with those of unidentified members of the *Hypocreales*, whereas with the TEF1α sequences, no matching sequences were found (query cover of < 20% for five of the six strains).

Phylogenetic analyses. The four Bayesian phylogenetic analyses had effective sample sizes higher than 100 for all parameters, showing sufficient sampling and acceptable mixing of the runs. The obtained consensus trees are illustrated (Figs. 3~6) and were each generated from the 15002 remaining trees after burn-in.

In the phylogenetic tree of nectriaceous fungi (Fig. 3), we could distinguish the *Fusarium* and *Fusarium*-like clade as defined by Gräfenhan *et al.* [1]. Similar to their study, these groups were separated from each other by a large number of species classified in different genera. Though, in contrary to what is seen in the tree of Gräfenhan *et al.* [1], which was constructed using sequences of RPB2 and the larger subunit of ATP citrate lyase, *Fusarium* sensu Gräfenhan *et al.* [1] formed no monophyletic clade in our ITS-RPB2 tree of nectriaceous fungi. The aberrant taxa were the same as those who received a provisional status as "*Fusarium*" in the reference phylogeny of O'Donnell *et al.* [4] (used by Geiser *et al.* [2] for their definition of the *Fusarium* genus) and are now members of *Bisifusarium*, the new genus installed by Lombard *et al.* [3].

The BCCM/IHEM strains with presumed *Fusarium*-like identities were all embedded in the *Fusarium*-like clade (Fig. 3). IHEM 2040 and IHEM 2989 were respectively most closely related to the reference strain of the *Fusicolla* genus and the "*Fusarium*" *ciliatum* reference strain. The strains IHEM 2105, IHEM 2413, IHEM 2440, and IHEM 5322 formed a well-supported (i.e., 100% posterior probability) phylogenetic lineage in the *Fusarium*-like clade, distinct from all the monophyletic *Fusarium*-like genera as defined by Gräfenhan *et al.* [1]. This unique, distinct lineage was also observed and supported by a maximum posterior probability in all our phylogenetic trees of *Fusarium*-like species only (Figs. 4~6).

Similar as in our tree of nectriaceous fungi (Fig. 3), IHEM

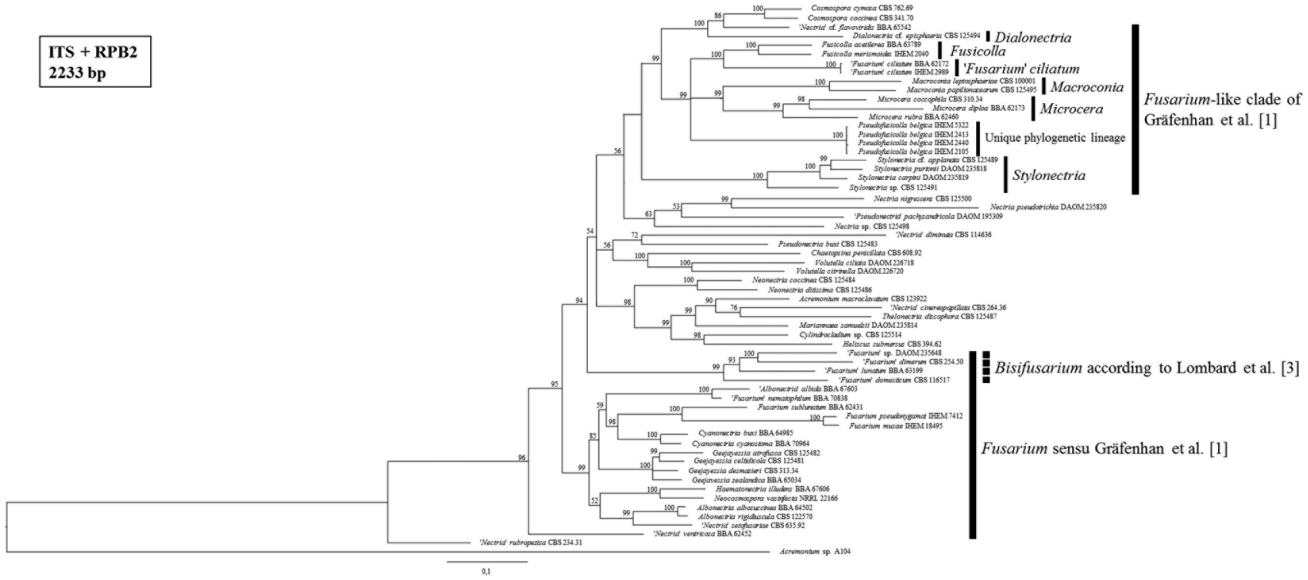

Fig. 3. Consensus tree from the Bayesian phylogenetic analysis, using the internal transcribed spacer (ITS) and second largest subunit of RNA polymerase II (RPB2) reference sequences of nectriaceous fungi published by Gräfenhan *et al.* [1] and including the strains of the Belgian fungal culture collection BCCM/IHEM used in this study (i.e., IHEM 2040, IHEM 2989, IHEM 5322, IHEM 2105, IHEM 2413, and IHEM 2440). Posterior probabilities (%) are represented at the nodes of the tree. An *Acremonium* sp. strain was chosen as outgroup. We can distinguish the *Fusarium* and *Fusarium*-like clade as defined by Gräfenhan *et al.* [1], being separated from each other by a large number of species from different genera. The *Fusarium* sensu Gräfenhan *et al.* [1] clade in our tree is not monophyletic and the aberrant taxa, for which the genus *Bisifusarium* was installed by Lombard *et al.* [3], are indicated by a dashed line. Our six strains with presumed *Fusarium*-like identities are all embedded in the *Fusarium*-like clade, for which the different genera, as defined by Gräfenhan *et al.* [1], are shown.

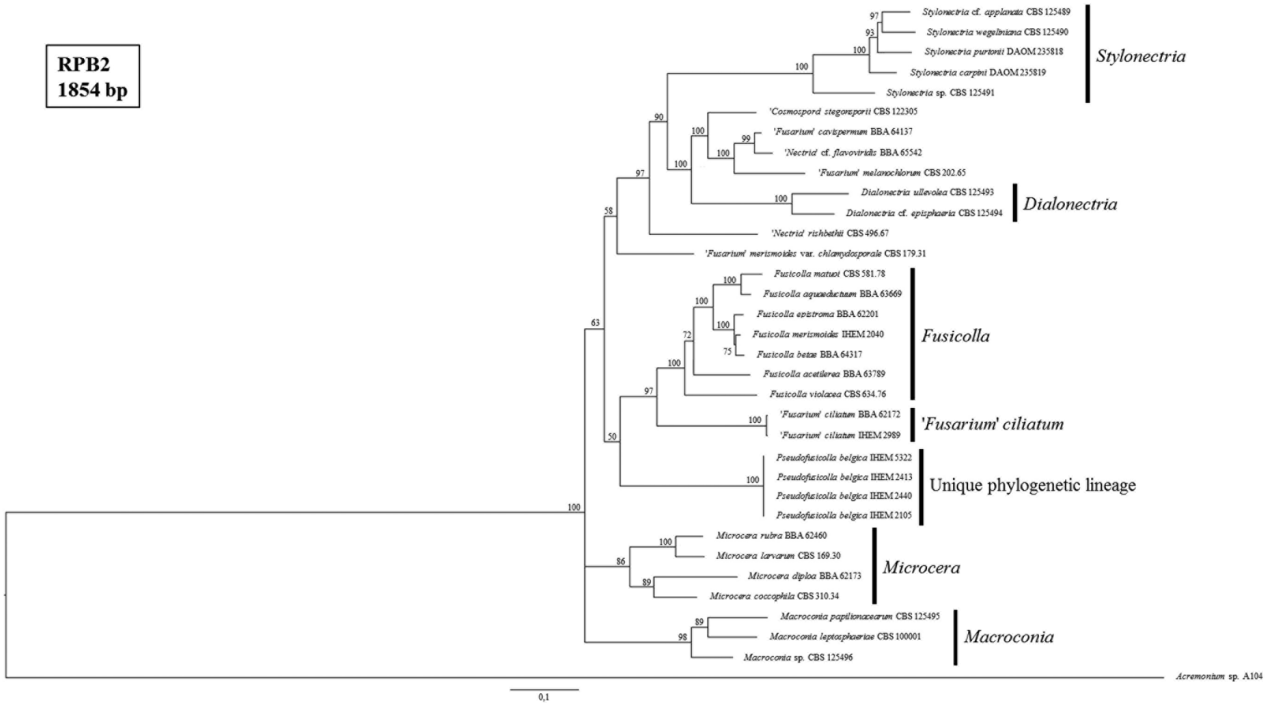

Fig. 4. Consensus tree from the Bayesian phylogenetic analysis, using the second largest subunit of RNA polymerase II (RPB2) reference sequences of species from the *Fusarium*-like clade published by Gräfenhan *et al.* [1] and including the strains of the Belgian fungal culture collection BCCM/IHEM used in this study (i.e., IHEM 2040, IHEM 2989, IHEM 5322, IHEM 2105, IHEM 2413, and IHEM 2440). Posterior probabilities (%) are represented at the nodes of the tree. An *Acremonium* sp. strain was chosen as outgroup. The different genera of the *Fusarium*-like clade, as defined by Gräfenhan *et al.* [1], are indicated.

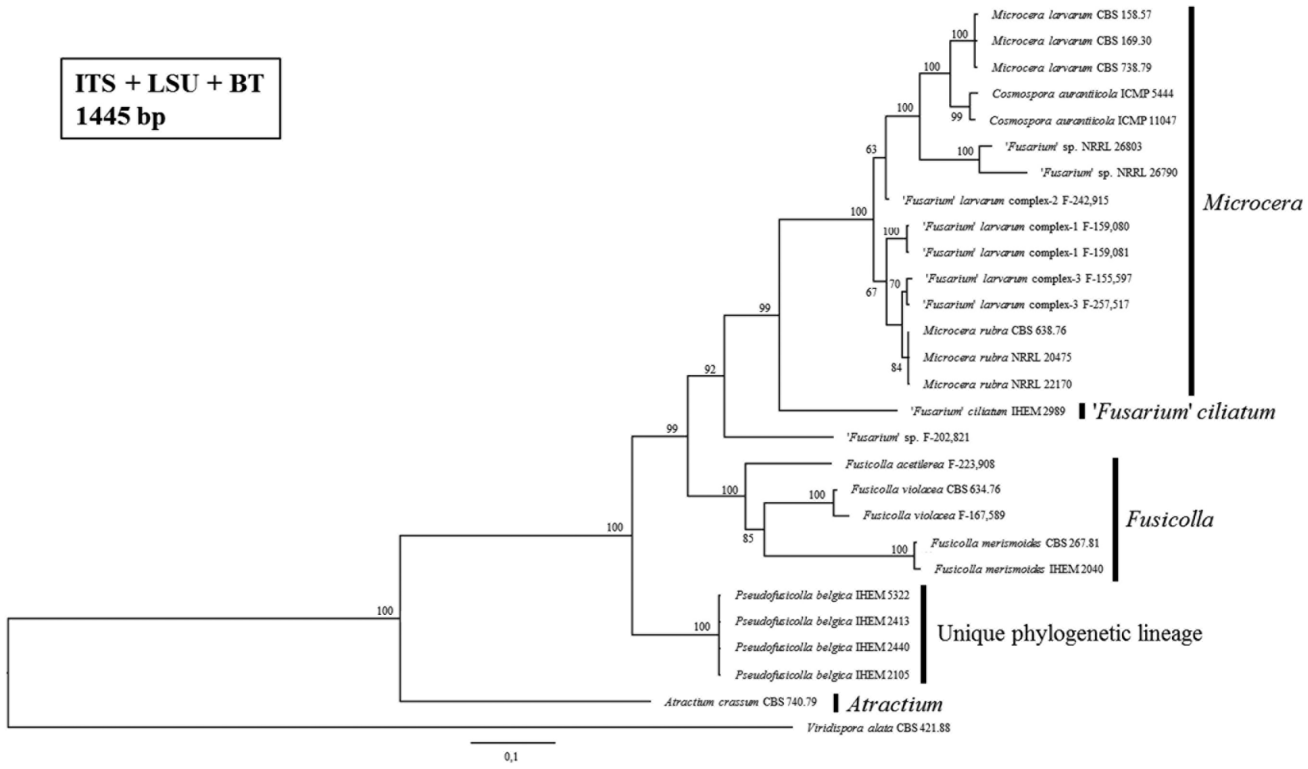

Fig. 5. Consensus tree from the Bayesian phylogenetic analysis, using the internal transcribed spacer (ITS), ribosomal large subunit (LSU), and beta-tubulin (BT) reference sequences of species from the *Fusarium*-like clade published by Bills *et al.* [5] and including the strains of the Belgian fungal culture collection BCCM/IHEM used in this study (i.e., IHEM 2040, IHEM 2989, IHEM 5322, IHEM 2105, IHEM 2413, and IHEM 2440). Posterior probabilities (%) are represented at the nodes of the tree. A *Viridispora alata* strain was chosen as outgroup. The different genera of the *Fusarium*-like clade, as defined by Gräfenhan *et al.* [1], are indicated.

2989 clustered together with the "*Fusarium*" *ciliatum* reference strain in a separate and well-supported clade in both the ITS and RPB2 tree of *Fusarium*-like species only (Figs. 4 and 6). This was not the case in the ITS-LSU-BT tree (Fig. 5), for which no "*Fusarium*" *ciliatum* reference strain could be included due to the absence of a BT reference sequence. These observations are in agreement with the identification obtained for IHEM 2989 by performing sequence similarity searches in GenBank. Consequently, IHEM 2989 is identified as "*Fusarium*" *ciliatum*.

IHEM 2040, on the other hand, appeared to be most closely related to *Fusicolla merismoides* in the ITS-LSU-BT tree as well as the ITS tree of *Fusarium*-like species only (Figs. 5 and 6). This was not the case in the ITS-RPB2 tree (Fig. 3) nor in the RPB2 tree (Fig. 4), for which no *Fusicolla merismoides* reference strain could be included due to the absence of a RPB2 reference sequence. Again, these observations are in agreement with the provisional identifications obtained for IHEM 2040 by performing sequence similarity searches in GenBank. Consequently, IHEM 2040 is identified as *Fusicolla merismoides*.

Mating experiments. All sexual crosses were negative and pionnotal growth was observed after a few days on VJA, together with a sparse, white aerial mycelium.

Taxonomic description.

Pseudofusicolla

Triest, gen. nov. (Fig. 1A~1I). MycoBank No. MB 811910.

Etymology: Latin "*Pseudofusicolla*" = like *Fusicolla*; *Pseudofusicolla* is a genus in the *Fusarium*-like clade as defined by Gräfenhan *et al.* [1] and shows morphological resemblances with *Fusicolla*.

Description: Dense sterile, white to pale orange, aerial mycelium on PDA and pionnotal growth on SNA with or without fragments of carnation leaf. On SNA: conidiogenesis starting after a few days from phialides, consisting of branched conidiogenous cells or conidiogenous cells scattered along branched hyphae; microconidia strongly falcate, aseptate or one-septate; macroconidia strongly falcate, smooth-walled, aseptate to three-septate; chlamydospores present. Teleomorph unknown.

Type species: ***Pseudofusicolla belgica*** Triest, sp. nov. (Fig. 1A~1I). MycoBank No. MB 812587.

Description: On PDA: colony diameter 22~23 mm after 14 days at 23°C; sterile hyphal growth and dense, white to pale orange, aerial mycelium, reverse cream colored. On SNA with or without fragments of carnation leaf: slow

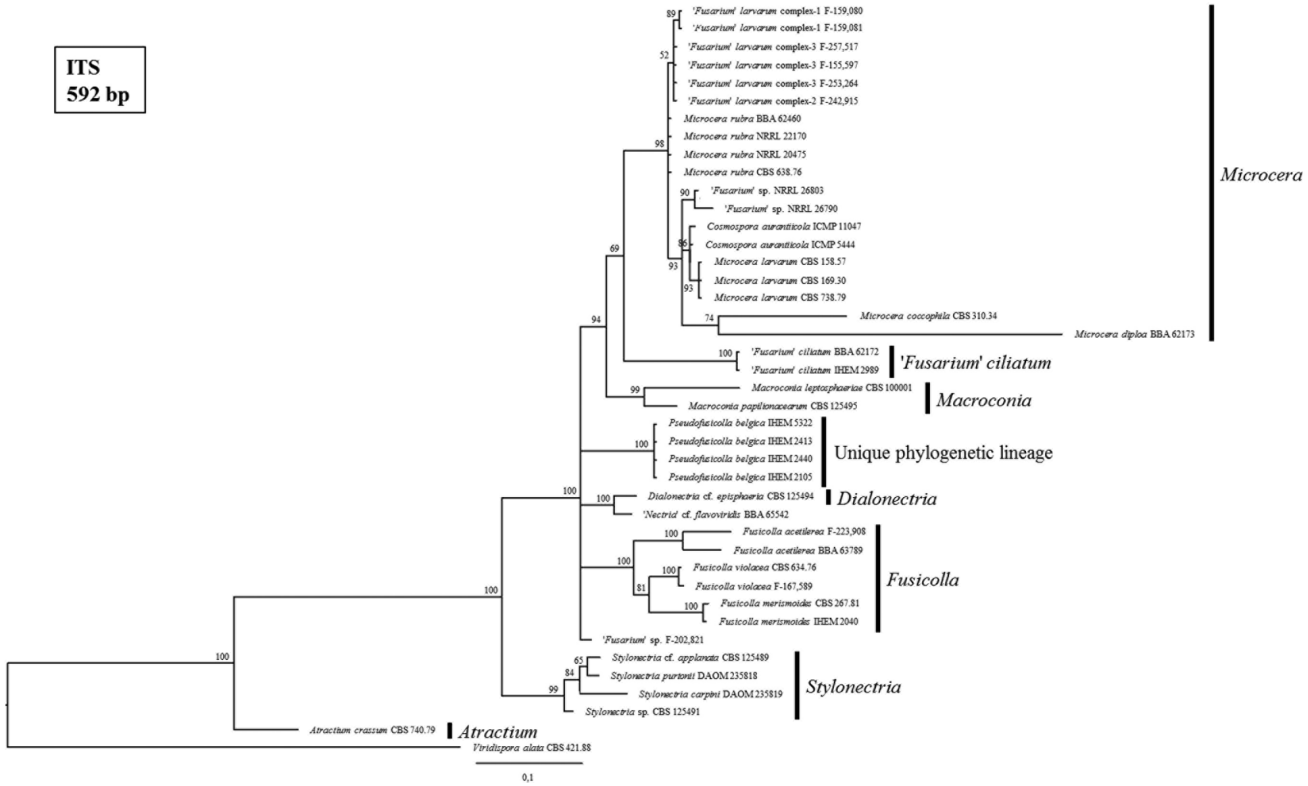

Fig. 6. Consensus tree from the Bayesian phylogenetic analysis, using the internal transcribed spacer (ITS) reference sequences of species from the *Fusarium*-like clade published by Gräfenhan *et al.* [1] as well as Bills *et al.* [5] and including the strains of the Belgian fungal culture collection BCCM/IHEM used in this study (i.e., IHEM 2040, IHEM 2989, IHEM 5322, IHEM 2105, IHEM 2413, and IHEM 2440). Posterior probabilities (%) are represented at the nodes of the tree. A *Viridispora alata* strain was chosen as outgroup. The different genera of the *Fusarium*-like clade, as defined by Gräfenhan *et al.* [1], are indicated.

pionnotal growth, no aerial mycelium; conidiogenesis starting after a few days from phialides, consisting of branched conidiogenous cells or conidiogenous cells scattered along branched hyphae; microconidia abundantly produced, strongly falcate, aseptate or one-septate, 5~16 × 2~3.5 µm; macroconidia abundantly produced, strongly falcate, smooth-walled, aseptate to three-septate (mostly one-septate, seldom three-septate), 17~32 × 2~3.5 µm, apical cell blunt or hooked, basal cell poorly developed; chlamydospores abundantly produced, singly and in chains (intercalary and lateral). Teleomorph not observed.

Type strain: Belgium, Brussels, isolated from recycled water from spray humidifier, collected by the BCCM/IHEM collection in 1983, permanently inactivated but living strain preserved by the BCCM/IHEM collection (holotype, IHEM 2413).

Additional specimens examined: Belgium, Antwerp, isolated from recycled humidifier water from air-conditioning, collected by the BCCM/IHEM collection in 1990, IHEM 5322; Belgium, Brussels, isolated from recycled humidifier water from air-conditioning, collected by the BCCM/IHEM collection in 1983, IHEM 2105; Belgium, isolated from humidifier water from air-conditioning, collected by the BCCM/IHEM collection in 1984, IHEM 2440; permanently inactivated but living strains preserved by the BCCM/IHEM collection.

Habitat: Recycled water from spray humidifier and air-conditioners.

Known distribution: Belgium.

Etymology: "*belgica*" refers to Belgium, i.e., the country in which all *Pseudofusicolla belgica* sp. nov. strains, presented in this research, were isolated.

Notes: *Pseudofusicolla belgica* sp. nov. forms a distinct and well-supported phylogenetic lineage in multilocus phylogenies of the *Fusarium*-like clade. *Pseudofusicolla* gen. nov. is installed in order to retain the monophyly of the different genera as defined in the *Fusarium*-like clade according to Gräfenhan *et al.* [1] and Lombard *et al.* [3].

DISCUSSION

The *Fusarium*-like clade, of which most members were formerly classified in the *Fusarium* sections *Arachnites*, *Eupionnotes*, *Macroconia*, *Pseudomicrocera*, and *Submicrocera* [2], is clearly phylogenetically distinct from *Fusarium* sensu stricto. This was shown by Gräfenhan *et al.* [1] as well as Lombard *et al.* [3] and is also confirmed in the present study (Fig. 3).

Re-identification of six "*Fusarium*" strains from the BCCM/IHEM fungal culture collection revealed a *Fusarium*-like

identity for each of them, supported both morphologically and phylogenetically. All strains were slow growing and produced pionnotes, i.e., typical morphological characteristics of many *Fusarium*-like species (Table 1, Figs. 1A and 2A). Since the majority of *Fusarium*-like species have descriptions which are often not well-documented or confirmed [1], reliable morphological identification up to the species level or even genus level was not possible. Because sequence data and phylogenetic analysis seems indispensable for species identification in the *Fusarium*-like clade, a multilocus phylogenetic approach, using reference sequences of type material, was applied.

We identified one strain as *Fusicolla merismoides* (i.e., IHEM 2040). *Fusicolla*, which has for a long time been considered a synonym of *Fusarium*, was installed by Gräfenhan *et al.* [1] as a separate genus containing most of the former "*Fusarium*" *merismoides* varieties of which some were raised to species rank. Another strain was identified as "*Fusarium*" *ciliatum* (i.e., IHEM 2989), a known member of the *Fusarium*-like clade provisionally retained as "*Fusarium*" by Gräfenhan *et al.* [1].

The remaining four strains (i.e., IHEM 2105, IHEM 2413, IHEM 2440, and IHEM 5322) were also embedded in the *Fusarium*-like clade of our phylogenetic tree of nectriaceous fungi (Fig. 3), but their sequences, which were identical for all tested DNA markers, showed limited similarity with those published by Gräfenhan *et al.* [1] or in public databases in order to perform species/genus identification. Moreover, they formed a distinct and maximum posterior probability supported lineage in this phylogenetic tree as well as in the ones of *Fusarium*-like species only (Figs. 4~6). Based upon these phylogenetic observations and in order to retain the monophyly of the different genera as defined in the *Fusarium*-like clade according to Gräfenhan *et al.* [1] as well as Lombard *et al.* [3], we installed a new genus, *Pseudofusicolla* gen. nov., and a new species, *Pseudofusicolla belgica* sp. nov., to describe this unique phylogenetic lineage. Morphologically, *Pseudofusicolla belgica* strains resemble somewhat to immature strains of *Microcera larvarum* and *Dialonectria* spp., which also form relatively small and strongly falcate aseptate to two-septate macroconidia [1, 19]. Though, when mature, *Microcera larvarum* and *Dialonectria* spp. predominantly produce three-septate macroconidia, whereas this type of macroconidia is only rarely observed in the cultures of our *Pseudofusicolla belgica* strains (Fig. 1G). The predominant occurrence of one-septate macroconidia (Fig. 1H) appears to be discriminatory for the species as opposed to the other members of the *Fusarium*-like clade, which generally form macroconidia that are three-septate or more, as was seen in our cultures of IHEM 2989 and IHEM 2040 (Table 1, Fig. 2B and 2C). Microconidia were also detected in all SNA cultures of *Pseudofusicolla belgica* (Fig. 1F), though similar as in *Fusicolla matuoi* and other *Fusicolla* spp., these form a continuum in conidial shape and length with the macroconidia [1, 7]. Moreover, our *Pseudofusicolla belgica* strains produced chlamydospores (Fig. 1I). But, no teleomorph was found after performing crossing experiments.

Five of the six IHEM *Fusarium*-like strains discussed in this study had been isolated from humidifier water of either a spray or air-conditioners from different localities in Belgium (Table 1). Only the "*Fusarium*" *ciliatum* strain (i.e., IHEM 2989) had been isolated from the leaf of a beech tree. Additional sampling is required to gather strains other than the historical strains presented in this study (cfr. collected over 20 years ago) as well as to tell us something more about the ecological niches of *Fusarium*-like species. For example: do humidifiers act as reservoirs for *Fusarium*-like species? Our generated and published DNA marker sequences will aid this search. However, sequence similarity searches in GenBank with the ITS sequences of our isolates, showed us that a substantial part of the *Fusarium*-like species diversity might be hidden due to the inability to culture them.

In conclusion, we found a unique phylogenetic lineage in the *Fusarium*-like clade after re-examining collection material from the Belgian fungal culture collection BCCM/IHEM and described this new taxon as *Pseudofusicolla belgica*, thereby expanding our knowledge about the *Fusarium*-like species diversity. More isolates will need to be gathered in order to further reveal the actual phylogenetic species diversity in the *Fusarium*-like clade.

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