A new species of *Fusicolla* (*Hypocreales*), *F. ossicola*, from Belgium

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Abstract: *Fusicolla ossicola* sp. nov. is described and illustrated based on material collected on bone of wild boar in Belgium. This species is placed in the genus *Fusicolla* based on morphological characters such as changing color in lactic acid and the fusarium-like asexual morph produced in culture. A phylogenetic comparison of its ITS and LSU sequences was made with those of known *Fusicolla* species and 13 nectriaceous species having a fusarium-like asexual morph. *Fusicolla ossicola* is characterized by pale yellow to pale brownish orange, non-stromatic ascomata, not changing color in 3% KOH but becoming bright orange in lactic acid and a fusarium-like asexual morph. Furthermore, the systematic position and identity of *Cosmospora matuoi* Hosoya & Tubaki are discussed.
Keywords: Ascomycota, bone, cosmospora-like, fusarium-like, *Hypocreales*, ribosomal DNA, taxonomy.

Résumé : *Fusicolla ossicola* sp. nov. est décrite et illustrée d'après du matériel récolté sur os de sanglier en Belgique. Le placement de cette nouvelle espèce dans le genre *Fusicolla* repose sur les caractères morphologiques, tels que le changement de couleur dans l'acide lactique et le stade asexué fusarium-morphe obtenu en culture. La comparaison phylogénétique de ses séquences ITS et LSU avec celles des espèces de *Fusicolla* connues et 13 espèces de *Nectriaceae* ayant un stade asexué de type fusarium. *Fusicolla ossicola* est caractérisée par des ascomes jaune pâle à orange brunâtre pâle, sans stroma, ne changeant pas de couleur dans KOH à 3% mais devenant orange vif dans l'acide lactique et un stade asexué de type fusarium. De plus, la position systématique et l'identité de *Cosmospora matuoi* Hosoya & Tubaki sont discutées.
Mots-clés : ADN ribosomal, Ascomycota, cosmospora- et fusarium-morphe, *Hypocreales*, taxinomie.

Introduction

During a survey of hypocrealean fungi, an intriguing specimen was collected by Bernard Clesse on bone of wild boar (*Sus scrofa*) in Belgium, which permitted a detailed morphological characterization and successful single ascospore isolation. This specimen was determined to be a new species assigned to the *Nectriaceae* based on its ascomata changing color in lactic acid and its fusarium-like asexual morph. The placement of this species in the *Nectriaceae* is confirmed by phylogenetic comparison of its ITS and LSU sequences with those of 13 other nectriaceous species having a fusarium-like asexual morph (Table 1, Fig. 1). Based on morphological characters of sexual and asexual morphs as well as phylogenetic analysis, this fungus represents a previously undescribed species in the genus *Fusicolla*. Furthermore, the status of *Cosmospora matuoi* Hosoya & Tubaki and *Fusicolla matuoi* (Hosoya & Tubaki) Gräfenhan & Seifert are discussed.

Materials and methods

The specimen was examined, using the methods described in Lechat & Fournier (2015a). DNA extraction, amplification, and sequencing were performed using methods described in Lechat & Fournier (2016).

Table 1. Species having fusarium-like asexual morph and GenBank accession numbers of sequences used in the phylogenetic analyses.

Species	GenBank Accession Number	
	ITS	LSU
Albonectria rigidiuscula	HM054158	HM042403
Bionectria ochroleuca	KC460538	GQ50600
Corallonectria jatrophae	KC479759	KM231611
Cosmospora stegonsporii	KP114076	KC291755.
Dialonectria episphaeria	HQ897811	KM231697
Fusarium cyanostromum	HQ728144	HM626673
Fusarium sambucinum	KC445242	U85523
Fusicolla acetilerea	KF494020	U88108
Fusicolla aquaeductuum	KM231823	KM231699
Fusicolla matuoi	KM231822	KM231698
Fusicolla melogrammae	KX897140	-
Fusicolla merismoides	KU214553	HQ377264
Fusicolla ossicola	MF628022	MF628021
Fusicolla violacea	KM231824	KM231700
Geejayessia celtidicola	HM626656	HM626668
Macroconia gigas	EF121863	EF121869
Macroconia cupularis	EF121864	EF121870
Microcera larvarum	KC354705	KC338992
Microcera rubra	NR_111604	KM231702
Stylonectria applanata	HQ897805	KM231689
Varicosporella aquatica	KP192669	KP192671

Taxonomy

Fusicolla ossicola Lechat & Rossman, *sp. nov.* Fig. 2
MB 823487

Diagnosis: Similar to *Fusicolla melogrammae* differing in ascomata turning bright orange in lactic acid, shorter and wider ascospores, asexual morph with smaller conidia, colony cream to pale orange in culture, without carmine, and its occurrence on bone.

Holotype: Belgium, Couvin (province de Namur, Région wallonne), Pesche, "Fond de l'Eau", on bone of wild boar (*Sus scrofa*), *leg*. Bernard Clesse, 01 Apr. 2015, CLL15074 (LIP), ex-type culture CBS140161, GenBank ITS: MF628022, LSU: MF628021.

Etymology: The epithet refers to *ossis* (bone), on which this species was collected.

Ascomata superficial, solitary or in groups of 3–10, crowded on host surface with base remaining slightly immersed in substratum, non-stromatic, subglobose to widely pyriform, 250–300 µm high, 240–280 µm diam. (Me = 280 × 260 µm, n = 10), uniloculate, smooth, pale yellow to pale brownish orange, not changing color in 3% KOH, becoming bright orange in lactic acid, laterally pinched when dry, with a rounded apex, 40–50 µm high, 80–100 µm diam at base, composed of subglobose to cylindrical, pale yellow cells. Perithecial surface cells forming a *textura angularis* in surface view with cells up to 15 µm in greater dimension, covered by thick-walled hyphal elements arising from base of perithecium, pale yellow, aseptate, 3–4.5 µm diam, rounded at free end, developing to form a crown around ostiolar region. **Ascomatal wall** 18–25 µm thick, composed of a single region of ellipsoidal, subangular cells 8–12 × 6–10 µm with pale yellow to orange wall 1–1.5 µm thick, becoming flattened and hyaline inwardly. **Asci** unitunicate, cylindrical, short stipitate, (70–) 80–85(–90) × 8–11µm (Me = 80 × 10 µm, n = 20), cylindrical to narrowly clavate, with 8 obliquely uniseriate ascospores, apically truncate when immature, becoming rounded when mature, with a faint apical ring–like thickening, interspersed with early deliquescing, widely moniliform paraphyses, up to 12 µm diam at base. **Ascospores** ellipsoidal, rounded at ends, 1-septate, (9.5–)10–12(–13) × (4.8–)5–5.5(–6) µm (Me = 11× 5.4 µm, n = 30), hyaline, becoming pale golden brown, orange en masse, spinulose, not constricted at septum.

Asexual morph: fusarium-like.

Cultural characteristics: Colony after two weeks on PDA, 25–35 mm diam, aerial hyphae rare, slimy; cream to pale orange in center, with white, radiating strands in middle area, white at margin, producing a fast growing fusarium-like asexual morph. No microconidia produced; macroconidia hyaline, smooth, long-fusiform, falcate, acute at ends, (0–)1–3-septate, 12–28(–30) µm long, 3–3.5 µm when more than 15 µm long, and up to 4.5 µm wide when less than 15 µm long.

Discussion

The ascomata of species belonging the *Nectriaceae* are usually brightly colored and change color in 3% KOH or lactic acid as defined by Rossman *et al*. (1999) and Schroers (2001). However, some nectriaceous fungi are known to have pale colored ascomata not changing color in 3% KOH, such as the genera *Albonectria* and *Pseudonectria* (Rossman *et al*., 1999) as well as *Varicosporella* and *Varicosporellopsis* (Lechat & Fournier, 2015b, 2016) and *Fusicolla* (Bonorden, 1851). Morphologically, the new species described herein resembles *Fusicolla melogrammae* Lechat & Aplin (Lechat & Aplin, 2016) in having pale colored ascomata turning orange in lactic acid and spinulose, ellipsoidal ascospores becoming golden brown when mature, but differs from it in its asexual morph whose colony

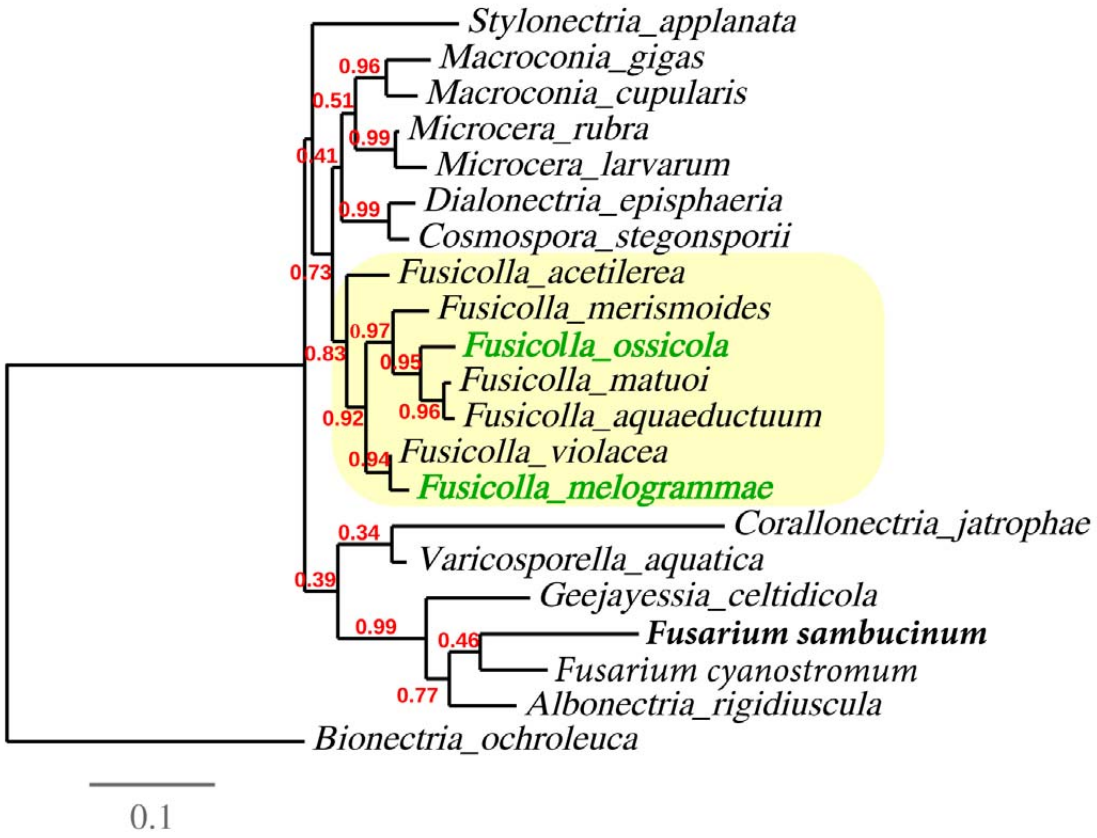

Fig. 1 – Maximum likelihood phylogeny of the new species based on combined ITS1-5.8S-ITS2 and LSU sequences, rooted with *Bionectria ochroleuca* in the *Bionectriaceae*.

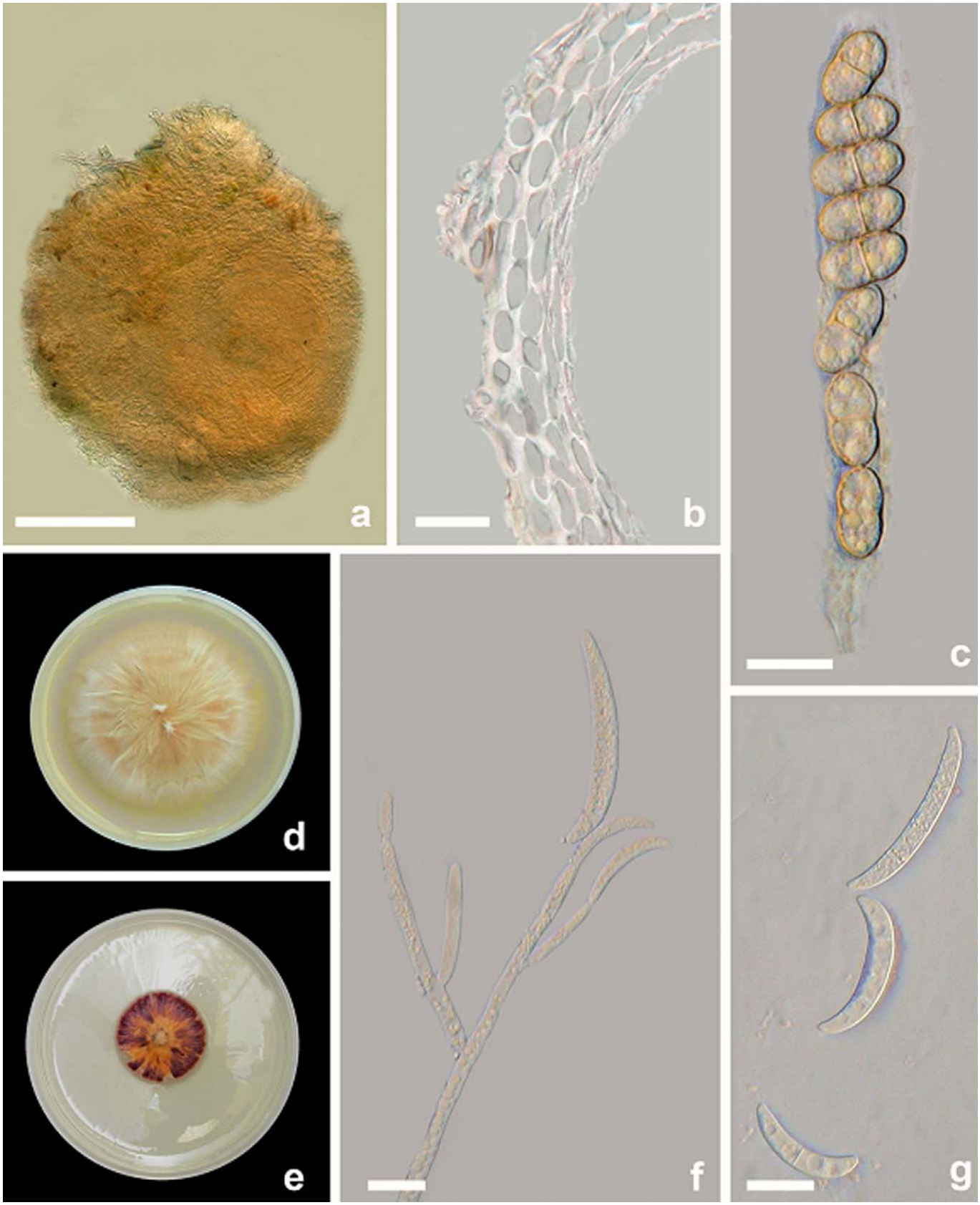

Fig. 2 – a-g: *Fusicolla ossicola* (Holotype CLL15074); a: Close-up of perithecium in water; b: Lateral ascomatal wall in vertical section; c: Ascus and ascospores; d-e: Cultures in Petri dish 55 mm diam, after two weeks; d: *F. ossicola*; e: *F. melogrammae*; f: Conidiophores and conidia; g: Conidia. Scale bars: a: 100 µm, b: 20 µm, c, f, g: 10 µm.

shows very different coloration in culture (Fig. 2) and by its occurrence on bone, an unusual host for the *Nectriaceae*, with only *Cosmospora viridescens* (C. Booth) Gräfenhan & Seifert previously reported on this host (Herrera *et al*., 2015). The fast growing cultures of *Fusicolla ossicola* and *F. melogrammae* are unusual in cosmospora-like fungi, which are usually slow growing, this characteristic separating these species of *Fusicolla* from other cosmospora-like fungi. Our phylogenetic analysis showed that *F. ossicola* is nested in the *Fusicolla* clade, which appears quite distant from *Fusarium sambucinum*, the type species of *Fusarium*. *Fusicolla ossicola* is close to *F. merismoides* (Corda) Gräfenhan, Seifert & Schroers whose sexual morph is unknown and *F. matuoi* (Hosoya & Tobaki) Gräfenhan & Seifert. *Fusicolla merismoides* differs from *F. ossicola* in having shorter and wider macroconidia, while *F. matuoi* differs in having two shapes of conidia, which can be strongly curved and almost C-shaped or lightly curved, up to 6-septate and significantly longer.

The genus *Fusicolla* was introduced by Bonorden (1851) with *Fusicolla betae* (Desm.) Bonord. as type species to accommodate the basionym *Fusisporium betae* Desm. (1830), which he describes as an asexual morph, without ascomata. Description of the genus *Fusicolla* provided by Gräfenhan *et al.* (2011) differs from that of the type designated by Bonorden (1851), and seems based on *Cosmospora matuoi* Hosoya & Tubaki, which was erroneously considered at that time to be the sexual morph of *Fusarium matuoi* as discussed below. Accordingly, all *Fusicolla* species accepted by Gräfenhan *et al.* (2011) are only known as asexual morphs. Only the two recently described species *F. melogrammae* Lechat & Aplin and *F. ossicola* Lechat & Rossman sp. nov. (this paper) are known to have sexual morphs.

Fusicolla is characterized by ascomata superficial, non-stromatic, pale yellow to pale brownish orange, not changing color in 3% KOH, becoming orange in lactic acid, ascomatal wall 18–25 µm thick, of a single region and a fusarium-like asexual morph.

Hosoya & Tubaki (2004) proposed *Cosmospora matuoi* for a sexual morph specimen (TNS-F-11126) from Japan, occurring on twigs of *Albizia julibrissin* (*Mimosaceae*), which was linked to an asexual morph assigned to *Fusarium matuoi* Hosaya & Tobaki. However, their description and illustrations of the sexual morph *Cosmospora matuoi* do not match morphological characteristics of the genera *Cosmospora* or *Fusicolla* and differ from them in having ascomata almost completely immersed in an erumpent, prosenchymatous stroma. Presence of a prosenchymatous stroma excludes this fungus from the genus *Cosmospora*, which is characterised by non-stromatic ascomata and is restricted to species having an acremonium-like asexual morph as defined by Gräfenhan *et al.* (2011). This stroma also excludes this fungus from the genus *Fusicolla* as defined above. Morphological differences separating *Cosmospora matuoi* from *F. melogrammae* and *F. ossicola* led us to think that the specimen studied by Hosoya & Tubaki (2004) was erroneously linked to *Fusarium matuoi* Hosoya & Tobaki.

Based on molecular data, Gräfenhan *et al.* (2011) combined *Fusarium matuoi* Hosoya & Tobaki into *Fusicolla matuoi* (Hosoya & Tobaki) Gräfenhan & Seifert, that is well supported in our phylogenetic analysis and we agree with this decision. Unfortunately, there is no type culture for *Cosmospora matuoi*, and although its asexual morph remains unknown, we think that this specimen is not the sexual morph of *Fusicolla matuoi* (Hosoya & Tobaki) Gräfenhan & Seifert. We did not see the *Cosmospora matuoi* sexual morph, for which there is no molecular data available, but based on Hosoya & Tubaki's description and illustrations, we think that this species would be best placed in the genus *Geejayessia* Schroers, Gräfenhan & Seifert (Schroers *et al*., 2011) for which it possess all morphological characteristics. Accordingly, we propose the new combination ***Geejayessia matuoi*** (Hosoya & Tobaki) Lechat & Rossman, *comb. nov*. MycoBank MB 823288 to accommodate the basionym *Cosmospora matuoi* Hosoya & Tubaki, *Mycoscience*, 45 (4): 262 (2004).

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