

Flammoclaadiella decora, a new combination to accommodate the hypocrealean fungus *Nectria decora*

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Abstract: The new combination *Flammoclaadiella decora* is proposed to accommodate *Nectria decora*, whose taxonomic position is unsettled. Detailed description and illustrations of this species are presented, based on material occurring on ascomata of *Massaria inquinans* on *Acer pseudoplatanus* in France. The placement of this fungus in the genus *Flammoclaadiella* is based on its sexual and asexual morphs deviating from those of typical *Bionectriaceae* and *Nectriaceae* and phylogenetic comparison of ITS1-5.8S-ITS2 and LSU sequences with genera and species belonging to families in the *Hypocreales*.

Keywords: Ascomycota, *Flammoclaidiaceae*, *Hypocreales*, ribosomal DNA, taxonomy.

Résumé : la nouvelle combinaison *Flammoclaadiella decora* est proposée pour *Nectria decora* dont la position taxinomique n'est pas résolue. Une description détaillée et les illustrations de cette espèce sont présentées d'après du matériel récolté en France sur des ascomes de *Massaria inquinans* sur *Acer pseudoplatanus*. Le placement de ce champignon dans le genre *Flammoclaidiella* repose sur ses stades sexué et asexué, éloignés de ceux des *Bionectriaceae* et *Nectriaceae* typiques, et sur la comparaison phylogénétique des séquences ITS1-5.8S-ITS2 et LSU avec les genres et espèces appartenant à diverses familles parmi les Hypocréales.

Mots-clés : ADN ribosomal, Ascomycota, *Flammoclaidiaceae*, *Hypocreales*, taxinomie.

Introduction

The fungus *Nectria decora* (Wallr.) Fuckel has long been challenging and intriguing because of its fungicolous lifestyle, its small pale orange, villose, gregarious ascomata and association with an asexual morph assigned to *Fusarium ciliatum* Link (BEENKEN, 1997). Whereas the supposed fusarium-like asexual morph suggested affinities with *Nectriaceae*, the small pale orange ascomata not changing colour in 3% KOH nor in lactic acid are more suggestive of the *Bionectriaceae* as defined by ROSSMAN *et al.* (1999). Moreover, based on morphological comparison, no genus in either of these families appear suitable to accommodate it. In order to elucidate the familial status of *Nectria decora*, a fresh collection was cultured, sequenced, and submitted to phylogenetic analysis.

A preliminary BLAST search based on ITS sequence showed 100% identity with the recently described species *Flammoclaidiella aceris* Crous, L. Lombard & R.K. Schumach. (CROUS *et al.*, 2015). Phylogenetic analysis of ITS sequences was carried out on a dataset including *Fl. aceris*, representatives of 23 genera in the *Bionectriaceae*, *Cordycipitaceae*, *Nectriaceae*, and *Ophiocordycipitaceae* with *Melanopsamma xylophila* (*Stachybotryaceae*) as outgroup, while LSU sequences were compared to 15 genera of *Nectriaceae*, including *Nectria cinnabarina* (Tode) Fr. (type species), some species of *Cordycipitaceae* and *Ophiocordycipitaceae*, and 15 bionectriaceous genera with one niessliaceous species as outgroup. Based on congruent results from our ITS- and LSU-based phylogenetic analyses, "*Nectria*" *decora* appeared identical to *Fl. aceris* in the isolated family *Flammoclaidiaceae* Crous, L. Lombard & R.K. Schumach., sister to the *Bionectriaceae* (CROUS *et al.*, 2015). Furthermore, the systematic position and identity of *F. decora* asexual morph, historically assigned to *Fusarium ciliatum*, are discussed.

Materials and methods

The specimens were examined, cultured, sequenced and phylogenetically analysed using the methods described in LECHAT & FOURNIER (2015).

Taxonomy

Flammoclaidiella decora (Wallr.) Lechat & J. Fourn., *comb. nov.* – MycoBank 823794 – Figs. 1, 2, 5

Basionym: *Sphaeria decora* Wallr., *Fl. crypt. Germ.*, II: 842 (1833).

≡ *Nectria decora* (Wallr.) Fuckel, *Jahrbücher des Nassauischen Vereins für Naturkunde*, 23–24: 179 (1870).

- ≡ *Calonectria decora* (Wallr.) Sacc., *Michelia*, 1 (3): 311 (1878).
- = *Sphaeria agnina* Desm., *Annales des Sciences Naturelles, Botanique*, 6: 72 (1846).
- = *Nectria massariae* Pass., *Fungi europaei* no. 1827 (1874).
- = *Flammoclaidiella aceris* Crous, L. Lombard & R.K. Schumach., in Crous *et al.*, *Sydowia*, 67: 104 (2015).

GenBank: ITS MF611693, LSU MF614949.

Description:

Ascomata crowded on ostiolar region of immersed ascomata of *Massaria* species, seated on a prosenchymatous hypostroma arising from inside dead ascomata, in groups of 3–20, broadly obpyriform to subglobose, (260–)280–330(–350) × 260–300 μm, (Me = 300 × 280 μm, n = 10), orange, appearing pale orange to pale luteous due to hyaline hyphal elements covering ascomatal wall, collapsing by lateral pinching or not collapsing when dry, not changing colour in 3% KOH or lactic acid. Perithecial apex, when mature, rounded with a bright orange papilla up to 55 μm high, 180 μm diam., composed of a palisade of clavate, thick-walled cells 20–52 × 3–4 μm, merging with periphyses. Ascomatal surface composed of subglobose, angular cells 10–15 μm diam., covered by smooth, hyaline, septate, moniloid hyphal elements arising from basal hypostroma, evolving to terminal, subglobose to globose cells reaching 3–9 μm diam. **Hypostroma** prosenchymatous, composed of hyaline, smooth, septate, hyphae 3–4 μm diam., originating from asexual morph, proliferating on ascomatal surface. **Ascomatal wall**, excluding hyphal elements, 25–30(–35) μm thick, composed of two regions: outer region 15–20 μm wide, of subglobose to ellipsoidal cells 4.5–10 × 3–5.5 μm, with pale orange walls 1–2 μm thick; inner region 10–12 μm wide, of elongate, flattened cells 10–18 × 2–4 μm, with hyaline walls 1–1.5 μm thick. **Asci** (85–)95–110(–130) × (14–)16–20(–22) μm (Me = 105 × 18 μm, n = 20), clavate, shortly stipitate, apex rounded without apical apparatus, containing 8 ascospores obliquely uniseriate or biseriate above and uniseriate below, completely filling each ascus, interspersed with moniliform paraphyses up to 18 μm diam., filled with numerous orange oily droplets. **Ascospores** (22–)26–30(–32) × 6–8(–9) μm (Me = 28 × 7 μm, n = 30), narrowly ellipsoidal, attenuated at ends to fusiform, equally transversally 3-septate, slightly constricted at septa when mature, hyaline, finely verrucose.

Cultural characteristics: After three weeks on PDA, colony reaching 20–25 mm diam., pale yellow in centre, slimy, pale cream to pinkish in medium area with rare pale greenish brown spots, white at margin. Mycelium composed of smooth, septate hyphae 2–3 μm diam. Conidiophores 2.5–3 μm diam, branched with a simple coni-



Fig. 1 – a-g: *Flammoclaidiella decora* (CLL16020) a, b: Habit of ascomata on bark; c: Asexual morph emerging from an ostiole of *Massaria inquinans*; d, e: Vertical section of ascomata of *M. inquinans*, showing white mycelium developing from inside and producing hypostroma, asexual morph and ascomata; f: Vertical section through an ascoma; g: Vertical section of ascomatal wall showing the outer coating composed of moniloid, hyphal elements arising from base (f, g in water). Scale bars: a–e = 200 μ m; f = 30 μ m; g = 10 μ m.

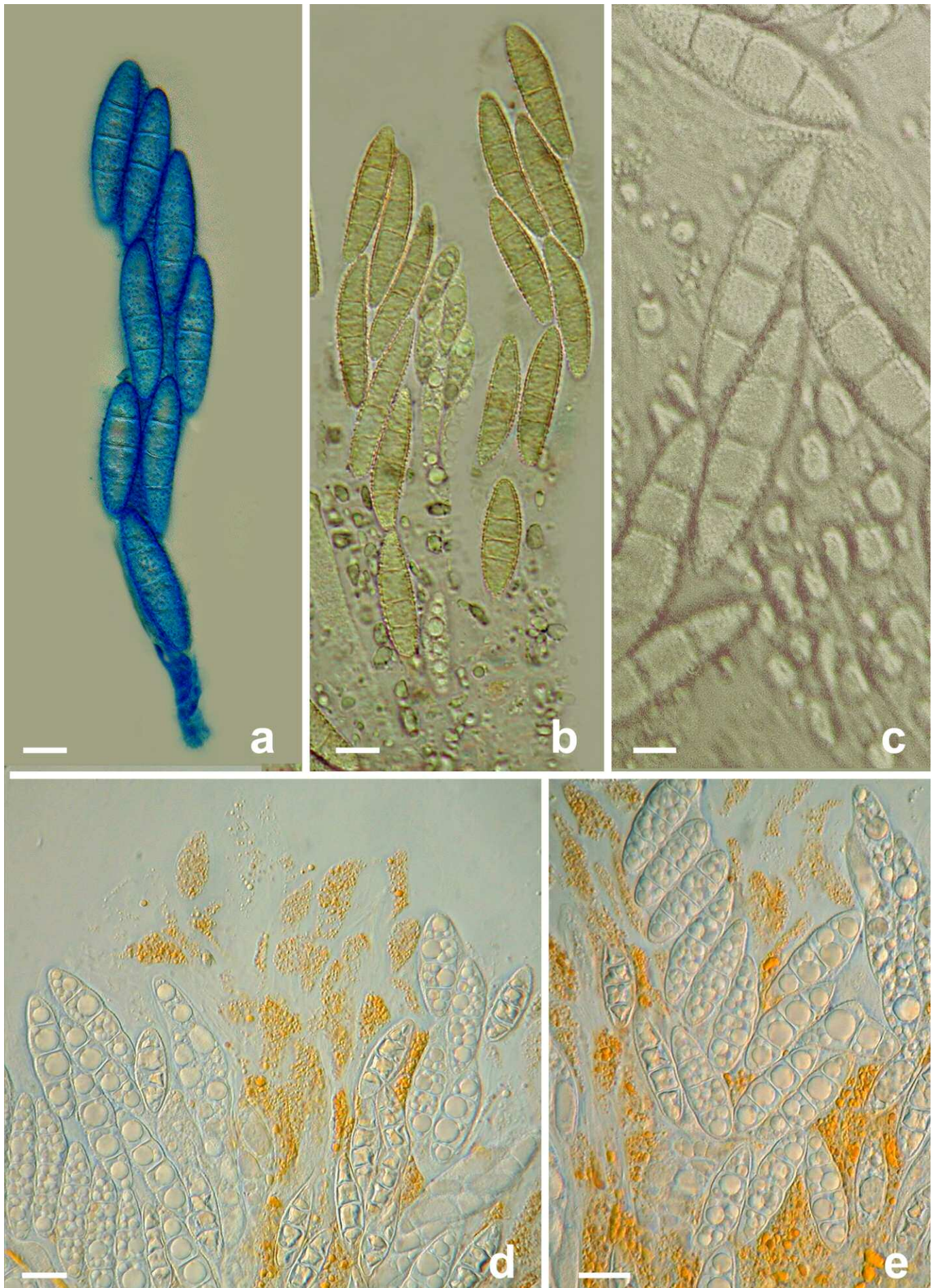


Fig. 2 – a-c: *Flammoclaidiella decora*, asci and ascospores showing verrucose ornamentation a: in lactic cotton blue, b: in Melzer's reagent, c: in lactic acid; d: Asci and ascospores, and moniliform paraphyses filled with orange oily droplets, in water. Scale bars: a, b, d, e =10 μ m; c = 5 μ m.

diogenous cell producing subcylindrical conidia, irregularly curved, rounded at tip, attenuated and truncate at base, 1–3-septate, smooth, (30–)37–65(–78) × 2–2.5 µm.

Asexual morph in natural environment: Sporodochia arising from inside of dead ascomata of *Massaria* species, covering ostiolar region, white when fresh, becoming orange yellow and occasionally flame-shaped when dry. Conidiogenous cells simple, producing long, solitary, subcylindrical, flexuous conidia appearing fasciculate, 37–140(–160) × 2.5–3 µm, rounded at tip, 1–3-septate, truncate at base when measuring up to 50 µm long, becoming 6–10-septate and attenuated toward base when over 50 µm long.

Material examined: FRANCE, Jura, Esserval-Tartre, on *Massaria inquinans* on a corticated branch of *Acer pseudoplatanus*, 25 Nov. 2016, leg. J.-M. Moingeon. Culture CBS 142776, ITS and LSU GenBank sequences: MF611693 and MF614949.

Discussion

Nectria-like species having white to orange or brown perithecial wall not changing colour in 3% KOH or lactic acid belong to the *Bionectriaceae*, as defined by ROSSMAN *et al.* (1999) and SCHROERS (2001). These fungi have various asexual morphs and are accommodated in numerous genera, among which some are of uncertain systematic position, of which the hectic nomenclatural history of

N. decora is a good example. “*Nectria*” *decora* was first described as *Sphaeria decora* by WALLROTH (1833) and transferred to the genus *Nectria* (Fr.) Fr. as *Nectria massariae* Pass. by PASSERINI (1874), later to *Calonectria* De Not. by SACCARDO (1878), before being re-instated in *Nectria* by ROSSMAN (1983) who designated Fuckel, *Fungi rhenani* #986 at BR as the neotype. More recently, *Nectria decora* and its asexual morph were described in detail and illustrated by BEENKEN (1997), who showed that this fungus is fungicolous, parasitizing living ascomata of *Massaria* spp. and illustrated parasitized ascospores. In spite of numerous attempts we were not able to observe such parasitized ascospores during our study. BEENKEN (1997) followed WOLLENWEBER (1926) in linking *N. decora* to “*Fusarium*” *ciliatum* Link as sexual and asexual morphs. As demonstrated by GRÄFENHAN *et al.* (2011), “*Fusarium*” *ciliatum* belongs to the *Nectriaceae*, on a sister branch to *Fusicolla* Bonord., in agreement with previous observations by BOOTH (1971), who regarded “*F.*” *ciliatum* as close to “*Fusarium*” *aquaeductuum* (Radlk. & Rabenh.) Lagerh. & Rabenh. (= *Fusicolla aquaeductuum* (Radlk. & Rabenh.) Gräfenhan, Seifert & Schroers) (GRÄFENHAN *et al.*, 2011). Our phylogenetic results are congruent, placing “*F.*” *ciliatum* in the *Nectriaceae*, close to *Fusicolla aquaeductuum* and far from *Fl. decora* (Fig. 6). This is further supported by the morphology of the conidia which are subcylindrical with rounded to truncate ends, unlike the macroconidia of true *Fusarium* typified by *Fusarium sambucinum* Fuckel (= *Gibberella pulicaris* (Fr.) Sacc.) that are curved with attenuated, often hooked tips.

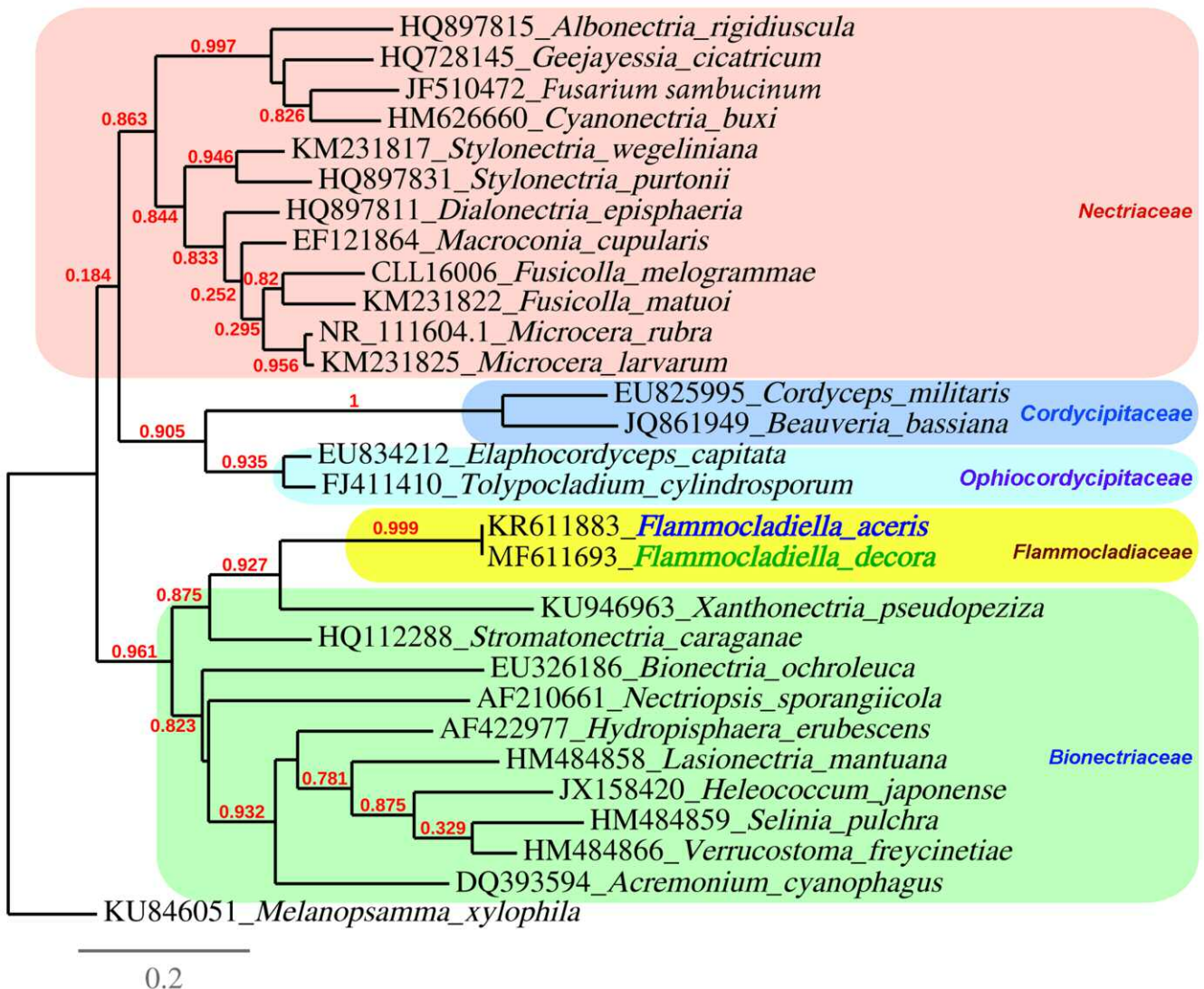


Fig. 3 – Maximum likelihood phylogeny of *Flammocladiella decora* inferred from ITS1-5.8S-ITS2 sequences, rooted with *Melanopsamma xylophila*.

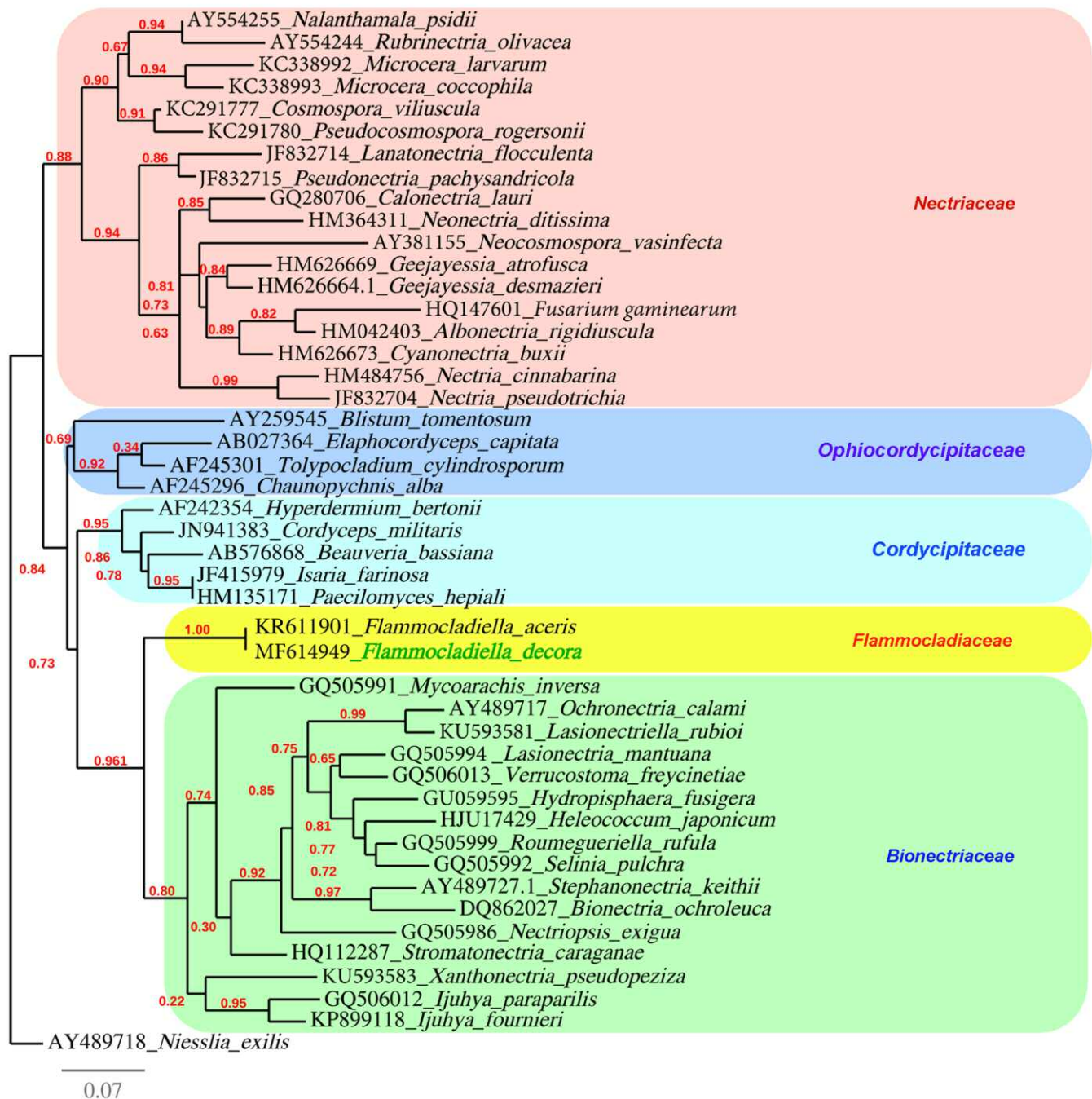


Fig. 4 – Maximum likelihood phylogeny of *Flammocладиella decora* inferred from LSU sequences, rooted with *Niesslia exilis*.

Accordingly, we think that the asexual morph of *Fl. decora* has been erroneously assigned to "*F. ciliatum*".

The genus *Flammocладиella* was introduced by Crous *et al.* (2015) with *Fl. aceris* as type species in the *Flammocладиaceae* for a fungus occurring on twigs of *Acer platanoides*, without specifying a fungicolous association. We have not seen the type specimen, but according Dr. P. Crous (comm. pers.), this species does occur on immersed ascomata of *Massaria vomitoria* Berk. & M.A. Curtis, confirming its fungicolous lifestyle. Because the tight clusters of ascomata of *Flammocладиella decora* obscure the ostioles of the fully immersed ascomata of *Massaria* spp., they appear superficial on bark (Fig. 1), thus their relationship with the underlying parasitized fungus may be overlooked if a vertical section is not performed, thus the superfluous creation of *Fl. aceris* by Crous *et al.* (2015).

The asexual morph of *Flammocладиella decora* (Fig. 5) and its synonym *Fl. aceris* produces long, subcylindrical conidia, and all morphological characteristics of their sexual morphs are indistinguishable. Phylogenetic trees show that *Fl. decora* and *Fl.*

aceris group together in the *Flammocладиaceae* clade, confirming that both specimens belong to the same species. Accordingly, based on morphological characters and phylogenetic analyses of ITS and LSU sequences (Figs. 3-4), the new combination *Flammocладиella decora* (Wallr.) Lechat & J. Fourn. comb. nov. is proposed to accommodate *Nectria decora* (Wallr.) Fuckel, resolving its long-time unsettled status.

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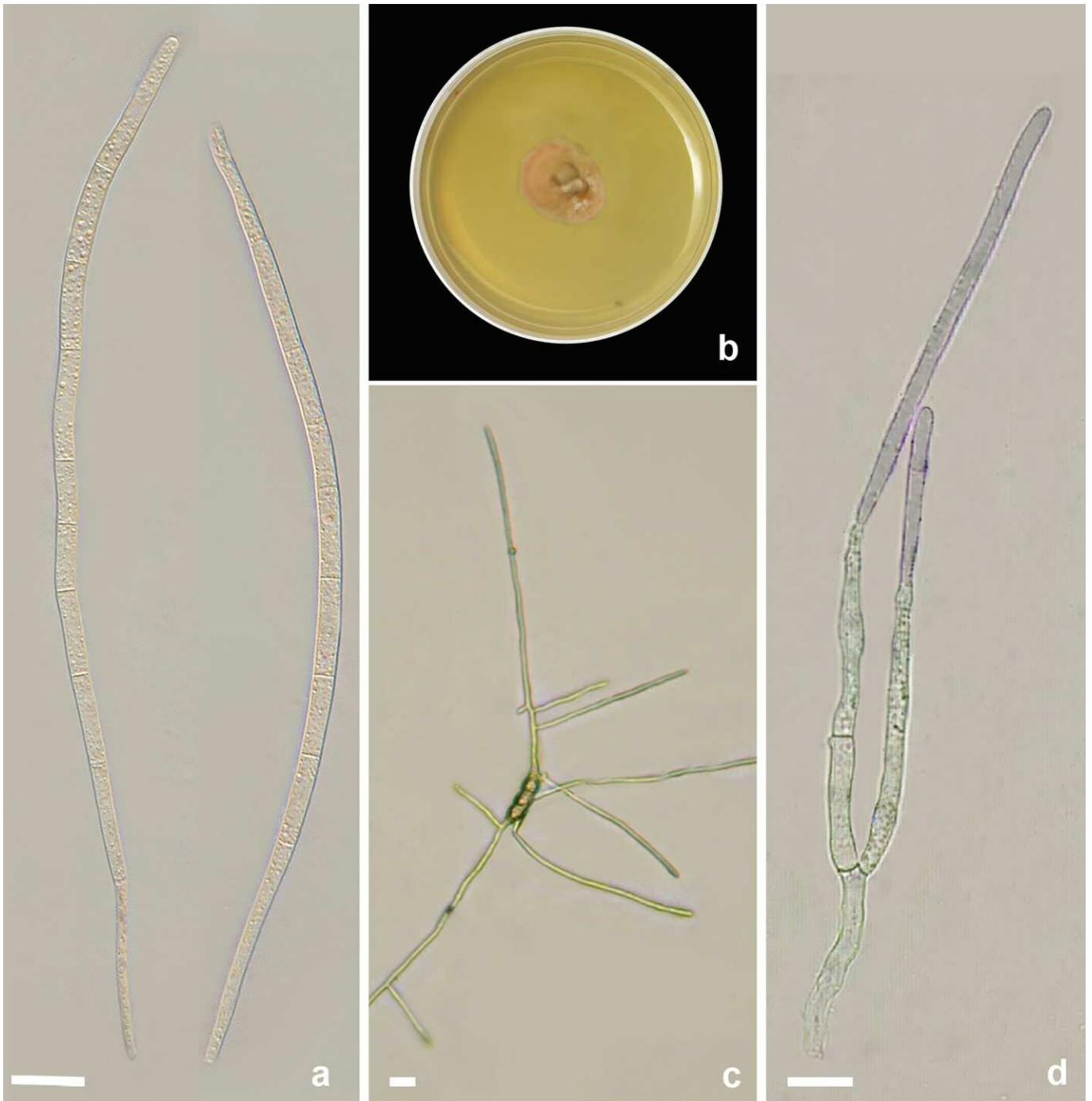


Fig. 5 – a-d: *Flammoclaidiella decora* (CLL16020) a: Conidia from natural environment in water; b: Culture at three weeks; c: Ascospore germinating in culture; d: Conidiophores and conidia in culture. Scale bars: a, c, d=10 μ m.

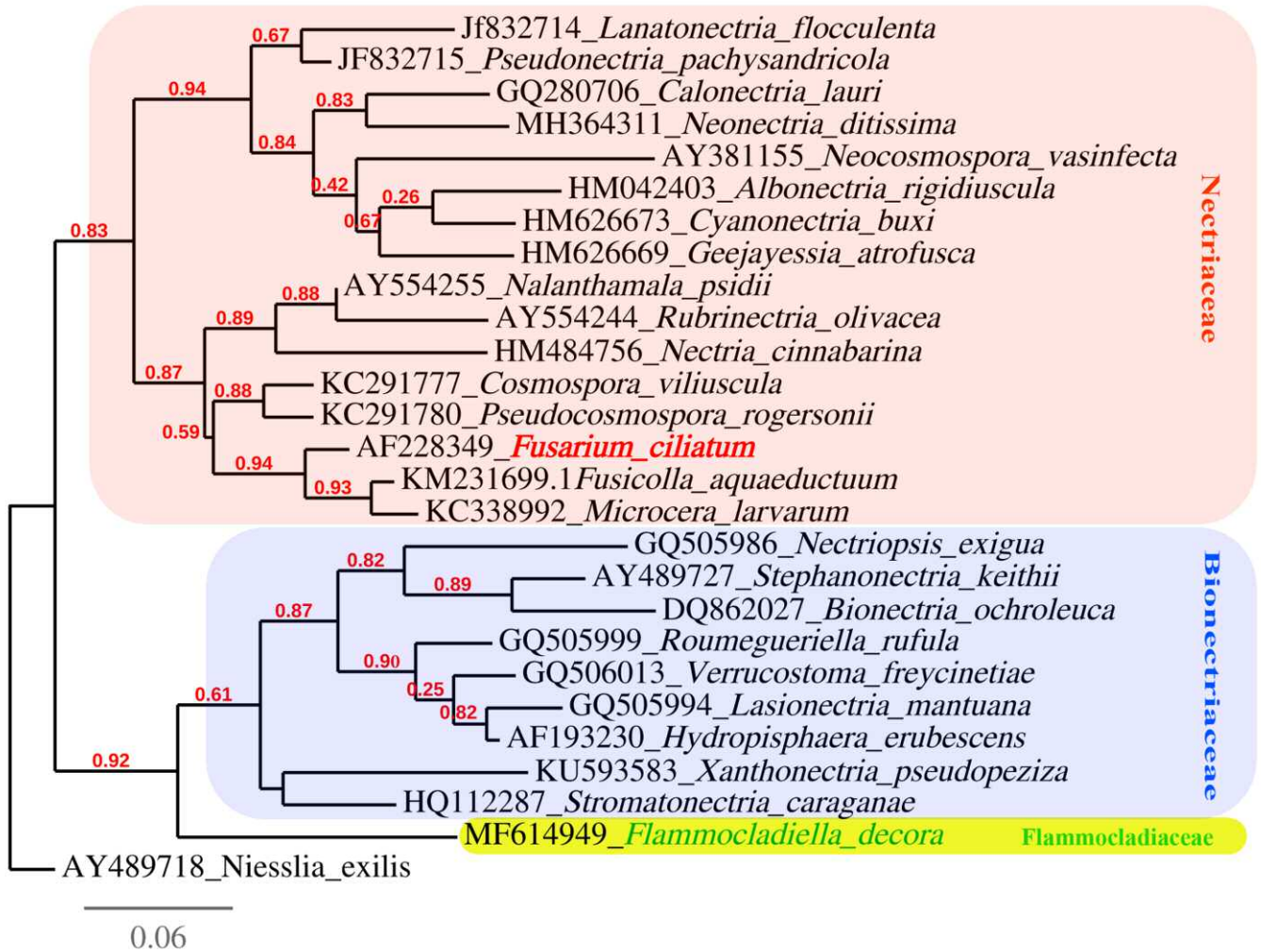


Fig. 6 – Maximum likelihood phylogeny of *Fusarium ciliatum* inferred from LSU sequences, rooted with *Niesslia exilis*.

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