



Stylonectria hygrophila



Stylonectria hygrophila* Mombert & Crous, *sp. nov.

Etymology: The name refers to the occurrence of this species in a marshy locality.

Classification: *Nectriaceae*, *Hypocreales*, *Sordariomycetes*.

Ascomata perithecial, gregarious, in groups of 2–16, or solitary, erumpent to superficial with base remaining immersed in a hypostroma of fusarium-like sporodochia, arising from dead pyrenomycetes, broadly pyriform, 240–308 µm high, 234–288 µm diam. (av. 274–261 µm, n = 10), with a discoid papilla, red to dark red, turning dark purple in 5 % KOH, yellow in lactic acid, not collapsing when dry. *Ascomatal wall* smooth, 32–40 µm thick, composed of two regions: outer region 22–29 µm thick, of irregularly shaped thick-walled cells of *textura intricata* to *textura epidermoidea*; inner region 12–16 µm thick of thin-walled, flattened cells of *textura prismatica* to *textura angularis*. *Asci* unitunicate, subcylindrical, 64–79 × 5.6–6.9 µm, 8-spored, apices rounded and simple, uniseriate; evanescent, narrowly moniliform paraphyses, interspersed between asci. *Ascospores* ellipsoidal, 1-septate, often constricted at septum, (9.4–)9.6–11.3(–12.3) × (4.3–)4.6–5.4(–5.9) µm, (av. = 10.4 × 5 µm, n = 76), (Q = 1.7–2.1–2.5), smooth, thick-walled, hyaline at first, becoming pale golden brown at maturity (measurements from discharged mature ascospores). *Mycelium* consisting of hyaline, smooth, branched, septate, 2–2.5 µm diam. hyphae. Crystalline sporodochia superficial on CLA. *Conidiophores* solitary or aggregated in clusters to form sporodochia reduced to conidiogenous cells or 1–2-septate, branched, subcylindrical, hyaline, smooth, 10–40 × 2–3 µm. *Conidiogenous cells* hyaline, smooth, monophialidic, straight to curved, subcylindrical with apical taper, 10–20 × 2–3 µm, with inconspicuous collarette. *Conidia* solitary, aggregating in mucoid mass, hyaline, smooth, fusoid, apex subobtuse, curved, base tapered to truncate hilum, (0–)1-septate, (10–)22–27(–30) × (2–)2.5(–3) µm; larger conidia develop a slight constriction in the basal cell, appearing as a poorly developed foot cell, but mostly absent, along with *chlamydospores* and *microconidia*.

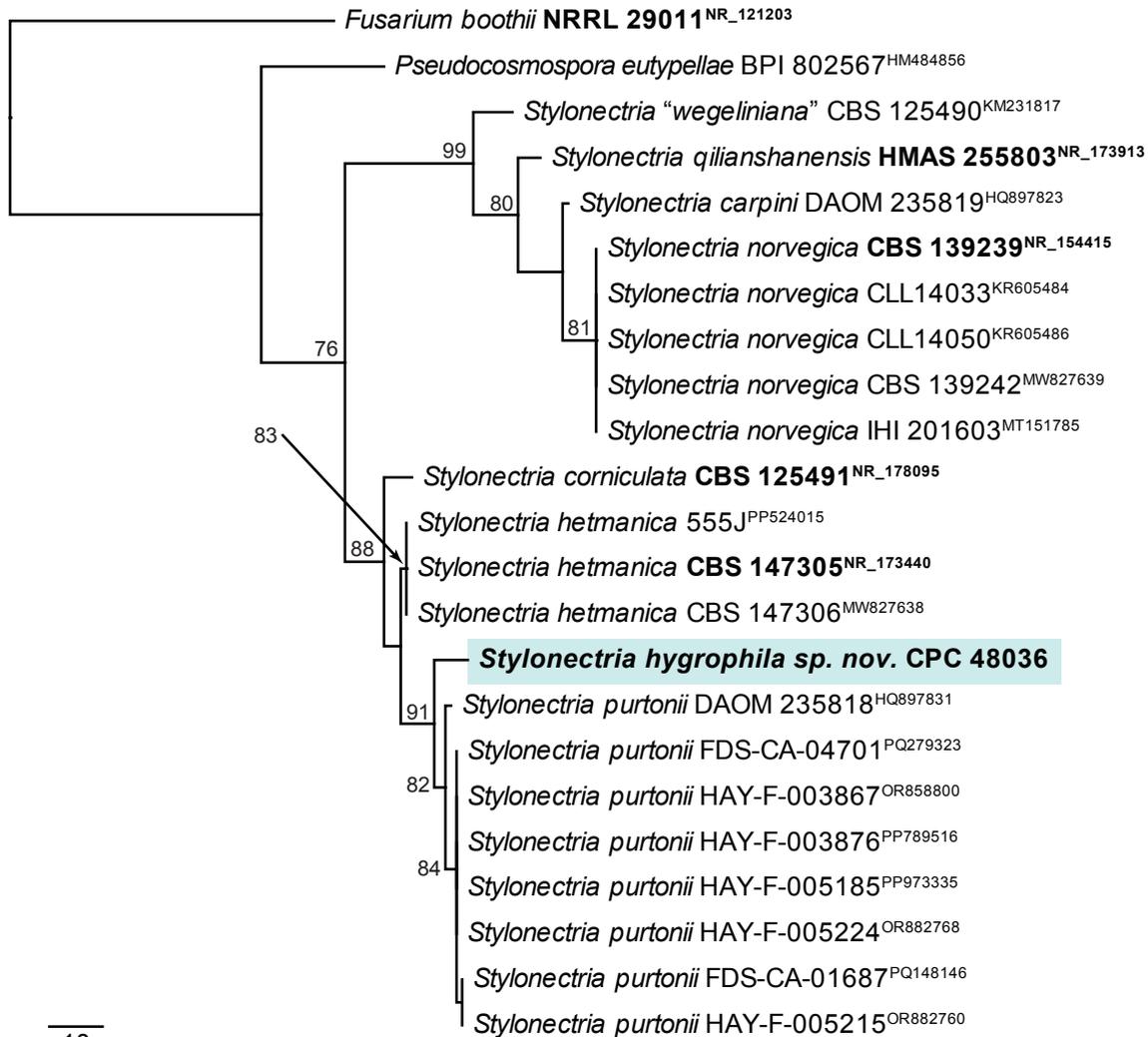
Culture characteristics: Colonies flat, spreading, with sparse aerial mycelium and smooth, lobate margin, reaching 17 mm diam. after 2 wk at 25 °C. On MEA, PDA and OA surface and reverse saffron.

Typus: **France**, Haute-Saône (70), Saint-Germain, Réserve Naturelle de la Grande Pile, on unidentified *Diaporthales*, on dead twigs of *Betula pubescens* (*Betulaceae*), 47.73370° N 6.50264° E, 326 m a.s.l., 12 Mar. 2024, A. Mombert, CBNM0079 (**holotype** CBS H-25722; ex-type culture CPC 48036 = CBS 153465; ITS and LSU sequences GenBank PV664935.1 and PV664961.1).

Notes: *Stylonectria*, a fungicolous member of the *Nectriaceae*, occurs on old stromata of pyrenomycetes (Gräfenhan *et al.* 2011). The genus is characterized by having red cosmospore-like perithecia with a conspicuous flattened apical disc, giving rise to fusarioid asexual morphs in culture. *Stylonectria hygrophila* is phylogenetically distinct from species presently recognized in the genus. The phylogenetically closest species is *S. purtonii*, which is mainly distinguished by narrower ascospores 8–11 × 3.5–4.5 µm and shorter conidia 20–24 × 1.5–2 µm, (Lechat *et al.* 2021).

Based on a megablast search of NCBI's GenBank nucleotide database, the closest hits using the **ITS** sequence had highest similarity to *Stylonectria* sp. [strain MYB204, GenBank PQ699599.1; Identities = 507/509 (99 %), no gaps], *Stylonectria purtonii* [voucher HAY-F-003867, GenBank OR858800.1; Identities = 508/518 (98 %), no gaps], and *Stylonectria hetmanica* [strain CBS 147305, GenBank NR_173440.1; Identities = 494/507 (97 %), three gaps (0 %)]. Closest hits using the **LSU** sequence are *Stylonectria applanata* [strain CBS 125489, GenBank KM231689.1; Identities = 822/829 (99 %), no gaps], *Stylonectria wegeliniana* [strain CBS 125490, GenBank KM231690.1; Identities = 819/829 (99 %), no gaps], and *Stylonectria corniculata* [strain CBS 125491, GenBank NG_088024.1; Identities = 814/829 (98 %), no gaps].

Colour illustrations: Forest in Réserve Naturelle de la Grande Pile, France. Asci and ascospores; perithecia; conidiophores and conidiogenous cells giving rise to conidia on SNA. Scale bars: perithecia = 300 µm, all others = 10 µm.



10

The single most parsimonious tree obtained from a maximum parsimony phylogenetic analysis (PAUP* v. 4.0a; Swofford 2003) of the *Stylonectria* ITS nucleotide alignment. The tree was rooted to *Fusarium boothii* (NRRL 29011; GenBank NR_121203) and the scale bar indicates the number of changes. Parsimony bootstrap support values from 1000 replicates and > 74 % are shown at the nodes and the novelty described here is highlighted with a coloured block and **bold** font. Culture collection or specimen voucher numbers and GenBank accession numbers (superscript) are indicated for all species. Sequences from material with a type status are indicated in **bold** font. Alignment statistics: 23 strains including the outgroup; 529 characters including alignment gaps analysed: 379 constant, 94 variable and parsimony-uninformative and 56 parsimony-informative. Tree statistics: Tree Length = 231, Consistency Index = 0.870, Retention Index = 0.904, Rescaled Consistency Index = 0.786. The alignment and tree were deposited at figshare.com (doi: 10.6084/m9.figshare.29050751).

Cosmospora nemaniae





Cosmospora nemaniae Mombert & Crous, *sp. nov.*

Etymology: Named for *Nemania*, the host genus it was isolated from.

Classification: Nectriaceae, Hypocreales, Hypocreomycetidae, Sordariomycetes.

Ascomata crowded on dead or effete stromata of *Nemania* cf. *colliculosa*, gregarious, subglobose, 200–276 µm high, 174–254 µm diam. (av. = 238 × 214 µm, n = 10), with a slightly acute papilla, smooth, red, becoming purple in 5 % KOH, yellow in lactic acid, laterally pinched when dry. *Ascomatal wall* smooth, 30–33 µm thick, composed of two regions: outer region 19–24 µm thick, composed of subglobose to ellipsoidal cells; inner region 7–12 µm thick, of thin-walled, ellipsoidal, elongate cells. *Asci* unitunicate, cylindrical, short-stipitate, 62–75 × 3.7–4.9 µm, containing 8 uniseriate ascospores, becoming narrowly clavate, 6–8 µm wide, with irregularly biseriolate ascospores. *Paraphyses* evanescent, narrowly moniliform, 2–3 µm diam., interspersed between asci. *Ascospores* (7.5–)8.1–9.9(–10.8) × (3.9–)4.2–4.9(–5.3) µm (av. = 9 × 4.5 µm, n = 83), Q = 1.6–2–2.6, ellipsoidal, equally 1-septate, constricted at septum, strongly verrucose, hyaline at first, becoming pale yellowish brown when mature (measurements from discharged, mature ascospores). *Mycelium* consisting of hyaline, smooth, branched, septate, 2.5–3 µm diam. hyphae. *Conidiophores* solitary, erect, hyaline, smooth, flexuous, subcylindrical, reduced to conidiogenous cells on hyphae, or verticillate with terminal and intercalary whorls of phialides, up to 300 µm tall, 3–4 µm wide, multiseptate. *Conidiogenous cells* monophialidic, hyaline, smooth, subcylindrical, with apical taper, flexuous, 30–60 × 3–4 µm, with slightly flared collarete at apex. *Conidia* of two types. Predominant conidia aseptate, hyaline, smooth, guttulate, fusoid-ellipsoid, straight to slightly curved, apex subobtuse, tapering to truncate hilum, (6–)7–8(–10) × 2.5–3 µm. Abnormal conidia subcylindrical, aseptate, hyaline, smooth, (10–)11–13(–15) × 3–3.5 µm.

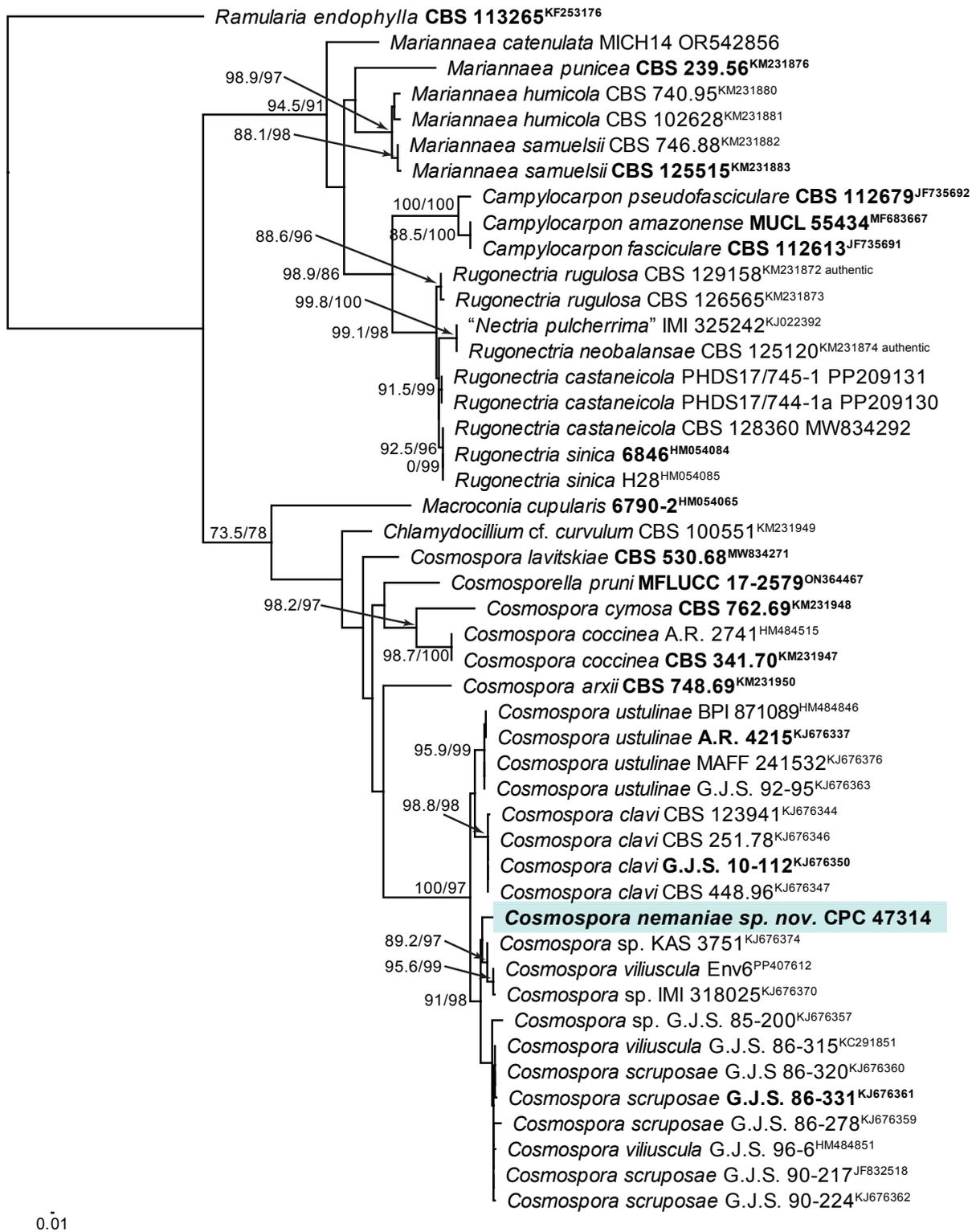
Culture characteristics: Colonies erumpent, spreading, with moderate aerial mycelium and smooth, lobate margin, reaching 10–15 mm diam. after 2 wk at 25 °C. On MEA, PDA and OA surface amber, with diffuse amber pigment, reverse honey.

Typus: France, Haute-Saône (70), Mantoche, bois d'Apremont, on dead or effete stromata of *Nemania* cf. *colliculosa*, 198 m a.s.l., 47.418709°N, 5.494462°E, 31 Oct. 2023, A. Mombert & C. Vernillet, AM2310311 [holotype CBS H-25702; culture ex-type CPC 47314 = CBS 153517; ITS, LSU, *tef1* (first part) and *tef1* (second part) sequences GenBank PV664938.1, PV664964.1, PV664035.1 and PV664045.1].

Colour illustrations: Forest at Mantoche, bois d'Apremont, France. Section through perithecial wall; perithecia; asci with ascospores; colony sporulating on SNA; conidiophores and conidiogenous cells giving rise to conidia; conidia. Scale bars: perithecia = 200 µm; all others = 10 µm.

Notes: Most cosmospora-like fungi have been shown to be mycoparasites of fungi in *Xylariaceae* and *Diatrypaceae* (Gräfenhan *et al.* 2011). Species of *Cosmospora* tend to be strongly host specific. Presently there are no species known from *Nemania* spp. *Cosmospora nemaniae* is characterized by subglobose ascomata with slightly acute papilla and strongly verrucose ascospores. The closest species is *C. ustulinae* and *C. xylariae*, which are distinguished in having narrowly obpyriform ascomata, and smaller ascospores (*C. ustulinae*, 6.0–8.5 × 2.5–5.0 µm, and *C. xylariae*, 8.5–9.5(–10) × 3.8–4.2 µm; Lechat & Fournier 2021). However, *C. nemaniae* is distinct from both of these species with an ITS similarity of 92.26 % and 91.77 % for *C. ustulinae* [as *Cosmospora* sp. '*ustulinae*'; ex-epitype strain AR4215, GenBank JN995619.1; Identities = 489/530 (92 %), 22 gaps (4 %)] and *C. xylariae* [holotype voucher JF19001, GenBank MZ955629.1; Identities = 457/498 (92 %), 22 gaps (4 %)], respectively. No *tef1* sequence of the latter species was available for inclusion in the phylogenetic tree.

Based on a megablast search of NCBI's GenBank nucleotide database, the closest hits using the ITS sequence had highest similarity to *Cosmospora coheniae* [strain BRIP 75895a, GenBank PQ806952.1; Identities = 638/650 (98 %), no gaps], *Cosmospora ustulinae* [as *Cosmospora* sp. '*ustulinae*'; strain AR4215, GenBank JN995619.1; Identities = 560/571 (98 %), no gaps], and *Cosmospora vilioscula* [strain G.J.S. 86-315, GenBank KC291748.1; Identities = 558/570 (98 %), no gaps]. Closest hits using the LSU sequence are *Cosmospora vilioscula* [strain CBS 455.96, GenBank GQ506003.1; Identities = 849/854 (99 %), no gaps], *Pseudocosmospora vilior* [strain CBS 126109, GenBank MH875480.1; Identities = 849/854 (99 %), no gaps], and *Cosmospora khandalensis* [strain CBS 356.65, GenBank NG_069711.1; Identities = 848/854 (99 %), no gaps]. Closest hits using the *tef1* (first part) sequence had highest similarity to *Cosmospora vilioscula* [strain Env6, GenBank PP407612.1; Identities = 413/437 (95 %), five gaps (1 %)], *Cosmospora vilior* [strain G.J.S. 90-217, GenBank JF832518.1; Identities = 370/404 (92 %), 14 gaps (3 %)], and *Cosmospora scruposae* [strain G.J.S. 86-278, GenBank KJ676359.1; Identities = 240/264 (91 %), five gaps (1 %)]. Closest hits using the *tef1* (second part) sequence had highest similarity to *Cosmospora aquatica* [voucher S-350, GenBank MN200275.1; Identities = 829/865 (96 %), no gaps], *Neocosmospora solani* [as *Fusarium solani*; strain MB10A, GenBank LT615305.1; Identities = 880/936 (94 %), no gaps], and *Nectria haematococca* [strain GJS89-70, GenBank AY489624.1; Identities = 899/959 (94 %), two gaps (0 %)].



Most likely phylogram obtained from the maximum likelihood analysis with IQ-TREE v. 2.4.0 (Kalyaanamoorthy *et al.* 2017, Minh *et al.* 2020, Mo *et al.* 2023) of the *Cosmospora tef1* (first part) nucleotide alignment. Bootstrap support values from 1000 non-parametric bootstrap replicates are shown at the nodes (> 74 % are shown), preceded by the SH-aLRT test value (only shown if the node has > 74 % bootstrap support). Culture collection or specimen voucher numbers and GenBank accession numbers (superscript) are indicated for all species. Sequences from material with a type status are indicated in **bold** font. The tree was rooted to *Ramularia endophylla* (CBS 113265; GenBank KF253176) and the novelty described here is highlighted with a coloured block and **bold** font. Alignment statistics: 47 strains including the outgroup; 381 characters including alignment gaps analysed: 345 distinct patterns, 234 parsimony-informative, 59 singleton sites, 88 constant sites. The best-fit model identified for the entire alignment in IQ-TREE using the TESTNEW option was: TPM2u+F+G4. The scale bar shows the expected number of nucleotide substitutions per site. The alignment and tree were deposited at figshare.com (doi: 10.6084/m9.figshare.29050751).

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