Stylonectria norvegica (*Nectriaceae*), a new species from Norway

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Summary: *Stylonectria norvegica* sp. nov. is described from three collections on pyrenomycetous hosts on *Quercus*, *Betula* and *Alnus* in Norway. The fusarium-like asexual state was obtained in culture and the ITS1-5.8S-ITS2 loci were sequenced. This new species is described and illustrated and its affinities and differences with other species in the genus are discussed.
Keywords: Ascomycota, fungicolous, *Hypocreales*, ITS, taxonomy.

Résumé : *Stylonectria norvegica* sp. nov. est décrit à partir de trois récoltes sur des pyrénomycètes variés en Norvège. Le stade asexué de type *Fusarium* a été obtenu en culture et les loci ITS1-5.8S-ITS2 ont été séquencés. Cette nouvelle espèce est décrite et illustrée, de même que ses affinités et ses différences avec les autres espèces connues du genre sont discutées.
Mots-clés : Ascomycota, fongicole, *Hypocreales*, ITS, taxinomie.

Introduction

Stylonectria Höhn. was erected by Höhnel (1915) to accommodate a supposed pycnidial phialidic asexual morph of *Nectria applanata* Fuckel, that he designated as the type species, *S. applanata* Höhn. As established first by Booth (1959) and subsequently recognized by Rossman *et al.* (1999) and Gräfenhan *et al.* (2011), the supposed pycnidia were in fact perithecia filled with discharged ascospores, making the name *Stylonectria* eligible for a sexual morph.

Stylonectria was included by Booth (1959) in his broad concept of *Nectria* Fr. and Rossman *et al.* (1999) synonymized it with *Cosmospora* Rabenh. Gräfenhan *et al.* (2011) presented a phylogenetic revision of the genus *Cosmospora* that was shown to be polyphyletic, leading to the resurrection of several genera, including *Stylonectria*.

As currently conceived, *Stylonectria* is morphologically characterized by pale yellow, dark orange to dark red ascomata, each with a wide, distinctly flattened discoid apex, occurring in dense groups up to 20–30 on a hyphal hypostroma seated on effete stromata of mostly diaporthalean pyrenomycetes. The ascomatal wall, composed of two regions, turns dark red to purple in 3% KOH and yellow in lactic acid, a feature characteristic of the *Nectriaceae*. The hypostroma is an old sporodochium previously bearing the fusarium-like asexual state, usually yielding microconidia and falcate macroconidia, the latter mostly 0–1-septate.

In addition to the type species *S. applanata* known from stromata of *Melogramma bulliardii* Tul. & C. Tul. on *Corylus*, Gräfenhan *et al.* (2011) retained three species, viz.: *S. carpini* Gräfenhan on *Carpinus betulus*, on old pyrenomycetes, including *Melanconis spodiaea* Tul. & C. Tul.; *S. purtonii* (Grev.) Gräfenhan on coniferous wood, possibly on *Valsa* sp. according to Booth (1959), and on old pyrenomycetes on *Hippocrepis emerus*; and *S. wegeliniana* (Rehm) Gräfenhan, Voglmayr & Jaklitsch on old ascomata of *Hapalocystis bicaudata* Fuckel on *Ulmus*. Except for *S. wegeliniana* having significantly larger warted ascospores than other species of *Stylonectria*, the morphology of the sexual states is not different enough to distinguish species. Defining these species relies on phylogenetic comparison coupled with the morphology of asexual morphs obtained in culture as well as host affiliation.

During a pyrenomycetes workshop in south Norway in October 2014, a nectrioid ascomycete resembling *Stylonectria* was collected by two of the authors. These specimens were cultured and sequenced. This fungus represents a previously undescribed species in the genus *Stylonectria*. A morphological description of its asexual and sexual morphs is presented with illustrations, a maximum likelihood phylogeny of *Stylonectria* based on ITS1-5.8S-ITS2 sequences is provided, and the status of the new species within *Stylonectria* is discussed.

Materials and methods

Specimens were examined using the method described by Rossman *et al.* (1999). Microscopic observations and measurements were made from material mounted in water and ascospore ornamentation was observed in lactic cotton blue without heating. Cultures of the living specimen were made on PDA (Potato Dextrose Agar) with 5 mg/l of streptomycin in Petri dishes 9 cm diam. Centrum contents with asci and ascospores were removed from a perithecium with a fine needle and placed in a drop of sterile water that was stirred with a sterile needle. The drop with ascospores was placed on PDA using a sterile micropipette under an inverted microscope, then the Petri dishes were incubated at 25 °C.

DNA extraction, amplification, and sequencing: Mycelium from the surface of cultures was transferred to Eppendorf tubes and sent to the Canadian centre for DNA Barcoding (CCDB) in Guelph, Canada, for barcoding. There, DNA was extracted using the glass fiber plate DNA extraction protocol by Ivanova *et al.* (2015). PCR amplification followed Kuzmina & Ivanova (2015). Sequencing was performed using the primers ITS1-5 for the internal transcribed spacer (ITS) region according to White *et al.* (1990).

Phylogenetic analyses were performed online at www.phylogeny.lirmm.fr (Dereeper *et al.*, 2008). Maximum likelihood phylogenetic analyses were performed with PhyML 3.0 aLRT (Zwickl, 2006), using the GTR + I + Γ model of evolution. Branch support was assessed using the nonparametric version of the approximate likelihood-ratio test, implemented in PhyML (SH-aLRT) (Anisimova & Gascuel, 2006).

Taxonomy

Stylonectria norvegica Lechat, J. Fourn. & Nordén *sp. nov.* — Mycobank MB 813734. Plates 1–2, Fig. 1

Diagnosis: Differs from its closest relative, *Stylonectria carpini*, by 10% difference in ITS sequences, in not producing microconidia in culture, and in having smaller macroconidia (8.5–)10–13(–14) × 1.8–2.5 µm vs. 15–45 × 3–3.5 µm.

Holotype: NORWAY, Aust-Agder, Arendal, Stea, on dead sporodochia of fusarium state on pyrenomycete (presumably *Amphiporthe* sp.), on dead, corticated *Quercus* branch, 4 Oct. 2014, *leg.* C. Lechat, Holotype CLL14047, ex-holotype culture CBS 139239, GenBank KR605485.

Etymology: The epithet *norvegica* refers to Norway, the country where this species was collected.

Ascomata in groups of 5–25(–30), erumpent to superficial with base remaining immersed in a hypostroma of fusarium-like sporodochia, arising from dead pyrenomycetes, yellow, dark orange to bright red, turning dark purple in 3% KOH and yellow in lactic acid, subglobose to obpyriform, not collapsing or laterally pinched upon drying, 250–330(–350) µm high, 200–300 µm diam, apex broadly discoid, flattened 50–70 µm high × 160–220 µm diam, slightly constricted below, with a minute, pallid, conical central papilla. **Ascomatal wall** in vertical section 35–40 µm thick at sides, 25–30 µm thick at base, of two intergrading regions: outer region 25–35 µm thick, of thick-walled cells lacking a definite shape, in surface view appearing as intertwined hyphae; inner region of thin-walled hyaline, flattened cells; apex of palisadic, thick–walled, elongate cells with slightly swollen tips. **Asci** unitunicate, cylindrical to narrowly clavate, short-stipitate, containing eight ascospores obliquely uniseriate or irregularly biseriate above and uniseriate below, (60–)65–75(–78) × 6–7(–8) µm, apically truncate when immature, becoming rounded, when mature, without ring. **Ascospores** (8–)8.5–9.5(–11) × 3.5–4.2(–4.5) µm (X = 9 × 4 µm, n = 30), ellipsoid, equally two–celled, constricted at septum, thick-walled, hyaline to pale yellowish-brown, with 2–3 small guttules in each cell, smooth-walled.

Asexual state: fusarium-like on sporodochia.

Cultural characteristics: After one week at 25 °C, colony 3–4 cm diam, pale orange, producing a slow-growing fusarium-like asexual morph, sporulating at white to cream margin. No microconidia produced. Macroconidia cylindrical to long-fusiform, falcate, acute at both ends, 1-septate, (8.5–)10–13(–14) × 1.8–2.5 µm, smooth, hyaline.

Paratype specimens: NORWAY, Ytre Lauvrak, on sporodochia of fusarium-like morph on dead stromata of *Diatrypella favacea* (Fr.) Ces. & De Not., on *Betula* sp., 3 Oct. 2014, *leg*. C. Lechat, pers. herb. CLL14033, culture CBS139234, GenBank KR605484; Aust-Agder, Arendal, Nedenes, on sporodochia of fusarium-like morph on dead, unidentified pyrenomycete, on *Alnus* sp., 3 Oct. 2014, *leg*. C. Lechat, pers. herb. CLL14050, culture CBS 139242, GenBank KR605486.

Discussion: *Stylonectria norvegica* conforms to *Stylonectria* as defined by Gräfenhan *et al.* (2011) in having yellow, dark orange to bright red nectrioid ascomata, clustered on a hyphal hypostroma seated on dead stromatic pyrenomycetes, with distinctive flattened-discoid apices, KOH+, two-layered wall with thick-walled outer cells, and a fusarium-like asexual morph.

When compared with other species in *Stylonectria*, *S. norvegica* clearly differs from *S. wegeliniana* by the smaller, smooth ascospores but has ascospores dimensions in the same range as *S. applanata*, *S. carpini* and *S. purtonii*. Our phylogenetic dendrogram (Fig. 1) shows that the three collections of *S. norvegica* cluster together sister to *S. carpini* but are more distant from *S. applanata* and *S. purtonii*. *Stylonectria norvegica* differs from *S. carpini* by lacking microconidia in culture and by having smaller macroconidia (8.5–)10–13(–14) × 1.8–2.5 µm vs. 15–45 × 3–3.5 µm (according to

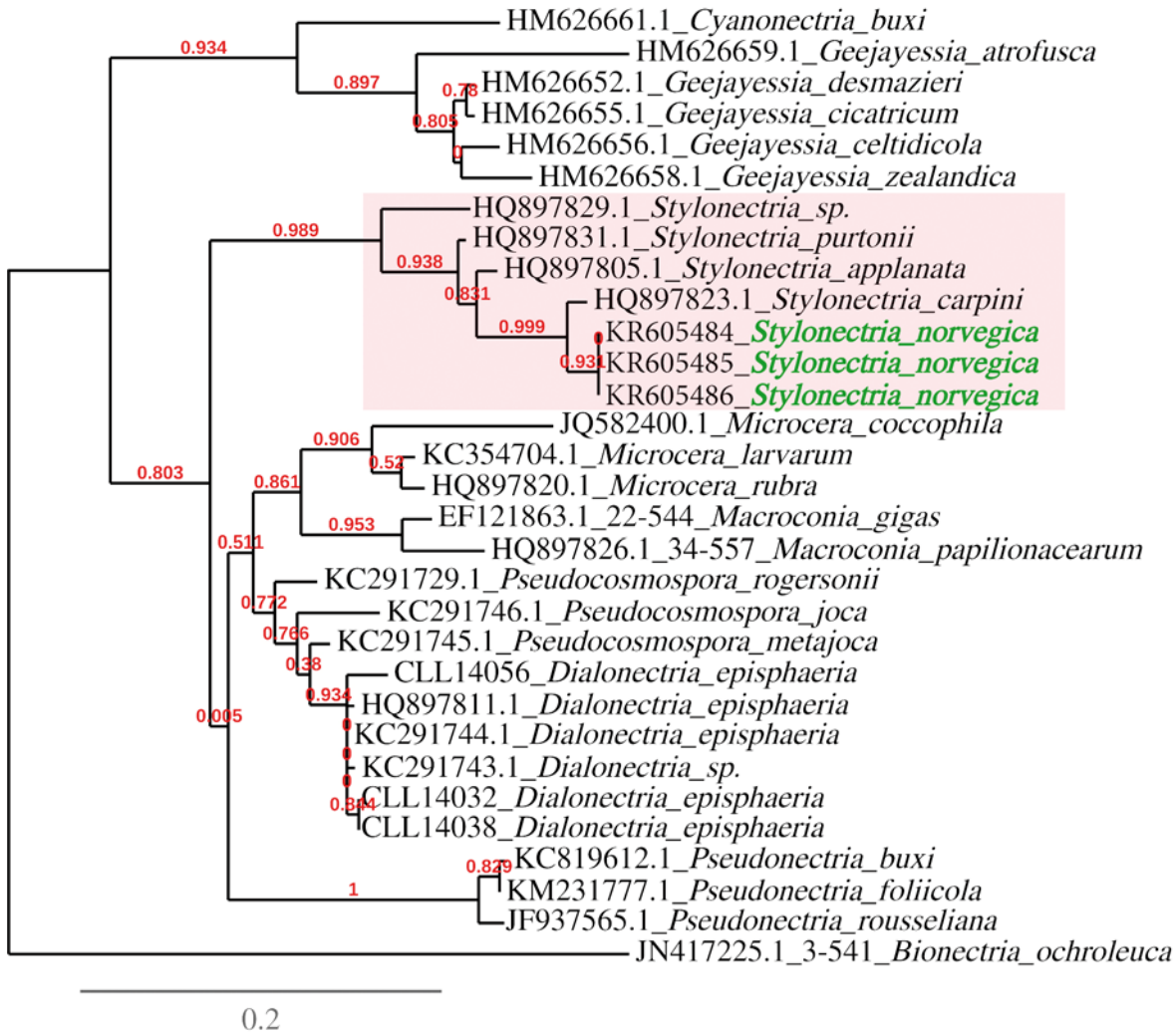

Fig. 1 – Maximum likelihood phylogeny of *Stylonectria norvegica* from ITS1-5.8S-ITS2 sequences rooted with *Bionectria ochroleuca*.

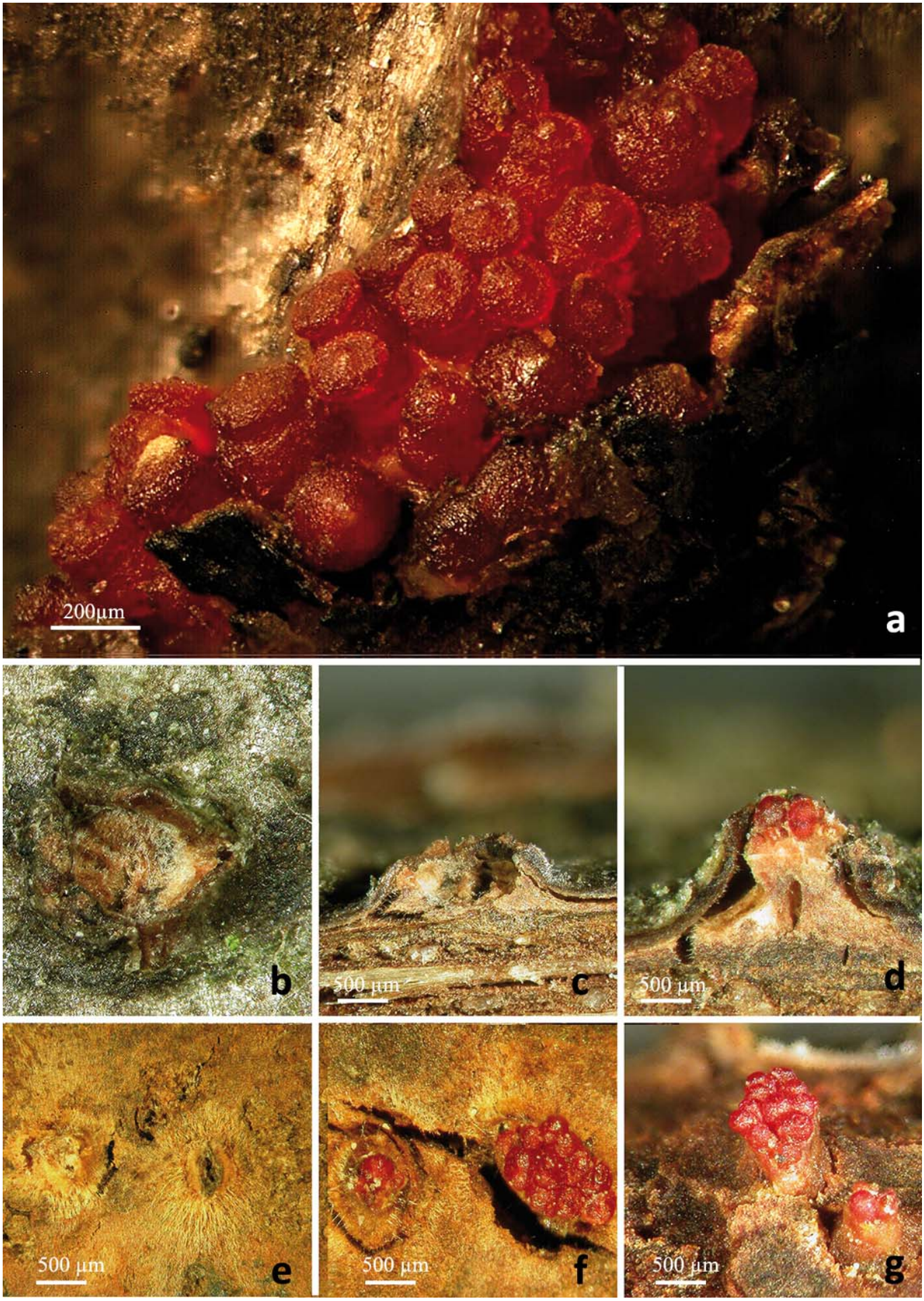

Plate 1 — *Stylonectria norvegica*

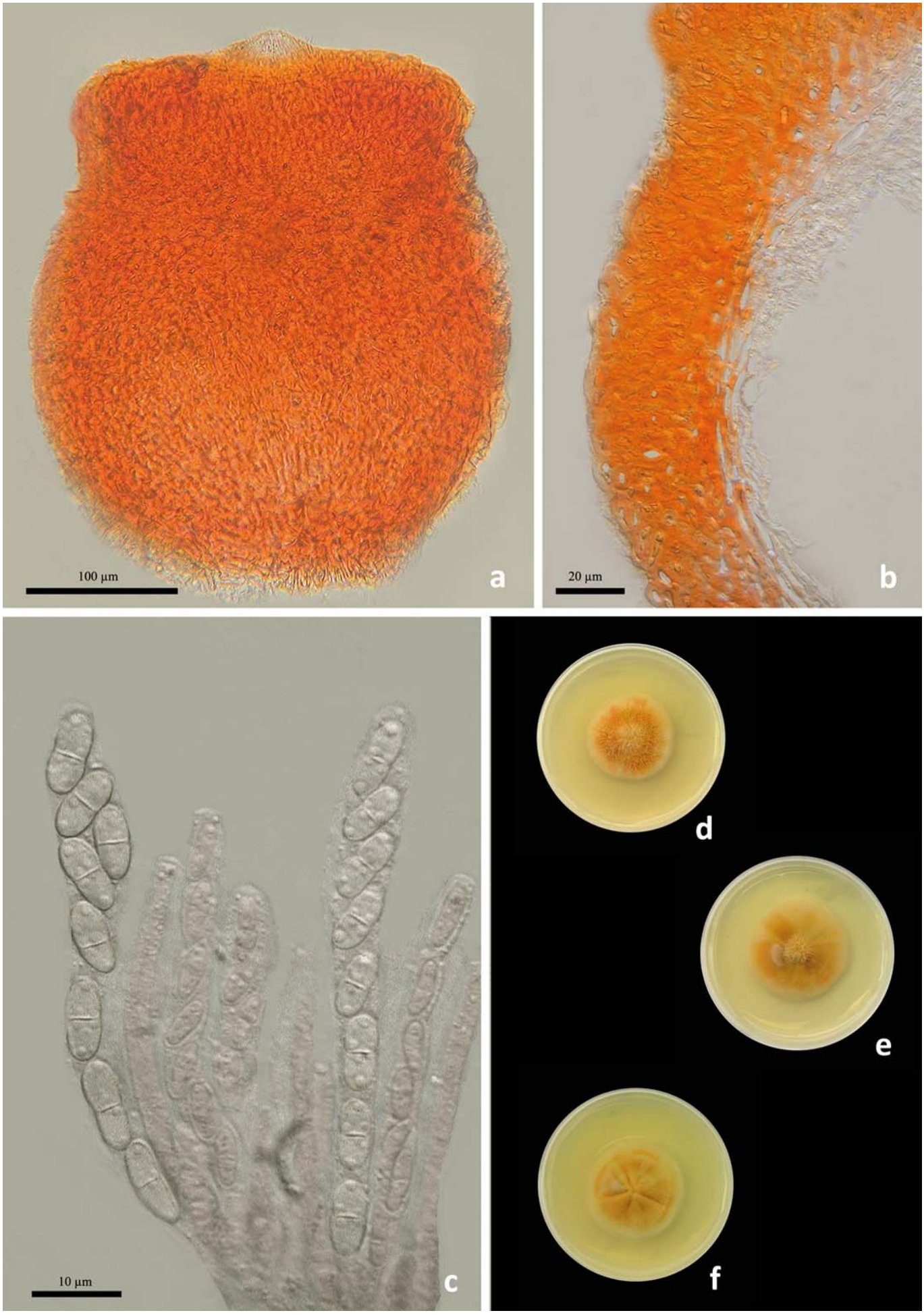

Plate 2 — *Stylonectria norvegica*

Legend of Plate 1. a-g: *Stylonectria norvegica* (Holotype) in the natural substratum; a: Ascomata with typically flattened-discoid apices; b: Sporodochium of the fusarium-like morph in surface view; c: Sporodochium of the fusarium-like morph in vertical section; d: Ascomata seated on sporodochium; e-g: Sporodochia and ascomata on substratum after removal of the periderm.

Legend of Plate 2. a-g: *Stylonectria norvegica*, a: Close-up of an ascoma in side view, in water; b: Lateral ascomatal wall in vertical section, in water; c: Asci and ascospores, in water; d-f: Cultures after nine days: d: CLL14047, e: CLL14033, f: CLL14050.

Booth, 1971). Moreover, pairwise alignment indicates only 90% similarity between ITS sequences of *S. carpini* and *S. norvegica*. According to Gräfenhan *et al.* (2011), *S. carpini* is affiliated with pyrenomycetes occurring on *Carpinus*, while *S. norvegica* occurs on pyrenomycetes on *Alnus glutinosa*, *Betulus pendula* (*Betulaceae*) and *Quercus* sp. (*Fagaceae*) and is thus not host-specific. Species in *Stylonectria* are usually considered to be host specific, such as *S. carpini* on pyrenomycetes on *Carpinus* and *S. wegeliniana* on *Hapalocystis bicaudata* on *Ulmus glabra* but only a limited number of collections are known. Most hosts of *Stylonectria* are presumably diaporthalean, this assumption is hampered by the difficulty of identifying effete pyrenomycetes. The presence or absence of an apical ring in the asci may further differentiate species of *Stylonectria* but this characteristic is not documented for all species.

Another species previously placed in the broadly conceived *Cosmospora*, namely *Nectria magnusiana* Rehm ex Sacc., may belong in *Stylonectria*. Booth (1959) described its ascomata with flat discoid apices clustered on sporodochia producing microconidia and occurring on *Diatrypella favacea* and *D. quercina*. This species differs from *S. wegeliniana* by smaller, smooth-walled ascospores 10–15 × 4.6–6 µm (Booth, 1959), and from *S. purtonii*, *S. applanata* and their relatives by significantly larger ascospores.

Stylonectria is currently a small and poorly known genus but further additions of new species so far regarded as *Cosmospora* sp. might be expected in the future.

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