

Lasionectriella, a new genus in the *Bionectriaceae*, with two new species from France and Spain, *L. herbicola* and *L. rubioi*

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Abstract: *Lasionectriella rubioi* gen. and sp. nov. and *L. herbicola* sp. nov. are described and illustrated based on collections from France and Spain. The asexual morph of *L. rubioi* was obtained in culture and cultures of both species were sequenced. The genus is placed in the *Bionectriaceae* based on ascomata not changing colour in 3% KOH or lactic acid, acremonium-like asexual morph and phylogenetic comparison of LSU sequences with species in 13 genera of the *Bionectriaceae*. *Lasionectriella* is primarily characterized by subglobose, pale yellow, pale orange to dark brownish orange, non-stromatic ascomata with a conical apex and ascomatal surface covered by thick-walled hyphae proliferating from base and agglutinating to form a fringe around the upper margin of ascomata. It is morphologically and phylogenetically compared with the most similar genera *ljuhya*, *Lasionectria* and *Ochronectria*.

Keywords: acremonium-like, Ascomycota, *Hypocreales*, *ljuhya*, *Lasionectria*, ribosomal DNA, taxonomy.

Résumé : *Lasionectriella rubioi* gen. et sp. nov. et *L. herbicola* sp. nov. sont décrits et illustrés d'après des récoltes effectuées en France et en Espagne. La forme asexuée de *L. rubioi* a été obtenue en culture et les cultures des deux espèces ont été séquencées. Le genre est placé dans les *Bionectriaceae* d'après les ascomes ne changeant pas de couleur dans KOH à 3% ou dans l'acide lactique, la forme asexuée acremonium-morphe et la comparaison des séquences LSU avec des espèces représentant 13 genres de *Bionectriaceae*. *Lasionectriella* caractérisé par des ascomes subglobuleux, jaune pâle, orange pâle à orange-brunâtre foncé, non stromatiques, à sommet conique et la surface couverte d'hyphes à paroi épaisse se développant depuis la base et s'agglutinant pour former une frange autour de la marge de l'ascome. *Lasionectriella* est morphologiquement et phylogénétiquement comparé avec les genre *ljuhya*, *Lasionectria* et *Ochronectria*.

Mots-clés : acremonium-morphe, Ascomycota, *Hypocreales*, *ljuhya*, *Lasionectria*, ADN ribosomal, taxinomie.

Introduction

In the course of a survey of hypocrealean fungi in Europe, three collections on *Ruscus aculeatus* L. (*Liliaceae*) and one on unidentified herbaceous stem were intriguing because of their unusual type of vestiture. They were assigned to the *Bionectriaceae* based on their pale yellow, pale orange or brownish-orange ascomata not changing colour in 3% KOH or lactic acid, which was confirmed by phylogenetic comparison of their LSU sequences with those of 13 other bionectriaceous genera (Fig. 3). As they feature an ascomatal wall less than 25 µm thick, composed of thick-walled cells, covered by thick-walled hairs aggregated into a fringe around the upper margin of perithecia, they appeared to closely resemble *Lasionectria* (Sacc.) Cooke (COOKE, 1884) and *ljuhya* Starbäck (STARBÄCK, 1899). However, a thorough morphological comparison of our collections with these genera showed a difference in type of vestiture and in our phylogenetic dendrogram they appeared distant from the *Lasionectria* clade and the *ljuhya* clade and instead clustered with *Ochronectria* Rossman & Samuels on a separate branch. As our collections are morphologically clearly different from the monotypic tropical genus *Ochronectria* characterized by smooth thick-walled ascomata, the wall of which comprises a middle layer rich in orange oily droplets, we feel justified to introduce the new genus *Lasionectriella* to accommodate our collections. As the three collections on *Ruscus* deviate from that on herbaceous stem by larger and spinulose ascospores, they are accommodated in two different species, viz. *L. rubioi* and *L. herbicola* respectively.

Both species were successfully cultured but only *L. rubioi* yielded an acremonium-like asexual morph, while the culture of *L. herbicola* remained sterile. The differences of *Lasionectriella* with *Lasionectria* and *ljuhya* are discussed and an illustrated morphological comparison of *Lasionectriella* with *Ochronectria* is provided.

Materials and methods

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

Taxonomy

Lasionectriella Lechat & J. Fourn., *gen. nov.*
Mycobank 815673.

Diagnosis: Differs from *Lasionectria* and *ljuhya* by a conical apex and thick-walled hairs covering the ascomatal wall, proliferating from base and agglutinating to form a fringe around the upper margin of perithecia.

Type species: *Lasionectriella rubioi* Lechat & J. Fourn.

Etymology: *Lasionectriella* refers to the resemblance of its hairy ascomata with *Lasionectria*.

Lasionectriella rubioi Lechat & J. Fourn., *sp. nov.* – Fig. 1
Mycobank 815674.

Diagnosis: Ascumata superficial, non-stromatic, subglobose, cupulate upon drying, pale yellow, pale brownish orange to reddish brown, not changing colour in 3% KOH or lactic acid, covered by thick-walled hairs arising from base, agglutinated and proliferating to form a fringe around upper margin of perithecia; ascomatal wall 18–25 µm thick composed of thick-walled, globose or ellipsoidal to flattened cells; asci 8-spored, fusoid-clavate without apical apparatus; ascospores 10–12 × 2–2.5 µm, equally two-celled, spinulose.

Holotype: FRANCE, Deux-Sèvres: Villiers-en-Bois, Forêt de Chizé, 1 Mar. 2015, on dead stem of *Ruscus aculeatus*, leg. C. Lechat, CLL15078 (LIP). Ex-type culture CBS 140157, GenBank KU593581.

Additional specimens examined (paratypes): FRANCE, Deux-Sèvres: Villiers-en-Bois, Forêt de Chizé, 30 Mar. 2012, on dead stem of *Ruscus aculeatus*, leg. C. Lechat, CLL12011 (LIP); Culture: CBS 132543. SPAIN, Asturias: Soto de los Infantes, 27 Jan. 2008, on dead stem of *Ruscus aculeatus*, leg. E. Rubio, CLL7155 (LIP).

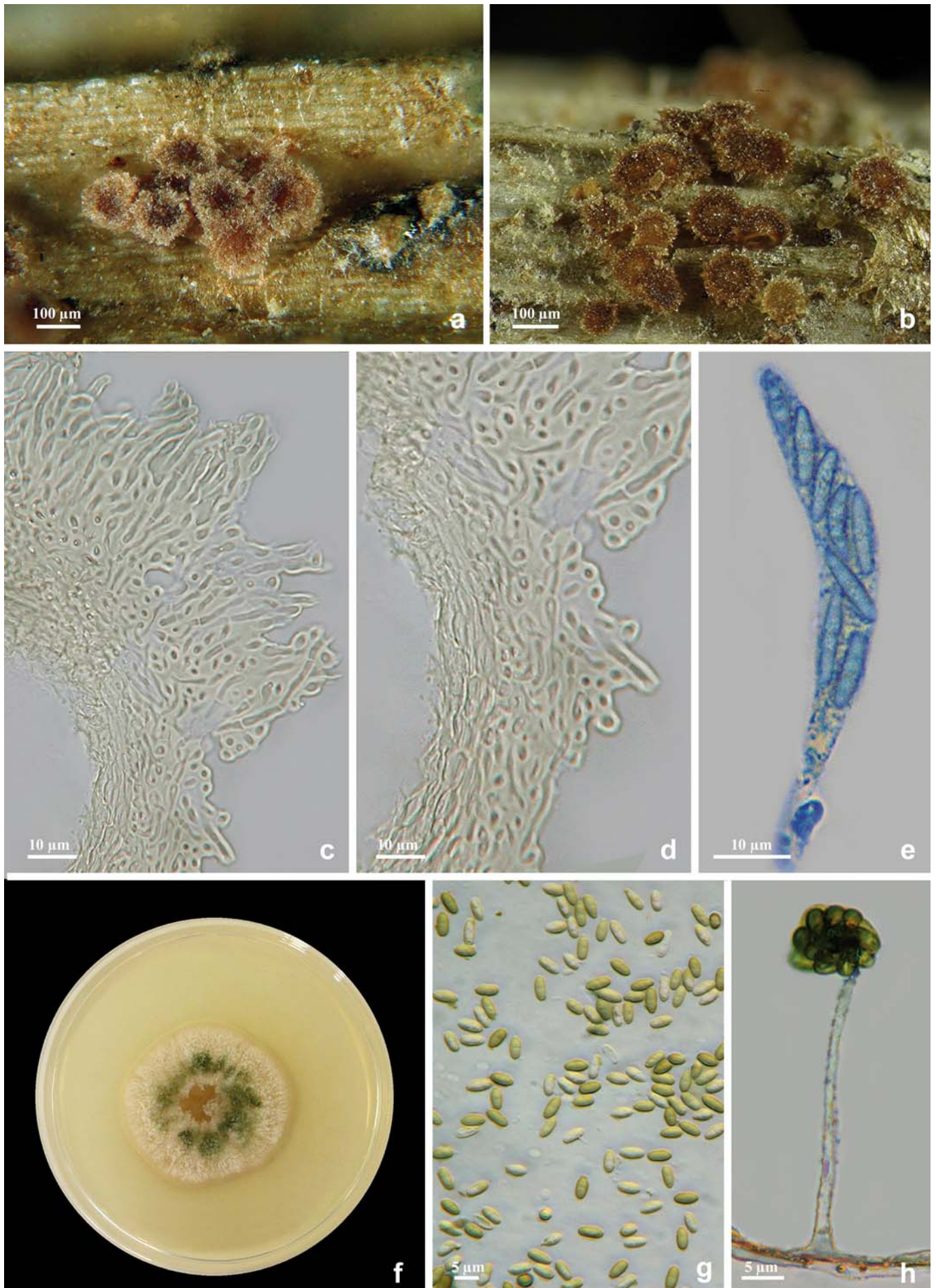


Fig. 1 – a-h: *Lasionectriella rubioi* (holotype CLL15078). a-b: Ascomata on host substratum, c: Ascomatal wall in vertical section, d: Tip of hairs covering ascomatal surface: Ascus and ascospores, f: Culture after two weeks, g: Conidia, h: Conidiophore and conidia.

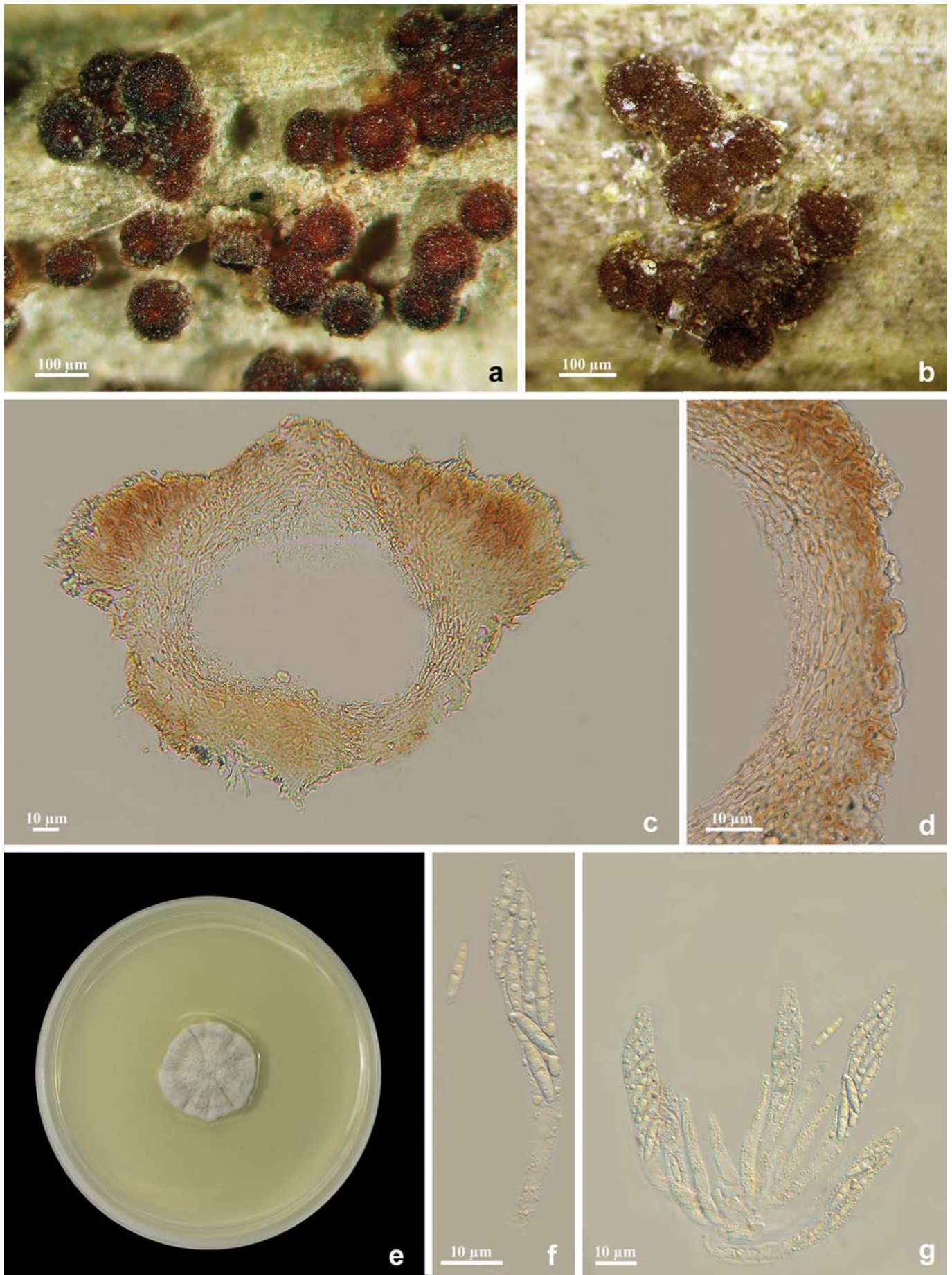


Fig. 2 – a-h: *Lasionectriella herbicola* (holotype CLL15077). a-b: Ascomata on host substratum, c: Vertical section through ascoma, d: Vertical section through ascomatal wall, e: Culture after two weeks, f–g: Asci and ascospores.

Etymology: The epithet *rubioi* refers to the distinguished Spanish mycologist Enrique Rubio Dominguez who collected the first specimen of *Lasionectriella*.

Ascomata gregarious, solitary or crowded in groups of 3–8, superficial, subglobose, apex conical, 140–170 µm high × 140–180 µm diam ($X = 165 \times 175 \mu\text{m}$, $n = 10$), white when immature, later dark orange to brownish-orange, collapsing cupulate when dry, not changing colour in 3% KOH or lactic acid. Hyphae covering ascomatal surface 2–3 µm diam, developing from ascomatal base, proliferating and agglutinating to form a fringe around upper margin of perithecia, hyaline to pale yellowish when dry, thick-walled, wall 1–1.5 µm thick, swollen and rounded at tip, septate, intertwined and difficult to separate. **Perithecial wall** 15–25 µm thick, composed of two regions: outer region 10–15 µm wide, of globose to ellipsoid cells 2–5 × 2.5–4.4 µm with hyaline to pale yellow wall; inner region 5–10 µm wide, of elongate, flattened, hyaline cells 5–8 × 1.5–2.5 µm. **Asci** 55–65 × 6–8 µm ($X = 60 \times 7.5 \mu\text{m}$, $n = 30$), clavate to fusoid, apex flattened, without ring, with 8 irregularly biseriolate ascospores. **Paraphyses** moniliform, 8–12 µm wide at base, inserted between asci. **Ascospores** (8.5–) 9–12 (–14) × 2.2–2.5 (–2.8) µm ($X = 10.4 \times 2.4 \mu\text{m}$, $n = 30$), hyaline, fusoid, rounded at ends, straight to lightly curved, equally 2-celled, constricted at septum, spinulose, with two yellow drops in each cell.

Asexual morph: acremonium-like.

Cultural characteristics: Colony after two weeks at 25°C on PDA 25–30 mm diam., greyish green in centre, white at margin, reverse greenish yellow; after three weeks colony reaching 40–45 µm diam., greyish green in centre, white in median area and green at margin, producing no coloration in medium. Mycelium with hyphae branching, septate, hyaline to pale brown, smooth, 3–5 µm diam. Conidiophores borne on aerial hyphae, macronematous, mononematous, unbranched, flexuous, hyaline, faintly roughened, 2.5–3.5 µm diam. Conidiogenous cells monophialidic, 23–45 µm long, 1.5–1.8 µm wide at apex with a flared collarette, 2–2.2 µm wide at base. Conidia grouped at tip of phialide to form a mucous head, aseptate, narrowly ellipsoidal to subcylindrical with rounded apex, attenuated at base with an apiculate hilum, walls smooth, at first hyaline, becoming dark green, appearing nearly black in mass, 3.5–5.5 × 1.8–2.2 µm ($X = 4.5 \times 2 \mu\text{m}$, $n = 30$).

Lasionectriella herbicola Lechat & J. Fourn., *sp. nov.* – Fig. 2
Mycobank 815675.

Diagnosis: Differs from *L. rubioi* by smooth smaller ascospores 8–10 × 2–2.5 µm.

Holotype: FRANCE, Deux-Sèvres: Villemanan, Chanc, 23 Apr. 2015, on dead herbaceous stems, *leg.* Suzanne Buissonnet, CLL15077 (LIP). Ex-type culture CBS 140156. GenBank: KU593582.

Etymology: The epithet *herbicola* refers to the substrate on which this species was collected.

Ascomata gregarious, solitary or crowded in groups of 2–9, superficial, subglobose, apex conical, 120–150 µm high × 130–160 µm diam ($X = 145 \times 150 \mu\text{m}$, $n = 10$), dark brownish-orange, collapsing cupulate when dry. Hyphae covering ascomatal surface 2–3 µm diam, developing from ascomatal base, proliferating and agglutinating to form a fringe around upper margin of perithecia, hyaline to pale yellowish brown, thick-walled, wall 1–1.5 µm thick, cylindrical and rounded at tip, septate, tightly intertwined and impossible to separate. **Perithecial wall** 20–25 µm thick, composed of two regions: outer region 15–20 µm wide, of subglobose to ellipsoidal, thick-walled cells 4–10 × 2.5–3.5 µm with hyaline to pale yellow wall 1–1.5 µm thick; inner region 5–7 µm wide, of elongate, flattened,

hyaline cells 5–8 × 1–1.5 µm, with sparse orange, oily droplets between the cells and hymenium. **Asci** 45–55 × 5–7 µm ($X = 50 \times 6 \mu\text{m}$, $n = 30$), clavate to fusoid, apex flattened, without ring, with 8 irregularly biseriolate ascospores. **Paraphyses** narrow, moniliform, 4–6 µm wide at base, inserted between asci. **Ascospores** (7.5–) 8–10 (–11) × 2.2–2.5 (–2.8) µm ($X = 9 \times 2.3 \mu\text{m}$, $n = 30$), hyaline, fusoid, rounded at ends, straight to lightly curved, equally 2-celled, smooth, with two yellow drops in each cell.

Asexual morph: unknown.

Cultural characteristics: Colony after two weeks at 25°C on PDA 25–30 mm diam., white in centre, greyish white to greyish yellow in median area and white at margin, radially furrowed, reverse pale yellow to pale yellowish brown; floccose aerial mycelium white to pale yellow in median area, white at margin, without coloration of the medium. No conidia produced in culture after four weeks.

Discussion

Lasionectriella is characterised by the combination of pale brownish orange to pale reddish brown ascomata with wall composed of thick-walled cells not changing colour in 3% KOH or lactic acid, collapsing cupulate upon drying, a conical apex surrounded by a crown of hyaline to pale yellow, thick-walled entangled hairs and an acremonium-like asexual morph. This set of characters strongly suggests affinities with the *Bionectriaceae* as defined by ROSSMAN *et al.* (1999), especially with the genera *Ijuhya* and *Lasionectria*.

Ijuhya primarily differs from *Lasionectriella* and *Lasionectria* in having ascomata usually with a flat, discoidal apex and fasciculate hairs agglutinated to form triangular teeth arranged in a stellate fringe around the upper margin of the perithecia.

Lasionectria is morphologically the genus most closely related to *Lasionectriella* in having hairs scattered over the ascomatal surface. However, ascomatal hairs in *Lasionectria* are solitary or bound in triangular fascicles while in *Lasionectriella* they cover the whole ascomatal surface, developing from ascomatal base, proliferating and agglutinating to form a fringe around the upper margin of perithecia. The value of these morphological differences of ascomatal vestiture is supported by our phylogenetic analysis showing *Lasionectriella* is fairly distant from both *Ijuhya* and *Lasionectria* (Fig. 3). We also considered comparing *Lasionectriella* to the monotypic genus *Stephanonectria* Schroers & Samuels which likewise belongs to the *Bionectriaceae* and features a crown-like structure around the ostiolar area (SCHROERS *et al.*, 1999). However, in *S. keithii* (Berk. & Broome) Schroers & Samuels perithecia are stromatic, apical crown is cellular, not hyphal as in *Lasionectriella*, ascospores are ornamented with short striae and asexual morph, referred to myrothecium-like, differs from the known asexual morph of *Lasionectriella* in being penicillate and in yielding hyaline conidia. This set of morphological characters deviating from *Lasionectriella* is supported by the position of *S. keithii* in our phylogram which clusters with *Bionectria ochroleuca* (Schw.) Schroers & Samuels on a distant branch. Moreover, *Myrothecium* appears very distant from *Stephanonectria*, which suggests that the asexual morph of *Stephanonectria* is closer to *Clonostachys* (= *Bionectria*) than to *Myrothecium* (Fig. 3).

This molecular analysis, comparing 13 genera in the *Bionectriaceae*, shows that the closest genus to *Lasionectriella* is *Ochronectria*. *Ochronectria*, a monotypic genus known only from the tropics, has an acremonium-like asexual morph (ROSSMAN *et al.*, 1999), like *Lasionectriella*, but is distinguished by glabrous ascomata seated on a thin basal subiculum, ascomatal wall 45–60 µm thick, three-layered, comprised of thin-walled cells, containing abundant orange oily droplets in the median region, and multiseptate and striate ascospores (Fig. 4). Despite apparent phylogenetic affinities, *Ochronectria* cannot be equated with *Lasionectriella* because of these strong morphological differences. However, the presence of sparse orange oily droplets in hymenium and ascomatal wall observed in

L. herbicola is reminiscent of *Ochronectria*, which also has orange droplets but considerably more abundant than in *L. herbicola*. It is unknown whether this character explains the apparent phylogenetic relationship between both genera. Thus, based on morphological, cultural and molecular data, the new bionectriaceous genus *Lasionectriella* is introduced to accommodate the new species *L. herbicola* and *L. rubioi*.

We do not regard the occurrence of moniliform basal paraphyses reported here from both species of *Lasionectriella* as a differential character for the genus. While the absence of true basal paraphyses is regarded as typical of *Hypocreales* (ROSSMAN *et al.*, 1999), we repeatedly observed such moniliform paraphyses in either bionectriaceous or nectriaceous collections, provided that the material is fresh and barely mature. These sparse, fragile, thin-walled paraphyses likely deliquesce rapidly upon maturation of the centrum and cannot be detected in dry herbarium material.

Lasionectriella herbicola is primarily distinguished from *L. rubioi* by smaller and smooth ascospores and possibly by a different host affiliation. Further differential characters which should be confirmed by examination of more specimens are the overall darker ascomatal colour of *L. herbicola* and the presence of sparse orange droplets in hymenium and ascomatal wall. Finally, the fact that, under the same conditions of culture, *L. rubioi* yields an asexual morph while *L. herbicola* does not is likely a further differential character between the two species.

The repeated occurrence of *L. rubioi* on *Ruscus aculeatus* suggests a strong host-preference for this plant, if not host-specificity. It is noteworthy that several *Hypocreales* seem to be specific for this host, including *Coccinonectria rusci* (Lechat, Gardiennet & J. Fourn.) L. Lombard & Crous (LOMBARD *et al.*, 2015) and others still under investigation.

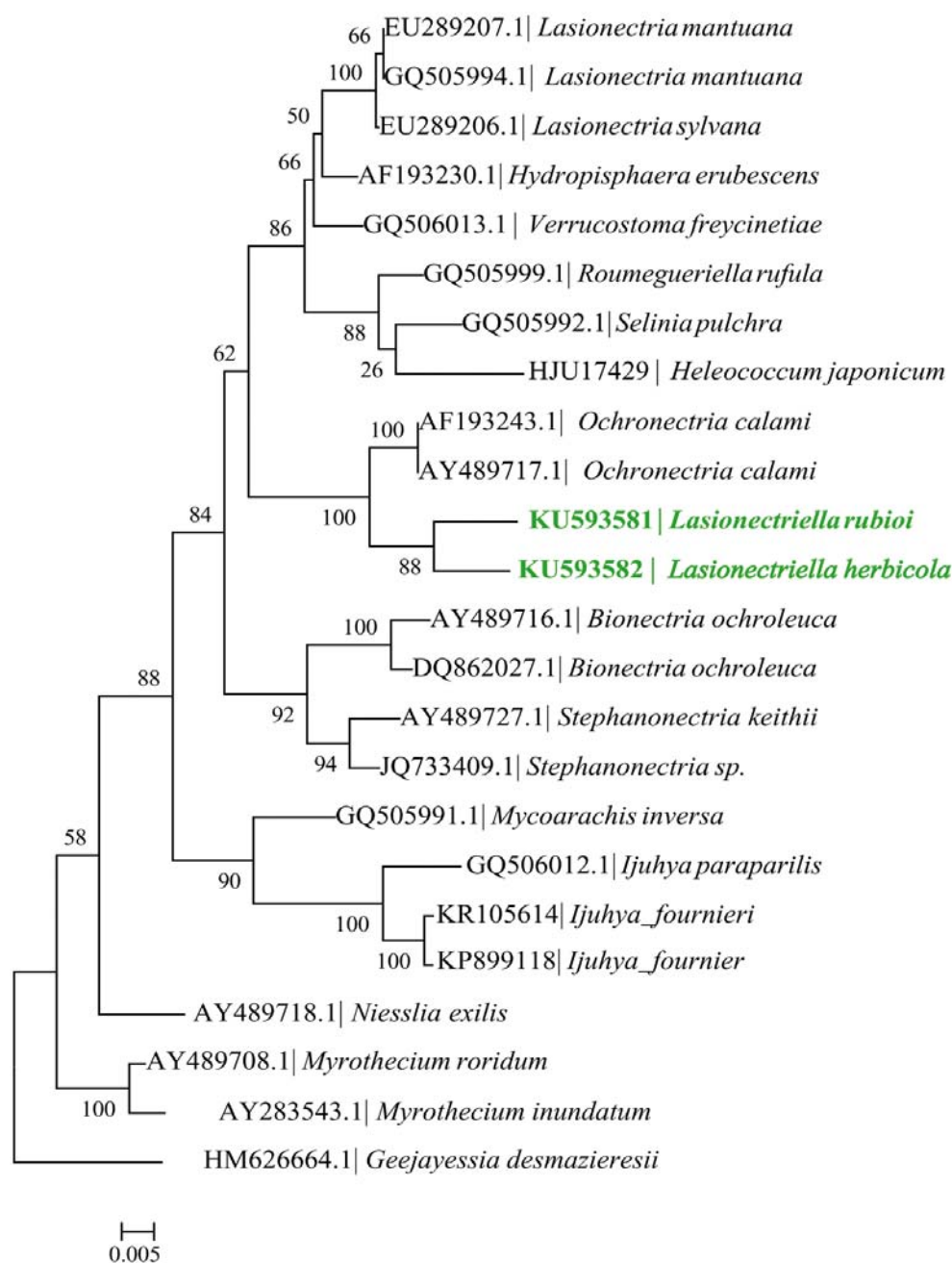


Fig. 3 – Maximum likelihood phylogeny of *Lasionectriella* spp. inferred from comparison with LSU sequences of 13 genera in the *Bionectriaceae*, rooted with *Geejayessia desmazieresii* (*Nectriaceae*).

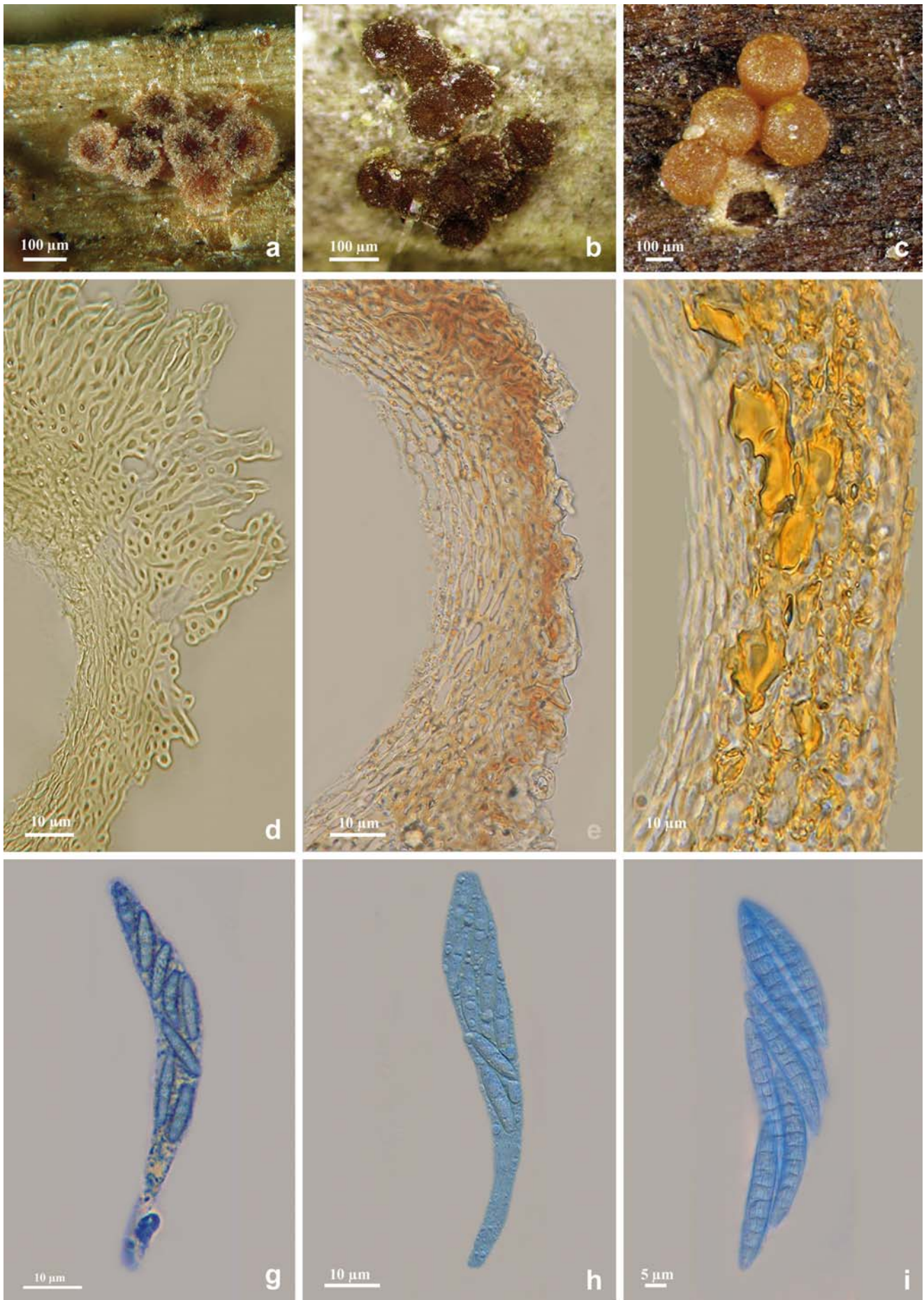


Fig 4 – a-i: Comparison of *Lasionectriella* spp. with *Ochronectria*. a-c: Ascomata in natural environment; a: *Lasionectriella rubioi* (holotype), b: *Lasionectriella herbicola* (holotype), c: *Ochronectria calami**; d–f: vertical section through ascomatal wall, g–i: Asci and ascospores in lactic blue. *FWI, Martinique, Sainte-Marie, Forêt départementale-domaniale, La Philippe; 22 Aug. 2012, on spathe of *Cocos nucifera* L. CLLM12059 (LIP).

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