Geonectria, a new genus in the *Bionectriaceae* from France

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Abstract: *Geonectria subalpina* gen. and sp. nov. is described and illustrated based on three collections on soil from subalpine zone in the French Alps. The genus is placed in the *Bionectriaceae* based on morphological characteristics of the sexual morph and phylogenetic comparison of LSU sequences with species in 14 genera in the *Bionectriaceae*. *Geonectria* is primarily characterized by subglobose, nonstromatic, orange ascomata, becoming brownish orange, laterally collapsing or not collapsing when dry, roughened in upper half by superficial, scattered clusters of evanescent yellow hyphae, ellipsoidal to widely fusiform, 1-septate, finely longitudinally striate ascospores and habitat on soil in subalpine zone. It is recognized as a distinct genus by its peculiar ecology and its isolated phylogenetic position within the *Bionectriaceae*.
Keywords: Ascomycota, French Alps, *Hypocreales*, new taxa, ribosomal DNA, taxonomy.

Résumé : *Geonectria subalpina* gen. et sp. nov. est décrite et illustrée d'après trois récoltes faites sur le sol, en zone subalpine dans les Alpes françaises. Le placement du nouveau genre dans les Bionectriacées repose sur les caractères morphologiques du stade sexué et sur la comparaison phylogénétique de la séquence LSU avec celles d'espèces appartenant à 14 genres dans les *Bionectriaceae*. *Geonectria* est principalement caractérisé par des ascomes subglobuleux, non stromatiques, de couleur orange, devenant orange brunâtre et s'effondrant latéralement ou non en séchant, porteurs sur la moitié supérieure d'amas épars, superficiels, constitués d'hyphes évanescentes, de couleur jaune, par des ascospores ellipsoïdales à largement fusiformes, uniseptées, finement longitudinalement striées et son habitat au sol, en zone subalpine. Il est reconnu comme un genre distinct par son écologie particulière et sa position phylogénétique isolée au sein des *Bionectriaceae*.
Mots-clés : ADN ribosomal, Alpes françaises, Ascomycota, *Hypocreales*, taxinomie.

Introduction

In the course of a survey of Ascomycota in the French Alps in late August 2017, initiated by Ascomycete.org, a distinctive taxon characterized by non-stromatic, subglobose, orange ascomata and striate ascospores was repeatedly collected on soil in the subalpine zone. The ascomata do not change colour in KOH or lactic acid and at first glance recall a member of the genus *Bionectria* Speg. but the asexual morph obtained in culture is not clonostachys-like as defined by Schroers (2001) for *Bionectria* rather it is acremonium-like. Moreover, our molecular analysis of LSU sequences places this fungus on an isolated and long branch within the *Bionectriaceae*. Morphological comparison with the genera in the *Bionectriaceae* likewise show its distinctiveness (Hirooka et al., 2010; Lechat & Fournier, 2016; Lechat *et al.*, 2016; Lechat & Fournier, 2018; Rossman *et al.*, 1999; Schroers, 2001). Accordingly, we conclude that this fungus represents a previously undescribed genus in the *Bionectriaceae* and we propose the new genus *Geonectria* to accommodate the new species *Geonectria subalpina*.

Materials and methods

Dry specimens were rehydrated and examined using the method described by Rossman *et al.* (1999). Microscopic observations and measurements were made in water. The holotype specimen and paratypes were deposited in LIP herbarium (University of Lille, France) and living cultures in the CBS Collection of the Westerdijk Fungal Biodiversity Institute (Utrecht, The Netherlands). Cultures of the living specimen were made on PDA (Potato Dextrose Agar) with 5 mg/l of streptomycin in Petri dishes 5 cm diam., incubated at 25°C. DNA extraction, amplification, and sequencing were performed by ALVALAB (Santander, Spain): Total DNA was extracted from dry specimens blending a portion of them using a micropestle in 600 µL CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min at 65°C. A similar volume of chloroform: isoamylalcohol (24:1) was added and carefully mixed with the samples until their emulsion. It was then centrifuged for 10 min at 13.000 g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in 70% cold ethanol, centrifuged again for 2 min and dried. It was finally resuspended in 200 µL ddH_2O. PCR amplification was performed with the primers LR0R and LR5 (Vilgalys & Hester, 1990) to amplify the 28S nLSU region. PCR reactions were performed under a program consisting of a hot start at 95°C for 5 min, followed by 35 cycles at 94°C, 54°C and 72°C (45, 30 and 45 s respectively) and a final 72°C step 10 min. Chromatograms were checked searching for putative reading errors, and these were corrected.

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 non-parametric version of the approximate likelihood-ratio test, implemented in PhyML SH-aLRT (Anisimova & Gascuel, 2006). Nomenclature follows MycoBank (CBS-KNAW Fungal Biodiversity Center, Utrecht, The Netherlands).

Taxonomy

Geonectria Lechat & J. Fourn., *gen. nov.* – MB 824881

Diagnosis: Ascomata on soil, superficial, non-stromatic, subglobose, orange, not changing colour in 3% KOH and lactic acid; ascospores hyaline, ellipsoidal to widely fusiform, 1-septate, finely longitudinally striate; asexual morph acremonium-like.

Type species: *Geonectria subalpina* Lechat, J. Fourn., M. Vega & Priou

Etymology: The prefix *geo-* derives from Greek γῆ = soil, ground and refers to the unusual ecology of the fungus.

Geonectria is primarily distinguished from other bionectriaceous genera by its occurrence on bare soil in subalpine zone. Its distinctiveness is supported by its isolated phylogenetic position within the *Bionectriaceae*.

Geonectria subalpina Lechat, J. Fourn., M. Vega & Priou, *sp. nov.* – MB 824882 **Fig. 1-3**

Diagnosis: Ascomata on soil, superficial, non-stromatic, subglobose, orange, not changing colour in 3% KOH and lactic acid; ascospores 17–19 × 5.5–6 µm, hyaline, finely longitudinally striate; asexual morph acremonium-like.

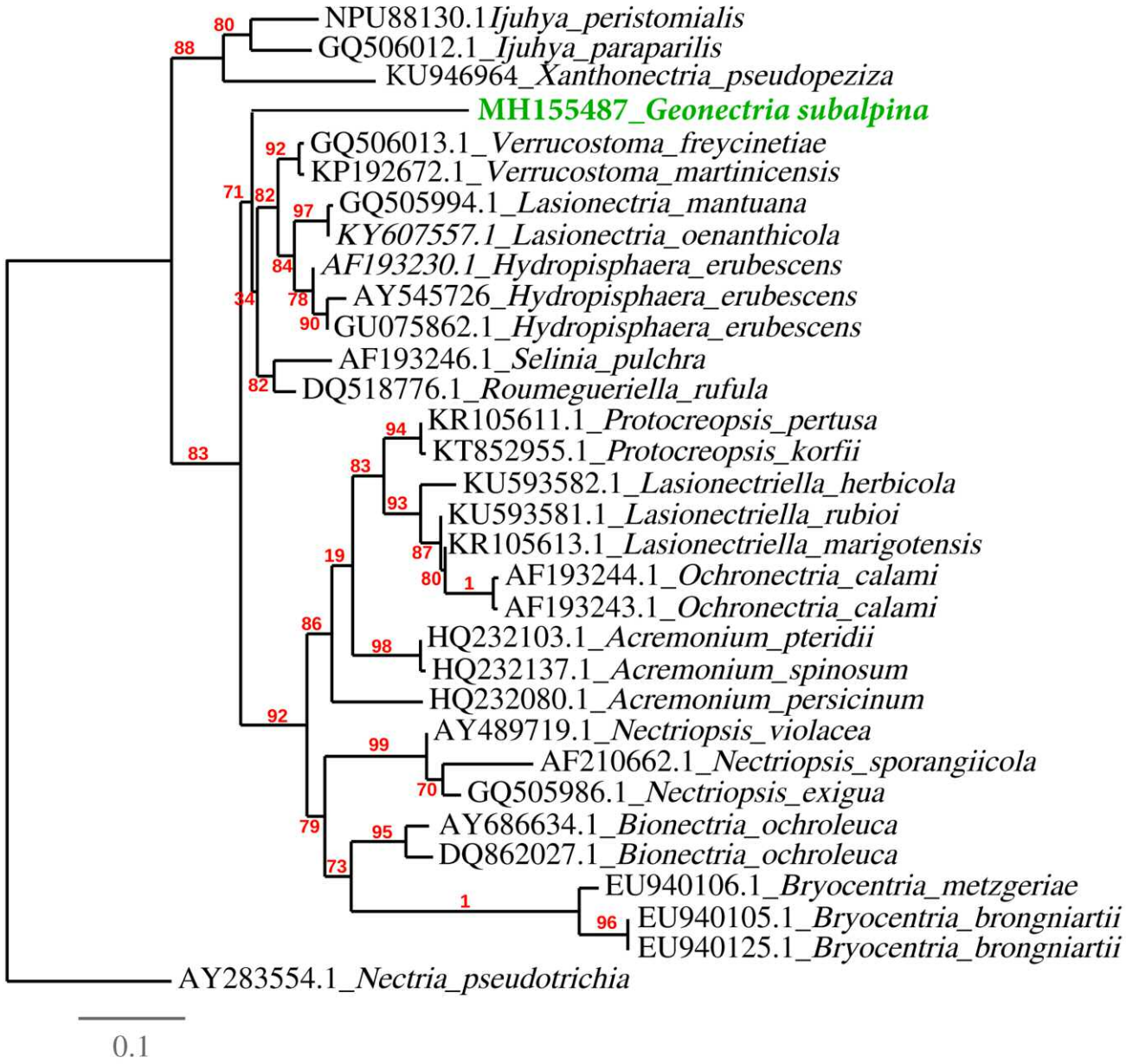

Fig. 1 – Maximum likelihood phylogeny (-lnL = 3085.08568) of *Geonectria subalpina* inferred by PhyML 3.0, model HKY85 from a 1120 bp matrix of 28S rRNA sequence, rooted to *Nectria pseudotrichia* (*Nectriaceae*).

Etymology: The epithet refers to occurrence of this species in the subalpine zone.

Holotype: France, Savoie, Beaufort, La Gittaz, 45° 42′ 41.4″ N 6° 39′ 31.4″ E, 1630 m asl, on soil, near bryophyte *Dicranella schreberiana* (Hedw.) Dixon, 29 Aug. 2017, *leg*. M. Vega, CLL 17023 (LIP) (= MV20170829-04), ex-type culture CBS 143540, GenBank LSU sequence: MH155487.

Ascomata solitary or in groups of 2–5, on the ground, superficial, non-stromatic, laterally collapsing or not collapsing upon drying, subglobose, 300–380 µm high, 280–350 µm wide, orange, becoming brownish orange when dry, not changing colour in 3% KOH or lactic acid, with a minute, slightly darker and shiny conical papilla. **Ascomatal surface** glabrous, roughened in places by superficial clusters of bright yellow hyphal elements 1–1.5 µm diam., becoming pale yellow, powdery and evanescent when dry. **Perithecial wall** 55–65 µm thick, composed of two regions: outer region 35–50 µm wide, of large, subglobose to globose or ellipsoidal, subangular cells 10–23 × 5–20 µm, with pale yellow walls 1–2 µm thick; inner region 10–15 µm wide, of elongate cells 6–12 × 3–6 µm, with hyaline walls 1 µm thick. **Asci** (50–)55–70(–75) × 10–12 µm (Me = 60 × 11 µm, n=20), unitunicate, short-stipitate, clavate, rounded at apex, with a discoid apical apparatus stained dark blue in lactic cotton blue, and (3–6–)8 biseriate ascospores. **Interthecial elements** not seen. **Ascospores** (16–)17–19(–21) × 5.5–6(–6.5) µm (Me = 18.5 × 5.8 µm, n=30), ellipsoidal to widely fusiform, 1-septate, hyaline, finely longitudinally striate, striae slightly sinuous and often inconspicuous.

Cultural characteristics: After two weeks at 25°C on Difco PDA containing 5 mg/L streptomycin, colony 2–2.5 cm diam, yellow to pale orange, diffusing a pale brown coloration into medium, aerial mycelium white, producing an abundant acremonium-like asexual morph at margin; conidiophores simple, 1.5–2.5 µm diam., flexuous, smooth, arising from smooth, septate hyphae 2–3 µm diam, with a simple conidiogenous cell 15–35 µm long, 2–3 µm diam. at base, subulate with a slightly flared collarette, producing ellipsoidal to subcylindrical, hyaline, smooth, non-septate conidia (2.5–)3–5.5(–6.5) × 1.5–2.5(–3) µm, with rounded apex, attenuated at base with a basal abscission scar, grouped at tip of phialides to form a mucous head.

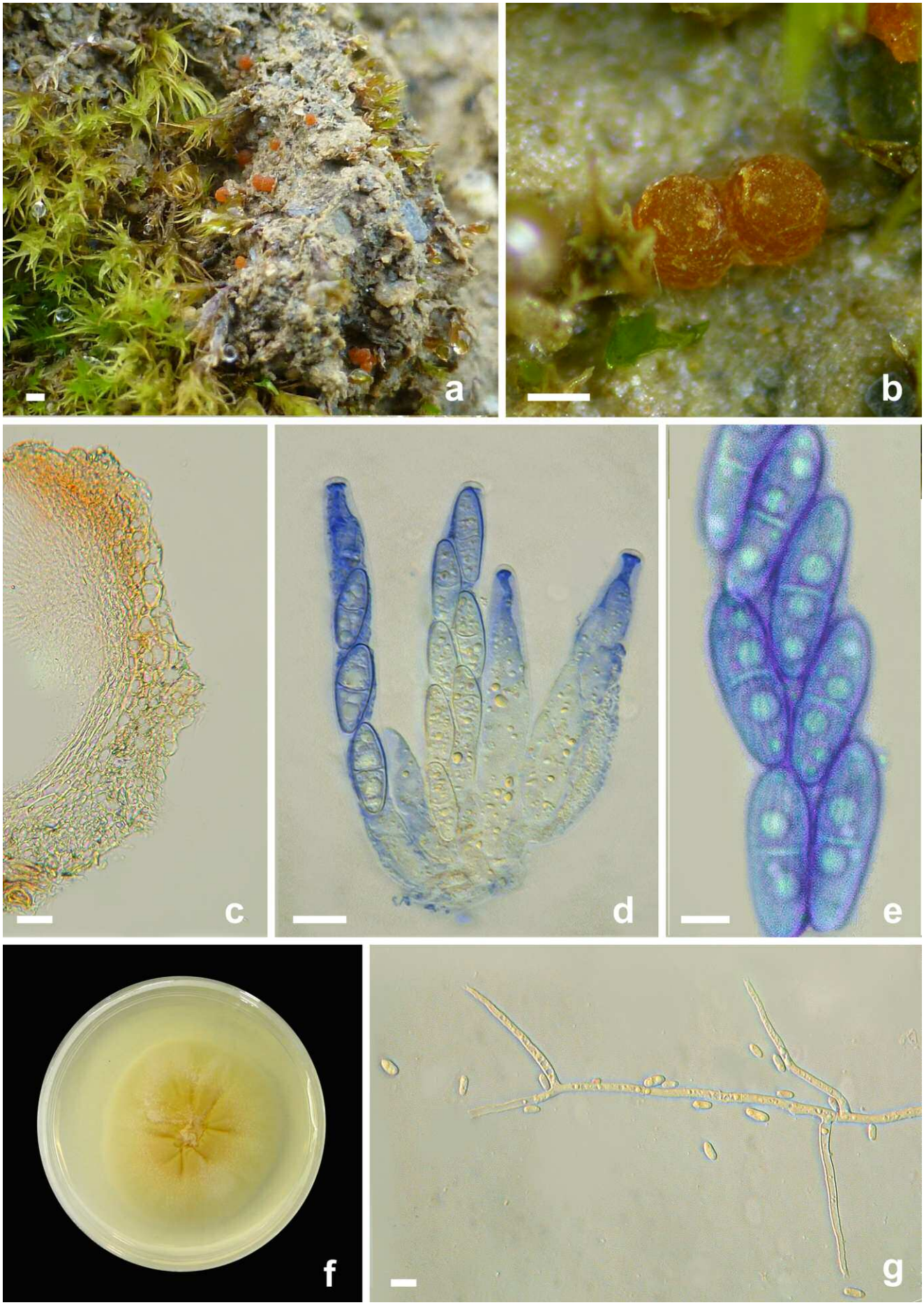

Fig. 2 – a-g: *Geonectria subalpina* (Holotype); a: Ascomata in natural environment; b: Fresh ascomata showing scattered yellow scurf on upper ascomatal wall; c: Lateral ascomatal wall in vertical section (in water); d: Asci and ascospores in lactic cotton blue; e: Ascospores in lactic cotton blue; f: Culture at three weeks; g: Conidiophores and conidia in water. Scale bars: a = 500 µm; b = 200 µm; c = 20 µm; d, e = 10 µm; g = 5 µm

Fig. 3 – a-c: *Geonectria subalpina* (CLL17144). Ascomata in natural environment growing on the ground without obvious contact with the bryophyte. Scale bars: a-c = 200 µm.

Additional specimens examined: France, Savoie, Aime-la-Plagne, Cormet d'Arêches, ~45° 36' 59" N 6° 36' 13.6" E, 2105 m asl, on soil, 27 Aug. 2017, *leg*. M. Vega, CLL17144 (= JPP17183) (LIP). Haute-Savoie, Les Contamines-Montjoie, Col du Joly, 45° 46' 59.4" N 6° 40' 34.0" E, 1974 m asl, on soil, 30 Aug. 2017, *leg*. M. Vega, MV20170830-03 (LIP).

Discussion

The fungus described above can be assigned to the *Bionectriaceae* by its superficial, non-stromatic, subglobose orange ascomata not changing colour in 3% KOH or lactic acid. The acremonium-like asexual morph obtained in culture distinguishes it from *Bionectria* which is characterized by a clonostachys-like asexual morph (Schroers, 2001). It is likewise different from the bionectriaceous genera with known acremonium-like asexual morph such as *Ijuhya* Starb., *Lasionectria* (Sacc.) Cooke, *Lasionectriella* Lechat & J. Fourn., *Nectriopsis* Maire, *Protocreopsis* Doi and *Stilbocrea* Pat. in lacking superficial hairs arranged in fascicles or forming a dense hyphal coating (Rossman *et al*., 1999; Lechat & Fournier, 2016). The genera *Hydropisphaera* Dumort, *Ochronectria* Rossman & Samuels and *Xanthonectria* Lechat et al. were considered because of their glabrous ascomata and acremonium-like asexual morphs. Glabrous species of *Hydropisphaera* lack the evanescent scurf of our fungus, they usually collapse upon drying, and they occur on woody or herbaceous substrates. *Ochronectria* and *Xanthonectria* likewise lack a fugacious scurf and feature a different wall anatomy with the presence of conspicuous orange oily droplets and they possess long-fusiform pluriseptate ascospores (Rossman *et al*., 1999; Lechat *et al*., 2016). The subtropical to tropical genus *Verrucostoma* Hirooka, Tak. Kobay. & Chaverri was also considered because its ascomata feature apical protuberances and its asexual morph is acremonium-like. Unlike the fungus dealt with here, apical protuberances in *Verrucostoma* are pseudoparenchymatous and adherent to the ascomatal wall (Hirooka *et al*., 2010; Lechat *et al*., 2015). All genera cited above occur on herbaceous or woody substrates, unlike the fungus described above which was repeatedly collected on bare soil as reflected in the name of the new genus *Geonectria*.

As it was often collected in the vicinity of a moss, we also considered *Bryocentria* Döbbeler which is known to be parasitic on bryophytes (Döbbeler, 2004) and belongs to the *Bionectriaceae* (Stenroos *et al*., 2010). Besides its parasitic lifestyle, *Bryocentria* is characterized by small, up to 250 µm wide ascomata often featuring apical setae and small, usually less than 10 µm long ascospores with a central cyanophilous transverse band, thus different from our fungus. The molecular analysis carried out in the present study (Fig. 1), comparing *Geonectria* with members of 14 genera in the *Bionectriaceae* clearly supports: 1) the placement of *Geonectria* in the *Bionectriaceae*, 2) the distinctiveness of *Geonectria* in relation to the other genera discussed above.

Geonectria is shown to be isolated on a long branch basal to a subclade including *Hydropisphaera*, *Lasionectria*, *Roumeguierella*, *Selinia* and *Verrucostoma*. The closest genus is *Verrucostoma*, and the pairwise alignments indicate that *Geonectria* has only 95% similarity with *V. freycinetiae* Hirooka, Tak. Kobay., & Chaverri and *V. martinicense* Lechat, J. Fourn. & Courtec. The phylogenetic tree likewise shows that the bryophilous genus *Bryocentria* is distant from *Geonectria*.

Ascomata of *G. subalpina* were collected either on bare soil or in close vicinity of the moss *Dicranella schreberiana*, which raised questions about a possible connection between the fungus and the moss. As ascomata were never observed growing directly on moss tissues, we can rule out a parasitic lifestyle as in *Bryocentria*. However, we cannot rule out a possible connection between the fungus and dead or living rhizoids of the moss, as it has been documented for some bryophilous *Pezizales* (Döbbeler, 1997). If it proves to be so, it would be a unique configuration of host-fungus relationship within *Hypocreales*. The apparent correlation of the fungus with the moss may also just reflect similar ecological requirements without significant interaction between them.

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