

Lectotypification of *Typhula graminum* and description of *T. berthieri* sp. nov.

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Abstract – Two syntypes of *T. graminum* were examined, one of which is designated as lectotype and is described in detail. The synonymy between *T. graminum* and *T. incarnata*, as currently supported, seems doubtful. The white basidiomata and the presence of conidiomata with sympodulospores on the sclerotia were not reported by Berthier and Remsberg, who studied the original material of *T. graminum*, nor for *T. incarnata*. A review of the history of *T. graminum* is made and the synonymy is fully discussed. *T. berthieri* sp. nov. is proposed for *T. graminum* sensu Berthier. Berthier (1976) used the name *T. graminum* for a species growing on *Molinia* and with striate epidermoid layer, and therefore excluding the type of *T. graminum*. The holotype and an isotype are selected amongst the collections cited by Berthier. All the materials agree with Berthier's description, except for the presence of scarce clamps and for a cuticle of up to 8 µm thick in old sclerotia, two characteristics not mentioned by Berthier.

Basidiomycota / Typhulaceae / systematics / taxonomy

Résumé – Deux syntypes de *T. graminum* ont été examinés, parmi lesquels il a été désigné un lectotype lequel est décrit en détail. La synonymie entre *T. incarnata* et *T. graminum*, aujourd'hui acceptée, est douteuse. Les basidiomes blancs et la présence de conidiomes avec sympodulospores sur les sclérotés ont été cités par Berthier et Remsberg, qui ont étudié le matériel original de *T. graminum* mais pas celui de *T. incarnata*. Nous récapitulons brièvement la nomenclature et le concept spécifique pour *T. graminum*; sa synonymie est discutée en détail. Nous proposons *T. berthieri* sp. nov. pour désigner *T. graminum* sensu Berthier. En effet, Berthier (1976) a employé le nom *T. graminum* pour une autre espèce avec couche épidermoïde striée et qui pousse sur *Molinia*. Nous avons sélectionné un holotype et un isotype parmi les collections citées par Berthier. Nous précisons deux caractères non mentionnés par Berthier pour ce taxon : la présence de rares boucles et une cuticule mesurant jusqu'à 8 µm d'épaisseur pour les vieux sclérotés.

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INTRODUCTION

The genus *Typhula* (Pers.: Fr.) Fr. includes around 68 species (Kirk *et al.*, 2001), and is mainly distributed in the temperate regions. Due to the very minute basidiomata, most of the species are seldom recorded and the collections are usually very scarce. Many names have been published, most of them without an explicit type designation. Berthier's monograph (1976) contributed to a better understanding of the genus, describing many species in detail and resolving several confusing species interpretations. However, a number of taxa still require typification or nomenclatural revision.

During the revision of the genus in the Iberian Peninsula, original material of several *Typhula* names was examined and described in detail. The original material of *T. graminum* P. Karst., was studied by Remsberg (1940) and Berthier (1976), but was never properly typified. A lectotype has therefore been designated.

In relation to this last name, a new species, *T. berthieri*, is described for *T. graminum* sensu Berthier, since the latter has been used in a sense that does not include its type. Both *T. graminum* and *T. berthieri* have a well distinguished clavula, pubescent stipe, amyloid spores, subepidermic sclerotia with normal epidermoid layer and grow on gramineous plants, features which made Berthier (1974, 1976) classify them within the subgenus *Microtyphula*.

MATERIALS AND METHODS

Herbaria are abbreviated according to Holmgren & Holmgren (1998). Material from the following herbaria have been used in this study: BIO, G, H and UPS. Colour codes are from Munsell (1990). The adopted terminology for basidiomata and sclerotia follows both Berthier (1976) and Kirk *et al.* (2001). Microscopic measurements were made in Congo Red in KOH 5%. Melzer's reagent was used to check for the amyloid and dextrinoid reactions. Spore measurements were made from the side view. The abbreviations of the journals and authors are according to Bridson & Smith (1991) and Kirk & Ansell (2003), respectively.

TAXONOMY

1. *Typhula graminum* P. Karst., *Not. Sällsk. Fauna Fl. Fenn. Fohr.* 9: 373. 1868.

Protologue: *T. graminum* n. sp. Pusilla, subglabra, clavula fusioidea, alba, stipite distincto, capillari, pellucido, longissimo. *Sclerotium fulvum* Fr. sistit mycelium hibernale. Fertilis ad folia vetusta Calamagrostidis in Myllyperä m. Oct. 1867 lecta; mycelium frequentissimum.

LECTOTYPUS (designated here): Herbarium of P.A. Karsten no. 1478 (H).

Description of the lectotype based on our own observations:

Clavula obtuse, ochre (10YR 8/2, 8/3), 0.1 mm wide. Stipe cylindrical, ochre (10YR 8/2, 8/3), brownish at the base (5YR 5/8), 0.05 mm wide, glabrous, with some hairs at the base clasping the sclerotium. Sclerotia oblong, 0.4-1 × 0.2-0.3 mm, minutely wrinkled, reddish brown to dark brown.

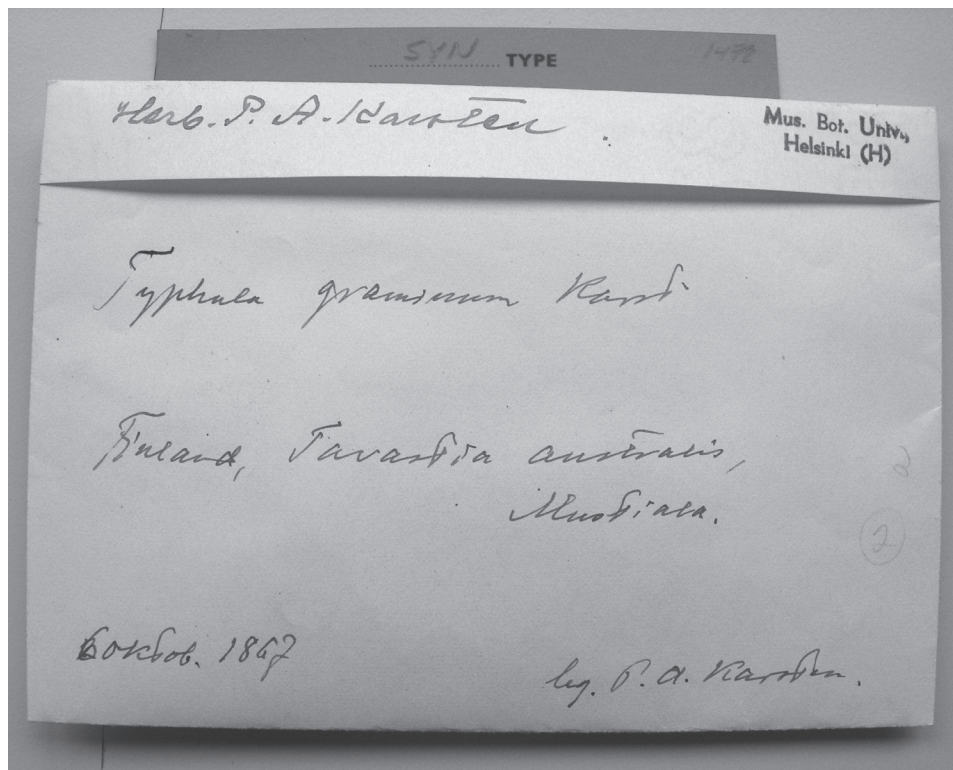


Fig. 1. Envelope of a original collection of *T. graminum* (Karsten 1478, H), bearing a recent label saying "syntype".

Medulla of the clavula formed by parallel arranged hyphae, cylindrical, 5-9.5 μm , thin-walled, non-gelified, collapsed. Basidiospores ellipsoidal to cylindrical, thin-walled, 9.5-12 (16) \times 4-5 μm . Subhymenium collapsed. Surface of the stipe formed by cylindrical hyphae, 4-6.5 μm wide, thin-walled, non-gelified, clamped. Caulinar hairs scattered, cylindrical to conical, sometimes branched and septate, 40-44 \times 4.5-6.5 μm . Sclerotium with a normal epidermoid layer, without cutis, with interlocking lobate cells, sometimes only sinuous, thin-walled, golden-brown to brown (Fig. 2). Cuticle 2-4 μm thick, smooth. Medulla formed by densely interwoven hyphae, non-gelified, thin-walled, 3-6.5 μm wide, without crystals. Conidiophores are sympodially branched and resemble sand particles covering few sclerotia (Fig. 3, 4a). Abundant sympodulospores, cylindrical, thin-walled, 3.5-5 \times 1.2-1.8 μm (Fig. 4b).

Additional material examined

Typhula graminum P. Karst.: **Finland:** Tavastia australis, Tammela, Mustiala, ad Calamagr., 06/10/1867, leg. P. A. Karsten, no 1477 (H, sub *Pistillaria graminum*).
Typhula incarnata Lasch: **Germany:** Driesden, leg. Lasch (UPS-Fries, holotype).
Sweden: Uppland, Stockholm, Experimentalfältet, on *Agrostis* sp. leaves, 20/06/1882,

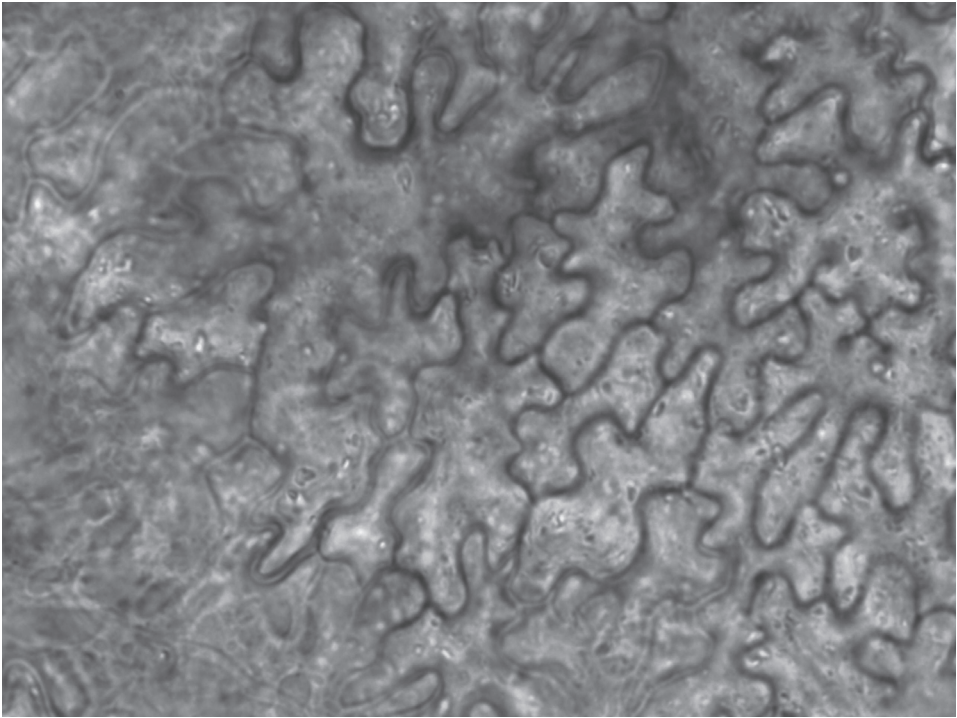


Fig. 2. *T. graminum* (Karsten 1478, lectotype, H). Lobated and interlocking cells of the epidermoid layer.

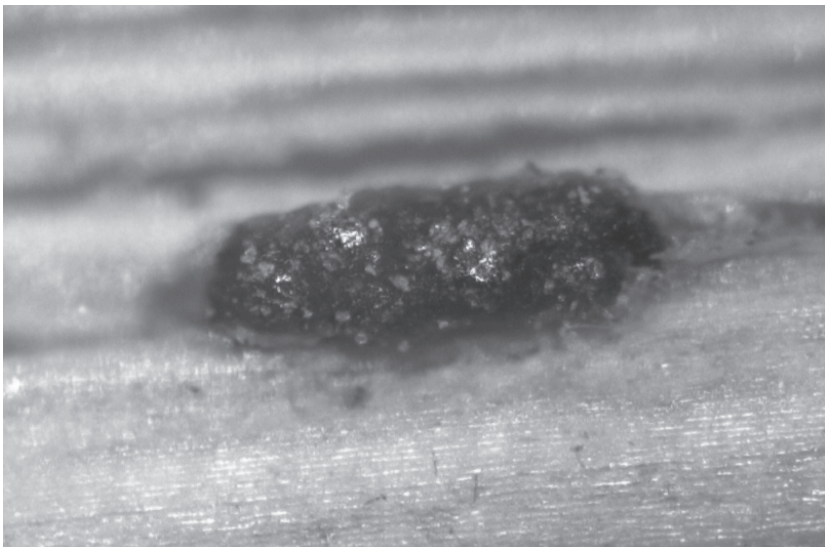


Fig. 3. *T. graminum* (Karsten 1478, lectotype). Conidiophores over a sclerotium.

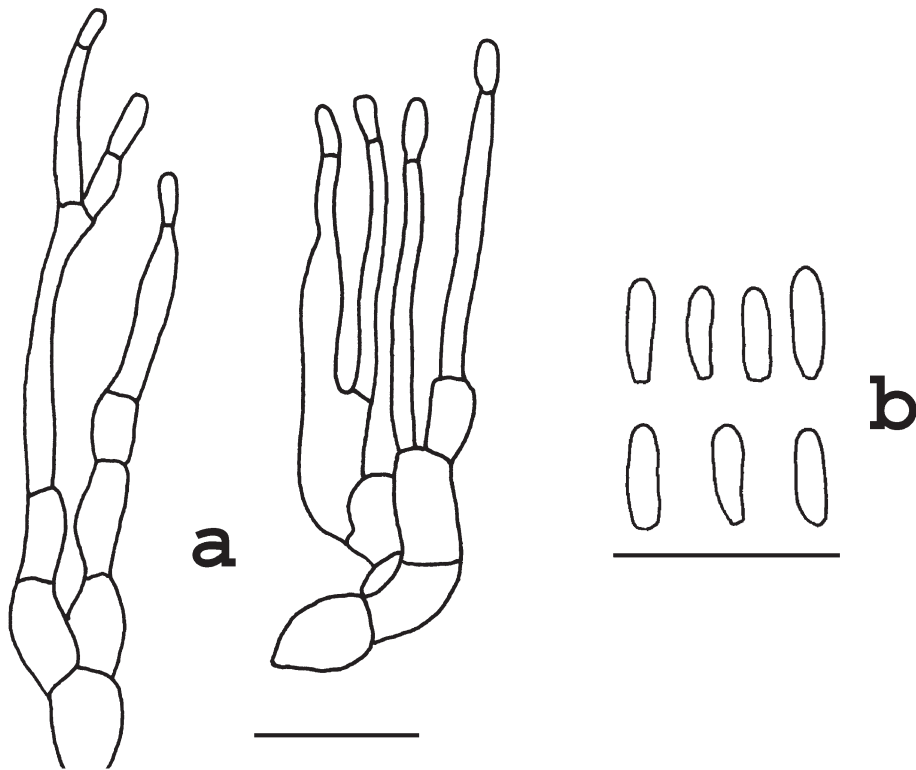


Fig. 4. *T. graminum* (Karsten 1478, lectotype). **a.** Conidiophores. **b.** Sympodulospores. Scale bar = 10 μ m.

leg. J. Eriksson, (UPS). **United States:** Idaho, Sandpoint, Substation farm, on leaves of *Hordeum sativum*, 28/03/1932, leg. R. Remsberg, no. 2515A (UPS, sub *Typhula itoana*).

Typhula ishikariensis Imai: **United States:** Idaho, Hill city, 05/1931, det. R. Remsberg, no. 27221 (UPS, sub *Typhula idahoensis*, isotype).

Commentary: No type was designated by Karsten in the protologue. The original description of *T. graminum* was based on material collected in October 1867 at Myllyperä, growing on *Calamagrostis* leaves. Two collections gathered at “Tavastia australis, Tammela, Mustiala”, namely no. 1478 and no. 1477, are kept in the herbarium H and coincide with the date and substratum. This little discordance of the locality might be explained by the fact that Myllyperä, Mustiala and Tammela are three close localities which Karsten often referred to as Mustiala, the main city of the area. Collection no. 1478, determined as *Typhula graminum*, bears a recent label saying “syntype” (Fig. 1) and it is composed of several gramineous leaves with numerous sclerotia, few stipes and a tiny clavula piece. It is dated 6th October 1867. Collection no. 1477, determined as *Pistillaria graminum* (a name never effectively published) and not bearing any type status label, is more scarce. It has few sclerotia and no basidiomata. It is also dated 6th October 1867. The

locality, date and substratum are therefore identical in both collections and they probably belong to the same gathering. It can be assumed that the description of the protologue is based on both collections, the reason why they both can be considered as syntypes (McNeill *et al.* 2006, art. 9.4). Berthier examined both collections and wrote down “type” in the correction label of collection no. 1478, probably due to it being a more abundant collection. However, as far as we know, no formal designation has been proposed. For this reason we propose collection no. 1478 as a lectotype, since it is labelled *Typhula graminum* and is in better condition than no. 1477.

The most remarkable characteristics of the original material of *T. graminum* are its long and white basidiomata, short caulinar hairs and subepidermic sclerotia with smooth and thin cuticle. The presence of sympodiospores in the lectotype, not observed by Remsberg (1940) nor by Berthier (1976) is very noteworthy. These conidiophores form minute conidiomata and are, consequently, easily overlooked. Their occurrence might be facultative, but as in *T. crassipes* Fuckel and *T. micans* (Pers.: Fr.) Fr., could have taxonomic importance (Patouillard, 1885; Berthier, 1976). The conidia of *T. crassipes* and *T. micans* are annelloconidia and therefore, the presence of sympodiospores on the sclerotia has never been mentioned in the literature dealing with *Typhula*. However, they are known to occur in the cultures of some species of *Typhula* (Berthier, 1976; Metzler, 1984). Since sympodiospores are missing in many sclerotia, they are not taxonomically determinant when not present.

In the classification proposed by Berthier (1976), *T. graminum* fits within the subgenus *Microtyphula*. Within this group, *T. graminum* seems related to other species that inhabit gramineous plants, such as *T. incarnata* Lasch (= *T. elegantula* P. Karst. = *T. itoana* Imai), *T. ishikariensis* Imai (= *T. borealis* Ekstrand; = *T. hyperborea* Ekstrand) and *T. idahoensis* Remsberg (sometimes synonymized with *T. ishikariensis*), that are well documented as parasites (Tasugi, 1929; Remsberg & Hungerford, 1933; Imai, 1937; Matsumoto *et al.*, 1996; Vergara *et al.*, 2004). *T. incarnata* possesses a white stipe with pink clavulae, reddish brown sclerotia (Fries, 1838; Remsberg, 1940; Berthier, 1976) and, according to Årsvoll & Smith (1978), an epidermoid layer often with lobate interlocked cells. *T. ishikariensis* is mainly distinguished from *T. incarnata* by its whitish to brownish basidiomata, dark brown sclerotia, and epidermoid layer of the sclerotia with mainly non-interlocked cells (Årsvoll & Smith, 1978). Due to the non-brownish basidiomata, partly reddish brown sclerotia and the presence of interlocked cells in the epidermoid layer, the original material of *T. graminum* is closer to *T. incarnata*.

Although the name *T. graminum* has been widely used by many authors, only Remsberg (1940) and Berthier (1976) examined the original material of *T. graminum*. The name *T. graminum* has been persistently used to designate *T. incarnata*, a pathogenic species on which many papers have been published (Eriksson, 1879; Volk, 1937; Killermann, 1934). Some authors even explicitly synonymised *T. graminum* and *T. incarnata* (Tasugi, 1929; Remsberg & Hungerford, 1932). However, Imai (1937) and Remsberg (1940) were the first to split *T. graminum* from *T. incarnata* (sub *T. itoana*). The former stated that *T. graminum* differed on account of its white imperfect fructification in culture, while *T. incarnata* formed perfect pinkish fructifications. Remsberg (l.c.) described the presence of a gelatinised layer underneath the epidermoid layer of the original material as a useful characteristic to distinguish it from *T. incarnata*

(sub *T. itoana*). Furthermore, she stated that *T. graminum* possessed white basidiomata, while *T. incarnata* had pinkish ones.

Some years later, Røed (1969) carried out mating tests with material from Asia, Europe and America in order to elucidate the relationship between *T. graminum* and *T. incarnata*. This resulted in the synonymy of the names *T. graminum* and *T. incarnata* which was later rejected by Berthier (1976), who claimed that Karsten's material of *T. graminum* differed from *T. incarnata* because of its white and smaller basidiomata. Nevertheless, the sclerotium of *T. incarnata* was described as gelified underneath the epidermoid layer, which coincides with Remsberg's observations on the original material of *T. graminum*. Årsvoll & Smith (1978) suggested that the shape of the cells of the epidermoid layer could be taxonomically very valuable. They found the cells of *T. incarnata* to be very interlocked, differing from those of *T. idahoensis*.

In more recent works, the discussion about the identity of *T. graminum* seems forgotten and despite *T. incarnata* being currently the subject of intense research, no mention of *T. graminum* has been made (Matsumoto *et al.* 1996, Hsiang & Wu 2000, Vergara *et al.* 2004).

According to our experience and observations on *Typhula*, some of the characteristics that have been used to distinguish *T. incarnata* from close species are not taxonomically important. First, the gelatinisation of the cells underneath the epidermoid layer has been observed to be more conspicuous in young sclerotia. Furthermore, a little gelatinisation was detected in *T. berthieri* (described below as a new species), and could also be a feature shared by related species. Secondly, although Berthier (1976) proved that the shape of the cells of the epidermoid layer can be used to distinguish some species, we consider that it has a high plasticity. In the same sclerotium, either small and polygonal cells or larger and interlocked cells can be found in different zones. This fact was already noticed by Årsvoll & Smith (1978). Therefore, we regard that both sclerotial characteristics cannot be used to reliably discern *T. graminum* from *T. incarnata*.

The white coloured clavulae of *T. graminum* (Karsten 1868) are clearly different from those of *T. incarnata*, the colour of which has been described as pink (Fries 1838, Karsten 1870, Remsberg 1940, Knudsen 1997, Maas Geesteranus 1976, Årsvoll & Smith 1978) or pinkish (Berthier 1976). Although the colour can fade in mature basidiomata, the pinkish tones of *T. incarnata* seem to be always noticeable. On the other hand, the presence of sympodulospores on the sclerotia has never been mentioned in *T. incarnata*. They have only been cited in cultures, being morphologically similar to those we have observed on the sclerotia of *T. graminum*. Therefore, those two features support that *T. graminum* can be a different species than *T. incarnata*, as previously stated by Remsberg (1940) and Berthier (1976). However, future research using cultural studies, mating tests and molecular data would be desirable to confirm the status of *T. graminum*.

2. *Typhula berthieri* sp. nov.

= *Typhula graminum* sensu Berthier, *Bull. Mens. Soc. Linn. Lyon* 45(6): 109. 1976; Siepe, *Beitr. Kenntn. Pilz. Mitteleuropas* 14: 131-137. 2005; Olariaga & Salcedo, *Anales Biol., Fac. Biol., Univ. Murcia* 27: 44. 2005.

HOLOTYPE: Berthier's herbarium at Geneva, G 53994 (sub *Typhula graminum*).

ISOTYPE: Berthier's herbarium at Genève, G 53993 (sub *Typhula graminum*).

Etymology: the name is dedicated to J. Berthier, author of the best monograph of *Typhula* ever published.

Latin diagnosis: *Carpophora tota alba*, 1-4 mm, nascentia e sclerotio. Clavula 0,3-1,7 × 0,15-0,25 mm, ovoidea vel cylindrata, obtusa. Stipes longior quam clavula, pubescens. Sclerotium immersum vel erumpens, oblongum, 0,6-1,5 × 0,25-0,5 mm, brunneum rubellum. Sporae ellipsoideae, amyloideae, 7,5-10 × 3,8-4,4 μ. Basidia 4-sporigera. Stipes corticatus, non gelatus. Pili in stipite presentes, conici vel cylindrici, 22-62 × 5-8 μ. Hyphae non fibulatae vel raro fibulatae. Sclerotium non gelatum, cum epidermoideo ordinario. Cuticula striata. In caulibus emortuis *Moliniae*. A speciebus similaribus differt cuticula striata et hyphis non fibulatis vel raro fibulatis.

Holotypus: Galia, Haute Savoie, Samoëns (la Charnia), J. Berthier lectus, 04/09/1965, in caulibus emortuis *Moliniae*, in herb. G 53994 conservatus.

Isotypus: Idem, in herb. G 53993 conservatus.

Material examined

France: Haute Savoie, Samoëns (la Charnia), sur feuilles et tiges de *Molinia*, 29/08/1966, J. Berthier, G 53992. Ibidem, 04/09/1968, G 53990. Ibidem, 04/09/1965, G 53994 (Holotype). Idem, G 53993 (Isotype). Ain, Bourg, St. Etienne du Bois, sur feuilles de *Molinia*, 01/09/1965, J. Berthier, G 53991. **Spain**: Huesca, Torla, San Nicolás de Bujaruelo, 30TYN3632, 1600 m, *Molinia caerulea* and *Carex flacca* dead leaves, 17/09/2003, leg. I. Olariaga, BIO-Fungi 9899.

Commentary: In 1868, a white *Typhula* growing on *Calamagrostis* was described as *T. graminum* by Karsten. That name, sometimes misinterpreted as a synonym of *T. incarnata* as already discussed above, has been widely used to designate a pathogenic species causing economical losses (Killermann, 1934; Imai, 1937; Volk, 1937; Røed, 1969; Berthier, 1976). In 1950, Corner, not having examined any original material, erroneously ascribed an English collection growing on *Molinia* and lacking clamps to *T. graminum*. Corner's interpretation was mostly based on his English collection, but references to Karsten (1868) and Rembsberg (1940) were also given in his description.

Subsequently, the same species growing on *Molinia* was described in detail by Berthier (1976), who cited as its main diagnostic characteristics the striate sclerotial surface (Fig. 5), the absence of clamps and its habitat on *Molinia*. He named it *T. graminum*. However, he stated that none of the collections named by Karsten as *T. graminum* possessed striate cuticle or lacked clamps, belonging therefore to another taxon than that inhabiting *Molinia*. The name *T. graminum* has also been adopted to denominate the *Typhula* species growing on *Molinia* in subsequent works (Knudsen, 1997; Siepe, 2005; Olariaga & Salcedo, 2005).

After examining Berthier's material, we agree with his characterization of the species. However, although scarce, we have observed clamps in collections G 53991 and G 53994, which proves that the species can also reproduce sexually. In regard to the thickness of the cuticle, it was found to be variable. Young reddish brown sclerotia have 2.5-4 μm thick cuticle, with weak striation. On the contrary, old dark brown sclerotia possess a thicker cuticle, up to 8 μm, which is notably striate. Moreover, the cuticle, at least in old sclerotia, is thicker than in closely related species such as *T. caricina* P. Karst., *T. incarnata* or *T. idahoensis* and, thus, cuticle thickness can be considered as a taxonomically informative feature. Despite these slight differences in comparison with Berthier's description, we remain convinced that *T. berthieri* merits a separate rank as a distinct and well characterized species.

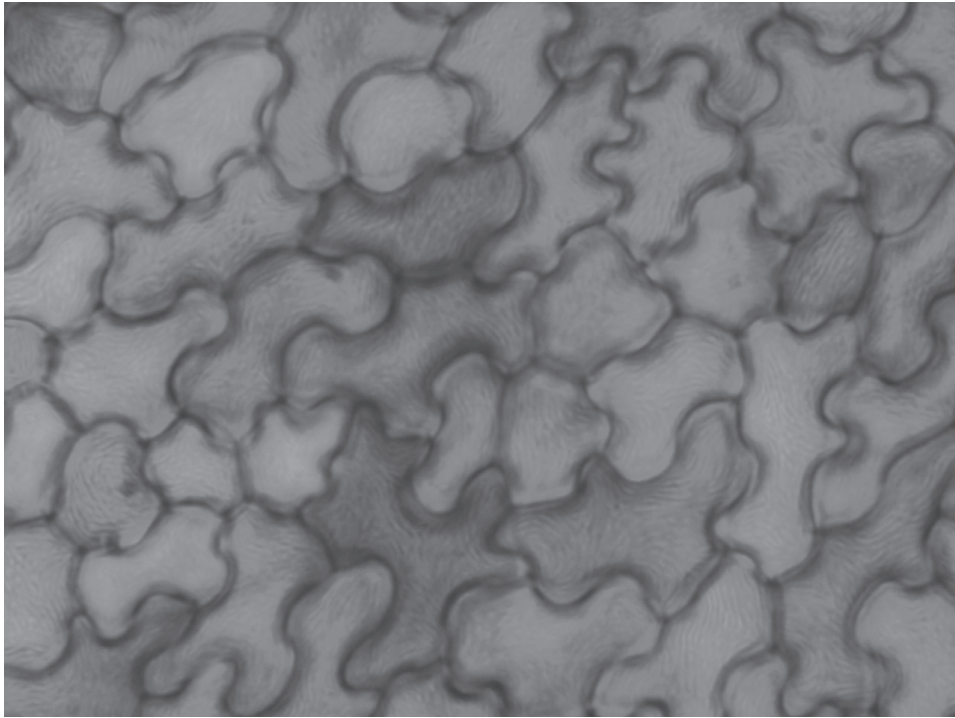


Fig. 5. *T. berthieri* (G 53994, holotype). Surface of the epidermoid cells exhibiting the striation of the cuticle.

As the name *T. graminum* has been profusely used to refer to *T. incarnata* (Eriksson, 1879; Killermann, 1934; Imai, 1937; Volk, 1937), the possibility to conserve it in Berthier's sense with a new type (McNeill *et al.*, 2006, art. 14.9) has been ruled out. Instead, we regard it desirable to describe a new species for the taxon treated as *T. graminum* sensu Berthier (1976). Corner's description was mixed and Berthier (1976) was the first to characterize and describe thoroughly the species growing on *Molinia*, which is why we regard it more appropriate to select a type amongst Berthier's material. In Berthier's monograph, besides some illustrations (Plate 4 fig. B; Plate 21 A, B, C, D, E; Fig. 12 of 2nd table of photographs), references to 4 collections are made (CL 6, CL 35, CL 124 and CL 25). Currently, those collections are kept in Geneva (G), and are numbered G53993 (duplicate of CL6), G 53994 (CL6), G 53992 (CL 35), G 53990 (CL 124) and G 53991 (CL 25). All collections are in good condition, and except for G 53991, are abundant collections. Amongst Berthier's collections, we select as holotype G 53994, an abundant collection with 4 slides having spore-prints. The duplicate G 53993 is selected as an isotype.

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