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Notes on Mollisioid Ascomycetes from the Beartooth Plateau, Rocky Mountains USA

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Abstract: During a visit to the Beartooth Plateau, Rocky Mountains USA, several collections were made of inoperculate Ascomycetes. This article deals with some Mollisioid fungi, i.e., members of the family Dermateaceae, which are closely related to the core genus *Mollisia* s. l. Four collections on *Salix* twigs proved to belong to two different species of *Mollisia*, new to the Beartooth Plateau. The new combinations *Mollisia salicis* and *Mollisia minutissima* are presented.

Keywords: Alpine, Beartooth Plateau, Dermateaceae, *Mollisia*, Mollisioid fungi, *Pyrenopeziza*, *Salix*

Introduction: In 2008 the author participated in ISAM 8 and collected Mollisioid ascomycetes on the Beartooth plateau, Montana and Wyoming, USA. Since very little is known about the occurrence of Mollisioid and other inoperculate fungi in the Rocky Mountains and especially on the Beartooth Plateau (Cripps, pers. comm.), this article contributes to the knowledge of the distribution of inoperculate ascomycetes in this region. This is the first part of the study on the specimens collected and reports two species found on twigs of *Salix* in alpine habitats.

Mollisioid fungi are defined as members of the inoperculate family Dermateaceae, Helotiales Nannfeldt ex Korf & Lizoň (2000) which are closely related to the genus *Mollisia*. Mollisioid fungi are notoriously difficult to identify, because of a lack of stable morphological characters. Several apparently closely related genera have been erected over the course of years, all sharing the presence of at least some rounded or rectangular brown-walled elements in the outer excipulum but differing in structures such as spores and paraphyses. Mollisioid fungi are further characterized by mostly hyaline spores and a soft excipulum tissue. In the scope of the project 'The Ascomycetes of Great Britain and Ireland' the author has made an overview of the genera of Dermateaceae including Mollisioid fungi of Great Britain, based on morphological characters (Nauta and Spooner 1999a, b, c, Spooner and Nauta 1999, Nauta and Spooner 2000a b, c). Little has been changed in the taxonomical concepts since then, despite several studies based on sequences of genes in the rDNA region by Wang et al. (2006a, b) and the studies for the AFTOL project (<http://tolweb.org/tree/phylogeny.html>). This is mainly a result of the fact that only a few genera of Dermateaceae are treated in those studies. Although as shown by Wang et al. (2006a, b) the family Dermateaceae is a heterogeneous family better restricted to the group of genera closely

related to *Dermea*, with the genus *Mollisia* in a different clade, for the time being it is considered best to keep it in Dermateaceae by default.

In order to clarify the chaos, a study was conducted by the author in 1985 into the use of cultural characters in the taxonomy of Mollisioid fungi. This study did not sufficiently elucidate the major problems in this group, viz. the lack of stable characters (Nauta, in prep.). In 2007 the author was given the opportunity at CBS to study molecular data of this group in order to find more taxonomically relevant and stable characters. This study is now about to reveal a new phylogeny for this group (Nauta, in prep.).

The collections which were made in 2008 on the Beartooth plateau were also taken into account for this study. Unfortunately a living culture could be grown from only one from these four collections and subsequently DNA only could be extracted and studied for two genes. This was enough, however, to place the species in the Mollisioid fungi, which was also clear from the morphological data, and it also enabled a closer look at the relationships of this particular species.

Material and methods: Collections were dried the same day they were collected at 40° C and studied later. Collections are kept at the National Herbarium of the Netherlands (L). Microscopical examination and spore measures were made in 5% KOH, with later addition of lactic acid in order to make the slides semi-permanent. Amyloidity (J+/J-) was studied in Melzer's reagent.

Pure cultures were made later at CBS by placing apothecia of the moistened dried ascomycetes in the lid of petri dishes and letting the spores shoot onto MEA-agar (Crous et al. 2009) with the addition of antibiotics to prevent development of bacteria. After 7 days the

relevant colonies were transferred onto MEA-agar.

DNA was isolated from the cultures with Ultraclean Microbial DNA isolation kit (Mo Bio), and kept in a refrigerator for further use.

Results and Discussion: Only one collection of the four Mollisioid fungi collected on *Salix* was grown successfully in pure culture so DNA could be successfully isolated. The other three were not in sufficiently viable condition for growth. No attempts were made to isolate DNA directly from the apothecia, due to their poor condition. Morphological studies revealed that all four collections belong to Mollisioid fungi, based on the structure of the outer excipulum. For identification, the author's own database was used, which contains all published species of *Mollisia* and related genera worldwide, along with their relevant morphological characters.

Not much is known about North American species of Mollisioid fungi. Some species are mentioned in the work of Seaver (1951), and two from the Alaskan arctic are mentioned in Kobayashi et al. (1967). In Europe the work of Nannfeldt (1932) is invaluable, together with several studies by different authors such as Gremmen (1952-1957), and Le Gal and Mangenot (1956-1966).

According to unpublished results of the author based on sequences of four genes, *Mollisia* is a large genus that needs to be subdivided. However, not entirely according to the divisions previously used by ascomycetologists like Spooner and Nauta that were based on morphology of the apothecia. In the course of taxonomy many small genera have been split off from the core genera of Mollisioid fungi such as *Mollisia*, *Pyrenopeziza*, *Belonopsis* and *Niptera*. *Pyrenopeziza* is still a genus which comprises the core species like the Type *P. chailletii*. However, many species previously classified as *Pyrenopeziza* are better placed in *Mollisia*.

The four collections studied belong to two different species of *Mollisia*, both possessing a subiculum. As generally accepted now, species with a subiculum are placed in *Mollisia*. *Tapesia*, as these species were formerly called, is synonymous to *Mollisia*, necessitating conservation of the name *Mollisia* over *Tapesia* to prevent undesired name changes (Hawksworth and David 1989). Preliminary analyses of sequences of one of the four collections studied in comparison to 300 of our own collections of *Mollisia* and related genera show that the collections from the Beartooth Plateau belong to the core species of *Mollisia*.

Descriptions of species studied on *Salix*

Mollisia salicis (Feltg.) Nauta, comb. nov.
(Fig. 1 A, B)

Basionym: *Pseudophacidium salicis* Feltg., Rec. Mém. Trav. Soc. Bot. Luxemb. 16: 97 (1903)

Synonyms: *Hysteropeziza salicis* (Feltg.) v. Hohn., Sitz.ber. Akad. Wiss. Wien 127: 581 (1918)

Pyrenopeziza salicis (Feltg.) Nannf. Nov. Act. Reg. Soc. Sci. Ups. Ser. IV (8-2): 160 (1932)

Apothecia on extensive dark brown to blackish subiculum, in dried condition irregularly discoid to folded, up to 1.3 cm in diameter, sometimes confluent; hymenium dark grey, sometimes with yellowish tinge; receptaculum black, granulose, with utmost margin whitish fimbriate.

Outer excipulum composed of rows of rounded rectangular brown-walled elements of 7-9-10 x 5-7-8 µm, outer-most elements thick-walled, encrusted, brown, clavate, 14-22 x 5-8 µm. Medullary excipulum composed of repent hyphae with elements of 10 x 2.5 µm. Margin composed of elongate brown-walled paraphyses, ca. 4 µm wide. Asci 70-80 x 7-9 µm, 8-spored, J+, intermixed with pale paraphyses up to 2 µm wide at top. Spores smooth, fusoid, 9-12 x 1.5-2 µm, sometimes curved.

Fig. 1. A, B. *Mollisia salicis* MN 7772, spores in asci and excipulum cells. C, D. *Mollisia minutissima* MN 7768 excipulum and spores in asci. Bars = 10 μ m.

Material studied: USA. Montana, Carbon County, Beartooth Plateau, Wyoming Creek, 5.8.2008, MN 7772, on *Salix* twigs. Wyoming,

Park County, Beartooth Plateau, Frozen Lake, 6.8.2002, MN 7763, on *Salix planifolia* twigs.

Remarks: Considering the morphology of this taxon, this species belongs without doubt to the genus *Mollisia*. According to unpublished molecular studies of four genes for over 300 collections (Nauta, in prep.) the genus *Pyrenopeziza* is limited to a number of species closely related to and resembling *P. chailletii* Fuck. Although it may seem preliminary to combine this taxon in *Mollisia*, pending further study. Decisions concerning (sub) generic names for the subdivision of *Mollisia*, it is the best solution for the time being. The morphology of the excipulum structure of the species is typical of *Mollisia*. The presence of a subiculum points towards the genus *Tapesia*, which is considered synonymous to *Mollisia*. Also, the unpublished results of molecular studies (Nauta, l. c.) show that the Type species of *Tapesia*, *T. fusca*, and *Mollisia cinerea* (type species of *Mollisia*) belong to the same cluster as core species of *Mollisia*.

Mollisia minutissima (Fuck.) Nauta,
comb. nov. (Fig. 1 C, D)

Basionym: *Tapesia minutissima* Fuckel Symb. App. I: 48. 1871.

Apothecia mostly on subiculum of dark brown thick-walled hyphae, in dried condition irregular, up to 0.8 mm; hymenium very dark grey, receptaculum very dark brown, slightly granulate, margin not differing from receptaculum.

Excipulum composed of rows of rounded rectangular dark brown-walled elements of 5 x 4 µm, no outgrowths. Asci 54 x 6.5 µm, rounded top, 8-spored, J- or slightly J+; intermixed with thin-walled pale paraphyses with rounded top, 2 µm wide, overtopping asci by 9-15 µm. Spores smooth, ellipsoid, sometimes irregular, with one pointed end, 7.5-8.5 x 2.5-3 µm.

Material studied: USA. Montana, Carbon County, Beartooth Plateau, Quad Creek,

4.8.2008, MN 7768, on *Salix*, USA; MN 7773, idem.

Remarks: This species is remarkable for the combination of broad spores and the presence of a subiculum, a combination which does not occur often in the Mollisioid fungi. This species is not well known, but the original description fits this taxon very well.

Also, this species belongs morphologically in the core species of *Mollisia*. The unpublished molecular data confirm this. This taxon clusters with nrITS and nrLSU data from *Mollisia cinerella* (Norway, Genbank AJ430228.1), *Mollisia cinerea* (Oregon, Genbank DQ491498.1) and *Mollisia fusca* (CBS 234.71; pers. obs.). This is reason enough to combine this species into *Mollisia*.

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