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## ***Cyathicula brunneospora* and *Pirottaea atrofusca*, two new *Helotiales* from Tian Shan (Kazakhstan)**

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**Abstract**—Two new species from Tian Shan are described: *Cyathicula brunneospora* on *Anthoxanthum alpinum* and *Carex griffithii* and *Pirottaea atrofusca* on *Festuca coelestis*. New combinations *Cyathicula melanospora* and *Cyathicula* sect. *Scelobonium* are proposed.

**Key words**—discomycete, taxonomy, graminicolous fungi, caricicolous fungi

### **Introduction**

Ascomycete fungi of the western part of Tian Shan have been described only sporadically. Schwartzman (1962) listed fungi from such genera as *Cenangium* Fr., *Erysiphe* R. Hedw. ex DC., *Geoglossum* Pers., *Gyromitra* Fr., *Helvella* L., *Lophodermium* Chevall., *Mitrula* Fr., *Morchella* Dill. ex Pers., *Naemacocyclus* Fuckel, *Otidea* (Pers.) Bonord., *Phyllactinia* Lév., *Podosphaera* Kunze, *Rhytisma* Fr., *Sarcoscypha* (Fr.) Boud., *Sphaerotheca* Lév., *Terfezia* (Tul. & C. Tul.) Tul. & C. Tul., *Uncinula* Lév., and *Venturia* Sacc. Raitviir (2004) described some hyaloscyphaceous fungi from Tian Shan: *Cistella tianschanica* Raitv. and *Hyalopeziza tianschanica* Raitv. In 2005 we collected fungi from the upper limits of where plants grow in Central Tian Shan. We noted mostly saprotrophic and parasitic species, including *Cainia graminis* (Niessl) Arx & E. Müll., *Lophodermium alpinum* Rehm, *Lachnellula arida* (W. Phillips) Dennis, *Comoclathris planispora* (Ellis) Harr, *Wettsteinina oreophila* Shoemaker & C.E. Babc., and others. Amongst our materials we also found new and very rare species belonging to basidiomycete and ascomycete groups. In this article we describe two new species, which belong to *Helotiales*.

## Methods

Dried material was examined, measured and prepared under a zoom stereo microscope (Nikon SMZ 1500, Olympus SZ 61), and also with a light microscope (LM) Olympus BX-51 equipped with an oil immersion lens, at magnifications of 1000x and 2000x (using a magnification changer), and in some cases using Nomarski contrast (DIC). Line drawings were designed using a drawing tube. Microscopical observations and measurements were made on material in 3% KOH. Freehand-made longitudinal sections of apothecia were observed in water or 3% KOH. Lugol's solution (IKI: 1% iodine, 3% KI in water), Melzer's reagent (MLZ) and 5% KOH were used to describe the reactions of apical rings and character of setae for *Pirottaea*. Gelatinous sheaths of free ascospores were observed in India ink. Materials are deposited at the National Museum in Prague (Czech Republic) and the W. Szafer Institute of Botany of Polish Academy of Sciences in Kraków (Poland).

*Cyathicula brunneospora* M. Chleb. & Chleb., sp. nov.

FIGURES 1-3

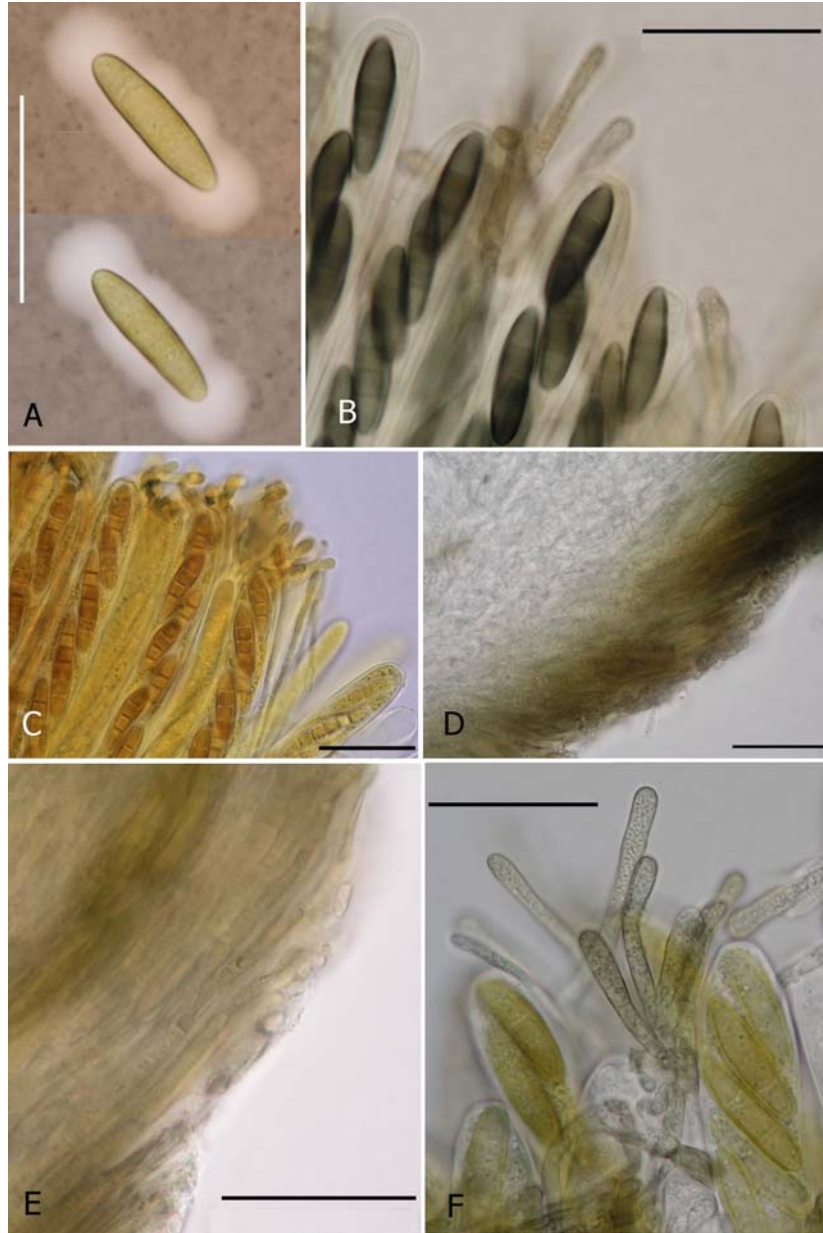
**Diagnosis:** *A specie Belonidium melanosporum* Rehm ascis inamyloideis, sporis permanenter brunneis atque laevibus, absentia strato excipuli ex cellulis magnis prismaticis biserialiter ordinatis discrepat.

**Etymology:** The specific epithet refers to the brown ascospores.

**SPECIMENS EXAMINED:** KAZAKHSTAN, TIAN SHAN: Zailijskij Alatau Mts., VALLEY OF ISSYK (YSSYK) RIVER, UPSTREAM FROM THE POINT WHERE ISSYK DIVIDES INTO TWO BROOKS, AT THE MORaine OF THE SOUTH BROOK, N 43° 07' 45", E 77° 30' 32", 3490 m elev., on fallen dead culms of *Anthoxanthum alpinum* Á. Löve & D. Löve, 7 August 2005, coll.: M. Suková (Chlebická), **HOLOTYPE-PRM 907424**. TIAN SHAN: Zailijskij Alatau Mts., ISSYK VALLEY, N 43° 07' 52.5", E 77° 30' 25", 3445.5 m elev., on *Carex griffithii* Boott, 3 August 2005, coll.: A. Chlebicki, **PARATYPES: KRAM "F": 55679, 55680, 55681, 55682, 55683.**

**Description:** Apothecia cupulate, 0.2-0.4(-0.7) mm high, 0.3-0.7(-1.1) mm in diam. when dry, shortly stipitate; receptacle olive brown or dark blackish brown, at margin and less densely at flanks covered with pale powder, stalk pale brown or dark brown, disc dark olive-brown or dark brown, apothecia opening when mature and staying open or becoming closed on drying. **Excipulum:** Outer layer of ectal excipulum dark brown, inner layer and medulla hyaline, hymenium dark brown. The ectal excipulum composed of parallel hyphae in a gelatinous matter, oriented at a low angle or almost parallel to the surface (textura oblita). The medullary excipulum composed of

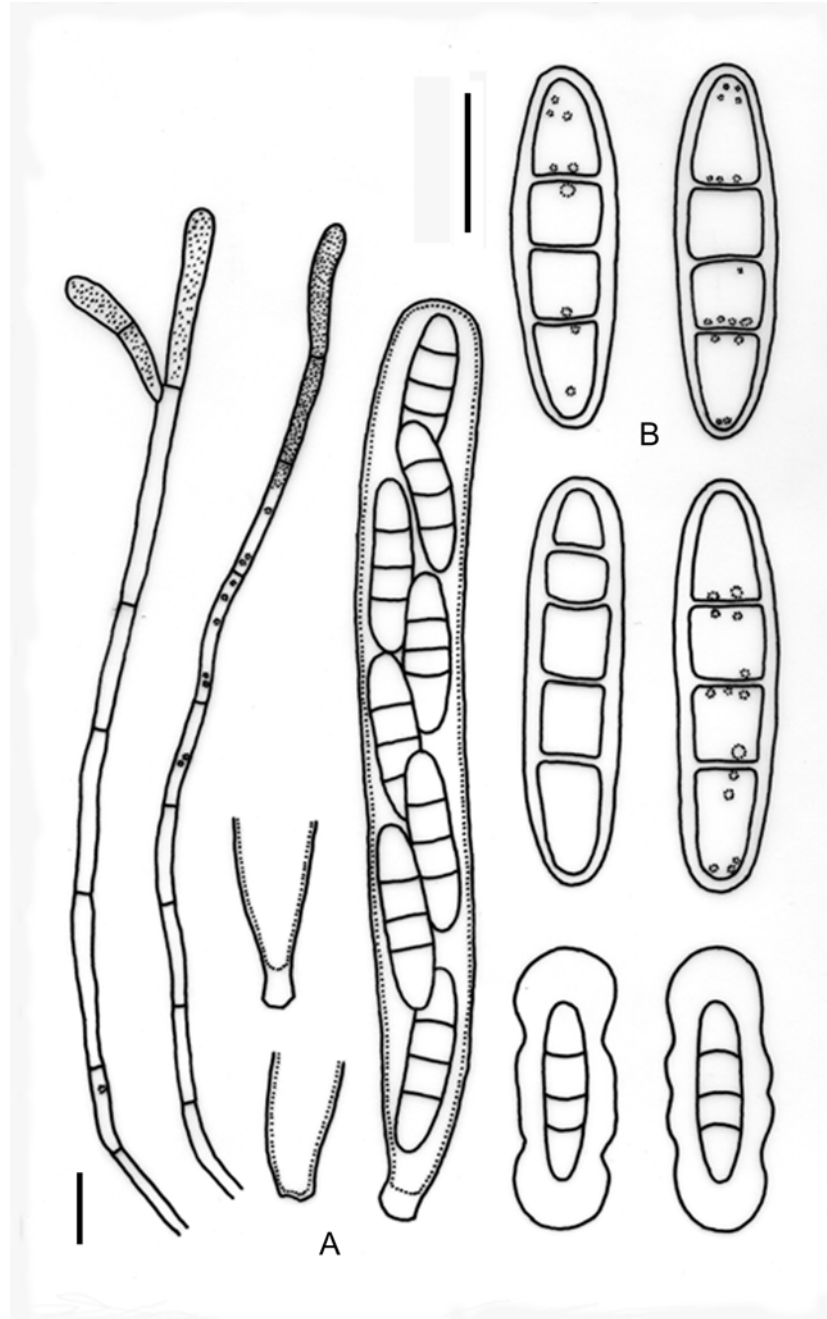
**Fig. 1.** *Cyathicula brunneospora*: **A:** ascospores with gelatinous sheaths (India ink); **B:** ascus apices (5% KOH/MLZ); **C:** hymenium (5% KOH/IKI); **D:** ectal excipulum and medulla (water); **E:** ectal excipulum (water); **F:** paraphyses and asci (water). Scale bars **A-F:** 30 µm.



freely intertwined hyphae (textura intricata). **Asci** 113-119 x 13.5-16 µm, cylindrical-clavate, non-stipitate or rarely very shortly stipitate, arising from croziers, 8-spored, with biseriate ascospores, apical ring without any reaction in MLZ, KOH/MLZ, IKI, KOH/IKI. **Ascospores** elliptic-fusoid, pale brown when young in asci, dark brown when mature (brown when observed in water), always smooth, 3-septate, very rarely up to 7-septate, 23-29.5 x 5.9-7.2 µm, surrounded by a hyaline, gelatinous sheath, which is constricted at the end spore cells, 5.8-8.6 µm thick at spore ends and 3.5-5.3 µm thick at the middle spore septum. **Paraphyses** cylindrical-clavate, hyaline and septate in their lower parts (1.9-2.6 µm broad), branched in their upper parts, with enlarged, 3.8-4.5 µm broad, smooth, pale brown tips (**Figs.** 1-3).

**Comments**—*Cyathicula brunneospora* is close to *Cyathicula melanospora* (syn. *Crocicreas melanosporum*). The latter occurs in arctic and alpine habitats on culms of *Poaceae*, stems of *Juncaceae* and dicotyledonous herbs and is known from Finland, Switzerland, Austria and USA (Carpenter 1981, Müller 1977, Rehm 1893, Vesterholt 2000). *Cyathicula brunneospora* is known only from the vicinity of the type locality (Asia, Kazakhstan), however, it is possible that other Asian material of the species is deposited in herbaria and currently identified as *Crocicreas melanosporum*. Ascospores of *Cyathicula melanospora* are hyaline when young (Rehm 1882, Carpenter 1981) and roughened in maturity (Carpenter 1981). In *Cyathicula brunneospora* we observed that ascospores are permanently smooth and at least pale brownish in youth. The main difference is in the amyloidity of the ascoapical ring. It is without reaction in MLZ nor IKI (also after pretreatment) in *C. brunneospora*, and light blue in MLZ (Carpenter 1981) and violet in IKI in *C. melanospora* (Rehm 1882, Rehm 1893: 747). It differs from the *C. melanospora* of Carpenter's description and drawing also because *C. brunneospora* lacks the thin layer with two rows of big prismatic cells in excipulum. In *C. brunneospora* the mature apothecia are cup-shaped. According to Carpenter (1981) apothecia for *C. melanospora* remain closed when mature and he listed 15 collections under specimens examined, among them the type collection containing apothecia whose structure is said to agree with the protologue. The difference in apothecial opening between *C. brunneospora* and *C. melanospora*, however, become irrelevant, when checking Rehm. Rehm (1882) originally described the apothecia of *C. melanospora* as "perithecia" urceolate-turbinate and later (Rehm 1893) reported on the same collection that apothecia are at first closed and then opening.

**Fig. 2.** *Cyathicula brunneospora*: **A:** paraphyses, bases of asci, ascus (3% KOH) and ascospores with gelatinous sheaths (India ink); **B:** ascospores (3% KOH). Scale bars **A-B:** 10 µm. Note: Brown colour of ascospores is not depicted in these drawings.



In agreement with Baral (2005), we accept that *Crocicreas* Fr. (type species *C. gramineum* (Fr.) Fr.) is not congeneric with *Cyathicula* De Not. (type species *C. coronata* (Bull.) De Not. ex P. Karst.). Paraphyses of *Crocicreas gramineum* are of two intergrading morphologies, lanceolate and cylindrical (Carpenter 1981), while paraphyses of *Cyathicula* are cylindrical to clavate. According to Svrček (1985), species of *Cyathicula* with apothecia without marginal dents should be placed in a separate genus, *Conchatium* Velen. We do not accept Svrček's opinion and place our new species as well as *C. melanospora* (both with non-dentate apothecia) into the genus *Cyathicula*.

***Cyathicula melanospora*** (Rehm) M. Chleb. & Chleb., **comb. nov.**

**Basionym:** *Belonidium melanosporum* Rehm, Hedwigia 21: 100, 1882.

≡ *Belonium melanosporum* (Rehm) Sacc., Sylloge Fungorum 8: 496, 1889.

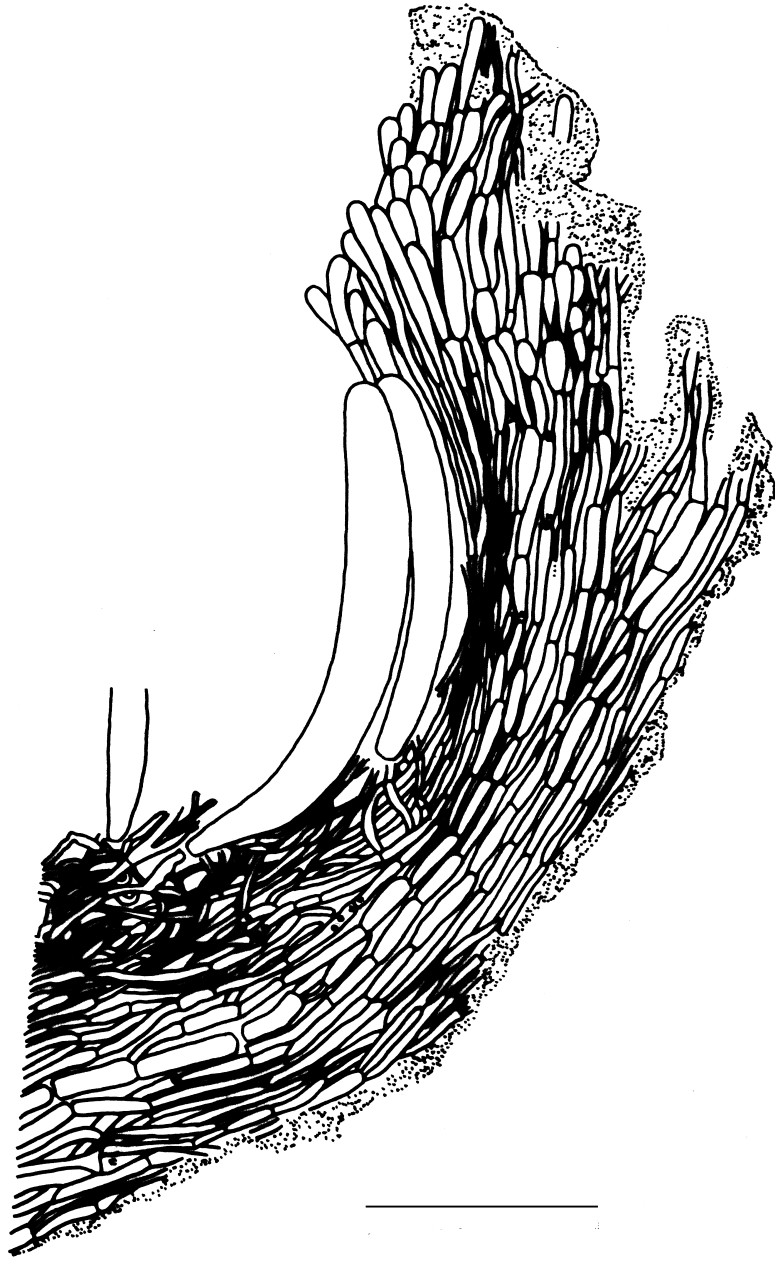
≡ *Belonioscypha melanospora* (Rehm) Rehm, Kryptogamen-Flora von Deutschland, Oesterreich und Schweiz, 2. Ed., 1(3): 746, 1893.

≡ *Scelobonium melanosporum* (Rehm) Höhn., Annalen des K.K. Naturhistorischen Hofmuseums Wien 20: 367, 1905.

≡ *Crocicreas melanosporum* (Rehm) S.E. Carp., Brittonia 32: 270, 1980.

In our opinion, *Cyathicula culmicola* (Desm.) S.E. Carp. & Dumont, *C. melanospora*, and *C. brunneospora* form a separate group within the genus *Cyathicula* based on their characteristic ascospores (relatively thick-walled and with a gelatinous sheath) and also apically enlarged, at least rarely branched paraphyses. Apices of paraphyses of the two last species are brown, while apices of paraphyses of *C. culmicola* are hyaline or pale brownish in various specimens (see e.g. Stadelmann 1978, Carpenter 1981), therefore we consider the colour of paraphyses as non-important at the generic level. The members of this group occur from colline (e.g. Velenovský's material of *C. culmicola* from the vicinity of Mnichovice examined by Carpenter 1981: 60-specimens PRM 150416, 150417 and 150467) to alpine altitudes and are known from grass culms, stems of *Juncaceae* and *Cyperaceae*, and several dicotyledonous plants. We would prefer to separate this group as a section of *Cyathicula*. To separate the group at generic level, it should need molecular taxonomic methods. The name *Belonioscyphae*, derived from Rehm's *Belonioscypha*, should be appropriate for this section from a taxonomical point of view. We agree with Stadelmann (1978) and Carpenter & Dumont (1978) and accept *Belonioscypha culmicola* (Desm.) Dennis as the lectotype species of the genus. Von Höhnelt (1918) and Nannfeldt (1932) considered another species—*Belonioscypha campanula* (Nees) Rehm—as the lectotype (“die Grundart”, pseudotypus), however, this lectotypification can be considered as based on mechanical method of selection (Art. 10.5.b. of the Saint Louis Code). The characters sketched in the original generic description

**Fig. 3.** *Cyathicula brunneospora*: Excipulum in longitudinal section (3% KOH). Scale bar: 50 µm.



of *Belonioscypha* by Rehm (1893) are in general agreement with our conception of the section. There are only a few differences: we here include only species with ascospores enclosed by gelatinous sheath, species with as well as without reaction of ascoapical ring in IKI and species with hyaline as well as brown paraphyses. According to Stadelmann (1978), further two species (*Belonioscypha miniata* Kanouse and *B. alpina* Stadelmann) have ascospores sometimes in gelatinous sheath, and they possibly could belong to the section, but we cannot litigate on character of ascospore wall from Stadelmann's drawings.

Saccardo (1889) had another concept: he considered the colour of the apothecia as a defining character. On this basis he distinguished between *Belonium* Sacc. (species with black or brown apothecia) and *Belonidium* Mont. et Durieu (species with apothecia of pale colours). Representatives of Rehm's *Belonioscypha* belonged in Saccardo's work to *Belonium* sect. *Scelobelonium* Sacc. and *Belonidium* sect. *Podobelonium* Sacc. He created both sections for the species of each genus with stalked apothecia.

Section *Scelobelonium* (Saccardo 1889) originally contained only *Belonium melanosporum* (Rehm) Sacc., one of the species of our proposed section within *Cyathicula*. *Belonioscypha* has priority over *Scelobelonium* at generic rank, but the latter has priority at the rank of section. We therefore retain the name *Scelobelonium* for our proposed section, although this involves far greater emendation than would be required for a section based on *Belonioscypha*. We include in the section also a species with pale-coloured apothecia, *Cyathicula culmicola*, which Saccardo placed under *Belonidium* sect. *Podobelonium* as *B. vexatum* De Not.

A lectotype species of *Podobelonium* (Sacc.) Sacc. & D. Sacc. has probably not been designated until Carpenter (1981), who designated *Peziza campanula* Nees. We endorse the opinion of Dennis (1956: 40), later proved by Cooke (1961), that *Peziza campanula* (*Calyptella campanula* (Nees) W.B. Cooke) is a basidiomycete; therefore the name *Podobelonium* has no relevance to our section.

***Cyathicula* sect. *Scelobelonium*** (Sacc.) M. Chleb. & Chleb., **comb. nov.**

**Basionym:** *Belonium* sect. *Scelobelonium* Sacc., Sylloge Fungorum 8: 496, 1889.

≡ *Scelobelonium* (Sacc.) Höhn., Annalen des K.K. Naturhistorischen Hofmuseums Wien 20: 367, 1905.

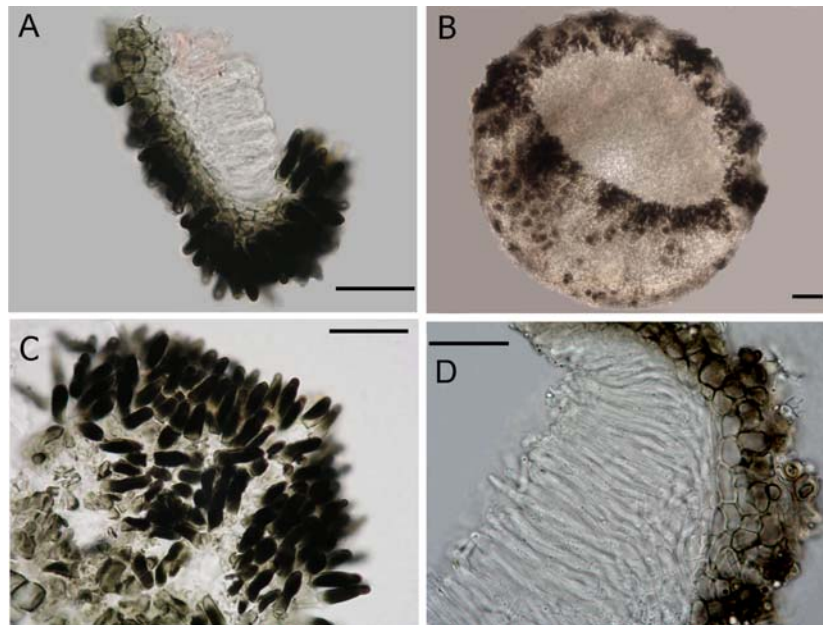
**Type species:** *Belonium melanosporum* (Rehm) Sacc.

**Syn.:** *Belonioscypha* Rehm, Kryptogamen-Flora von Deutschland, Oesterreich und Schweiz, 2. Ed., 1(3): 743, 1893. – Type species: *B. vexata* (De Not.) Rehm [= *B. culmicola* (Desm.) Dennis fide Saccardo (1889: 503), Dennis (1956: 39)], lectotype designated by Clements & Shear (1931).

**Our delimitation of the section:** Apothecia white, pale-coloured to blackish-brown, ascospores hyaline to brown, (septate), relatively thick-walled and with gelatinous sheath, paraphyses hyaline to brown, apically enlarged, at



least rarely branched. We include in the section *Cyathicula culmicola*, *C. melanospora* and *C. brunneospora*.



**Fig. 4. A-C:** *Pirottaea atrofusca*: **A:** excipulum in longitudinal section (3% KOH); **B:** apothecium rehydrated in water; **C:** setae on outer surface of apothecium (3% KOH); **D:** *Pirottaea* cf. *imbricata* PRM 877304: excipulum in longitudinal section (3% KOH). Scale bars **A-D:** 20  $\mu$ m.

***Pirottaea atrofusca*** Chleb. & M. Chleb., **sp. nov.**

FIGURES 4, 5

**Diagnosis:** *Apotheciis minutis 50-180  $\mu$ m diam., Pirottaeae imbricatae similis sed differt setis spathulatiformibus vel navicularibus, minoribus 4-13.5 x 4-4.5(5)  $\mu$ m, non-septatis et sporiis longioribus (8)9-11 x 1.5-2.2  $\mu$ m, atque habitatione in Festuca coelestis.*

**Etymology:** The specific epithet refers to the distinctly brown-black apothecia.

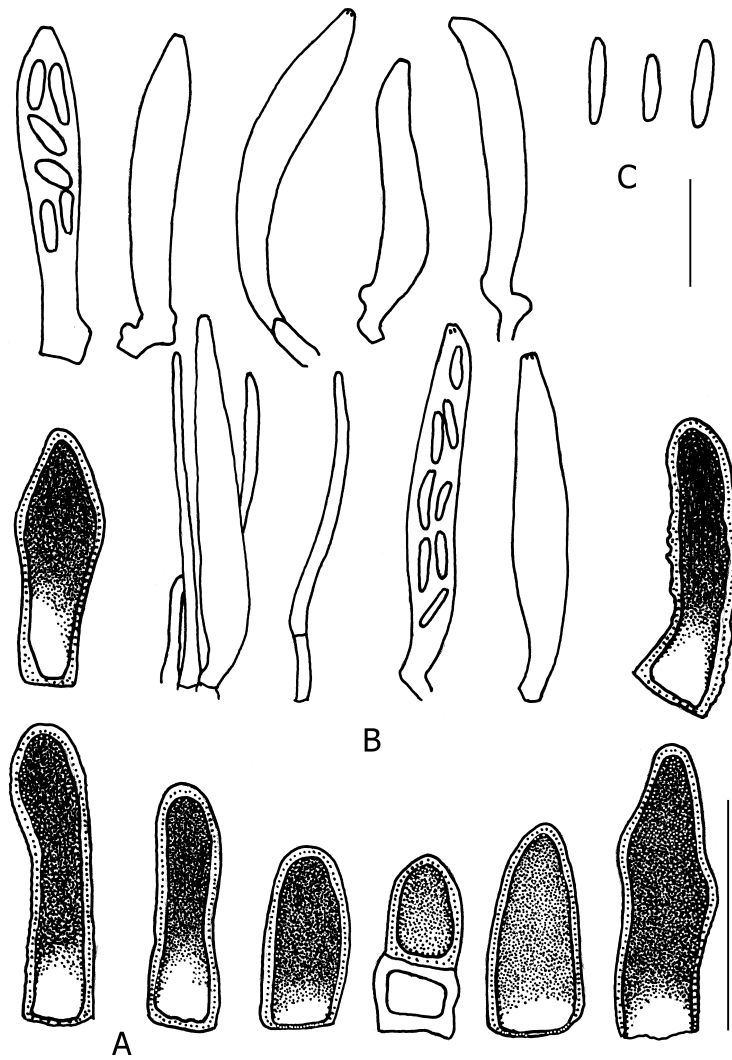
**SPECIMENS EXAMINED:** KAZAKHSTAN, TIAN SHAN: Zailijskij Alatau Mts., VALLEY OF ISSYK (YSSYK) RIVER, N 43° 07' 52.5", E 77° 30' 25", 3445.5 m elev., on leaves of *Festuca coelestis* (St.-Yves) Krecz. & Bobr., 3 August 2005, coll.: A. Chlebicki, **HOLOTYPE-KRAM** "F" 55672.

**Description:** **Apothecia** cup shaped, sessile or rarely very shortly stalked, (50)60-120  $\mu$ m in diam. on dry, rehydrated up to 180  $\mu$ m diam., 40-60  $\mu$ m high, dark brown to nearly black, disc cream-white to pale brown. **Setae**

densely distributed in upper part of apothecium (**Fig. 4**), arranged into short irregular streaks narrowing toward the base, brown in water, dark brown in 5% KOH, reddish brown in MLZ after 5% KOH, main part of seta dark brown, basal part distinctly paler (**Fig. 4, 5**), ± spatulate to naviculate (wider in the upper part) with slightly tapered or hemispherical head, solid part 4-13.5 x 4-4.5(5) µm, with refractive wall, not losing refractiveness in 5% KOH. **Grana** scarce to abundant, especially below streaks of the setae, dark and thick-walled. **Ectal excipulum** textura angularis, cells pale brown. **Asci** arising from croziers, ± cylindric-clavate, 8-spored, narrower in the upper part (32)38-42 x 4.5-5.2 µm (dead material in water), IKI+ pale blue, KOH/MLZ+ blue. **Ascospores** aseptate, hyaline, narrowly fusoid, (8)9-11 x 1.5-2.2 µm (dead in water), irregularly 2-seriate in dead asci. **Paraphyses** filiform, up to 1.2 µm broad, not exceeding the asci, 0-1 septate in lower part (**Fig. 5**).

**Comments**—The ectal excipulum of this fungus is composed of textura angularis whereas in the similar genus *Venturiocistella* Raitv. is composed of textura angularis-prismatica (Raitviir 2004). The textura of our fungus more closely resembles *Dermateaceae*, as the cells are brown and not so thin-walled. The presence of hairs or setae is also known from some *Dermateaceae*, therefore we place the fungus into this family. Our specimen is very similar to *Pirottaea imbricata* Nannf. (*Dermateaceae*). This species possesses mostly non-septate setae with obtuse ends. Nannfeldt (1985) pointed out that the apothecial margin of *P. imbricata* is bordered by short setae evolved from lateral hairs. Such origin of the setae can indicate connection with the genus *Pyrenopeziza* Fuckel. Apothecia of the genus *Pyrenopeziza* sensu Hütter (1958) are covered by marginal hairs (Müller 1989) and very rarely by protuberances called ‘grana’ (“Excipulumauswüchse”), or are devoid of any hairs, whereas lateral setae and grana are commonly found in *Pirottaea*. Setae can be considered as structures delimited with distinct, thick septum, which separate them from the ectal excipulum (Nannfeldt 1985, Müller 1989, Nauta & Spooner 2000). Lateral hairs are hyphalike, mostly hyaline or pale brown structures originated from grana or pale-walled excipular cells (Nannfeldt 1985) as in *Pyrenopeziza millegrana* Boud. The ‘hair’ structures of our specimen and specimen HB 4922a (cited below) can therefore be named true setae, sensu Nannfeldt. Hence we place the new species into the genus *Pirottaea*.

There are 52 described species of *Pirottaea* in Index Fungorum. Baral (2005) illustrated some specimens he tentatively referred to *P. imbricata*.



**Fig. 5.** *Pirottaea atrofusca*: **A:** setae (3% KOH); **B:** asci and paraphyses (water); **C:** ascospores (water). Scale bars A-C: 10  $\mu$ m.

Especially his drawing no. HB 4922a, illustrating the specimens collected by Karl Helm on *Laserpitium latifolium* L., possesses setae very similar to the setae of our fungus. Setae of both fungi have the same thickness from base to apex and the basal part of their lumen is paler. There are also some differences. The specimen from *Laserpitium* has slightly longer setae (9-21 x 3.5-5.3  $\mu\text{m}$ ) and distinctly bigger apothecia (450  $\mu\text{m}$  diam.). The next specimen HB 7551a possesses septate setae arranged in more regular streaks and abundant grana. The setae of *P. imbricata* (Nannfeldt 1985) are cylindrical, with hemispherical tips, and are strongly constricted at the septa. The setae of *P. atrofusca* are spatulate or naviculate with slightly tapered heads (see Tab 1). According to Nannfeldt (1985) and Holm & Nannfeldt (1992) the specimen of '*P. imbricata*' on *Valeriana* spp. proved to be an independent taxon. It is possible that each of the taxa presented in the Tab. 1 belongs to a separate species. Nannfeldt (1985) considered it difficult to draw precise limits between the genera *Pyrenopeziza* and *Pirottaea* because the large genus *Pyrenopeziza* is insufficiently investigated. It is also difficult to provide a clear distinction between the *Pirottaea imbricata* group and other lineages of the genus *Pirottaea*. According to Nannfeldt (1985) there are four independent lineages inside *Pirottaea*, each of which can be connected to hypothetical ancestors of *Pyrenopeziza* species. The *P. imbricata* group, where our species belongs, has been placed in his fourth lineage. He pointed out that "crucial features of this lineage are thick wall of the setae (including basal septum) and the "double" septa between all or most cells in septate setae". *P. atrofusca* possesses mostly non septate setae, whereas the true *P. imbricata* and specimen HB 7551a have both: non-septate and septate setae. There are also transitional species such as *P. geraniicola* Nannf., *P. paupercula* Nannf. and *P. trichostoma* (Kirschst.) E. Müll. & Arx, which possess distinctly septate marginal setae. Nannfeldt (1985) suggested that fungi from the genus *Pirottaea* are host specialized with narrow host-spectra. Members of the *P. imbricata* group inhabit *Laserpitium latifolium* (Baral 2005), *Cirsium oleraceum* Scop., *Valeriana sambucifolia* Eichw., *Cynanchum vincetoxicum* Pers. (Nannfeldt 1985) and *Festuca coelestis* (this article).

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Table 1. Comparison of selected characters of *Pirottaea atrofusca* and *P. imbricata*

| Characters              | <i>P. atrofusca</i><br>This article   | <i>P. imbricata</i><br>Nannfeldt<br>(1985)   | <i>P. imbricata</i><br>Baral (2005) HB<br>4922a  | <i>P. imbricata</i><br>PRM 877304  |
|-------------------------|---|--|--|--|
| Diam. of apothecia (µm) | Dry: (50)60-120<br>rehydrated: 180  | Dry: 100-150   | Fresh: up to 450   | Dry: 100-160<br>rehydrated: 160  |
| Setae (µm)              | Non-septate, 4-<br>13x4-4.5(5),<br>spathulate or<br>naviculate with<br>slightly tapered<br>head | Mostly non-<br>septate, 6-<br>15x3(4),<br>cylindrical<br>with<br>hemispherical<br>head | Non-septate, 9-<br>21x3.5-5.3<br>cylindrical to<br>naviculate with<br>slightly tapered<br>head | 0-2-septate, 9.6-<br>16x(3.1)4-4.8<br>cylindrical to<br>clavate with<br>rounded head |
| Ascus size<br>(µm)      | (32)38-42x4.5-5.2   | 30-40x4  | 39-51(57)x6-7.5  | 36-42x3.7-4.6  |
| Spore size<br>(µm)      | (8)9-11x1.5-2.2   | 6-8x1-1.5  | (6)7.5-10x2-2.2  | 7.3-9(10)x1.8-2  |
| Paraphyses<br>(µm)      | 1.2 wide, 0-1<br>septate  | No information   | 1.7-2.3 wide, 1-<br>septate  | 1.5-2.4 wide, 1-3<br>septate   |
| Host                    | <i>Festuca coelestis</i>  | <i>Cirsium<br/>oleraceum</i>   | <i>Laserpitium<br/>latifolium</i>  | <i>Valeriana<br/>sambucifolia</i>  |

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