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TWO SIBLING SPECIES OF THE GENUS BYSSONECTRIA (PEZIZALES). SUPPRESSION OF SPORE DEVELOPMENT UNDER ENVIRONMENTAL STRESS

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The first records of *Byssonectria cartilagineum* (Kanouse et Smith) Pfister and *B. terrestris* (Alb. & Schwein.: Fr.) Pfister (Pezizales) from Estonia are presented. This represents the first report of *B. cartilagineum* outside North America. Fresh collections were used to confirm distinction between *B. cartilagineum* and *B. terrestris* growing together. A comparison of characters based on living material is given.

The number of spores in asci and the size of spores in the fruitbodies of *B. terrestris* growing at the temperature of +4°C were studied during 11 days. In a young fruitbody the cytoplasm of the ascus is used to produce a small number of spores per ascus. The spores grow at a different rates so that some of them would be mature. This may be caused by stress. Later more asci with 8 mature spores are formed. The range of spore size variability in a mature fruitbody is smaller.

Introduction

Species distinction in the genus *Byssonectria* P. Karst rely on morphological characters (spore size and shape) as well as on ecological features (= *Inermisia* Rifai, Benkert, 1987; Dennis & Itzerott, 1973; Korf, 1971, 1972; Pfister, 1993,1995; Rifai, 1968; Svrček, 1969; Yao & Spooner, 1994,1996). However, in the case of ascospore size there may occur overlapping size ranges. This may result because ascospores are measured from herbarium material where fruitbodies may be in different stages of development. It has been stressed that since taxonomic research should be based on organs in compatible states and stages of development it is reasonable to use vital taxonomy (Baral, 1992). Pfister (1993) and Yao & Spooner (1996) suggest that measurement of discharged ascospores from fresh material can solve the problem of spore size range and delimitation of species within the genus *Byssonectria*.

The subiculum in the genus *Byssonectria* is, in fact, variable (Benkert, 1987; Yao & Spooner, 1996). It is possible that the development of the subiculum depends on the substrate (Yao & Spooner, 1996). According to Benkert (1987) it does not provide significant characters for taxonomic purpose.

The genus *Byssonectria* comprises four species: *B. cartilagineum* (Kanouse et Smith) Pfister, *B. fusispora* (Berk.) Rogerson & Korf, *B. seaveri* Pfister and *B. terrestris* (Alb. & Schwein.: Fr.) Pfister.

Pfister (1993) studied the genus *Byssonectria* from North America and established all four species. According to him ***B. terrestris*** (= Humaria *aggregata* (Berk. & Broome) Sacc.) has ascospores with a range of 18.4-25.6 x 8-9.6 µm and it forms a thin but prominent white subiculum. Typical *B. terrestris* grows on soil, on leaves and on mosses where animals have urinated. The operculate discomycete *Pseudombrophila guldeniae* Svrček (Brummelen, 1995) (= *Nannfeldtiella aggregata* Eckblad) is often found growing on the subiculum of *B. terrestris*. Benkert (1987) suggests that this is an evidence of parasitism. *B. terrestris* is circumboreal and is widely distributed (Pfister, 1993).

The simple morphology of the fruitbody and somewhat variable ascospore size have certainly contributed to the confusion of *B. cartilagineum* and *B.terrestris,* both growing in early spring (Pfister, 1993).

B. cartilagineum is characterized by ascospores with a range of 20-24 x 8-9.6 µm. It is associated with dung and has not been found together with *P. guldeniae.* The species is generally collected early in the season as snow is retreating, and it has a thick subiculum. All collections of *B. cartilagineum* originate from the western mountains of the USA and Canada.

B. fusispora is distinguished from *B. terrestris* by longer ascospores. *B. fusispora* is characterized by ascospores with a range of 24.0-29.0 x 7.0-11.0 µm, lack of a conspicuous subiculum and occurrence on burnt areas or on scanty soil with cyanobacteria. *B. fusispora* occurs most often at middle latitudes.

B. seaveri resembles *B. fusispora* most. It is characterized by still longer ascospores, 29-36 x 8-10 µm and is found in the USA in December.

We have found two clearly different species of *Byssonectria* growing together in early spring. One grows on top of the other and develops somewhat later. The aim of this study was to use the described freshly collected specimens to confirm distinction between the species as well as to study spore measurements and shape in different stages of development.

Material and methods

In the course of this study I examined a specimen from Estonia, Tartumaa, Rahinge, growing in a nemoral spruce forest, at 26.4°: 58.23°, on ground mixed with excrements of some rodent and needles of *Picea abies* (L.) Karst., among the mosses *Cirriphyllum piliferum* (Hedw.) Grout, *Rhodobryum roseum* (Hedw.) Limpr. and *Plagiomnium affine* (Bland.) T. Kop., 22.04.1995, leg. K. Kalamees (TAA 157755).

Fresh ascomata were measured with the microscope "Amplival" and drawn by means of the drawing apparatus PA-6, using the objective 40 and the immersion objective HI 100. Tap water was used as a mounting medium. The length (l) and width (w) of spores are presented in the following form: $(l_{min})l_{mean}(l_{max})$ x $(w_{min})w_{mean}(w_{max})$ µm, where l_{mean} and w_{mean} denote the mean values of lengths and widths of 20 spores from a specimen, and l_{min}, w_{min} and l_{max}, w_{max} denote the minimum and maximum absolute values of length and width, respectively.

I studied also spore size and the number of spores in the asci of living fruitbodies growing on the substrate in a moist chamber at the temperature of +4°C in the refrigerator for 11 days.

Results and discussion

1. The size and number of ascospores in the asci of fruitbodies of *B. terrestris* growing at low temperature --

Regarding fungi, stress has been defined as any form of continuously imposed environmental extreme which tends to restrict biomass production by most organisms in question (Cooke & Rayner, 1984).

Temperature has been shown to have an important morphological effect on the development of fungal fructifications. Ascocarp size in *Thelebolus* spp. is significantly reduced at temperature above those which allow optimal growth (15-20°C). At optimal temperatures or lower, ascocarp dimensions fell within a similar range. All isolates produced mature ascocarps at 0°C when incubation times were substantially lengthened. It is related to their ecology to grow at low temperatures. They are psychrophiles fungi (Wicklow & Malloch, 1971). Psychrophiles fungi are characterized as growing below 10°C (optimum below 20°C) (Hawksworth, Kirk, Sutton, Pegler, 1995)

The development of spores in *Byssonectria* spp. may be suppressed by low temperature when fruitbodies grow early in spring.

Since ascospore size has been used as a taxonomic character in the genus *Byssonectria* even though the species are characterized by great variability of spore size, I studied spore size and the number of spores in the asci of *B. terrestris* growing at low temperature (Table 1).

Table 1. Number of ascospores in the asci of fruitbodies of *B. terrestris* growing at the temperature of 4°C. Variability of length (l), width (w) and shape (Q - average ratio of spore length to spore width) of mature ascospores (μm).

row	date	asci	l_{min}	l_{mean}	l_{max}	w_{min}	w_{mean}	w_{max}	Q
1	26.4	4-8-spored	20.2	20.7	21.4	6.9	7.2	7.6	2.9
2	28.4	4-spored	23.9	24.5	25.2	7.6	8.0	8.2	3.1
3	28.4	6-8-spored	18.3	21.8	25.2	6.3	7.5	8.2	2.9
4	01.5	6-8-spored	22.7	24.5	25.2	7.2	8.4	9.2	2.9
5	01.5	discharged spores	21.4	23.5	25.2	6.9	7.9	8.2	3.0
6	06.5	mostly 8-spored	21.4	23.3	25.2	7.6	8.5	9.5	2.7

At first it was possible to measure only a few seemly mature spores in some asci of a young fruitbody. They were remarkably smaller (row 1) than those of an older fruitbody (rows 2-6). After two days 4- to 8-spored asci (rows 2,3) were found in one fruitbody. There were 4-spored asci with 4 mature spores. In 6-spored asci, besides mature spores, there occurred also 1 juvenile or aborted spore, while in 8-spored asci their number was 2. I measured the same fruitbody 3 days later. Now the asci were 5- to 8-spored (row 4), most of them had 8 mature spores. Fourspored asci were not found. The mean value of spore size in 4-spored asci was higher and size variability smaller (row 2) compared with 6-and 8-spored asci, (row 3) (only mature spores were measured).

In stress conditions spores grow at a different rate so that some of them would be mature and have maximum size. In a young fruitbody the material of the ascus is used for producing a small number of spores; later more asci appear with 8 mature spores.

Although discharged spores (row 5) have somewhat higher variability than the spores measured in the asci taken from the same fruitbody, their maximum values coincide (rows 4,5).

I measured spores in another fruitbody 5 days later. Its asci were mostly 8-spored and the maximum values of spore size coincided with those measured earlier (row 6). Since the spores had not grown bigger, the established maximum could be the genetically determined maximum value for this specimen.

The range of spore variability 18.4-25.6 x 8-9.6 µm in *B. terrestris* as given by Pfister (1993) is in good accordance with the variability measured by me in developing fruitbodies during 11 days: 18.3-25.2 x 6.3-9.5 µm. Spore size variability in a mature fruitbody was lower: 21.4-25.2 x 7.6-9.5 µm (rows 6,8).

The index of spore shape in our material varied irrespective of age (Q=2.7-3.1, Table 1). It was noted also by Pfister (1993) that ascospores of *B. terrestris* attained their characteristic shape early in development but matured in size relatively slowly.

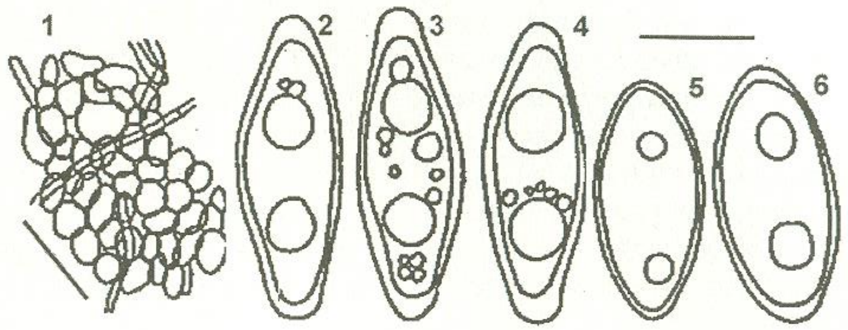
The ability of *B. terrestris* to produce mature ascospores at the temperature of 4°C is related to its growing in early spring. This species may be characterized as being 'psychrophilic' although the development of spores is at first suppressed by low temperature.

As shown above, when using spore measurements of young fruitbodies, grown in low temperature conditions, care should be taken in drawing taxonomic conclusions. This applies particularly to fungi growing at low temperature conditions in early spring, late autumn, or in mountains in the vicinity of snow.

2. Sibling species *B. cartilagineum* and *B. terrestris* --

Closely related species of plants, animal and fungi - sibling species - do not usually occur in the same site. However, this may be different in case of operculate discomycetes. Species of related genera as well as related species are often found growing close to one another provided the growth substrate, humidity and temperature conditions are adequate.

Recently we found two sibling species, *Geopora cervina* (Velen.) T. Schumach. and *G. tenuis* (Fuckel) T. Schumach., growing mixed on the ground. In the vicinity of *Aleuria aurantia* Pers. and *A. cornubiensis* (Berk. et Br.) Moravec (Moravec, 1994), two species occurring commonly close to each other, we encountered also *A. bicucullata* Boud.



Figs. 1-6. *Byssonectria cartilagineum* and *B. terrestris.*
1. Subiculum of *B. cartilagineum*. 2-4. Ascospores of *B. terrestris*. 5-6. Ascospores of *B. cartilagineum*. Bar = 100 µm in Figs. 1; bar = 10 µm in Figs. 2-6.

I had an opportunity to study the living fruitbodies of *B. cartilagineum* and *B. terrestris* growing close to each other. *B. cartilagineum* had a thick gelatinous light brown subiculum of Textura-globulosa (Fig.1) on which are situated densely clustered apothecia and in which develop young cleistohymenial apothecia as described by Pfister (1993). The subiculum was found on the ground mixed with the excrements of some rodent and needles of *Picea abies* (L.) Karst. Some mosses were growing through the subiculum: *Cirriphyllum piliferum* (Hedw.) Grout, *Rhodobryum roseum* (Hedw.) Limpr. and *Plagiomnium affine* (Bland.)T. Kop.

A very scanty greyish white subiculum of *B. terrestris* was growing on the ground near the subiculum of *B. cartilagineum*. The apothecia of *B. terrestris* developed somewhat later. Young fruitbodies of *B. terrestris* having juvenile spores were covered with the fertile spores of *B. cartilagineum*!

At first the fruitbodies of *B. terrestris* developed close to the margin of the subiculum of *B. cartilagineum*, but some days later they were seen also between the apothecia of *B. cartilagineum*. The apothecia were situated close together.

Differences in the colour of apothecia and in spore shape (Fig. 2-6) are the most useful characters for distinguishing between these two sibling species. As shown herein before, the shape of ascospores is different already in early phases of development. A comparison of the characters is given in Table 2.

Table 2. Comparison of *Byssonectria cartilagineum* and *B. terrestris.*

	B. cartilagineum	***B. terrestris***
Subiculum	light brown 5(D-4)*, 0.6 cm thick, Textura-globulosa interwoven with Textura-intricata	greyish white 1(B-1), thin, mycelial growth made up of hair-like hyphae Textura-intricata
Apothecia	densely crowded on the subiculum, initially cleistohymenial**	loosely grouped initially cleistohymenial
Disc	convex to plane, greyish orange 5(B-3)	slightly concave to plane, deep yellow 4(A-8)
Receptacle	diam 1-1.5 mm, cylindrical, cupulate, smooth	diam up to 6 mm, obconical, tomentose towards the base
Ectal-excipulum	outer surface Textura-globulosa, following layers formed of more ellipsoidal, perpendicular, thin-walled cells	outer surface Textuta-globulosa, following layers formed of more ellipsoidal, perpendicular, thin-walled cells
Margin	Textura-globulosa	Textura-globulosa
Medullary excipulum	Textura-intricata, thin-walled hyphae 4-20 µm in diam.	Textura-globulosa + Textura-intricata, elements thin-walled 14-16 x 16-40 µm
Paraphyses	straight, slender, containing coarse orange granules, diam up to 3.8- 5.6 µm at apex	usually curved, slender, containing fine yellow granules, up to 3,2-4.8 µm at apex ***
Asci	8-spored	4-8-spored
Spores	smooth, usually 2-guttulate, fuso-ellipsoid Q=2.4 (20.2)20.8(22.1) x (8.2)8.7(9.5) µm	smooth, usually 1-2 big guttules and several smaller ones, thick-walled and truncate at poles, fusoid Q=2.8 (21.4)24.1(25.2) x (7.6)8.6(9.5) µm

* Methuen Handbook of Colour (1978); ** term by Brummelen (1967); *** after 6 days in moisture conditions in the refrigerator the apex of paraphyses enlarged up to 6.3-10 µm depending on physiological reaction to absorb water and swell up (Kullman, 1995). Therefore the diameter of paraphyses at the apex is not a useful taxonomic character.

Although this collection of *B. cartilagineum* is the first record outside North America the species is probably more widely distributed, because when studying herbarium material, *B. cartilagineum* and *B. terrestris* can be easily confused. However, the characters of living material growing in the same place showed that they are clearly distinct species.

B. terrestris is close to *B. fusispora*. The latter has bigger ascospores. Proceeding from the study of Benkert (1987) on spore measurements of 27 specimens, it is evident that *B. fusispora* described by him from Germany comprises both species. He characterized *B. fusispora* as having highly variable of ascospore size, 21-27(32) x (6.5)7.5-9.5(10) µm. Some specimens had ellipsoid ("elliptische"), and others fusoid ("spindelförmige"), spores. He presented two extreme specimens, 22-25 x 9-10 µm and 26-32 x 7-8 µm (both growing on a skin-like subiculum) but he also found all transitional forms. On the basis of continuous variability of spore size it can be concluded that these species are not diverged in Central Europe. It is possible that *B. terrestris*, *B. fusispora* and even *B. seaveri* have diverged in the course of speciation, forming a homologous series based on spore length. However, considering the high variability one of the British specimens with spores 21.0-28.0 x 7.0-9.0 µm examined by R. W. G. Dennis (Yao & Spooner, 1996), which overlaps with the spore range of *B. terrestris* and *B. fusispora* given by Pfister (1993), they may occur as natural hybrids in Europe. This problem needs further research.

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