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Two new Ascomycetes on twigs and leaves of Silver birches (*Betula pendula*) in Estonia

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Abstract: *Cryptosporella betulae* (*Gnomoniaceae, Diaporthales*) and *Pleomassaria siparia* (*Pleomassariaceae, Pleosporales*), the first both in teleo- and anamorphic (*Disculina betulina*), the second only in its anamorphic stage (*Prosthemium betulinum*) are recorded for the first time in Estonia. In summer 2008 first disease symptoms were observed; in summer 2009 *C. betulae* was identified as a new species and as an obvious pathogen, causing these symptoms on young Silver birches (*Betula pendula*). At the following winter both anamorphic fungi were found abundantly sporulating on thin branches and twigs, fallen into the snow from mature Silver birches. In May 2010 the teleomorphic *C. betulae* was first time recorded on the birch leaves, fallen last autumn. Pathogenicity of *P. betulinum* is still not known. Taxonomic relevance of the fungi together with the possible connection of the findings with the recent meteorological extremities in Estonia is shortly discussed.

Kokkuvõte: Kaks Eestile uut kottseent arukase (*Betula pendula*) okstel ja lehtedel.

Esmakordselt Eestis registreeriti arukaskedel seeneliigid *Cryptosporella betulae* (*Gnomoniaceae, Diaporthales*) ja *Pleomassaria siparia* (*Pleomassariaceae, Pleosporales*), esimest nii teleo- kui ka anamorfses arengujärgus (*Disculina betulina*), teist vaid anamorfina (*Prosthemium betulinum*). 2008. a suvel märgati noorte arukaskede tüvekestel Järvseljal esimesi võrse-laiktõve tüüpi haigestumise sümptomeid, 2009. a suvel määrati neil ilmse patogeenina *C. betulae*, järgneval talvel leiti mõlemat seeneliiki, ülekaalukalt aga just esimest, lumes sporuleerimas vanade kaskede võradest varisenud pikkadel peentel okstel. 2010. a mais leiti *C. betulae* teleomorfse arengujärgu viljakehi eelmisel sügisel varisenud kaselehtedelt. Liigi *P. betulinum* patogeensus on seni teadmata ja suguline arengujärk Eestist leidmata. Artiklis tutvustatakse lühidalt ka nimetatud mikroseente süstemaatilist kuuluvust ning viidatakse nende esmaleidude võimalikule seosele äsjaste meteoroloogiliste ekstreemsustega Eestis.

INTRODUCTION

The health situation of several tree species has worsened in Estonia after a series of meteorologically unfavourable years. Even the indigenous Scots pine (*Pinus sylvestris* L.), which is generally characterized as a cold- and drought-resistant species, has been directly inflected by the series of recent extremities (Drenkhan & Hanso, 2006). Several new disease agents of forest trees have been registered for the first time in this country, being obviously released by the same meteorological extremities. Investigation level of the mycobiota of another indigenous, also cold- and drought-resistant and also economically important forest tree species, Silver birch (*Betula pendula* Roth.), is much less decent than that of Scots pine. Therefore, since last year, more attention has been paid to the mycobiota and pathology of birches.

MATERIAL AND METHODS

The investigations, first triggered by the diagnostic examination of the worsened health situation of young Silver birches in Järvselja-Rõkka (South-East Estonia), thereafter continued by the microscopic investigation of thin branches, most abundantly fallen into the snow from the crowns of adult birches during stormy winds in December 2009, led us to the registering of two new for Estonia fungal species, at first only in their anamorphic stages. In May 2010 also the teleomorph of one of the new species was registered on fallen leaves of birches.

The samples of symptomatic twigs and branches were brought to the laboratory of forest pathology of the Institute of Forestry and Rural Engineering (IFRE), Estonian University of Life Sciences, in clean plastic bags, kept in refrigerator at 0–3°C and investigated during the next

three days after collecting the samples under the stationary binocular lens CETI and microscope Nikon Eclipse 50i. The program IMAGE J 1.410 was used for the micro-morphological measurements. The fungi were isolated onto the malt extract agar (MEA OXOID malt extract LP0039 20 g l^{-1}, OXOID agar no. 3 LP0013 15 g l^{-1}) directly from pycnidia of *Disculina betulina* on twigs of young Silver birches from Järvselja and from pycnidia of *Prosthemium betulinum* on thin branches from Vara-Alajõe. The cultures were kept in the dark, at the room temperatrure (20–22°C).

RESULTS

Cryptosporella betulae (Tul. & C. Tul.) L.C. Mejía & Castl. [syn. *Ophiovalsa betulae* (Tul. & C. Tul.) Petr.] (*Gnomoniaceae, Diaporthales*) and *Pleomassaria siparia* (Berk. & Broome) Sacc. (*Pleomassariaceae, Pleosporales*) were recorded, first in their anamorphic stages *Disculina betulina* (Sacc.) Höhn. and *Prosthemium betulinum* Kunze, respectively.

The first (vegetation period) record of the anamorphic *Cryptosporella betulae***:**

Tartu county, Järvselja-Rõkka (South-East Estonia, 58°24'N, 27°29'E, alt 45 m), in a FAHM experimental area of the University of Tartu, on brownish necrotic lesions on stems of planted young Silver birches, some leader shoots of the host trees being broken at the places of lesions (Fig. 1). Conidial dimensions (N=31) of *D. betulina*: 26.4–44.1 (48.3)×1.7–5.6 µm, the mean 36.6×3.7 µm. First announcement about the disease symptoms arrived in IFRE in August, 2008; first sample was taken in 17 April 2009; leg. A. Sõber, det. M. Hanso & R. Drenkhan.

The following (hibernation period) records of the anamorphic *Cryptosporella betulae***:**

The hibernation period samples were collected in January, 2010 in South-East Estonia from three sample areas on a 40 km long NE – SW transect through Tartu. All the three records were made after stormy winds of December 2009 from abundantly fallen into the snow long, thin branches and twigs (Fig. 2) of grown-up or even old Silver birches. Fruiting bodies of other fungal species were rare in these samples.

Tartu town, Rõõmu tee park (58°22.9'N, 26°46.0'E, alt 35 m), 11 Jan 2010, leg. M. Hanso, det. M. Hanso & R. Drenkhan. (TAAM 158344). Conidial (Fig. 3) dimensions of *D. betulina* in the samples from Tartu (N=31): 29.4–53.6×3.9–5.4 (6.3) µm, the mean 41.7×4.8 µm.

Tartu county, Nõgiaru park (58°18.7'N, 26°32.8' E, alt 65 m), 14 Jan 2010, leg. & det. R. Drenkhan. Conidial dimensions of *D. betulina* in the samples from Nõgiaru (N=30): 29.7–54.0×2.7–5.5 µm, the mean 39.0×4.2 µm.

Tartu county, mixed coniferous-deciduous forest in Vara-Alajõe (58°31.7'N, 26°57.1'E, alt 53 m), 17 Jan 2010, leg. & det. M. Hanso. In the sample from Vara-Alajõe the conidial dimensions of *D. betulina*: (N=31): (35.0)37.4–58.6×2.7–5.1 µm, the mean 48.8×4.1 µm.

The first (spring time) record of teleomorphic *Cryptosporella betulae***:**

Tartu county, mixed coniferous-deciduous forest in Vara-Alajõe (58°31.7'N, 26°57.1'E, alt 53 m), perithecia (Fig. 4) on the fallen leaves, 10 May 2010, leg. & det. M. Hanso (TAAM 158344). The dimensions of asci and ascospores (Fig. 5) of *D. betulina*: asci (N=30): 46–73×4.5–7.8 µm, the mean 59.3×6.2 µm, ascospores (N=31): 11.8–21.0×2.1–4.2 µm, the mean 14.6×3.0 µm.

Cultures:

In cultures of *Cryptosporella betulae*, before drying out of the media, sporeless primordia of fruiting structures (Fig. 6) were formed on whitish aerial mycelium at the room temperature (20–22°C) in the dark.

The first (hibernation period) record of anamorphic *Pleomassaria siparia***:**

The anamorph of the second new for Estonia species *P. siparia*, named *Prosthemium betulinum*, was found only in Vara-Alajõe, 16 January 2010, leg. & det. M. Hanso (TAAM 158345). The dimensions of branches (arms, single appendixes, Fig. 7) of the *P. betulinum* staurosporous conidia (N=14): 20.7–48.2×(8.9) 10.2–20.1 µm, the mean 37.1×15.5 µm.

No teleomorphic *P. siparia* was recorded on fallen leaves or on twigs and branches of Silver birches in the hibernation or spring time samples of 2010 in Vara-Alajõe or elsewhere.

Fig. 1. Broken, symptomatic stem of young Silver birch on Järvselja-Rõkka FAHM experimental area (photo K. Aasamaa).

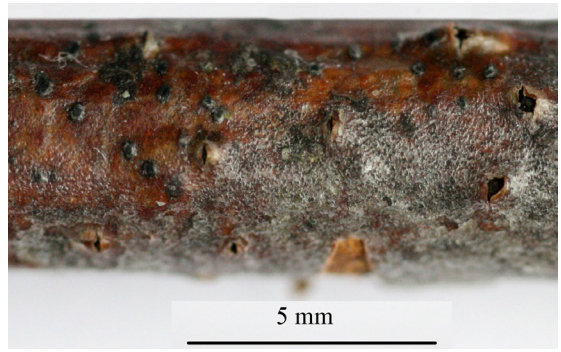

Fig. 2. Simultaneously occurring young, ripe and old pycnidia of *Disculina betulina* on a twig of Silver birch (January, 2010) (photo K. Drenkhan).

Fig. 3. Conidia of *Disculina betulina* (photo K. Drenkhan).

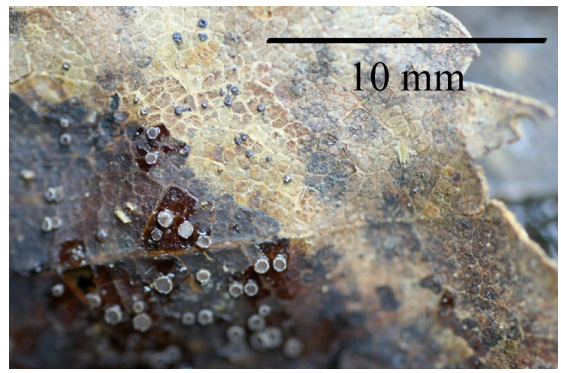

Fig. 4. Perithecia of *Cryptosporella betulae* on a fallen, hibernated Silver birch leave (photo K. Drenkhan).

DISCUSSION

Together with several other species, *Cryptosporella betulae* was only recently transferred from the genus *Ophiovalsa* to the genus *Cryptosporella* (Mejía et al., 2008). The fungus has been characterized earlier as a saprotrophic (Green, 2004) or even endophytic species (Kowalski & Kehr, 1992), effectively releasing glucose from cellulose (Bandre & Šašek, 1981). An extensive artificial watering during the entire vegetation period has been carried out on the FAHM experimental plot in Järvselja-Rõkka, obviously creating by that a provocative environment for the arrival of different diseases. Really, this fungus could be characterized there as an infectious disease agent, causing necrotic areas on stems and twigs of young (<10 years old) Silver birches, sometimes being connected even with the stem

Fig. 5. Asci and ascospores of *Cryptosporella betulae* (photo K. Drenkhan).

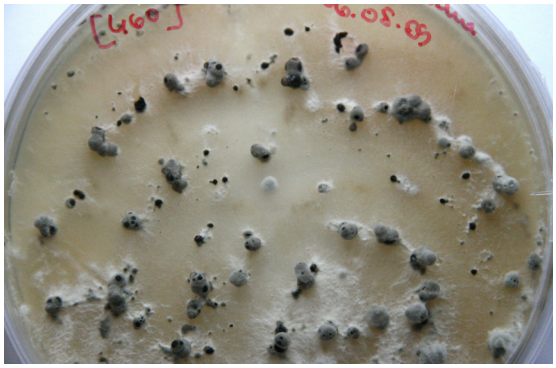

Fig. 6. Culture of *Disculina betulina* with sporeless primordia of fruiting structures on malt extract agar (plate diam. = 90 mm) (photo K. Drenkhan).

breakages. However, occurrence of the disease symptoms and of the fungus in watered, as well as in control (non-watered) experimental plots decreases the suspicions of the causal relations between the extensive watering of birches and the disease incidence.

Large variation of spore dimensions of *Disculina betulina* in different samples from South-East Estonia [N=123: (26.4)29.4–58.6×(1.7)2.7–5.6 (6.3) µm] may be caused by the generally

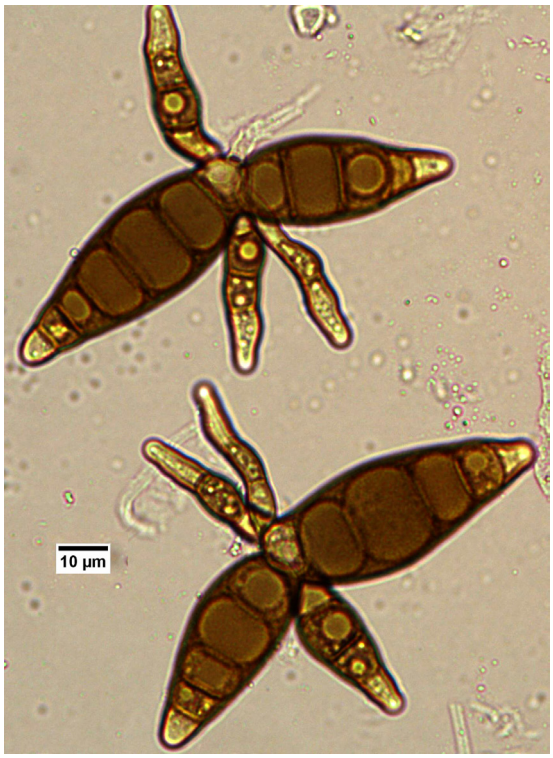

Fig. 7. Staurosporous conidia of *Prosthemium betulinum* (photo K. Drenkhan).

exceptional for fungi season and environment of sporulation – hibernation period and snow, respectively. In Finland the dimensions of spores of *D. betulina* were 45–53×3–5 µm (Paavolainen et al., 2001). Large variation of spore dimensions of *Prosthemium betulinum* in Vara-Alajõe was obviously caused by the measurement technique as we measured the ripe as well as unripe arms of spores (Fig. 7). The dimensions of largest arms (branches) of *P. betulinum* staurosporous conidia were in Finland 40–54×14–20 µm (Paavolainen et al., 2001), and of a taxonomically close species *P. asterosporium* were in Japan (35)37–50.5×9.5–14 µm, mean 43.2–11.8 µm (spores grown on RSA medium *in vitro*, cf. Tanaka et al., 2005).

Cryptosporella betulae and *Pleomassaria siparia* have been shown to occur at winter time also in England (Ellis & Ellis, 1997; Dennis, 1977) and in southern Finland (Paavolainen et al., 2001), although the pustules of *D. betulina* could be found in Finland all-around the year.

Records of these two fungi and especially of the predominating *Cryptosporella betulae* may continue the series of hard disease events on different tree species in Estonia (Hanso & Drenkhan, 2008a & 2008b, 2009, Drenkhan & Hanso, 2009) during several years after the deep and long droughts and exceptionally harmful autumns of 2002 and 2006 (Drenkhan & Hanso, 2006; Hanso & Drenkhan, 2007). It was considered in southern Finland that from several stress-causing environmental factors specifically pathogenic fungi (incl. *Disculina betulina*) had been involved in the process of crown thinning of birches (Paavolainen et al., 2001).

The taxonomy of the teleomorphic and anamorphic genera in the *Gnomoniaceae* is somewhat confused (Green & Castlebury, 2007). The occurrence of *Gloeosporium betulinum* on the leaves of birches in Tallinn was recorded by Normet (1986). To avoid misunderstanding: *Gl. betulinum* is a synonym of another anamorphic species in *Gnomoniaceae*, namely of *Discula betulina* (Westend.) Arx (*D. betulae* Pennyc.), which teleomorphic stage is *Gnomonia intermedia* (Green & Castlebury, 2007; Pennycook, 2007), and not *Cryptosporella betulae*. Therefore our findings appear as the first records of the species *Cryptosporella betulae* (in its both, teleo- and anamorphic stage) in Estonia.

Pleomassaria siparia is a common colonizer of birch twigs, which range extends from Europe (Dennis, 1977; Ellis & Ellis, 1997) to Japan (Tanaka et al., 2005). Another anamorphic species, *Prosthemium asterosporium* (*Pr. asterosporum*?) which is close to *Pr. betulinum*, was described on twigs of birches in Poland and Germany (Kowalski & Holdenrieder, 1996). Morphologically, *Pr. asterosporium* differs from *Pr. betulinum* by the simultaneously maturing conidial arms (in *Pr. betulinum* the individual arms are always of different ages, cf. Fig. 7) and by the constriction of the basal cells of these conidial arms.

After that, molecular investigations of *Pleomassaria siparia* in Finland led to the dividing also of the teleomorphic species into two biological species, unfortunately with morphologically indistinguishable characteristics (Paavolainen et al., 2000). These fungi can be morphologically distinguished only in their asexual states and therefore are described as *Pl. siparia* "type A" (with anamorph *Prosthemium asterosporium*) and *Pl. siparia* "type B" (with anamorph *Pr. betulinum*). Consequently, *Pl. siparia* "type B" was found in Estonia. No data could be found in literature about the earlier records of *Pl. siparia* in Estonia. The teleomorphic stage of the fungus is still not recorded in this country.

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