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Selinia pulchra (G. Winter) Sacc. (Bionectriaceae, Ascomycota): a new genus and species record for Ukraine

Lytvynenko YuI[1], Dzhagan VV[2] and Nyshenko VV[1]

[1] General Biology and Ecology Department, Sumy State Pedagogical University named after A.S. Makarenko, Sumy, 40002, Ukraine
[2] Educational and Scientific Centre (Institute of Biology and Medicine), Taras Shevchenko National University of Kyiv, Kyiv, 01601, Ukraine

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Abstract

Data on a new for Ukraine coprophilous fungus – *Selinia pulchra* (G. Winter) Sacc. (Bionectriaceae) are reported. It was collected in the north-east of the country (Sumy Region). Based on the collected specimen, distinctive morphological characters of the species are provided in detail. The description is illustrated by micrographs obtained using light and scanning electron microscopy. For the described specimen, its locality, substrate and collection date are indicated. In addition to morphological characters, ecological peculiarities and data on the general distribution of this species are briefly discussed.

Key words – coprophilous fungi – distribution – Hypocreales – Sordariomycetes – Sumy Region

Introduction

Selinia P. Karst. is a cosmopolitan genus of Sordariomycetes which includes coprophilous species. The genus is characterized by small perithecioid ascomata, immersed in a reddish-brown stroma but becoming erumpent. Stromata semi-immersed or almost immersed in the substratum is clearly subdivided into two parts and contains several perithecia. *Selinia* species have non-amyloid, unitunicate, short-stalked, ventricose to cylindric-clavate ephemeral asci and one-celled, hyaline, thick-walled, ellipsoid to fusiform ascospores, up to 64 μm long and 26 μm wide, with a gelatinous sheath surrounding the ascospores.

The genus *Selinia* was erected by Karsten (1876) to accommodate for the previously described species – *Hypocreopsis pulchra* G. Winter (Winter 1875). Karsten pointed out that generic name *Hypocreopsis* G. Winter was previously occupied by *Hypocreopsis* P. Karst. (Karsten 1873). This concept was accepted by Saccardo (1883) and a new combination in *Selinia* was proposed for *H. pulchra*. Subsequently, the description of this species was discussed in detail and supplemented by Hansen (1876) (as *H. pulchra*), von Arx & Müller (1955) and Udagawa (1980) (as *S. pulchra*).

The number of species of the genus *Selinia* is still uncertain. According to various data, the genus comprises of 2 (Kirk et al. 2008) to 5 species (Index Fungorum Database 2019). In addition to the above-mentioned *S. pulchra*, there are three new species published by Spegazzini:

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Corresponding Author: Yulia I. Lytvynenko – e-mail – lytvynenko2014@gmail.com

S. antarctica (Spegazzini 1887a), *S. intermedia* (Spegazzini 1887b) and *S. subtropica* (Spegazzini 1909). Subsequently, Khan & Krug (1989) have also described *S. africana*. However, some authors pointed out that *S. subtropica* Speg. (Khan & Krug 1989, Ranalli & Mercuri 1995, Doveri 2004) and *S. intermedia* Speg. (Rossman et al. 1999, Richardson 2006) could be probably synonyms with *S. pulchra*. Rossman et al. (1999) and Bell (2005) provided the key for 4 and 2 species, respectively. There is no monograph on the genus yet.

This genus is so peculiar that its systematic position was widely discussed and differently interpreted by particular authors. Winter (1875) considered his new genus as closely related to *Hypocrea*. Saccardo (1883) regarded this genus in the family Hypocreaceae. Clements & Shear (1931) placed *Selinia* in the Hypocreaceae within the order Hypocreales. von Arx & Müller (1955) proposed the new family Seliniaceae in the Sphaeriales to accommodate *Selinia* and another similar genus *Seliniella*. Dennis (1978) accepted the Seliniaceae in the Sphaeriales. Udagawa (1980) considered a taxonomic position of this genus near the Hypocreaceae as a representative of the primitive pyrenomycetes. Rossman et al. (1999) had placed *Selinia* in the new family Bionectriaceae within the order Hypocreales based on molecular phylogenetic analysis using rDNA gene sequences. Rossman et al. (2001) pointed out that this genus is unique in the Hypocreales in having large, uniseptate, thick-walled ascospores and belonging to the *Hydropishaeria-Selinia* clade. In taxonomic works of recent years, this genus is considered in the Bionectriaceae (Kirk et al. 2008, Maharachchikumbura et al. 2015).

Selinia comprises cosmopolitan coprophilous species which are common on the dung of both wild and domestic herbivores. This genus is widespread almost all over the world but mainly uncommon or infrequent in most countries. In 2019 during the cultivation of fecal material collected during mycological surveys in the north-east of Ukraine (Sumy Region) we found one species of this genus – *Selinia pulchra*. These genus and species are newly reported in Ukraine. In Eastern Europe, until recently *S. pulchra* was known only in Russia (Prokhorov & Armenskaya 2001).

Materials & Methods

Sampling and isolation

The ascomata of *Selinia pulchra* were found 26 March 2019 on cattle (*Bos taurus* L.) dung incubated for 42 days. Samples of excrement were collected during mycological surveys in the Sumy Region of Ukraine (N51°13′79.0″, E33°88′25.3″) in 12 July 2018.

The moist-chamber method of incubation was used to detect and obtain the ascomata (Richardson 2001) from samples of dung. Samples of excrement were placed in a Petri dish on a filter paper, moistened with water and more added subsequently, if necessary, to maintain sufficient moisture of the substrate. The incubation was carried out at room temperature (18–20 ºC) under natural light. The samples were examined frequently at intervals of a few days using a stereomicroscope.

The specimen is deposited at the Mycological Herbarium of the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine (KW-M).

Species identification

Microcharacters were examined in fresh material with a stereomicroscope MBS-10 (JSC "LZOS", Russia) and a compound microscope XSM-40 (Ningbo Sunni Instruments Co., Ltd., China). Dimensions of microstructures were measured using Tsview7 modular software (Fuzhou Tucsen Imaging Technology Co., Ltd., China). Photomicrographs were taken with 3.0mp Digital Microscope Camera (Fuzhou Tucsen Imaging Technology Co., Ltd., China).

The microstructures mounted in distilled water were examined at magnifications up to 600×. Thirty spores and twenty asci per specimen were measured. For spore sizes the following abbreviations are used: L = spore length, L_{av} = average spore length, W = spore width, W_{av} = average spore width, Q = quotient between spore length and width (L/W), Q_{av} = average quotient.

The amyloid reaction of apical apparatus and walls of the asci were determined by treating with Meltzer's reagent (MLZ). To highlight spore ornamentation, hyaline gelatinous sheath and some excipular details, Congo Red (CR) and Lactophenol Cotton Blue (LPCB) were used.

For scanning electron microscopy (SEM), small dried pieces of dung with stromata were mounted on the metal stubs. The samples were coated with an ultrathin coating of silver using vacuum vessel VUP-5M (OJSC "Selmi", Ukraine) and examined with a scanning electron microscope SEO-SEM Inspect S50-B (Sumy electron optics, Ukraine). Scanning electron microscopy (SEM) studies were conducted at the Center for collective use of scientific equipment "Laboratory of Materials Science for Helium Energy, Sensory and Nanoelectronic Systems" at Sumy State University (Ukraine).

Results

Taxonomic description of *Selinia pulchra* based on the Ukrainian specimens is provided below. Its morphological characters are described in detail and illustrated by micrographs obtained using light and scanning electron microscopy. For the examined specimen, its substrate, locality, and collection date are indicated. The data on general distribution, ecological and morphological peculiarities of the species are briefly discussed.

The taxonomic arrangement, species synonyms and author citations are provided according to Maharachchikumbura et al. (2015) and Index Fungorum Database (2019).

Selinia pulchra (G. Winter) Sacc., in Saccardo, Syll. Fung. **2**: 457, 1883. Figs 1–2

≡ *Hypocreopsis pulchra* G. Winter, Hedwigia **14**(2): 26, 1875 (basyonym). ≡ *Winteria pulchra* (G. Winter) Sacc., Michelia **1**(3): 281. 1878. ≡ *Seliniana pulchra* (G. Winter) Kuntze, Rev. gen. pl. **2**: 869, 1891.

Systematic position – Sordariomycetes O. E. Erikss. & Winka; Hypocreomycetidae O. E. Erikss. & Winka; Hypocreales Lindau; Bionectriaceae Samuels & Rossman; *Selinia* P. Karst.

Description – *Stromata* 5–6 mm in diam., pulvinate or irregular, semi-immersed, slightly tomentous, containing 5–9 perithecia, with protruding glabrous ostioles; consisting of two parts, external part (ectostroma) reddish-orange at first, soon reddish brown, consists of several layers of heavily pigmented thick-walled cells, internal part (endostroma) spongy and whitish, composed of cells measuring 5–7 μm in diam., textura epidermoidea. *Ascomata* perithecioid, 780–820 × 457–680 μm, subglobose, flask-shaped, membranous, smooth, pale orange; neck subcylindrical or tuberculate, dark orange, 290–360 × 255–310 μm, projecting ostiole measuring 65–85 μm in diam., inner covered with prominent periphyses. Peridium membranaceous, *ca* 40 μm thick, layered, with an exostratum made up of thick-walled, angular to rounded cells, filled with intracellular, orange pigments; endostratum pseudoparenchymatous, made up of hyaline, polygonal cells, 21–30 × 15–22 μm, becoming progressively narrower toward the ostiolar canal, periphyses arising from and merging with elements of the neck, hyaline, filiform, septate, 29–62 × 2.6–4.4 μm. Ascomata immersed in stroma, becoming erumpent, granular margin around the ostioles opening, apex protruding, venters remaining immersed in the stroma. *Asci* eight-spored, clavate to cylindrical-clavate, with a short stipe, 260–325 × 45–55 μm; unitunicate, thin-walled, rounded or somewhat truncate at the apex, without an apical ring, ephemeral. *Ascospores* uni- or biseriate, one-celled, thick-walled, yellowish with granular contents when young and hyaline or pale yellow with two small oil drops when mature, narrowly ellipsoidal to fusiforme, sometimes slightly asymmetrical, roundish or slightly flattened, rarely hardly pointed at the ends; (50.5–) 52.3–55.5 (–57.2) × (20.5–) 21.8–26.9 (–29.8) μm, L_{av}=52.8 μm, W_{av}=24.6 μm, Q=1.90–2.61, Q_{av}=2.17. Episporium thick, smooth or sometime indistinctly ornamented, measuring 1.3–1.9 μm wide, surrounded by an incomplete gelatinous sheath, 2.1–4.2 μm wide. *Paraphyses* hyaline, cylindric-filiform, septate, 5.5–7.5 μm in diam., ephemeral, soon reduced to a shapeless material.

Material examined – Ukraine, Sumy Region, Buryn District, Mykhailivka village, grassland, N51°13′79.0″, E33°88′25.3″, cattle dung, 12 July 2018, V. V. Nyshenko (KW-M71267).

General distribution – *Europe*: Denmark, Finland, France, Germany, Iceland, Italy, the

Netherlands, Russia (European part), Spain, Sweden, Ukraine, UK; *Asia*: Japan, Russia (Western Caucasus), Taiwan; *Australasia*: Australia, New Zealand; *North America:* Canada, USA; *South America:* Argentina, Brazil, Chile, Falkland Islands; *Caribbean*: Dominica, Puerto Rico; *Another locations*: St. Helen island (Atlantic ocean) (von Arx & Müller 1955, Dennis 1978, Udagawa 1980, Wang 1994, Ranalli & Mercuri 1995, Rossman et al. 1999, Jahn et al. 2000, Nordic macromycetes 2000, Prokhorov & Armenskaya 2001, Doveri 2004, Bell 1983, 2005, Richardson 2006).

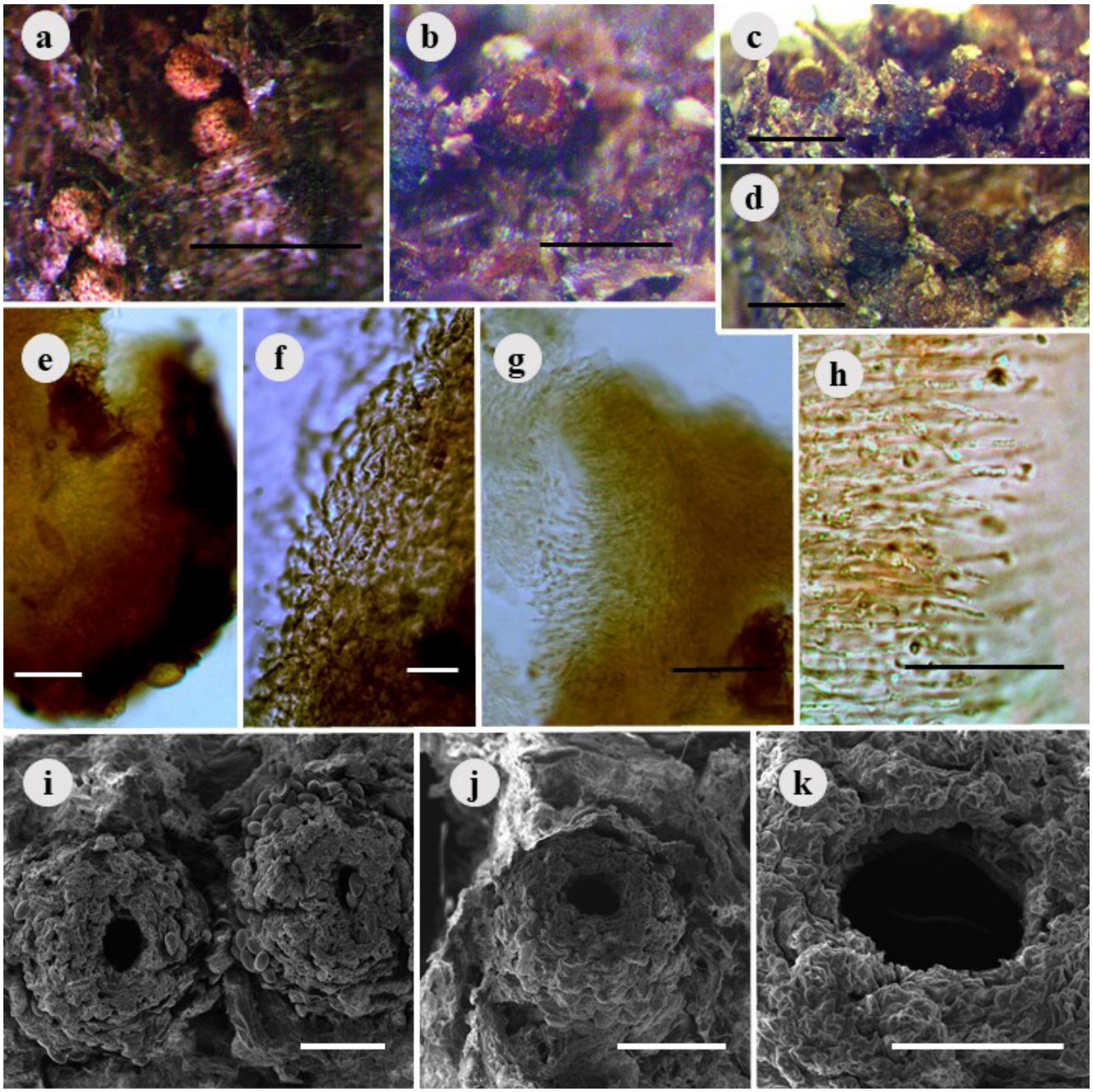

Fig. 1 – *Selinia pulchra*. Structural features of ascomata. a Necks of young perithecia. b–d Necks of mature perithecia. e Peridium with an exostratum and endostratum. f Cells of endostratum. g Ostiolar canal with periphyses. h Periphyses. i Perithecial necks with ascospores (by SEM). j–k Necks with ostioles (by SEM). Scale bars: a = 1 mm, b-d = 0.5 mm, e-h = 25 μm, i, j = 100 μm, k = 50 μm.

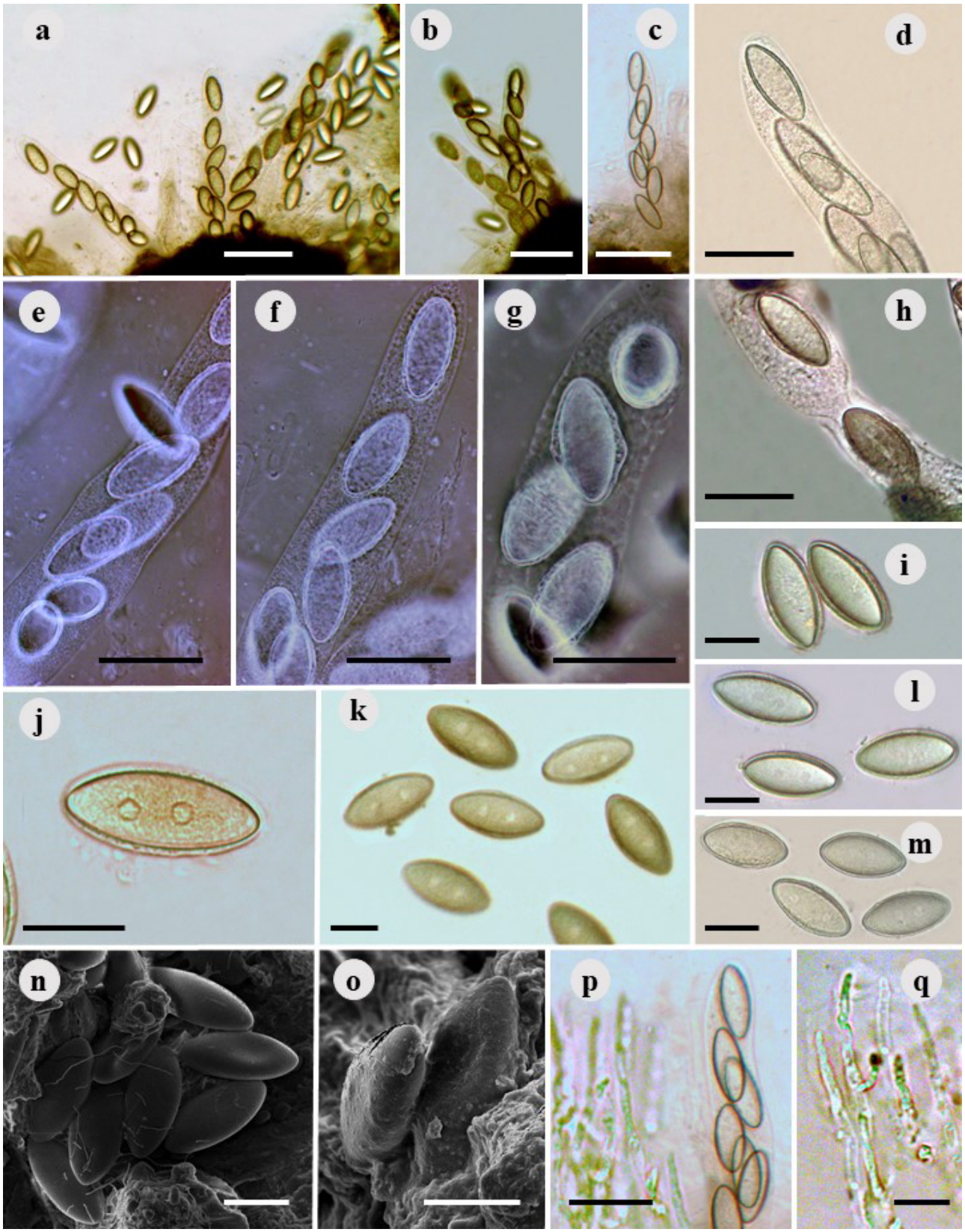

Fig. 2 – *Selinia pulchra*. Structural features of asci, ascospores and paraphyses. a–c Asci with ascospores in different stages of maturity. d Ascus apex with mature ascospores. e–f Asci with ascospores containing a granular material (photo negative). g Ascospores whith gelatinous sheath (photo negative). h Ascospores released from ascus. i–m Free mature ascospores. n-o Mature ascospores (by SEM). p Ascus and paraphyses. q Paraphyses. Scale bars: a-c = 100 µm, d-h, p = 50 µm, i-o, q = 25 µm.

Discussion

Selinia pulchra is the coprophilous species, which prefer the dung of herbivores, particularly of domestic animals (Doveri 2004, Watling & Richardson 2010). In the worldwide literature, it was described to be not only on cattle, deer, hare, horse, and sheep but also on opossum excrements (Bell 1975) and goose droppings (Watling & Richardson 2010). Despite a wide distribution of *S. pulchra*, these species do not generally occur often. Most authors describe it as infrequent (Udagawa 1980, Ranalli & Mercuri 1995, Richardson 2006), uncommon (Dennis 1978, Doveri 2004), rare or extremely rare (Larsen 1971, Nordic Macromycetes, 2000). Richardson (2006) even points out that "from over 900 samples collected over the last 12 years" all over the world his have only 14 records of *S. pulchra*. The examined material represents the second record of the species in east Europe. The first one was registered by Prokhorov & Armenskaya (2001).

According to various data, this species has fairly variable spore size. In particular, ascospore length and width vary within 50–56 × 20–30 µm (Saccardo 1883); 48–64 × 20–26 µm (von Arx & Müller 1955); 46–60 × 20–24 µm (Udagawa 1980); 58–65 × 24–30 µm (Bell 1983); 45–60 × 20–24 µm (Rossman et al. 1999); 56.7–63 × 22–25.2 µm (Doveri 2004); 54–60 × 20–26 µm (Bell 2005) and 45–55 × 19.5–24 µm (Richardson 2006). As noted by Richardson (2006) based on study of 14 collections of *S. pulchra*, the spore lengths are "represented by a continuum of overlapping values" and varies from 42 to 64 µm. The size of the ascospores of the samples we investigated is in accordance with Saccardo (1883). Most authors indicate that *S. pulchra* ascospores are smooth. Udagawa (1980), however, notes that spores can be smooth or sometimes ornamented. In our specimens, most ascospores also have smooth episporium (Fig. 2n), but we noted single indistinctly ornamented, somewhat rough ascospores (Fig. 2o).

Immature ascospores in our specimens are pale yellow with distinct granular contents (Fig. 2d-f), mature ascospores hyaline or yellowish (Fig. 2i-m), often with two oil drops (Fig. 2j, k). Khan & Krug (1989) pointed out that "yellowish granular contents in the spores" is one of the taxonomic features *Selinia africana*, by which this species differs from the others in the genus. Meanwhile, Hansen (1876) and von Arx & Müller (1955) indicated a yellow granular pigment in ascospores of *S. pulchra*. Moreover, *S. africana* differs from *S. pulchra* by smaller ascospores (26–41 × 12–22 vs. 45–64 × 20–30 µm).

The asci of *S. pulchra* are characterized with usually eight uni-, bi- or triseriate spores, but, as an exception, maybe less (von Arx & Müller 1955, Dennis 1978, Doveri 2004). Winter (1875) reported 4-8-spored asci in his collections. We have observed only 8-spored asci with uni- or, exceptionally, biseriate ascospores in our specimens (Fig. 2a-c, e, f). Doveri (2004) emphasized that the asci of this species are ephemeral. Hansen (1876) and Larsen (1971) noticed, that when mounted in water the asci are irregularly rupturing.

An interesting fact was observed by Hansen (1876) when ascospores were released from asci. In the water asci swell and rolls from side to side, after their wall is destroyed, usually in the upper part and ascospores soon to become free. We had observed the same.

Although for *S. pulchra* paraphyses are described, it is likely to be ephemeral and hardly observable. Winter (1875) pointed out that one of the distinctive features described by him species is the presence of numerous filiform, inflated and articulated paraphyses. von Arx & Müller (1955) did not discuss paraphyses at all. Udagawa (1980) cited data on "hyaline, fibrillose, about 2.5–5 µm in diam." paraphyses. Rossman et al. (1999), on the contrary, noted that paraphyses "were not seen in the specimens examined of *S. pulchra*". The exact description of the paraphyses was given by Hansen (1876) and Doveri (2004). Hansen described dense-filiform paraphyses, colorless, isolated or branched, sometimes anastomosing, with irregular swollen; lengths with asci or slightly shorter. Doveri characterized paraphyses as "cylindric-moniliform, septate, 4-17 µm diam. at the base, narrowing upwards but often inflated at the tips, branched and anastomosed, containing many hyaline vacuoles". Læssøe (1995) suggested, that the described paraphyses should not be regarded as true, since such paraphyses are not typical for Hypocreales. We have observed some cylindric-filiform remnants, which were mixed with asci, 5.5–7.5 µm diam., containing several hyaline vacuoles (Fig. 2p, q).

Selinia pulchra was isolated in pure cultures (Ranalli & Mercuri 1995, Udagawa 1980). Ranalli & Mercuri (1995) reported that in culture a *Tubercularia*-like anamorph and fertile perithecia were formed. The anamorphic stage is formed in irregular pulvinate sporodochia with a viscous slimy mass of conidia. The authors place it near *Tubercularia* but noted that there is a very big difference between anamorphs of the *Nectria cinnabarina* group (classic *Tubercularia* spp.) and *Selinia pulchra*. Previously, Winter (1875) also described unicellular conidia arising from hyphae aggregated in a felt-like lining on the outside of the stroma. But this type of anamorph has not been described and not commented so far.

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