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RESEARCH ARTICLE

Subulicystidium perlongisporum (*Trechisporales*), the first record in Ukraine

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Abstract. In this article we report the first record of *Subulicystidium perlongisporum*, a noteworthy corticioid fungus, which has never been previously recorded in Ukraine. A detailed morphological description of the examined specimen, along with the original photos, ecological specifics and global distribution details of the species are provided. The criteria for delimitation of long-spored species of *Subulicystidium* are discussed.

Keywords: *Basidiomycota*, corticioid fungi, *Hydnodontaceae*, long-spored species, rectangular crystals, subulate cystidia

Introduction

Subulicystidium is a genus of corticioid fungi established in 1968 by the Estonian mycologist Erast Parmasto, with the type species *Subulicystidium longisporum* (Pat.) Parmasto. According to the *Index Fungorum* database (https://www.indexfungorum.org/) (accessed 05 March 2024), the genus comprises 25 species, including 16 species described over the last six years (Ordynets et al., 2018; Liu et al., 2019, 2022). This genus comprises the species with resupinate arachnoid fruit-bodies with a smooth hymenophore, finely velutinous due to the numerous protruding cystidia, the monomitic hyphal system with clamps near all septa and unique morphology of the subulate cystidia (Duhem, Michel, 2001; Gorjón et al., 2011; Ordynets et al., 2018). The smooth thick crystalline sheath of cystidia is covered with two chains of the bow-tie-shaped crystals, which are seen in the light microscope as four chains of rectangular crystals along the cystidium body (Jülich, 1975; Keller, 1985; Ordynets et al., 2018). Such cystidia type has not been found in any other fungus. The presence of repetobasidia, as earlier referred to by some authors (Jülich, 1968; Liberta, 1980; Gorjón et al., 2011), was not confirmed by further morphological examinations. Ordynets et al. (2018) found out that it was a hyaline crystal collar at the base of the mature basidia, which is formed in some species. Another important feature is the basidiospores shape and dimensions. The length-to-width ratio (Q) of the spores is used in

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order to distinguish certain species within the genus. Traditionally, there were two morphological groups recognized with Q value higher and lower than four (Boidin, Gilles, 1988; Duhem, Michel, 2001; Gorjón et al., 2011; Ordynets et al., 2020). For the purpose of convenience, Ordynets et al. (2020) in their examinations of the genus *Subulicystidium* proposed to classify the species under this parameter as short-spored (Q < 4) and long-spored (Q > 4) species. Ordynets et al. (2018) suggested subdividing the short-spored species into three groups according to the basidiospore shape: species with fusiform, cylindric and allantoid basidiospores, respectively. Long-spored species have acicular, vermicular or fusiform basidiospores (Punugu et al., 1980; Maekawa, 1994; Bernicchia, Gorjón, 2010; Gorjón et al., 2011; Liu et al., 2019, 2022; Ordynets et al., 2020).

The genus *Subulicystidium* belongs to the family *Hydnodontaceae* Jülich, order *Trechisporales* K.H. Larss. The order *Trechisporales* has been recently proposed by Karl-Henrik Larsson (in Hibbett et al., 2007). Some taxonomic changes were introduced relying on the recent phylogenetic studies covering the order *Trechisporales* (Liu et al., 2022): a new order *Sistotremastrales* L.W. Zhou & S.L. Liu was distinguished, with the family *Sistotremastraceae* L.W. Zhou & S.L. Liu and two genera, *Sertulicium* and *Sistotremastrum*; a new genus *Allotrechispora* L.W. Zhou & S.L. Liu separated from *Trechispora* was formally arranged within the family *Hydnodontaceae*, order *Trechisporales*; the genus *Boidinella* was transferred to the order *Cantharellales* (incertae sedis). Moreover, Liu et al. (2022) suggested excluding the genera *Litschauerella* and *Sphaerobasidium* from the order *Trechisporales*. However, due to lack of sufficient molecular data they are still formally considered within this order. According to the current data, one family *Hydnodontaceae* and 12 independent genera, viz. *Brevicellicium*, *Brevicellopsis*, *Dextrinocystis*, *Fibrodontia*, *Litschauerella*, *Luellia*, *Porpomyces*, *Pteridomyces*, *Subulicystidium*, *Suillosporium*, *Trechispora* and *Tubulicium*, are accepted in the *Trechisporales* (Larsson, 2007; de Meiras-Ottoni et al., 2021; Spirin et al., 2021; Liu et al., 2022). The relationships among these genera within the order *Trechisporales* are not fully clarified (Liu et al., 2022). However, the genus *Subulicystidium* was strongly supported as a monophyletic lineage within *Hydnodontaceae* in various reports (Ordynets et al., 2018, 2020; Spirin et al., 2021; Liu et al., 2019, 2022).

The species of genus *Subulicystidium* form fruit-bodies over dead, heavily decayed wood at the late stages of destruction and over plant debris in various forest ecosystems (Bernicchia, Gorjón, 2010; Ordynets et al., 2018, 2020). Nevertheless, exact nutrition mode of the genus is still unclear (Hibbett et al., 2014; Ordynets et al., 2018). The investigations indicate that this genus reaches the utmost species diversity in the tropical regions (Ordynets et al., 2018, 2020; Liu et al., 2019, 2022).

Currently, ca. 290 species of corticioid fungi have been recorded in Ukraine (Akulov et al., 2003; Usichenko, 2009; Bernicchia, Gorjón, 2010; Ordynets et al., 2017; Shevchenko, 2017, 2018; Dudka et al., 2019; Bohoslavets, Prydiuk, 2023). As compared to other countries of Northern or Western Europe, the corticioid fungi diversity in Ukraine has been still insufficiently studied. No dedicated examinations of the genus *Subulicystidium* have ever been undertaken in Ukraine. Therefore, only extremely scarce details about occurrence of these fungi in the country are available.

Material and Methods

The examined specimen is deposited at the Mycological collection of the National Herbarium of Ukraine (KW-M) at the M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, Kyiv. The basidiocarp description was based on the dried specimen. The sections of basidiocarp were prepared in 5% KOH to observe and measure the microscopic features; Cotton blue was used to identify cyanophilous element of basidiocarp; Melzer's reagent was employed to check amyloidity and dextrinoidity. The micromorphological characteristics were examined with a Carl Zeiss Jena Amplival light microscope. The spore dimensions were obtained through at least 30 spore measurements per fruit-body. The other microstructures dimensions were obtained through 10 measurements per fruit-body. In the morphological descriptions, L stands for the arithmetic average for length of measured basidiospores, W — for the arithmetic average for width of measured basidiospores, Q — for variation in the ratio of L to W, and av. Q — for average ratio of L to W.

The species was identified according to Bernicchia and Gorjón (2010), Ordynets et al. (2018, 2020). The scientific name of the species is provided according to *Index Fungorum* database (https://www.indexfungorum.org/).

Results

Details on distribution patterns of *Subulicystidium* in Ukraine are extremely limited. Prior to our research, merely one species, *S. longisporum*, had been recorded for the country's territory. GBIF contains information about 30 occurrences of *S. longisporum* in different regions of Ukraine (https://doi.org/10.15468/dl.skrexp); particularly, 25 specimens are preserved in CWU(MYC) (Kharkiv, Ukraine) and three — in MB (Marburg, Germany). We have also managed to find two records of this species in the literature references: (1) Mountain Crimea, near Sudak, the northwest outskirts of Dachne, on fallen branch of *Cornus* sp., August 2001, leg. E.O. Yurchenko (Akulov et al., 2003); (2) Zakarpatska (Trans-Carpathian) Region, Carpathian Biosphere Reserve, outskirts of Mala Uholka, on decayed deciduous wood, September 11, 2013, leg. A. Ordynets (CWU6737) (Ordynets et al., 2018).

Mycological observations conducted in July 2023 in the Hlyboki Balyky Ecological Research Station resulted in discovering the corticioid fungus identified as *S. perlongisporum,* which is the first record of the species for Ukraine.

We provide below a detailed description and illustrations of the macro- and micromorphological structures of the collected specimen of *S. perlongisporum*, along with an overview of its ecological specifics, global distribution and criteria for delimitation of long-spored species of *Subulicystidium.*

***Subulicystidium perlongisporum* Boidin & Gilles**, Bull. trimest. Soc. mycol. Fr. 104(3): 197. 1988. — Fig. 1

Basidiome annual, resupinate, thin, up to 75 µm thick, loosely adnate, soft and fragile, arachnoid, porulose and pubescent under the lens because of the projecting cystidia, whitish grey when fresh, light grey to grey when dry (Fig. 1A). Margin not differentiated. ***Hyphal system*** monomitic, all septa with clamps. Subiculum thin, with richly branched hyphae 3.5–4.5 µm wide, thin-walled to slightly thick-walled, hyaline and smooth. Subhymenium thin, hyphae 3.0–3.5 µm wide, thin-walled, smooth. ***Cystidia*** numerous, subulate, terminal or pleural, 40–55 × 3.0–3.5 µm, projecting up to 30–35 µm, without basal swelling, heavily encrusted except the apex, covered by rectangular crystals arranged in longitudinal rows (Fig. 1B–D). ***Basidia*** urniform, 9–12 × 3.5–4.5 µm, thin-walled, with 4 sterigmata and a basal clamp, without encrustation. ***Basidiospores*** vermicular, hyaline, thin-walled, smooth (Fig. 1B(2)), inamyloid, indextrinoid, acyanophilous, guttulate, (11.9)16.6–20.7 (21.0) × (1.2)1.8–2.0(2.2) µm, L = 18.5, W = 1.6 µm, Q = 8.6–13.9, av. Q = 11.6.

Specimen examined. Ukraine. Kyiv Region, Obukhiv District, outskirts of Rzhyshchiv town, Hlyboki Balyky Ecological Research Station, 49°58'09.2" N 31°07'11.5" E, hornbeam-oak forest, on dead strongly decayed wood of *Carpinus betulus*, 27 July 2023, leg. M. Zykova (KW-M71581).

General geographical distribution. North (Central) America (Costa Rica, Cuba, Dominican Republic, Jamaica, Mexico), South America (Brazil), Africa (Canary Islands, Madagascar, Madeira, Reunion), Asia (China, Iran, Japan, Russia, Taiwan), Europe (Austria, Czech Republic, Estonia, France, Germany, Italy, Poland, Russia, Slovakia, Spain, Switzerland, Ukraine), Oceania (Vanuatu) (Boidin, Gilles, 1988; Duhem, Michel, 2001; Maekawa, 2002a, 2002b; Ghobad-Nejhad et al., 2009; Tellería et al., 2009; Bernicchia, Gorjón, 2010; Beltrán-Tejera et al., 2013; Urbizu et al., 2014; Saitta, Losi, 2016; Tejklová, Zíbarová, 2018; Ordynets et al., 2018, 2020, and references therein; Holec et al., 2019; Liu et al., 2019, 2022; Zíbarová, Pouska, 2020; Friebes et al., 2023).

Ecological features. According to Ordynets et al. (2018, 2020), fruit-bodies of this species are formed over fallen heavily decayed wood of deciduous trees. They may be also occasionally found on conifer wood, particularly of *Juniperus communis*, *Picea abies*, *Pinus sylvestris* (Duhem, Michel, 2001; Dämmrich, Rödel, 2017).

Discussion

The length of the basidiospores in the examined specimen does not exceed 21 µm, which is generally slightly less than the value provided in the protologue (16–25 µm, Boidin, Gilles, 1988) and other descriptions (17–23 µm, Maekawa, 1994; 16–25 µm, Bernicchia, Gorjón, 2010). Ordynets et al. (2020) found two sympatric lineages present in *S. perlongisporum* that morphologically slightly differed in the mean length of cystidia. In the examined specimen, this value was equal to 47 µm which is in average slightly less than that reported in the consulted descriptions (Maekawa, 1994; Bernicchia, Gorjón, 2010). The examined specimen better fits in the clade 1 as defined in Ordynets et al. (2020).

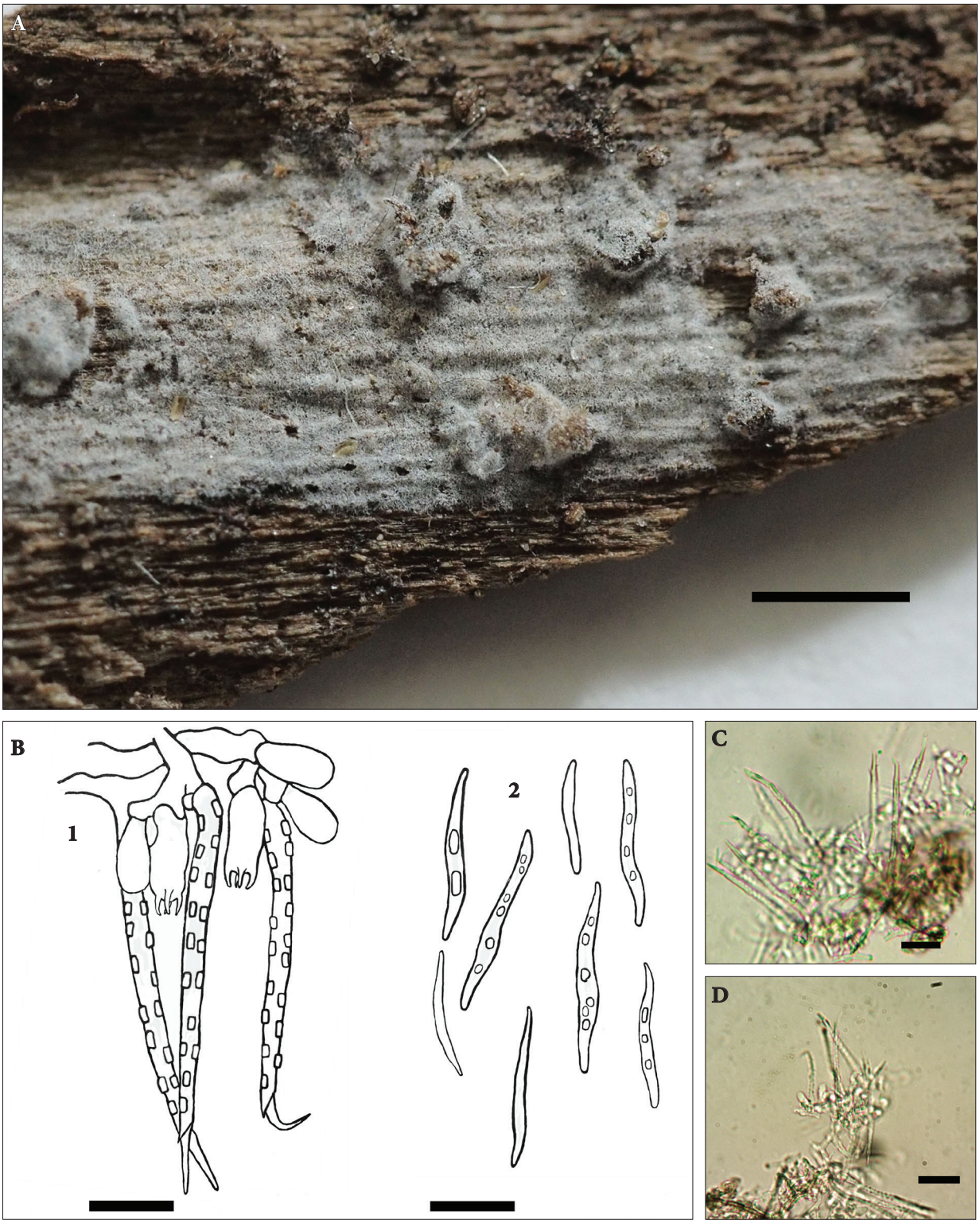

Fig. 1. Macro- and microscopic features of *Subulicystidium perlongisporum* (KW-M71581). A: general view of its basidiome (bar = 2 cm); B: hymenium (1) and basidiospores (2) (bar = 10 µm); C, D: hymenium (bar = 20 µm)

Table 1. **Morphological criteria for delimitation of long-spored species of *Subulicystidium***

Species	Basidiospores shape	Basidiospores dimensions (μm)	Q	Peculiarities of cystidium ornamentation	References
S. acerosum	Narrowly fusiform to slightly vermicular	14.5–20.0 × 1.8–2.2	8.3	Needle-like crystals in the middle part	Liu et al., 2019
S. cochleum	Acicular with twisted and curved distal end	20.0–27.0 × 2.0–3.0	9.0–10.0	Needle-like crystals in the middle part	Punugu et al., 1980
S. curvisporum	Acicular, spirally curved	27.0–35.0 × 2.0–2.5	13.5–14.0	Regularly covered with rectangular crystals except for the apex	Gorjón et al., 2011
S. daii	Fusiform to slightly vermicular	15.0–18.5 × 2.3–3.0	6.5–6.9	Regularly covered with rectangular crystals except for the apex	Liu et al., 2022
S. longisporum	Narrowly fusiform, usually sigmoid	12.0–16.0 × 2.0–3.0	4.5–7.0	Regularly covered with rectangular crystals except for the apex	Bernicchia, Gorjón, 2010
S. perlongisporum	Vermicular	17.0–23.0 × 2.0–2.5 (according to Maekawa); 16.0–25.0 × 1.5–2.5 (according to Bernicchia, Gorjón, 2010)	9.0–11.0	Regularly covered with rectangular crystals except for the apex	Maekawa, 1994; Bernicchia, Gorjón, 2010; Ordynets et al., 2020
S. tropicum	Fusiform to slightly vermicular	11.0–13.0 × 1.8–2.0	5.95	Regularly covered with rectangular crystals except for the apex	Liu et al., 2019

The genus-level phylogenetic analyses based on the ITS+28S dataset completed by Ordynets et al. (2018, 2020) showed that long-spored *Subulicystidium* were not closely related. *Subulicystidium perlongisporum* was most closely related to *S. robustius* K.H. Larss. & Ordynets and *S. rarocrystallinum* Ordynets & K.H. Larss., as well as to *S. boidinii* Ordynets, Striegel & Langer, *S. harpagum* Ordynets, Striegel & K.H. Larss., *S. parvisporum* Ordynets & Langer and *S. tropicum* S.H. He & S.L. Liu. Ordynets et al. (2020) consider that the presence of long basidiospores in different phylogenetic lines results from homoplasy within this genus.

As of the date, seven long-spored species have been described relying on the morphological and molecular data (*S. acerosum* S.H. He & S.L. Liu, *S. cochleum* Punugu, *S. curvisporum* Gorjón, Gresl. & Rajchenb., *S. daii* S.L. Liu & L.W. Zhou, *S. longisporum* (Pat.) Parmasto, *S. perlongisporum* and *S. tropicum*) (Parmasto, 1968; Boidin, Gilles, 1988; Gorjón et al., 2011; Ordynets et al., 2018, 2020; Liu et al., 2019, 2022). The major morphological feature that enables distinguishing these species is the shape and dimensions of basidiospores, and to the lesser extent — their length-to-width ratio (Table 1). Moreover, Ordynets et al. (2020) confirmed the data provided by Boidin and Gilles (1988) and Duhem and Michel (2001), whereunder the examined specimens of the genus *Subulicystidium* showed that the spore length is more variable than the spore width and the length-to-width ratio.

The longest basidiospores (27–35 × 2.0–2.5 μm) among the species described to date are reported in *S. curvisporum* (Table 1). Additionally to the length of the spores, *S. curvisporum* differs from its closely related species, *S. longisporum* and *S. perlongisporum*, in unique spirally curved basidiospores (Gorjón et al., 2011). *Subulicystidium cochleum* has somewhat shorter spores (20–27 × 2–3 μm), than that in *S. curvisporum*, featuring acicular basidiospores with twisted and curved distal end (Punugu et al., 1980; Ordynets et al., 2020).

Subulicystidium perlongisporum resembles *S. acerosum*, *S. longisporum* and *S. tropicum* by sharing the narrow basidiospores, but differs from *S. acerosum* in lacking the needle-like crystals on the cystidium body, from *S. longisporum* and *S. tropicum* it differs in having longer basidiospores (Maekawa, 1994; Liu et al., 2019; Bernicchia, Gorjón, 2010; Ordynets et al., 2020). *Subulicystidium tropicum* is also similar to *S. longisporum*, but differs in having slender basidiospores and a tropical distribution (Liu et al., 2019).

According to Liu et al. (2022), *S. daii* resembles *S. cochleum* and *S. perlongisporum* by the long and straight or slightly curved basidiospores; however, *S.*

cochleum differs in the presence of a bundle of needle-like crystals at the cystidial crystalline sheath ends, while *S. perlongisporum* differs in its narrower basidiospores. Also, *S. daii* is distinct from *S. acerosum* by the absence of needle-like crystals and by having wider basidiospores (Liu et al., 2019).

Subulicystidium acerosum and *S. cochleum* differ from the other long-spored species by sharing cystidia that are sheathed by needle-like crystals in the middle part (Ordynets et al., 2020). This crystal arrangement is different from most species in *Subulicystidium* that have rectangular crystals arranged in longitudinal rows (Ordynets et al., 2018). *Subulicystidium cochleum* is distinguishable from *S. acerosum* in having larger basidiospores (Punugu et al., 1980; Ordynets et al., 2018; Liu et al., 2019).

The global distribution patterns of some newly described *Subulicystidium* species still remain unclear. *Subulicystidium acerosum*, *S. daii* and *S. tropicum* are known only from the type locality from China (Liu et al., 2019, 2022), while *S. curvisporum* is recorded only in the woods of the Patagonian Andes in Argentina (Gorjón et al., 2011). *Subulicystidium cochleum* is distributed in the tropical and subequatorial regions. It was found in the Hawaiian Islands, Jamaica, Madagascar, Santa Lucia and Costa Rica (Punugu et al., 1980; Kisimova-Horovitz et al., 1997; Gilbertson et al., 2002; Ordynets et al., 2020). The examinations of long-spored species of *Subulicystidium* performed by Ordynets et al. (2020) significantly enriched the knowledge about their geographical distribution. *Subulicystidium longisporum* and *S. perlongisporum* are known from all continents, while for *S. cochleum* its transoceanic distribution was confirmed (Ordynets et al., 2020).

Subulicystidium perlongisporum is a species widely distributed worldwide, being known from all the continents; despite that, it has never been recorded in Ukraine so far. Whereas this species is confined to forest ecosystems, and subject to significant forestation of Ukraine, new findings should be expected in other regions of the country.

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ETHICS DECLARATION

The authors declare no conflict of interest.

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***Subulicystidium perlongisporum* (*Trechisporales*), перша знахідка в Україні**

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Реферат. У статті наведені відомості про першу знахідку *Subulicystidium perlongisporum* — цікавого кортиціоїдного гриба, який раніше не реєстрували на території України. Подано детальний морфологічний опис та фото дослідженого зразка, а також особливості субстратної спеціалізації і загальне поширення цього виду у світі. Наведено критерії, які використовуються для розмежування видів роду *Subulicystidium* із довгими спорами.

Ключові слова: *Hydnodontaceae*, *Basidiomycota*, види з довгими спорами, кортиціоїдний гриб, прямокутні кристали, шилоподібні цистиди