



Original Scientific Paper

## Lichenicolous fungi on *Verrucaria* s. lat. in Ukraine with the description of *Zwackhiomyces khodosovtsevii* sp. nov. and a key to the lichenicolous fungi on *Verrucaria* s. lat.

Valerii V. DARMOSTUK

Kherson State University, 27, Universytetska str. 73000, Kherson, Ukraine  
Correspondence: [valeriidarmostuk@gmail.com](mailto:valeriidarmostuk@gmail.com)

### ABSTRACT:

A revision of lichenicolous fungi on *Verrucaria* s. lat. in Ukraine is provided. As a result, 12 species of lichenicolous fungi on *Verrucaria* s. lat. are reported from Ukraine. Among them, *Zwackhiomyces khodosovtsevii* on *Verrucaria* cf. *nigrescens* is described as new to science and *Lichenopeltella coppinsii* on *V. muralis*, *Stigidium marinum* on *V. mucosa* as well as *S. rivulorum* on *V. dolosa*, are newly reported to Ukraine. Ten species are reported from xerotic terrestrial habitats mainly from Southern Ukraine. Only *Stigidium marinum* and *S. rivulorum* were found in a marine and freshwater habitat respectively. *Toninia subfuscae* should be removed from the Ukrainian list of lichenicolous fungi due to misidentification. *Didymosphaeria geminella* is considered as a new synonym for *Polycoccum dzieduszyckii*. A worldwide key for lichenicolous fungi on *Verrucaria* s. lat. is provided.

### Keywords:

biodiversity, new species,  
*Lichenopeltella*, *Stigidium*,  
*Zwackhiomyces*

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## INTRODUCTION

Lichenicolous fungi are a highly specialized group of fungi growing exclusively on lichens (DIEDERICH *et al.* 2018). They are mostly restricted to a single host genus rather than a species. Therefore, the study of lichenicolous fungi in individual genera is an important approach which serves to reveal the total diversity of these fungi. Such research is usually focused on a single host genus (ZHURBENKO 2012; ZHURBENKO & PINO-BODAS 2017) or sometimes on a single host species (ORANGE 2002; KHODOSOVTSSEV *et al.* 2018). This allows us to establish the diversity of lichenicolous fungi in more detail and to describe new species.

*Verrucaria* Schrad. is a polyphyletic cosmopolitan lichen genus comprising at least 300 species (LÜCKING *et al.* 2016). Although mostly saxicolous, species of the genus can also be found on soil, lignum and bark. Recent molecular phylogenetic studies allow for the clarification of

the once broad concept of *Verrucaria* s. lat. and allow us to distinguish phylogenetically more homogenous genera such as *Bagliettoa*, *Hydropunctaria*, *Placopyrenium*, *Verruculopsis*, etc. (GUEIDAN *et al.* 2009).

The aim of this research is to provide a revision of lichenicolous fungi growing on *Verrucaria* s. lat. in Ukraine as well as to introduce the new species *Zwackhiomyces khodosovtsevii* growing on *Verrucaria* cf. *nigrescens*.

## MATERIAL AND METHODS

The specimens were examined by a  $\times 10$  lens in situ and by standard microscope techniques using LOMO microscopes Optica and MICROMED-2. The microscopical examinations were done in water, 10% KOH (K), and Lugol's iodine solution, directly (I) or after pretreatment with KOH (K/I), and Brilliant Cresyl Blue (BCr). The measurements were taken in water with an accuracy of 0.2  $\mu\text{m}$  for ascospores, asci, conidia, conidiogenous cells,

conidiophores, ascomata, and pycnidial wall cells, and 5  $\mu\text{m}$  for ascomata and pycnidia. The results are given as (min.)  $\bar{x}$ -SD -  $\bar{x}$ +SD (max.), where  $\bar{x}$  is the average and SD is the standard deviation. Photographs were taken with a Levenhuk C510 NG camera. All the examined specimens are deposited in the lichenological herbarium of Kherson State University (KHER), M.G. Kholodny Institute of Botany NAS of Ukraine (KW-L) and the author's herbarium (herb. VD).

## RESULTS

### *Endococcus rugulosus* Nyl. s.str.

*Endococcus rugulosus* was described by NYLANDER (1855) on *Verrucaria macrostoma* s. lat. TRIEBEL (1989) also included specimens on several other host genera in her wide concept of the species, and finally, SÈRUSIAUX *et al.* (1999) clarified that the name *E. rugulosus* s. str. should be used only for specimens on hosts of the genus *Verrucaria*. *Endococcus rugulosus* s. str. is characterized by brown, distinctly verruculose ascospores  $10\text{--}12\text{--}(12.5) \times 5.5\text{--}7.5 \mu\text{m}$  as well as growth on *Verrucaria* spp. In Ukraine, this species was reported mostly on *Aspicilia* and *Circinaria* species (DARMOSTUK & KHODOSOVTSSEV 2017) with only a few records on *Verrucaria nigrescens* (GAVRYLENKO & KHODOSOVTSSEV 2009).

**Specimens examined: Ukraine, Kherson region**, Beryslav's'kyi district, near Burhunka village, N 46.79179°, E 33.23083°, on *Verrucaria nigrescens*, on limestone, 21 August 1994, A. Khodosovtsev (KHER 804); *ibidem*, 18 July 2008, A. Khodosovtsev & L. Gavrylenko (KHER 7689, 11785).

### *Lichenopeltella coppinsii* Earl.-Benn. & D. Hawksw.

Ascomata catathecia, superficial, black, orbicular,  $(80\text{--}95\text{--}120\text{--}140) \mu\text{m}$  diam. ( $n=10$ ), upper plate composed of dark reddish-brown quadrangular cells, lower plate composed of paler elongated cells, ostiole without setae, interascal filaments not seen, asci fisitunicate, subclavate, 8-spored,  $(37.6\text{--}43.6\text{--}52.0\text{--}54.6) \times (12.8\text{--}13.6\text{--}14.6\text{--}15.0) \mu\text{m}$  ( $n=15$ ), ascospores 1-septate, hyaline, ellipsoid with rounded apices, not constricted at the septum, with 2–4 flexuose setulae arising from the central part of the ascospores, up to 18  $\mu\text{m}$  long,  $(11.6\text{--}12.8\text{--}14.8\text{--}15.2) \times (4.4\text{--}4.6\text{--}5.0\text{--}5.2) \mu\text{m}$  ( $n=25$ ), length/width ratio  $(2.5\text{--}2.7\text{--}3.1\text{--}3.2)$ .

The material examined has slightly narrower asci than given in the protologue [ $(12.8\text{--}13.6\text{--}14.6\text{--}15.0) \mu\text{m}$  vs  $14.5\text{--}16 \mu\text{m}$  in EARLAND-BENNETT & HAWKSWORTH (1999)], also we did not find 0–3-septate ascospores and setulae arising from the apex. Until now *Lichenopeltella coppinsii* was known only from a few localities in Estonia, Germany and Great Britain (EARLAND-BENNETT & HAWKSWORTH 1999; TRIEBEL & SCHOLZ 2001; SUIJA *et al.* 2006). In our recent research this species was recorded as "*Stigmidium clauzadei*" (KHODOSOVTSSEV & DARMOSTUK 2016). This species is newly reported to Ukraine.

**Specimen examined: Ukraine, Kherson region**, Bilozers'kyi district, near Fedorivka village, N 46.80685°, E 32.79278°, on *Verrucaria muralis*, on limestone, 25 May 1995, A. Khodosovtsev (KHER 9511).

### *Lichenothelia renobalesiana* D. Hawksw. & V. Atienza

The material examined is characterized by sessile globose apothecoid ascomata with a central depression,  $(120\text{--}180\text{--}220\text{--}250) \mu\text{m}$  diam. ( $n=10$ ), 8-spored clavate asci and 1-septate, ellipsoid, hyaline to pale brown, constricted at the septum ascospore  $(21.0\text{--}21.6\text{--}23.2\text{--}24.0) \times (7.8\text{--}8.6\text{--}11.2\text{--}12.4) \mu\text{m}$  ( $n=25$ ), length/width ratio  $(1.7\text{--}2.0\text{--}2.6\text{--}2.9)$ , with a distinct gelatinous sheath up to 3  $\mu\text{m}$ . In the specimens studied, the ascospores were smaller than given in the protologue:  $(21.0\text{--}21.6\text{--}23.2\text{--}24.0) \times (7.8\text{--}8.6\text{--}11.2\text{--}12.4) \mu\text{m}$  vs  $25.5\text{--}28\text{--}30 \times 11.5\text{--}13.5\text{--}15 \mu\text{m}$  in ATIENZA & HAWKSWORTH (2008). However, we suggest that this is *Lichenothelia renobalesiana* due to the non-ostiolate broadly stipitate ascomata and ascospores with a distinct gelatinous sheath.

Our *Lichenothelia renobalesiana* is a rather widespread species in Europe and North America with a few records from Africa and Asia confined to the endolithic thalli of calcicolous species of Verrucariaceae (e.g. ATIENZA & HAWKSWORTH 2008; KOCOURKOVÁ & KNUDSEN 2009; URBANAVICHUS *et al.* 2011; KHODOSOVTSSEV & DARMOSTUK 2016). In Ukraine, it was reported in habitats with calcareous outcrops from a few administrative regions (KHODOSOVTSSEV & DARMOSTUK 2016; DARMOSTUK & KHODOSOVTSSEV 2020). Here, this species is reported for the first time to the Kherson region.

**Specimens examined: Ukraine, Autonomous Republic of Crimea**, Sudak district, near Sudak city, N 44.83727°, E 34.98430°, on *Verrucaria* sp., on limestone, 22 July 1999, A. Khodosovtsev (KHER 858); Mt Chatyrdag, N 44.73824°, E 34.29279°, alt. 900 m, on *Bagliettoa calciseda*, on limestone, A. Khodosovtsev (KHER 7006); **Chernivtsi region**, Kel'menets'kyi district, near Nahori-any village, N 48.54462°, E 26.66842°, on *Verrucaria nigrescens*, on argelites, 12 May 2018, V. Darmostuk & A. Khodosovtsev (KHER 12456, 12458); **Mykolaiv region**, Ochakiv's'kyi district, near Katalyno village, N 46.75793°, E 31.87410°, on *B. calciseda*, on limestone, 2 August 2018, V. Darmostuk (KHER 11669); **Kherson region**, Beryslav's'kyi district, near Chervonyi Maiak village, National Nature Park Kamyanska Sich, N 46.99056°, E 33.65238°, on *B. calciseda*, on limestone, 22 May 2020, A. Khodosovtsev (KHER 14584); Bilozers'kyi district, near Mykilske village, N 46.72405°, E 32.85845°, on *B. calciseda*, on limestone, 1 June 2017, V. Darmostuk & A. Khodosovtsev (KHER 14574).

### *Muellerella lichenicola* (Sommerf.) D. Hawksw.

*Muellerella lichenicola* is a common lichenicolous species which grows on a wide range of calcicolous lichens including *Verrucaria* spp.

**Specimens examined: Ukraine, Autonomous Republic of Crimea**, Leninskyi district, Chockrak, N 45.44049°, E 36.25260°, on *Verrucaria nigrescens*, on limestone, 9 July 1997, O. Redchenko (KW-L 66661); **Dnipropetrovsk region**, Kryvoriz'kyi district, near Chkalivka village, N 48.20595°, E 33.62972°, on *Verrucaria* sp., on limestone, 11 October 2008, A. Khodosovtsev, G. Naumovych, O. Smetana (KHER 9469).

***Opegrapha opaca* Nyl.**

Ascomata lirellate, black, unbranched, almost round to elliptical, (150–)200–280(–300)  $\mu\text{m}$  diam. (n=10), plane to slightly convex, aggregating to gall-like strongly convex clusters up to 1–1.2 mm diam. Exciple 20–25  $\mu\text{m}$  thick in the lateral part of the ascomata, dark brown in water, K–. Interascal filaments composed of thick-walled paraphyses 2–2.5  $\mu\text{m}$  diam., irregular and sparingly branched in the upper part. Asci 4–6-spored, clavate, (65.0–)70.0–83.4(–92.0)  $\times$  (12.6–)14.0–16.2(–18.8)  $\mu\text{m}$  (n=10). Ascospore 3-septate, hyaline to pale brown when mature, ellipsoid, with rounded ends, slightly constricted at the septa, smooth, with a distinct gelatinous sheath up to 2  $\mu\text{m}$  in young ascospores, (15.0–)15.4–17.4(–18.0)  $\times$  (4.6–)5.0–6.2(–7.0)  $\mu\text{m}$  (n=20) (only brown ascospores were measured), length/width ratio (2.2–)2.5–3.3(–3.5).

This overlooked and rarely reported species was recently lifted from the synonymy of *Opegrapha rupestris* Pers. (COPPINS *et al.* 2021). The examined specimens have wider ascospores than reported in COPPINS *et al.* (2021) [(4.6–)5.0–6.2(–7.0)  $\mu\text{m}$  vs (3.4–)3.8–4.8  $\mu\text{m}$ ], probably because they measured immature ascospores. The original description by NYLANDER (1853) also provides ascospores 7  $\mu\text{m}$  wide, while COPPINS *et al.* (2021) noted old brown ascospores up to 19  $\mu\text{m}$  long and 6  $\mu\text{m}$  wide. Therefore, any future examination of the specimens should measure mature brown and immature hyaline ascospores separately.

Up to now, *Opegrapha opaca* has been reported from Great Britain, France, Luxembourg, Spain and Israel, but it is probably more widely distributed in Europe (COPPINS *et al.* 2021). All Ukrainian specimens reported as *Opegrapha centrifuga* A. Massal. (GAVRYLENKO 2012; KHODOSOVTSEV *et al.* 2016) on *Verrucaria nigrescens* as well as *Opegrapha verrucariae* ined. (KONDRATYUK *et al.* 2014) proved to belong to *O. opaca*.

**Specimens examined** (all on *Verrucaria nigrescens*): **Ukraine, Kherson region**, Beryslav'skyi district, near Mykolaivka village, N 46.78703°, E 33.23808°, on limestone, 19 July 2008, A. Khodosovtsev & L. Gavrylenko (KHER 11783, 12189); Bilozers'kyi district, near Mykilske village, N 46.72405°, E 32.85745°, on limestone, 31 March 2017, A. Khodosovtsev & V. Darmostuk (KHER 11596); Velykooleksandriv'skyi district, near Zapovit village, N 47.09659°, E 32.96634°, on limestone, 2 May 2018, A. Khodosovtsev & V. Darmostuk (KHER 12678).

***Polycoccum marmoratum* (Kremp.) D. Hawksw.**

The examined specimens are characterized by semi-immersed to superficial globose ascomata, (160–)180–250(–290)  $\mu\text{m}$  diam. (n=10), 8-spored clavate asci and 1-septate verruculose dark brown ascospores with much larger upper cells, (23.0–)24.6–26.8(–27.8)  $\times$  (12.6–)13.2–14.6(–15.2)  $\mu\text{m}$  (n=25). Our specimens have slightly narrower ascospores than reported in HAWKSWORTH & DIEDERICH (1988) [(12.6–)13.2–14.6(–15.2)  $\mu\text{m}$  vs 14–18  $\mu\text{m}$ ].

*Polycoccum marmoratum* was reported from many European countries (HAWKSWORTH & DIEDERICH 1988), but it is a rarely collected species in Ukraine. Previously, it was reported from the Autonomous Republic of Crimea, Ternopil, and Kherson regions (KOPACHEVSKAYA 1986; SMERECHYNSKA 2006; GAVRYLENKO & KHODOSOVTSEV 2009). *Polycoccum marmoratum* reported by GAVRYLENKO & KHODOSOVTSEV (2009) from the Kherson region (KHER 7689) has to be removed from the regional list of lichenicolous fungi due to misidentification since the specimen belongs to *Endococcus rugulosus* s.str.

**Specimens examined: Ukraine, Autonomous Republic of Crimea**, Alushta district, Crimea Nature Reserve, N slope to the Alma river, N 44.71929°, E 34.24071°, on *Verrucaria* sp., on limestone, 29 July 1957, E. Kopachevskaya (KW-L 11575); Chatyr-Dag, N 44.75315°, E 34.29255°, on *Verrucaria* sp., on limestone, 20 July 1973, E. Kopachevskaya (KW-L 11575); near Alushta city, N 44.66708°, E 34.36725°, on *Verrucaria* sp., on limestone, 18 June 1978, I. Navrotska (KW-L 39803), Feodosia district, Karadag Nature Reserve, Suru-Kaya Mts. N 44.94491°, E 35.21717°, on *Verrucaria* sp., on limestone, 12 October 2001, A. Khodosovtsev (KHER 1658); Sudak district, near Soniachna Dolyna village, on *Verrucaria* sp., on limestone, 16 May 1960, A. Oxner & O. Blum (KW-L 38090); Yalta district, Ai-Petri Mts., N 44.44334°, E 34.05831°, on *Verrucaria* sp., on limestone, 18 June 1978, I. Navrotska (KW-L 31776); **Ternopil region**, Husyatyn'skyi district, near Krasne village, Medobory Nature Reserve, N 49.29103°, E 26.16516°, on *Verrucaria nigrescens*, on limestone, 10 July 2003, O. Smerechynska (KW-L 62441); Kremenets'kyi district, near Kremenets city, Divochy Stone, N 50.11841°, E 25.72710°, on *V. nigrescens*, on limestone, 21 July 2004, O. Smerechynska (KW-L 31773).

***Stigmatidium clauzadei* Cl. Roux & Nav.-Ros.**

The Ukrainian specimen fits the protologue of *Stigmatidium clauzadei* well (ROUX & NAVARRO-ROSINÉS 1994): it has globose semi-immersed ascomata 100–120  $\mu\text{m}$  diam., 'type b' pseudoparaphyses sensu ROUX & TRIEBEL (1994), 8-spored asci and 1-septate ellipsoid hyaline ascospores (13.4–)13.6–14.8(–15.0)  $\times$  (5.2–)5.4–6.0(–6.2)  $\mu\text{m}$  (n=25).

*Stigmatidium clauzadei* seems to be an overlooked species in Southern Ukraine. It was reported from the Kherson, Mykolaiv and Zaporizhzhia regions (KHODOSOVTSEV & DARMOSTUK 2016; KHODOSOVTSEV *et al.* 2019; DARMOSTUK & KHODOSOVTSEV 2020). *Stigmatidium clauzadei* reported by KHODOSOVTSEV & DARMOSTUK (2016)

from the Kherson region (KHER 9511) has to be removed from the regional list of lichenicolous fungi due to misidentification as the specimen belongs to *Lichenopeltella coppinsii*.

**Specimens examined** (all on *Verrucaria viridula*): **Ukraine, Mykolaiv region**, Novoodes'kyi district, near Mykhailivka village, N 47.39059°, E 31.62776°, on marl limestone, 26 May 2017, V. Darmostuk & A. Khodosovtsev (KHER 10799); **Zaporizhzhia region**, Melitopol's'kyi district, near Troitske village, Troitska ravine, N 47.06103°, E 35.43336°, on limestone, 7 July 2018, V. Darmostuk & A. Khodosovtsev (KHER 11964)

***Stigmatidium marinum*** (Deakin) Swinscow

Our specimen is characterized by semi-immersed, black, globose ascomata up to 120 µm diam., the absence of interascal filaments, 8-spored clavate asci (29.2–)34.8–38.0(–40.4) × (10.8–)12.2–13.4(–14.8) µm (n = 5), and 1-septate hyaline ellipsoid ascospores (9.8–)10.2–12.0(–12.8) × (4.2–)4.4–5.6(–6.0) µm (n = 15) without a distinct perispore. It fits the protologue well (SWINSCOW 1965). This fungus is not rare in the coastal zone of Europe and is known from several localities (SWINSCOW 1965; SCHIEFELBEIN *et al.* 2010). However, some authors suggest that it is a non-parasitic lichen (VAN DEN BOOM & APTROOT 1996; APTROOT *et al.* 2017). This species is newly reported to Ukraine.

**Specimen examined: Ukraine, Autonomous Republic of Crimea**, Yalta district, Martian Cape, N 44.51019°, E 34.25502°, on *Verrucaria mucosa*, on limestone, 2000, A. Khodosovtsev (KHER 849).

***Stigmatidium rivulorum*** (Kernst.) Cl. Roux & Nav.-Ros.

Our specimen matches the description provided by several authors (Zhurbenko & Hafellner 1999; Shivarov 2017) and is characterized by globose semi-immersed to superficial perithecia (40–)45–65(–85) µm diam. (n=10), 'type a' pseudoparaphyses sensu ROUX & TRIEBEL (1994), 8-spored clavate asci (28.2–)32.8–36.0(–38.4) × (13.8–)15.2–18.4(–20.2) µm (n = 10), and 1-septate hyaline ellipsoid ascospores (12.6–)13.2–14.0(–14.8) × (4.2–)4.6–6.0(–6.4) µm (n = 20) without a distinct perispore.

This species grows on freshwater Verrucariaceae in Europe (APTROOT *et al.* 1994; MOLITOR & DIEDERICH 1997; ZHURBENKO & HAFELLNER 1999; LUBEK & KUKWA 2017; SHIVAROV 2017; ROUX *et coll.* 2020). Our specimen was found on *Verrucaria dolosa* growing on limestone close to a waterfall. *Stigmatidium rivulorum* is newly reported to Ukraine and *V. dolosa* is a new host species.

**Specimen examined: Ukraine, Ternopil region**, Buchats'kyi district, Stinka village, N 48.91691°, E 25.23774°, on *Verrucaria dolosa*, on limestone, 10 May 2018, V. Darmostuk & A. Khodosovtsev (KHER 12584).

***Toninia subfuscae*** (Arnold) Timdal

This species was previously known in Ukraine from only one locality in the Odesa region (KHODOSOVTSEV *et al.*

2016). It is characterized by black, sessile apothecia, 0.2–0.3 mm in diam., disc plane to slightly convex, non-pruinose, epihymenium up to 10–15 µm thick, bluish to violet-blackish, K+ violetish, hypothecium reddish-brown, asci 8-spore, ascospores hyaline (1–2)-3-septate, ellipsoid to shortly bacilliform (14.6–)14.8–16.0(–16.2) × (3.0–)3.2–4.4(–4.6) µm (n=15). A detailed study of the specimen revealed a few pale grey squamules with one apothecium. Therefore, we suggest that this is *Toniniopsis aromatica* overgrowing on *Verrucaria nigrescens*. Although *Toninia subfuscae* is very similar to *T. aromatica*, it grows on *Lecanora* species (TIMDAL 1991). *Toninia subfuscae* has to be removed from the Ukrainian list of lichenicolous fungi.

**Specimen examined: Ukraine, Odesa region**, Lyman'skyi district, near Kairy village, N 46.92394°, E 30.98106°, on *Verrucaria nigrescens*, on limestone, 2 May 1994, A. Khodosovtsev (KHER 10147).

***Zwackhiomyces calcisedus*** Cl. Roux

This is a recently described species of an unidentified taxon of the family Verrucariaceae from France (ROUX *et coll.* 2020), also known on *Verrucaria nigrescens* from Ukraine (DARMOSTUK 2019). A detailed description of the Ukrainian specimen is provided in our previous work (DARMOSTUK 2019).

**Specimen examined: Ukraine**, Kherson region, Beryslavs'kyi district, near Burhunka village, N 46.80942°, E 33.21411°, on *Verrucaria nigrescens*, on limestone, 18 July 2008, A. Khodosovtsev & G. Naumovych (KHER 11784).

***Zwackhiomyces lithoicaea*** (B. de Lesd.) Hafellner & V. John

Vegetative hyphae indistinct. Ascomata perithecioid, scattered, globose to pyriform, black, sessile, (90–)125–140(–165) µm diam. (n=10); ascomatal wall pseudoparenchymatous, (15–)18–22(–25) µm wide (n=15); paraphysoids abundant, branched and anastomosing, up to 2.5 µm thick; asci fissitunicate, clavate, 4–6-spored, (45–)48–52(–54) × (14–)15–22(–25) µm (n=15); ascospores 1-septate, hyaline, slightly constricted at the septa, (16.8–)17.8–20.4(–24.6) × (5.6–)6.4–7.8(–8.7) µm, length/width ratio (2.2–)2.5–3.1(–3.3) (n=35).

This species is known from Europe and Asia (HAFELLNER & JOHN 2006; ERTZ *et al.* 2008). In Ukraine, *Z. lithoicaea* was previously reported from only the Kherson and Zaporizhzhia regions (DARMOSTUK *et al.* 2018; KHODOSOVTSEV & DARMOSTUK 2020). Here, it is reported as new to the Dnipropetrovsk and Mykolaiv regions.

**Specimens examined: Ukraine, Dnipropetrovsk region**, Nikopol's'kyi district, near Sholokhove village, N 47.67367°, E 34.03053°, on *Verrucaria* sp., on granite, 29 May 2009, I. Moysiyenko (KHER 12588); **Kherson region**, Beryslavs'kyi district, near Burhunka village, N 46.79318°, E 33.23031°, on *Verrucaria nigrescens*, on limestone, 19 July 2008, A. Khodosovtsev & G. Naumovych (KHER 7599); near Kachkarivka village, National Nature

Park Kamyanska Sich, N 47.08032°, E 33.72443°, on *V. nigrescens*, on limestone, 15 June 2020, A. Khodosovtsev (KHER 14091); Novovorontsovs'kyi district, near Osokorivka village, N 47.47337°, E 33.83982°, on *Verrucaria* sp., on limestone, 30 July 2017, V. Darmostuk & A. Khodosovtsev (KHER 10695); near Zolota Balka village, N 47.34448°, E 33.96809°, on *V. nigrescens*, on limestone, 3 June 2017, V. Darmostuk & A. Khodosovtsev (KHER 10816); **Mykolaiv region**, Domanivs'kyi district, National Nature Park Buzky Gard, Romanova Balka village, N 47.87867°, E 31.10866°, on *Verrucaria* sp., on granite, 5 July 2020, A. Khodosovtsev (KHER 14076); Snihurivs'kyi district, near Hrechanivka village, N 46.93715°, E 32.79675°, on *Verrucaria polysticta*, on limestone, 12 May 2018, V. Darmostuk & A. Khodosovtsev (KHER 12465); **Zaporizhia region**, Zaporiz'ka city, Khortytsa Island, N 47.8243°, E 35.0733°, on *Verrucaria fusconigrescens*, on granite, 29 June 2018, V. Darmostuk & A. Khodosovtsev (herb. VD 404).

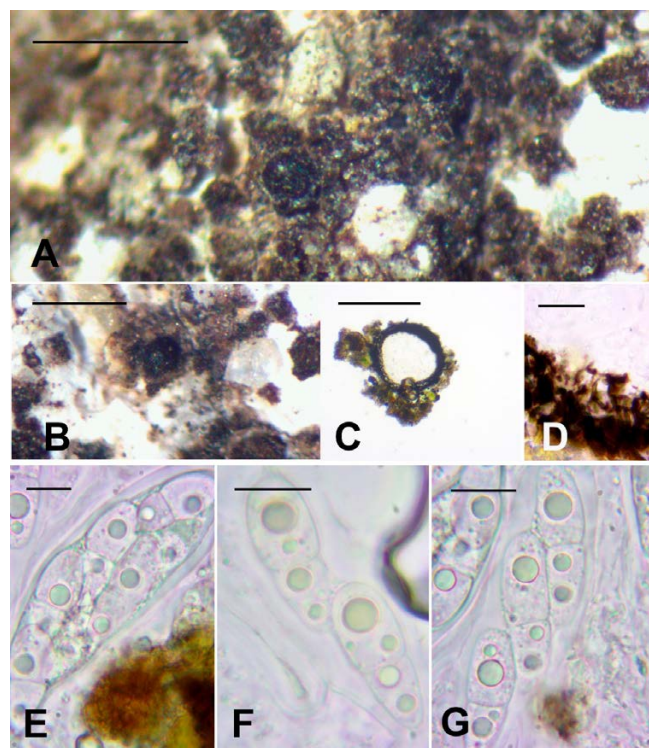
*Zwackhiomyces khodosovtsevii* Darmostuk sp. nov. (Fig. 1)

Mycobank MB840428

**Type:** Ukraine, **Kherson region**, Vysokopil's'kyi district, near Arkhangelske village, N 47.41255°, E 33.36254°, on *Verrucaria* cf. *nigrescens*, on limestone, 3 May 2018, V. Darmostuk and A. Khodosovtsev (KHER 11919 – holotype).

**Diagnosis.** Morphologically similar to *Zwackhiomyces lithoicae*, but differing in terms of larger ascomata (190–)205–220(–270)  $\mu\text{m}$  vs (90–)125–140(–165)  $\mu\text{m}$  diam., longer, (6–)8-spored asci, (65–)67–70(–74)  $\mu\text{m}$  vs (45–)48–52(–54)  $\mu\text{m}$  long, and larger ascospores (21.2–)25.4–29.6(–34.6)  $\times$  (8.6–)9.2–10.4(–11.6)  $\mu\text{m}$  vs (16.8–)17.8–20.4(–24.6)  $\times$  (5.6–)6.4–7.8(–8.7)  $\mu\text{m}$ .

**Description.** Vegetative hyphae not observed. Ascomata perithecioid, semi-immersed in initial states, superficial at maturity, scattered, black, subglobose, (190–)205–220(–270)  $\mu\text{m}$  diam. (n=15); ascomatal wall pseudoparenchymatous, dark brown in the outer part, brown in the middle, and hyaline in the inner part; (14–)16–18(–20)  $\mu\text{m}$  wide (n=20), with 5–7 layers of cells; cells rounded in the outer parts and  $\pm$  radially compressed in the inner part, (4.3–)6.8–7.8(–8.8) (n=25)  $\mu\text{m}$  wide; granular brown pigments extracellular, turning black-brown in K. Hymenial gel I–, K/I–. Paraphysoids abundant, branched and anastomosing, 1.5–2.5  $\mu\text{m}$  thick. Asci fissitunicate, clavate, (65–)67–70(–74)  $\times$  (18.0–)19.2–20.4(–22.2)  $\mu\text{m}$  (n=15), endoascus I–, BCr–, containing (6–)8 biserially arranged ascospores. Ascospores ellipsoid, 1-septate, hyaline, smooth to slightly verrucose, markedly constricted at the septum, (21.2–)25.4–29.6(–34.6)  $\times$  (8.6–)9.2–10.4(–11.6)  $\mu\text{m}$  (n=35), upper cell  $\pm$  rounded, lower cell narrower than the upper one and slightly attenuated, both cells with a few oil droplets; ascospore length/width ratio (1.9–)2.4–3.0(–3.6) (n=35), with a distinct halo 2.5–3.0  $\mu\text{m}$  thick in K. Conidiomata not observed.



**Fig. 1.** *Zwackhiomyces khodosovtsevii* (all from the holotype): A, B – superficial ascomata on the thallus of *Verrucaria* cf. *nigrescens*, C – cross-section of an ascomate, D – cross-section of the ascomatal wall, E – ascus with ascospores, F, G – ascospores. Scale bars: A, B – 0.5 mm, C – 200  $\mu\text{m}$ , D, E, F, G – 10  $\mu\text{m}$ .

**Host, ecology and distribution.** The new species is known from two localities in the Kherson region. It grows on *Verrucaria* cf. *nigrescens* on limestone pebbles in dry steppe habitats.

**Etymology.** The epithet honours Ukrainian lichenologist Prof. Alexander Khodosovtsev on the occasion of his 50<sup>th</sup> birthday.

**Notes.** Three *Zwackhiomyces* species were reported on Verrucariaceae (NAVARRO-ROSINÉS 1992; DIEDERICH *et al.* 2018). They are *Zwackhiomyces calcisedus*, *Z. lecanorae* and *Z. lithoicae*. *Zwackhiomyces lecanorae*, growing on *Lecanora* s. lat., was also reported from *Bagliettoa calciseda* and *B. parmigera* (ROUX 1978; NAVARRO-ROSINÉS 1992), while HOFFMANN & HAFELLNER (2000) state that records of the species on hosts other than *Lecanora* s. lat. are dubious. ROUX *et coll.* (2020) state that the record of ROUX (1978) probably belongs to *Z. calcisedus*. It differs from the new species as well as from other *Zwackhiomyces* species in terms of non-septate ascospores (vs 1-septate in *Zwackhiomyces khodosovtsevii*). The morphological differences between the new species, *Zwackhiomyces lithoicae* and *Z. calcisedus*, are presented in Table 1. *Zwackhiomyces khodosovtsevii* was published as “*Zwackhiomyces* sp.” in our previous research (DARMOSTUK 2019).

Several species of the genus have a similar combination of ascomata and ascospore sizes. Despite its similarities

**Table 1.** Key features of lichenicolous fungi growing on Verrucariaceae

	<i>Zwackhiomyces calcisedus</i>	<i>Zwackhiomyces lithoicaea</i>	<i>Zwackhiomyces khodosovtsevii</i>
<b>Ascomata diam.</b>	60–130	100–140	(190–)205–220(–270) µm
<b>Asci</b>	4–6-spored	8-spored	(6–)8-spored
<b>Asci length</b>	*(36–)38–42 (–46) µm	45–65 µm	(65–)67–70(–74) µm
<b>Asci width</b>	*(15–)17–20(–22) µm	13–15 µm	(18.0–)19.2–20.4(–22.2) µm
<b>Ascospore length</b>	(13.0–)14.6–17.0(–18.0) µm	15–20 µm	(21.2–)25.4–29.6(–34.6) µm
<b>Ascospore width</b>	(7.0–)7.7–8.5(–9.5) µm	5–6.5 µm	(8.6–)9.2–10.4(–11.6) µm
<b>References</b>	ROUX <i>et coll.</i> 2014; *DARMOSTUK 2019	GRUBE & HAFELLNER 1990	This paper

with the new species, id *Zwackhiomyces arenicola* R.C.Harris, which was described on an unidentified lichen on sandstone from the United States, it has bigger 8-spored asci 110–135 × 30–45 µm [vs (6–)8-spored asci (65–)67–70(–74) µm in *Z. khodosovtsevii*] and 1–3-septate pale brown mature ascospores (vs 1-septate hyaline ones in *Z. khodosovtsevii*) (HARRIS 1995). *Zwackhiomyces arenicola* was not included on the last worldwide check-list of lichenicolous fungi (DIEDERICH *et al.* 2018) and it probably represents another species that does not belong to *Zwackhiomyces*. *Zwackhiomyces aspiciliae* Halıcı & Candan can be distinguished by narrower asci and ascospores with twice as long and attenuated lower cells (HALICI & CANDAN 2009).

*Zwackhiomyces cervinae* is also similar to *Z. khodosovtsevii* in terms of the size of ascomata and ascospores with a distinct halo (CALATAYUD *et al.* 2007). It can be distinguished from the new species by longer 8-spored asci (90–110 µm vs (6–)8-spored asci (65–)67–70(–74) µm in *Z. khodosovtsevii*), thick interascal filaments (1.5–3.5 µm vs up to 1.5 µm in *Z. khodosovtsevii*) and pale brown mature ascospores (vs hyaline in *Z. khodosovtsevii*).

**Additional examined specimen (paratype): Ukraine**, Velykooleksandrivs'kyi district, near Zapovit village, N 47.09659°, E 32.96634°, on *Verrucaria* sp., on limestone, 2 May 2018, A. Khodosovtsev & V. Darmostuk (KHER 12675).

#### An additional nomenclature notes

*Didymosphaeria geminella* Lettau is characterized by immersed globose ascomata 100–150 µm, abundantly branched paraphyses, 2-spored asci and ellipsoid 1-septate brown ascospores 30–35 × 10–12 µm and growing on *Verrucaria viridula* (LETTAU 1958). The author discussed that *D. geminella* is similar to *Microthelia dzieduszyckii* Boberski, but differs in abundant paraphyses [vs no paraphyses in the protologue of *M. dzieduszyckii* (BOBERSKI 1887)]. Later Hawksworth transferred *M. dzieduszyckii* to the genus *Polycoccum* and updated the description adding information about the paraphyses (HAWKSWORTH *et al.* 1980; HAWKSWORTH & DIEDERICH 1988). According to this data we consider that *Didymosphaeria geminella* is a new synonym of *Polycoccum dzieduszyckii*.

*Polycoccum dzieduszyckii* (Boberski) D. Hawksw., in Hawksworth, James & Coppins, Lichenologist 12(1): 107 (1980)

= *Didymosphaeria geminella* Lettau, Feddes Repert. Spec. Nov. Regni veg. 61(2): 161 (1958) syn. nov.

Type: Bayern: Mühlthal bei München, auf dürrftigem Lager der *Verrucaria muralis*, leg. Schaffert (B?).

#### CONCLUSIONS

Forty-one specimens of lichenicolous fungi on *Verrucaria* s. lat. were revised. They are represented by 12 species. Among them, *Zwackhiomyces khodosovtsevii* on *Verrucaria* cf. *nigrescens* is described as new to science and *Lichenopeltella coppinsii* on *V. muralis*, *Stigmidium marinum* on *V. mucosa* as well as *S. rivulorum* on *V. dolosa*, are newly reported to Ukraine. Ten species are reported from xerotic terrestrial habitats mainly from Southern Ukraine. Only *Stigmidium marinum* and *S. rivulorum* were found in a marine and freshwater habitat respectively. *Toninia subfuscae* has to be removed from the Ukrainian list of lichenicolous fungi due to misidentification.

Up to now, 37 lichenicolous fungi are known to grow on *Verrucaria* s. str. worldwide (Supplementary Material 1). Despite the commonness of the host, the total number of lichenicolous species growing on *Verrucaria* s. str. is lower than other common genera such as *Cladonia* (138 species) (ZHURBENKO & PINO-BODAS 2017) or *Peltigera* (87 species) (HAWKSWORTH & MIADLIKOWSKA 1997; DIEDERICH *et al.* 2018). It is interesting to note that such species-poor genera like *Cetraria* (31 species, SUIJA *et al.* 2020) or *Xanthoria* (42 species, BRAUN *et al.* 2016; TSURYKAU & ETAYO 2017) are inhabited by comparably more lichenicolous fungi than the species-rich genus *Verrucaria*. It is possible that relatively fast growing foliose and fruticose taxa are easier to infect by lichenicolous fungi than slow growing saxicolous taxa.

Twenty-six species (70.2%) have a strict host preference and were found exclusively on *Verrucaria*. Among *Verrucaria*-dwelling species, only *Graphium samogiticum* is an anamorphic fungus. Teleomorphic species are represented mostly by species with perithecioid ascomata (27

species or 72.9%) whereas 10 species (27.0%) have apothecoid ascomata.

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**REZIME**

**Botanica**  
 SERBICA

## Lišajne gljive na *Verrucaria* s. lat. u Ukrajini sa opisom *Zwackhiomyces khodosovtsevii* sp. nov. i ključem za lišajne gljive na *Verrucaria* s. lat.

Valerii V. DARMOSTUK

Prikazana je revizija lišajnih gljiva na *Verrucaria* s. lat. u Ukrajini. Kao rezultat, zabeleženo je 12 vrsta lišajnih gljiva na *Verrucaria* s. lat. Među njima, *Zwackhiomyces khodosovtsevii* na *Verrucaria* cf. *nigrescens* je opisana kao nova za nauku i *Lichenopeltella coppinsii* na *V. muralis*, *Stigmidium marinum* na *V. mucosa*, kao i *S. rivulorum* na *V. dolosa*, su novoopisane za Ukrajinu. Deset vrsta je zabeleženo na kseričnim staništima, uglavnom iz južne Ukrajine. Samo su *Stigmidium marinum* i *S. rivulorum* nađene u morskim i slatkovodnim staništima, respektivno. *Toninia subfuscae* bi trebalo ukloniti sa liste lišajnih gljiva Ukrajine usled pogrešne identifikacije. *Didymosphaeria geminella* se smatra novim sinonimom za *Polycoccum dzieduszyckii*. Prikazan je globalni ključ za lišajne gljive na *Verrucaria* s. lat.

**Ključne reči:** biodiverzitet, nova vrsta, *Lichenopeltella*, *Stigmidium*, *Zwackhiomyces*

