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Didymocyrtis trassii sp. nov. and other lichenicolous fungi on *Cetraria aculeata*

Alexander KHODOSOVTSEV, Valeriy DARMOSTUK, Ave SUIJA and Alexander ORDYNETS

Abstract: Recently, nine species of lichenicolous fungi were found growing on *Cetraria aculeata* (*Parmeliaceae*) in a sand dune system in the Ukraine. One of them, *Didymocyrtis trassii*, is described here as new to science. This species is similar to *D. pseudeverniae* but differs in having smaller pycnidia, smaller obpyriform to clavate conidia as well as its DNA sequence. The new monotypic lichenicolous genus *Katherinomyces* is described here. *Acremonium lichenicola* s. l., *Eonema pyriforme*, *Didymocyrtis cladoniicola* and *Lichenoconium erodens* are reported for the first time on *Cetraria aculeata*. Furthermore, *E. pyriforme* is reported for the first time from lichen thalli. *Acremonium lichenicola*, *E. pyriforme* and *Taeniolella rolfii* are new for the mycobiota of the Ukraine. A key to the eleven known lichenicolous species on *Cetraria aculeata* is provided.

Key words: Ascomycota, Basidiomycota, coelomycetes, corticioid fungi, psammophytic communities, Ukraine

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Introduction

Cetraria aculeata (Schreb.) Fr. s. l. (including *C. steppae* (Savicz) Kärnef.) (*Parmeliaceae*, Lecanoromycetes) is a widespread, terricolous, fruticose lichen adapted to arctic, temperate and semi-arid conditions (Printzen *et al*. 2013). Its taxonomy, biogeography, phylogeny and symbiotic interactions were recently studied (e.g. Fernández-Mendoza *et al*. 2011; Printzen *et al*. 2012; Nadyeina *et al*. 2013; Lutsak *et al*. 2017). Despite the wide distribution of this bipolar lichen (Printzen *et al*. 2013), data regarding its associated fungi are limited. So far six lichenicolous species have been reported: *Clypeococcum cetrariae* Hafellner (Khodosovtsev & Darmostuk 2017), *Endococcus parmeliarum* Etayo (Brackel 2015), *Katherinomyces cetrariae* Khodos., *Sphaerellothecium aculeatae* Khodos. *et al*. (Khodosovtsev *et al*. 2016), *Lichenopeltella cetrariicola* (Nyl.) R. Sant. (Suija 2005; Brackel 2011; Kukwa *et al*. 2012) and *Taeniolella rolfii* Diederich & Zhurb. (Zhurbenko 2009).

In Southern Ukraine, *Cetraria aculeata* s. l. is one of the most frequent components of psammophytic lichen communities, which are indicators of deflation processes (Khodosovtsev *et al*. 2011). During fieldwork in a sand dune region in the Ukraine, we noted several types of damage (bleaching, turning reddish, etc.) or even death of thalli of *Cetraria aculeata* caused by various lichenicolous fungi. The spread of fungal infections was especially evident in wet seasons, while in dry seasons we did not observe any symptoms. We previously reported three species from Ukrainian sand dunes, viz. *Clypeococcum cetrariae*, *Katherinomyces cetrariae* and *Sphaerellothecium aculeatae* (Khodosovtsev *et al*. 2016; Khodosovtsev & Darmostuk 2017), two of which were described as new. Yet in different parts of the sand dunes of Lower Dnipro, an additional coelomycetous

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fungus with large hyaline aseptate conidia was collected that at first glance resembled *Didymocyrtis foliaceiphila* (Diederich *et al.*) Ertz & Diederich. Both the morphology and ITS sequences of this novel fungus were used to clarify its phylogeny and it is here described as a new species. We also describe as new the genus *Katherinomyces*, present additional records of lichenicolous species growing on *C. aculeata* found during the study and provide an identification key to 11 lichenicolous fungi known to grow on *Cetraria aculeata*.

Materials and Methods

Sampling and cultures

Ninety specimens of *Cetraria aculeata* were collected in different habitats in the Black Sea lowland (Ukraine). During collections taking place in the the Lower Dnepr sand dunes between 2015 and 2017, we found the largest concentration of lichenicolous fungi on *Cetraria aculeata*. 28 infected specimens were examined in detail using standard light microscopy techniques under LOMO microscopes MBS–1 and MICROMED–2. Microscopical examination was performed on material mounted in water; 10% KOH (K), Lugol's iodine (I) either without or after a KOH pretreatment (K/I), or Brilliant Cresyl blue (BCr). Measurements were made in water with a precision of 0·1 µm for microscopical structures and 5 µm for ascomata, basidiomata and pycnidia. Measurements are given as $(\min-)\bar{x}-\mathrm{SD}-\bar{x}+\mathrm{SD}(-\max)$, where $\bar{x}$ is the mean and SD is the standard deviation. Photographs were taken with a Levenhuk C510 NG camera. All specimens examined are deposited in the herbaria of Kherson State University, Ukraine (KHER) and the Natural History Museum of the University of Tartu (TU).

DNA extraction, amplification and sequencing

For the extraction of genomic DNA, three to five conidiomata per specimen of the unknown *Didymocyrtis* were removed from the host thallus and placed into a 1·5 ml test tube. Total DNA was extracted using the High Pure PCR Template Preparation Kit (Roche Applied Science®) following the protocol provided by the manufacturer. The internal transcribed spacer (ITS) region was amplified and sequenced using the primers ITS0F and LA-W (Tedersoo *et al.* 2008), and ITS4 and ITS5 (White *et al.* 1990). The PCR reaction mix consisted of 5 µl 5× HOT FIREPol® Blend Master Mix (Solis BioDyne, Tartu, Estonia), 0·5 µl of both primers (both 20 µM), 3 µl of target DNA and distilled water added to a total volume of 25 µl. The PCR cycle included 36 cycles and the annealing temperature was set at 57 °C.

PCR products were visualized on a 1% agarose gel stained with ethidium bromide. For the purification of PCR products, 1 µl of FastAP and 0·5 µl of Exonuclease I (Thermo Scientific, Waltham, MA, USA) were added to each tube and the tubes were then incubated at 37 °C for 45 min; the enzymes were deactivated by heating at 85 °C for 15 min. Both complementary strands were sequenced by Macrogen Inc. (Amsterdam, the Netherlands).

We used Sequencher 4.10.1. (GeneCodes Corp.®, Ann Arbor, MI, USA) to check, assemble and manually adjust the resulting sequence fragments. The consensus sequences were compared with those publicly available in NCBI (https://www.ncbi.nlm.nih.gov/genbank) using a BLAST search to confirm their identity. The newly generated sequences are accessible in NCBI under Accession numbers MG519610–MG519614 (Table 1).

Sequence analysis, alignment and phylogenetic reconstructions

We performed a nucleotide-to-nucleotide comparison and sequence-based clustering including INSD (International Nucleotide Sequence Databases), UNITE and environmental sequences using UNITE Phylogenetic Module massBLASTer in PlutoF workbench (Abarenkov *et al.* 2010) based on the species hypothesis (SH) concept (Kõljalg *et al.* 2013). To visualize the relationships, the five newly generated ITS sequences were aligned together with 62 *Didymocyrtis* sequences (Lawrey *et al.* 2012; Ertz *et al.* 2015) derived from the National Center for Biotechnology Information (NCBI) using MUSCLE (Edgar 2004). The sequences were later manually trimmed with SeaView 4.6 (Gouy *et al.* 2010) and thus the final alignment consisted of 574 sites, of which 70 were variable and 59 informative. The best-fit model of DNA evolution for the ITS data set, TrN + I + G, was chosen based on the Akaike Information Criterion (AIC; Akaike 1973) as implemented in jModelTest 2.0.2 (Posada 2008). Phylogenetic reconstruction on the resulting alignment was carried out using the Metropolis-coupled Markov chain Monte Carlo (MCMC) approach in MrBayes v.3.2.6. (Ronquist *et al.* 2012) at the CIPRES Science Gateway (Miller *et al.* 2010). Two parallel simultaneous runs, each using four independent chains and starting from a random tree, were performed over 10 000 000 generations; tree sampling was carried out every 1000th generation. The first 25% of saved data was discarded as burn-in and the 50% majority-rule consensus tree and posterior probabilities (PP) were calculated from the rest. We used Tracer v.1.6.0 (Rambaut *et al.* 2014) in order to test if the stationarity of log-likelihood values was reached to stable equilibrium. As an alternative, a maximum likelihood (ML) approach was applied to the same data using PhyML (Guindon *et al.* 2010) with the GTR evolutionary model chosen and bootstrap support (BS) calculated over 500 replicates. The phylogenetic tree was

TABLE 1. *GenBank Accession numbers, laboratory codes and voucher information (herbarium code, collectors and the collection date) for the newly generated sequences of* Didymocyrtis trassii. *The ITS barcoding sequence from the isotype is marked in bold.*

GenBank Acc. no.	Laboratory code	Voucher	Collector(s) and coll. no.	Collection date
MG519610	VO270	TU84809	*Khodosovtsev & Darmostuk*, ex KHER 10330	18/11/2016
MG519611	VO271	TU84808	*Khodosovtsev & Darmostuk*, ex KHER 9324	21/11/2015
MG519612	AB296	TU84812	*Darmostuk*, ex KHER 10763	25/06/2015
MG519613	AB297	TU84810	*Khodosovtsev*, ex KHER 10762	15/06/2015
MG519614	AB298	TU84813	*Khodosovtsev*, ex KHER 10765	28/04/2017

TABLE 2. *The results of sequence-based clustering and nucleotide-to-nucleotide comparison. Query is equivalent to the Laboratory code (see Table 1 for voucher information). The assignment to species hypothesis is indicated by reference sequence (Ref. seq.; indicated by NCBI code) and UNITE SH-code (Kõljalg* et al. *2013). The similarity percentage (Prcnt), number of mismatched nucleotide positions (MisM), and the lengths of the Query (Qend) and Reference sequences (Rend) are provided.*

Query	Ref. seq.	SH-code	Prcnt	MisM	Qend	Rend
AB296	KT383833	SH527899·07FU	98·95	6	572	573
AB297	KT383833	SH527899·07FU	98·95	6	572	573
AB298	KT383833	SH527899·07FU	98·95	6	572	573
VO271	KT383833	SH527899·07FU	98·78	7	572	573
VO270	KT383833	SH527899·07FU	98·95	6	572	573

visualized and edited using FigTree 1.4.2 (Rambaut 2014). We used ITSx (Bengtsson-Palme *et al.* 2013) to extract variable ITS1, ITS2 and conserved 5.8S subregions from full rDNA ITS sequences.

Results

The initial SH-clustering settled the newly-generated sequences into species hypothesis SH527899.07FU (with reference sequence KT383833 representing *Didymocyrtis pseudeverniae* (Etayo & Diederich) Ertz & Diederich) (https://unite.ut.ee/bl_forw_sh.php?sh_name=SH527899.07FU#fndtn-panel1). The nucleotide-to-nucleotide comparison showed sequence similarity of 98·78–98·95% with constant single nucleotide polymorphisms (SNP) in six positions (Table 2): position 180 (T–G) of ITS1, and positions 39 (T–C), 119 (G–T), 125 (T–C), 144 (indel: T) and 146 (C–T) of ITS2. The analysis of ITS sequences supports a close relationship with *D. pseudeverniae* (PP = 0·99, BS = 80; Fig. 2).

We found that both taxa have relatively long and multiguttulate conidia in contrast to other *Didymocyrtis* species, which have smaller conidia typically with 1–2 apical guttules (Ertz *et al.* 2015). For comparison we examined a single specimen of *D. pseudeverniae* (TU75667) and found that conidia, similar to those of specimens growing on *Cetraria aculeata*, are surrounded by a thin halo (*c.* 1 µm; observed in BCr). Besides the host choice (*Pseudevernia* vs. *Cetraria*), we found that pycnidia and conidia were smaller in specimens growing on *C. aculeata* compared to those of *D. pseudeverniae*: pycnidia are 60–110 µm vs. 130–170 µm (Etayo & Diederich 1996), conidia 12·0–20·5 × 4·2–8·5 µm vs. 14–26 × 6–9 µm (Etayo & Diederich 1996). *Didymocyrtis foliaceiphila* is sister to both (PP = 0·95, BS = 86; Fig. 2) but conidia of this species are biguttulate and much smaller, (5·0–)5·8–7·1(–7·5) × (2·0–)2·2–2·7(–3·0) µm (Diederich *et al.* 2007).

Thus, taking into account the differences in morphology and ITS sequences, as well as the specialization of the host lichen with a different ecology, we describe a new lichen-inhabiting species growing on *C. aculeata*.

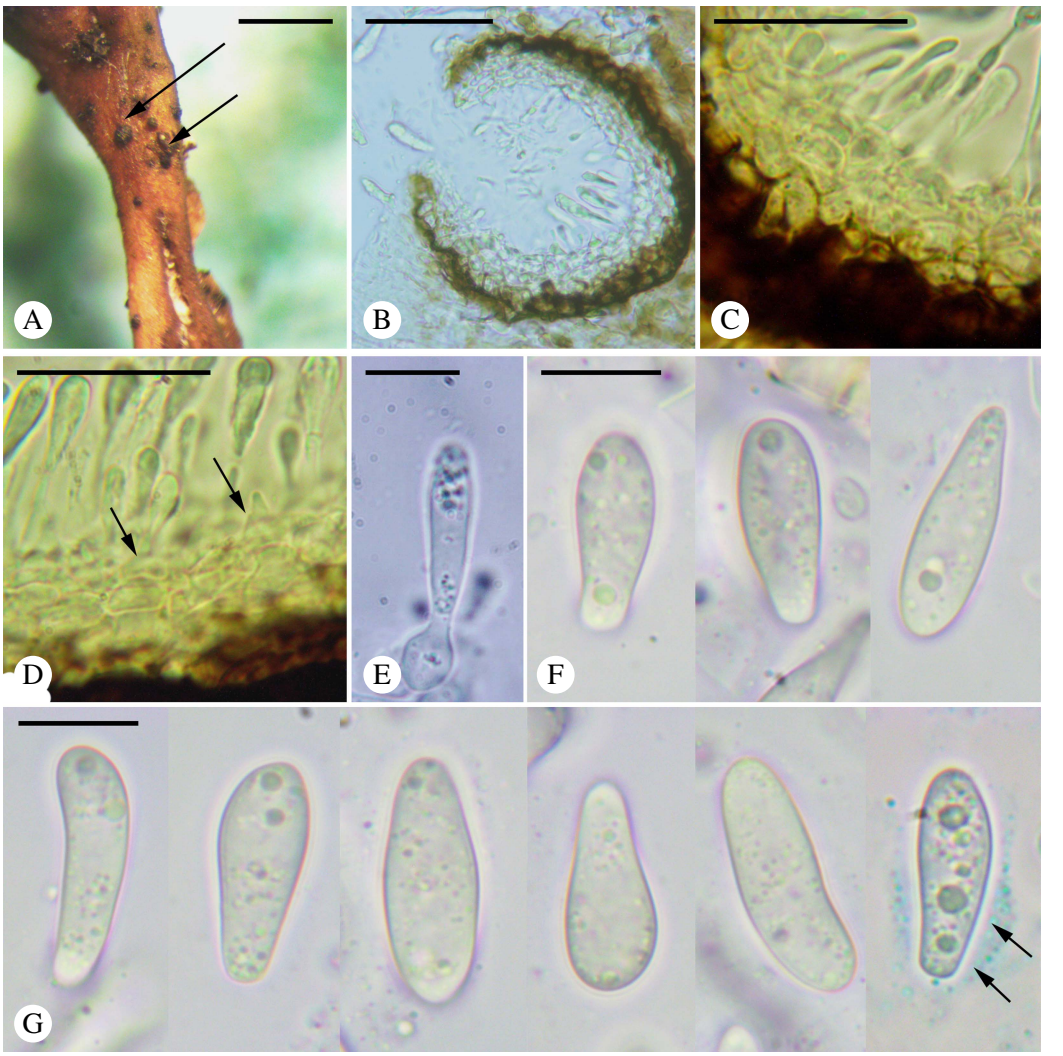

FIG. 1. *Didymocyrtis trassii* Suija, Darmostuk & Khodos. A, conidiomata (arrows) on the thallus of *Cetraria aculeata*; B, section through a conidioma; C, conidiomatal wall; D, conidiogenous cells (arrows); E, conidium with conidiogenous cell; F & G, conidia in water (last one in BCr with visible halo (arrows)). Scales: A = 1 mm; B = 50 µm; C & D = 25 µm; E–G = 10 µm. In colour online.

Taxonomy

Didymocyrtis trassii Suija, Darmostuk & Khodos. sp. nov.

MycoBank No.: MB 823931

Lichenicolous asexual fungus on *Cetraria aculeata*, similar to *Didymocyrtis pseudeverniae* in having large, obpyriform to clavate, multiguttulate and halonate conidia but differing by its smaller pycnidia, (60–)70–100 (–110) µm diam., and smaller conidia (12·0–)14·2–18·2 (–20·5) × (4·2–)5·0–7·2(–8·3) µm.

Type: Ukraine, Kherson Oblast, Goloprystansky District, way between villages Burkuty and Promin, 46° 21'53·1"N, 32°46'22"E, alt. 42 m, on *Cetraria aculeata*, in sand dunes, 21 November 2015, *A. Khodosovtsev* (KHER 9327—holotype; KHER 9326, 9325, TU84808—isotypes; ITS barcoding sequence Accession MG519611).

(Fig. 1)

Ascomata unknown. *Conidiomata* pycnidial, *Phoma*-type. *Vegetative hyphae* light

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brown, immersed in the host thallus, 1–2 µm thick.

Pycnidia scattered, semi-immersed, black, subglobose, ostiolate, (60–)70–100(–110) µm diam. ($n = 15$); wall 25–35 µm thick, composed of 3–4 external brownish layers and 2–3 internal hyaline layers; cells pseudoparenchymatous, thick-walled, (3·5–)3·5–5·5(–7·5) µm ($n = 20$); brown pigment turning olivaceous black in K. *Conidiophores* absent. *Conidiogenous cells* ampulliform, aseptate, hyaline, smooth-walled, (5·0–)6·4–9·2(–10·0) × (3·0–)3·5–5·5(–6·0) µm ($n = 20$). *Conidia* holoblastic, obpyriform to clavate, straight or slightly curved, usually narrow in the lower part, hyaline, smooth-walled, halonate, apex rounded, base rounded to abruptly truncate, multiguttulate, (12·0–)14·2–18·2(–20·5) × (4·2–)5·0–7·2(–8·3) µm ($n = 100$), halo up to 2 µm thick, length/width (l/w) ratio (1·8–)2·2–3·2(–3·9).

Etymology. The epithet honours the late Estonian lichenologist and botanist Hans-Voldemar Trass (2 May 1928–14 February 2017).

Host and pathogenicity. The fungus is known only on *Cetraria aculeata*. According to Ertz *et al.* (2015), the lichenicolous species of *Didymocyrtis* may be host-specific (e.g. *D. pseudeverniae*, *D. xanthomendozae*) or not (e.g. *D. consimilis*, *D. cladoniicola*, *D. foliaceiphila*, *D. melanelixiae*). The new species seems to belong to the former group. *Didymocyrtis trassii* usually grows on the lower branches of thick cushions of *C. aculeata* on sand and does not cause any visible damage to the host thallus.

Distribution. *Didymocyrtis trassii* is known only from sand dunes in Southern Ukraine.

Notes. Some basally abruptly truncate conidia of *D. trassii* resemble conidia of *Abrothallus*, but the conidiogenous cells in this genus show one to three annellations and a refractive periclinal rim at the base of the conidia (Hawksworth 1981).

Recently, Ertz *et al.* (2015) showed that some *Polycoccum* species represent the sexual stage of *Didymocyrtis*. There are two *Polycoccum* species described from Parmeliaceae, viz. *Polycoccum montis-wilhelmii* Diederich on *Hypotrachyna* (Aptroot *et al.* 1997) and *P. crespoae* Váczi & D. Hawksw. on *Chondropsis* (Váczi & Hawksworth 2001). Both species are known from the Southern Hemisphere and their asexual stages are not yet known.

Specimens examined (all on *Cetraria aculeata* on sand dunes). **Ukraine:** *Kherson Oblast*: Goloprystansky District, Chalbas'ka arena, village of Burkuty, 46°22'02·9"N, 32°46'29·7"E, alt. 26 m, 9 iv 2008, *A. Khodosovtsev* (KHER 8734); 46°23'38·6"N, 32°48'35·7"E, alt. 13 m, 18 xi 2016, *A. Khodosovtsev, V. Darmostuk* (KHER 10329, 10330, 10675; TU84809); *ibid.*, 28 iv 2017, *A. Khodosovtsev* (KHER 10765; TU84813); village of Gladkovka, 46° 25'14·3"N, 32°35'38·8"E, alt. 15 m, 15 vi 2017, *A. Khodosovtsev* (KHER 10672; TU84810); village of Ivanivka, Botanical Reserve "Khrestova Saga", 46°24'37·2"N, 32°04'59·9"E, alt. 11 m, 25 vi 2017, *V. Darmostuk* (KHER 10673; TU84812); Black Sea Biosphere Reserve, Solonoozerny, 46°27'33"N, 31°57'38"E, 6 v 2017, *A. Khodosovtsev*, *V. Darmostuk* (KHER 10674; TU84811); Oleshkivsky District, village of Radensk, 46°20'56·2"N, 32° 34'19·5"E, alt. 22 m, 1 xi 2015, *A. Khodosovtsev* (KHER 8734); Kozachelagerska arena, 46°37'04·7"N, 32°58'12·4"E, alt. 23 m, pine forest, 5 iv 2008, *A. Khodosovtsev* (KHER 3665).

Specimen of Didymocyrtis pseudeverniae *examined*. **Norway:** *Nord-Trøndelag County*: Steinkjer municipality, Mokk, 64°02'27.6"N, 12°29'51.7"E, alt. 354 m., on *Pseudevernia furfuracea*, 4 viii 2015, *A. Suija* (TU75667).

Other lichenicolous species on *Cetraria aculeata* collected in sand dunes in Southern Ukraine

Acremonium lichenicola Gams s. lat.

Notes. Specimen KHER 10286 is characterized by conidiogenous cells 30–50 µm long, absent or very short unbranched conidiophores *c.* 2–5 µm long, and 0(–1) septate conidia (4·1–)5·1–7·3(–9·3) × (1·5–)1·8–2·8(–3·0) µm ($n = 40$). The relative frequency of 1-septate conidia was 6%. As lichenicolous species of *Acremonium* are in need of revision involving molecular data, we use the name *A. lichenicola* s. l. here. *Cetraria aculeata* is a new host for *A. lichenicola*.

Specimens examined. **Ukraine:** *Kherson Oblast*: Oleschkivsky District, landscape reserve "Sagi", 46° 37'04·03"N, 32°50'03·13"E, alt. 15 m, 5 x 2016, *A. Khodosovtsev, V. Darmostuk* (KHER 10286, 10288); village of Radensk, 46°33'57·1"N, 32°52'40.2"E, alt. 32 m, 20 v 2016, *A. Khodosovtsev* (KHER 9763).

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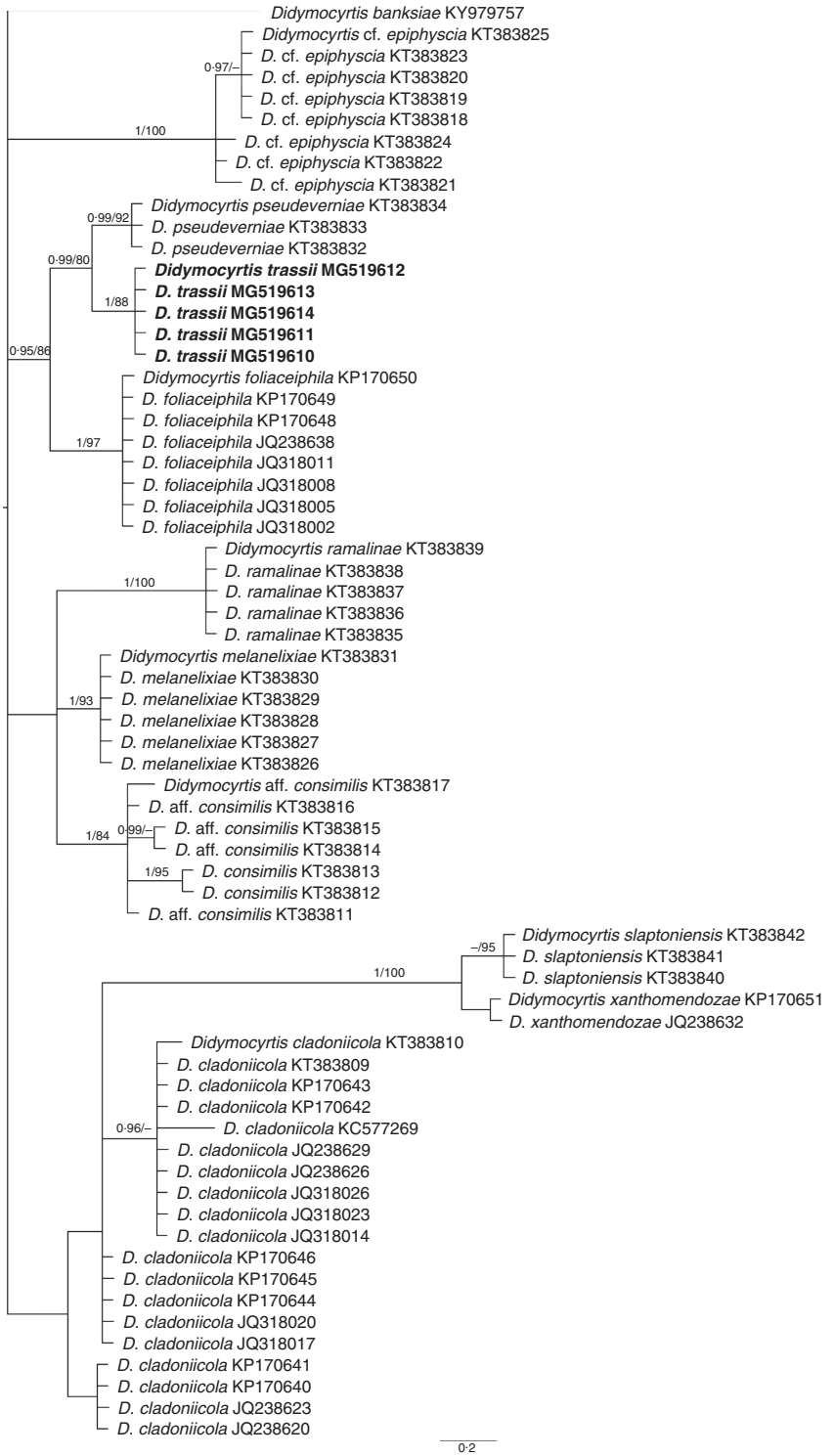

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Clypeococcum cetrariae Hafellner

Note. This species was recently collected on *Cetraria aculeata* in Lower Dnipro dunes (Khodosovtsev & Darmostuk 2017). The fungus is recognizable by its black necrotic spots covered by numerous pseudothecia.

Didymocyrtis cladoniicola (Diederich, Kocourk. & Etayo) Ertz & Diederich

(Fig. 3G & H)

Notes. The conidial size of our specimen KHER 10328 (Fig. 3) fits *D. cladoniicola*: (4·5–)4·9–5·7(–6·3) × (2·4–)2·6–3·2(–3·8) µm (n = 30), l/w ratio (1·4–)1·6–2·1(–2·3) on *Cetraria aculeata* vs. (3·8–)4·7–5·9(–7·3) × (2·0–)2·4–3·0(–3·5) µm, l/w ratio (1·4–)1·7–2·2(–2·8) in the original description on *Cladonia* species (Diederich *et al.* 2007). The species has been reported on *Flavoparmelia caperata*, *Parmelina tiliacea*, *Ramalina pollinaria*, *R. polymorpha* and *Squamarina cartilaginea* (Ertz *et al.* 2015). *Cetraria aculeata* is a new host for this species. The *C. aculeata* specimen (KHER 10328) infected by *D. cladoniicola* was found in a lichen community where the fungus was prevalent on *Cladonia rangiformis* and *C. foliacea.*

Specimens examined. **Ukraine:** *Kherson Oblast*: Goloprystansky District, Black Sea Reserve, Solonoozerny, 29 ii 2008, *O. Umanets* (KHER 8735); Oleshkivsky District, village of Radensk, 46°33'57·1"N, 32°52'40·2"E, alt. 32 m, 20 xi 2016, *V. Darmostuk* (KHER 10328).

Eonema pyriforme (M. P. Christ.) Redhead, Lücking & Lawrey

(Fig. 3A–F)

Notes. In addition to the basionym *Xenasma pyriforme* M. P. Christ., the species was known as *Athelia pyriformis* (M. P. Christ.) Jülich or *Athelidium pyriforme* (M. P. Christ.) Oberw. Larsson (2007) explained the problems of using any of these three names and suggested that an independent generic status be implemented for this resupinate member of *Agaricales*. This idea was eventually implemented by Lawrey *et al.* (2009) who introduced the generic name *Eonema* Redhead *et al.* We follow the latter nomenclatural concept in the present study.

In our specimen, basidiospores are slightly smaller, (5·3–)5·5–6·5(–7·0) × (3·3–)3·5–4·0 (–4·5) µm (n = 20), than in the material studied by other authors, cf. 7·0–8·5 × 3·5–5·0 µm in the type collection (Christiansen 1960), 7·0–9·5 × 3·6–5·5 µm (Jülich 1972) and 7–10 × 4–5 µm (Eriksson & Ryvarden 1973). However, all the measurements mentioned originate from Northern European collections (i.e. from a region much narrower than the distribution range of the species) and may not reflect the whole range of variability in the species. *Athelia phycophila* Jülich, a species believed to be lichenized or associated with mosses, has pyriform spores of a size that better matches our description (5·0–6·5 × 3·5–4·2 µm) but it has short clavate basidia (13–16 µm long) with short sterigmata (3·5–4·0 µm), typical in *Athelia*, and is known only from the type locality in Venezuela (Jülich 1972).

Eonema pyriforme has been reported from Belgium, the Czech Republic, Denmark, France, Germany, Italy, Portugal, the Netherlands, Norway, Russia (European part and Siberia), Spain, Sweden, Switzerland, the United Kingdom (Bernicchia & Gorjón 2010) and Canada (Lawrey *et al.* 2009) and is here reported from the Ukraine for the first time. The current distribution data may be incomplete due to the rarity on wood, a substratum usually inspected by corticiologists. Instead, *E. pyriforme* prefers forming fruit bodies on ferns, grasses and herbs (e.g. *Arenaria serpyllifolia*, *Poa annua*, *Pteridium aquilinum*) (Jülich 1972; Lawrey *et al.* 2009). We report *E. pyriforme* for the first time from lichen thalli. The exact nutrition mode of the species is

FIG. 2. Internal transcribed spacer (ITS)-based 50% majority-rule unrooted consensus tree based on a Bayesian approach and showing relationships between *Didymocyrtis trassii* and other *Didymocyrtis* species. The NCBI Accession numbers are given after each species, posterior probabilities (PP) ≥ 0·95 (before the slash) and Bootstrap support (BS) ≥ 75 (after the slash) are given on the branches.

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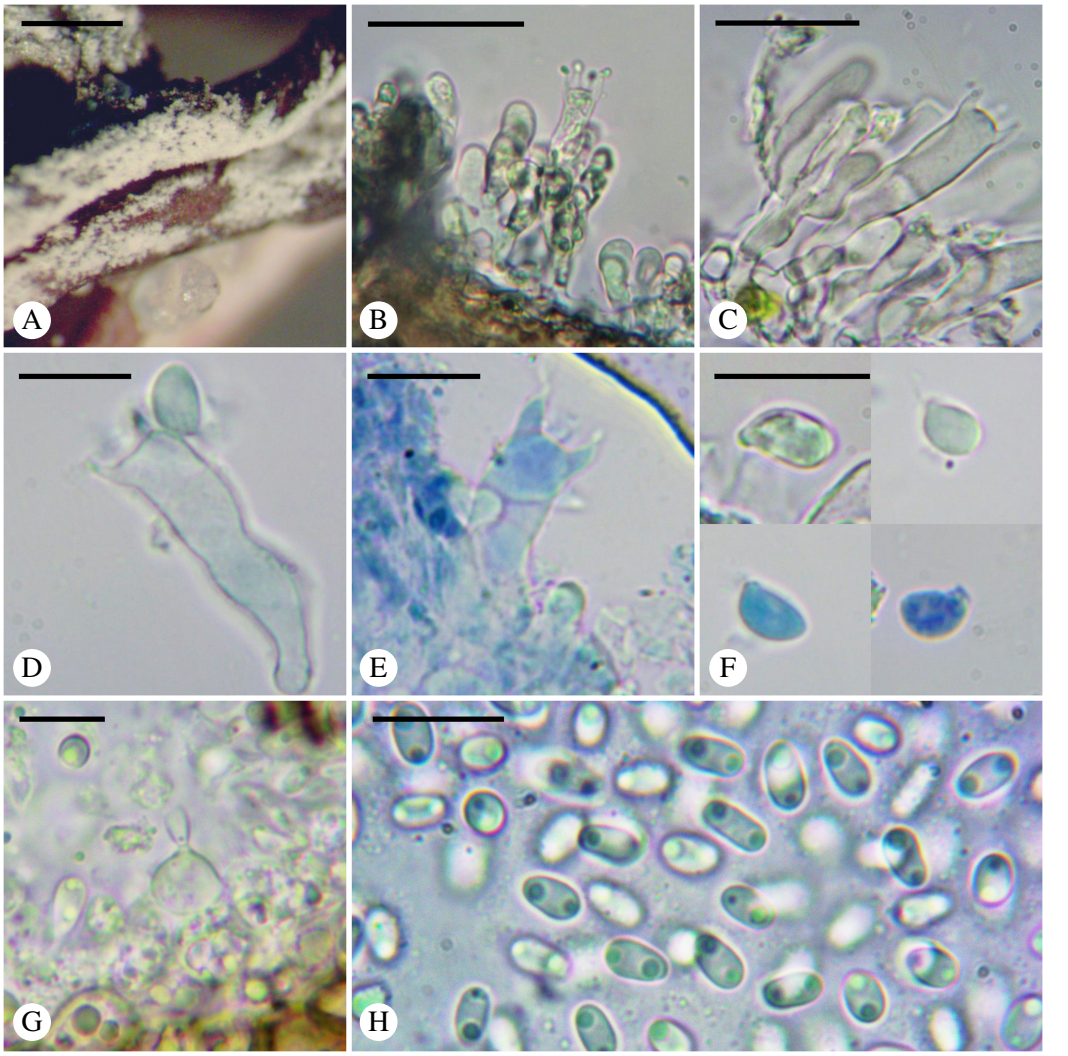

FIG. 3. A–F, *Eonema pyriforme* (KHER 9763); A, basidiocarp; B, section of basidiocarp (in water); C, hymenium and subhymenium (in water); D, basidium (in water); E, basidium (in cotton blue); F, basidiospores (in water and cotton blue). G & H, *Didymocyrtis cladoniicola* (KHER 8735): G, conidiogenous cell (in water); H, conidia (in water). Scales: A = 1 mm; B & C = 50 µm; D–F & H = 10 µm; G = 5 µm. In colour online.

still unclear but such a broad phylogenetic spectrum of substrata may suggest a saprotrophic lifestyle in forest debris and the use of various vertical supports for more efficient spore dispersal. *Eonema pyriforme* may therefore be considered a facultative lichenicolous fungus.

Specimen examined. **Ukraine:** *Kherson Oblast*: Oleshkivsky District, village of Burkuty, Lake Shelemetske, 46°33'57·1"N, 32°52'40·2"E, alt. 32 m, 20 v 2016, *A. Khodosovtsev* (KHER 9763).

Katherinomyces cetrariae Khodos. gen. et sp. nov.

MycoBank No.: MB 823932 (genus) and MB 823933 (species)

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(= *Katherinomyces cetrariae* Khodos. *nom. inval.* (Art. 40.1) in Khodosovtsev *et al.*, *Nova Hedwigia* **103:** 48 (2016)).

Lichenicolous fungus on *Cetraria aculeata*. Vegetative hyphae light brownish, immersed. Conidiomata stromatic with brownish walls. Conidiophores short, poorly developed, brown, 4–7 × 3–4 µm. Conidiogenous cells broadly ellipsoid, bacilliform or polygonal, brown, (5·7–)6·3–7·8 (–9·0) × (2·8–)3·3–5·1(–5·5) µm. Conidia irregular in shape, bacilliform, broadly ellipsoid, ovoid or rarely polygonal, holoblastic, aseptate, (4·3–)6·6–12·6(–16·3) × (2·8–)3·5–5·2(–6·0) µm.

Type: Ukraine, Kherson Oblast, Goloprystansky District, Chalbas'ka arena, village of Burkuty, alt. 26 m, 46° 22′02·9″N, 32°46′29·7″E, 9 April 2008, *A. Khodosovtsev* (KHER 5461—holotype).

Notes. The names *Katherinomyces* and *K. cetrariae* were invalidly published (Khodosovtsev *et al*. 2016) because the cited MycoBank identifier was that for the genus, while no identifier had been issued for the species (Art. 40.1). Here we validate the names following the new version of the *Melbourne Code* (McNeill *et al*. 2012). A detailed description and comparison of *Katherinomyces* with similar genera is provided in Khodosovtsev *et al*. (2016). The species is noticeable in the field owing to the reddish coloured branches of the host thallus.

Lichenoconium erodens M. S. Christ. & D. Hawksw.

Note. Lichenoconium erodens is one of the few lichenicolous species having a broad host spectrum (Hawksworth 1977). *Cetraria aculeata* is a new host for this species.

Specimen examined. **Ukraine:** *Mykolayiv Oblast*: Ochakovsky District, village of Pokrovka, Regional Landscape Park "Kinburns'ka Kosa", 46°28'48·4"N, 31° 39'55·9"E, alt. 2 m, 18 vii 2016, *V. Darmostuk* (KHER 10113).

Sphaerellothecium aculeatae Khodos., Klymenko & Gavrylenko

Note. This species was recently described on *C. aculeata* from Lower Dnipro dunes in the Ukraine (Khodosovtsev *et al*. 2016). It is more aggressive in wet seasons and causes bleaching and eventually death of the lichen thallus (Khodosovtsev *et al*. 2016).

Taeniolella rolfii Diederich & Zhurb.

Note. This species was first described on *Cetraria nigricans* from the Siberian Arctic (Diederich & Zhurbenko 1997) but is now known on different *Cetraria* species (including *C. aculeata*) from the British Isles, Canada, Finland, Greenland, Mongolia, Poland, Russia, Sweden and the USA (Diederich & Zhurbenko 2001; Hawksworth 2003; Zhurbenko 2009; Kukwa *et al*. 2010). The specimens studied form gall-like swellings on the tips of *C. aculeata* similar to lichen soralia. *Taeniolella rolfii* is reported here as new for the Ukraine.

Specimens examined. **Ukraine:** *Kherson Oblast*: Oleshkivsky District, near village of Luch, 46°22'41·7"N, 32° 47'10·3"E, alt. 35 m, 16 iv 2017, *A. Khodosovtsev* (KHER 10911); near village of Burkuty, 46°22'34·1"N, 32° 47'40·9"E, alt. 37 m, 25 iv 2017, *A. Khodosovtsev* (KHER 10912).

Key to the known lichenicolous fungi on *Cetraria aculeata*

1 Asci present 2
Asci absent 5

2(1) Ascomata catathecioid, ascospores hyaline with 3 pairs of setulae. ..... **Lichenopeltella cetrariicola**
Ascomata perithecioid, ascospores hyaline to brown when mature, without setulae ... 3

3(2) Ascomata with a dark clypeus ..... **Clypeococcum cetrariae**
Ascomata without a clypeus 4

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4(3) Net of vegetative brown hyphae present, ascospores hyaline in asci, 10·3–11·3 × 4·8–5·5 µm ..... **Sphaerellothecium aculeatae**
Net of vegetative brown hyphae absent, ascospores pale brown in asci, 7·0–9·5 × 5–6 µm ..... **Endococcus parmeliarum**

5(1) Basidiospores and basidia present, conidia absent ..... **Eonema pyriforme**
Basidiospores and basidia absent, conidia present 6

6(5) Conidia arising within pycnidioid conidiomata or stromata 7
Conidia not arising within pycnidioid conidiomata or stromata 10

7(6) Conidia hyaline 8
Conidia brown 9

8(7) Conidia multiguttulate, obpyriform to clavate, 12·0–20·5 × 4·2–8·3 µm ..... **Didymocyrtis trassii**
Conidia biguttulate, ellipsoid, 4·5–6·3 × 2·4–3·8 µm ..... **Didymocyrtis cladoniicola**

9(7) Conidia arising within pycnidial conidiomata, globose, verruculose, 2·0–3·5(–4·0) µm diam ..... **Lichenoconium erodens**
Conidia arising within pycnidia-like stromata, ornamented, bacilliform, ellipsoid or polygonal, 4·3–16·3 × 2·8–6·0 µm ..... **Katherinomyces cetrariae**

10 (6) Conidia colourless, smooth, 0(–1)-septate, 4·1–9·3 × 1·5–3·0 µm, not inducing galls ..... **Acremonium lichenicola** s.l.
Conidia brown, verrucose, 9·5–12·0 × 4–5 µm, (0–)1-septate, inducing soralium-like brownish galls ..... **Taeniolella rolfii**

Discussion

The widely distributed *Cetraria aculeata* has been the subject of extensive studies ranging from ecophysiology to phylogeography (review by Printzen *et al*. 2013). One further aspect for future studies is the severity and frequency of fungal infections of this lichen which can cover large areas of the landscape. We did not find any infected specimens of *C. aculeata* from carbonaceous substrata in the Black Sea region, while nine lichenicolous species were identified from Lower Dnipro sand dunes. It is possible that this observation reflects the small proportion of thalli in *C. aculeata* populations in petrophytic grasslands and its relatively large coverage (20–80%) of sand dunes. In total, eleven lichenicolous species are known to grow on *C. aculeata* (Suija 2005; Brackel 2011, 2015; Kukwa *et al*. 2012) and only two of these, *Endococcus parmeliarum* and *Lichenopeltella cetrariicola*, were not found in this study.

The severity of fungal infections is dependent on the infecting species but also on environmental (seasonal) conditions (Gilbert 1988; Beck *et al*. 2014). The most common species in the study area, *Sphaerellothecium*

aculeatae, is also the most aggressive, especially in wet seasons, and causes thallus bleaching and eventually death (Khodosovtsev *et al*. 2016). *Taeniolella rolfii*, growing on different *Cetraria* species (Diederich & Zhurbenko 1997), induces sporodochia on the tips of *C. aculeata* giving a sorediate appearance to the host thallus. *Clypeococcum cetrariae*, a rare species in the study area, forms blackish, necrotic spots on thallus branches. Infections by *Katherinomyces cetrariae* are recognizable by their reddish thallus branches. *Acremonium lichenicola*, *Didymocyrtis trassii* and *Eonema pyriforme*, which usually grow on the lower branches of thick cushions of *C. aculeata* over sand, do not cause visible damage to the lichen thallus. The lack of visible symptoms may be the reason why the newly described species *Didymocyrtis trassii* has remained unnoticed until recently.

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