Broad host range species in specialised pathogen groups should be treated with suspicion – a case study on Entyloma infecting Ranunculus

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INTRODUCTION

The smut fungi in a broad sense (Ustilaginomycotina) contain more than 1600 plant parasitic species in two major classes, the Ustilaginomycetes, the smut fungi in a strict sense and the Exobasidiomycetes, many of which do not cause typical smut symptoms with huge amounts of blackish spores being shed from sori in their host plants. Two more classes have been proposed recently (Wang et al. 2014), but as they might be embedded within the Exobasidiomycetes (Wang et al. 2015) or the sister group to the Ustilaginomycetes (Mishra et al. 2018), we do not treat them as separate classes here. Entyloma (Entylomatales, Exobasidiomycetes) is a species-rich genus with species that cause mostly inconspicuous, white to brown leaf spots. Entyloma currently comprises 172 species, restricted to dicotyledonous host plants belonging to 26 families (Vánky 2012, Denchev et al. 2013, Savchenko et al. 2013, 2014a, Rooney et al. 2017, Savchenko & Carris 2017). Because of their simple spore morphology, species delimitation in Entyloma is difficult (Savile 1947). A combination of spore morphology and host plant species is currently the most useful way to delineate species of Entyloma (Vánky 1994, 2012). Molecular phylogenetics has resolved species boundaries for many smut fungi (Vánky & Lutz 2007, Piątek et al. 2011, 2013, 2015a, b, 2016, Savchenko et al. 2013, 2014a, b, Vasighzadeh et al. 2014, Li et al. 2017, Kruse et al. 2018), including Entyloma (Begerow et al. 2002, Vánky & Lutz 2010, Savchenko et al. 2014a, Lutz & Piątek 2016). However, sequences of many Entyloma species are poorly represented in publicly available databases and many currently recognised species lack sequence data.

With about 600 species, Ranunculus is the largest genus of the family Ranunculaceae (Tamura 1995). Ranunculus species have a cosmopolitan distribution and mostly occur in temperate to arctic zones, where they grow in forests, meadows, peat bogs, on wet soils, as well as in lakes and rivers. Most species are herbaceous, some are annual, but the vast majority of species are perennial (Rastipishe et al. 2011). In the world monograph of smut fungi, Vánky (2012) recognised five different Entyloma species on Ranunculus s.lat., namely, E. ficariae, E. majewskii, E. microsporum, E. ranunculi-repentes, and E. verruculosum. Two species, Entyloma ficariae and E. majewskii, infect hosts in the genus Ficaria that is closely related to Ranunculus (Hörandi et al. 2005, Emadzade et al. 2010). Only three Entyloma species, E. microsporum, E. ranunculi-repentes, and E. verruculosum, were reported to infect species of the genus Ranunculus s.str. (Vánky 2012).
| Species         | Host          | Location details                                                                 |
|-----------------|---------------|----------------------------------------------------------------------------------|
| Entyloma bullosum | Ranunculus paludosus | Greece, Rhodes eastcoast, SE of Archangelos: c. 1.5 km S Stegna, Phrygana, northeast slope, N36°11'49" E28°08'06", elev. c. 70 m a.s.l. |
| E. eburneum | R. bulbosus | Germany, Baden-Württemberg Hegau, county Konstanz, NE of Neuhausen, near Schoren, dry grasland, MTB/Q: 8118/41, elev. c. 500 m a.s.l. |
| Entyloma bulbosus | R. bulbosus | Germany, Baden-Württemberg county Konstanz, peninsula Reichenau in the Undersea, E Oberzell, littoral, MTB/Q: 8320/2, elev. c. 400 m a.s.l. |
| Entyloma repens | R. repens | Germany, Baden-Württemberg county Sigmaringen, Leibertingen-Wildenstein, castle Wildenstein, N48°03'21" E09°00'0", MTB/Q: 7919/13, elev. c. 760 m a.s.l. |
| Entyloma repens | R. repens | Germany, Lower Saxony county Northeim, at the bottom of the Katlencastle, wayside near river, MTB/Q: 4326/21, elev. c. 110 m a.s.l. |
| Entyloma repens | R. repens | Germany, Hesse county Frankfurt at Main, Sachsenhausen, Landwehrstreet, South-Cementery, N50°05'20" E08°41'43", MTB/Q: 5918/11, elev. c. 150 m a.s.l. |
| Entyloma repens | R. repens | Germany, Saxony-Anhalt county Wittenberg, Zahna-Elster, E of Bad Zahna, Oßnitzbach, wet grasland, N51°55'24" E12°46'10", MTB/Q: 4042/41, elev. c. 100 m a.s.l. |
| Entyloma repens | R. repens | Germany, Bavaria Upper Bavaria, Chiemgauer Alps, county Rosenheim, Priener cabin, lift down towards Berg, Via Alpina, Firs forest, wayside, N47°41'41" E12°18'24", MTB/Q: 8339/22, elev. c. 1290 m a.s.l. |
| Entyloma repens | R. repens | Germany, Hesse county Rüsselsheim, county Groß-Gerau, Varkaustreet, forest cementery, wayside, N49°59'22" E08°26'10", MTB/Q: 6016/21, elev. c. 100 m a.s.l. |
| Entyloma repens | R. repens | Poland, Małopolska Province: near Bukowica Reserve, close to Wygiełzów, 10.09.2014 J. & M. Piątek 3653 KRAM F-59038 MH022814 – – |

Table 1: Stud specimens used for phylogenetic analysis.
Table 1 (cont.)

| Species | Host | Location | Date | Collector | GenBank no. |
|---------|------|----------|------|-----------|-------------|
| E. jolantae | R. oreophilus | Poland, Małopolska Province: Tatra Mts, Mała Dolinka valley – northern slopes | 25.08.2008 | J. & M. Piątek | KRAM F-59030, MF924688, MH022812, MF939250, MF939316 |
| E. kochmanii | R. lanuginosus | Italy, Liguria | Varavalley, c. 2 km NE of Caranza, Strada Provinciale from Caranza | 09.05.2016 | J. Kruse | GLM-F107660, MF924678, MH022802, MF939243, MF939309 |
| E. majewskii | Ficaria verna | Iran Tehran Prov., 60 km E Tehran, Mts Elburz, ‘Emamzadeh-Haskei’, | 17.05.1990 | D. Ershad, Efc34 | BRIP: HUV14888, MF924713, MH022837, MF939265, MF939331 |
| E. microsporum | R. repens | Germany, Lower Saxony county Hildesheim, Brüggen, Kirschweg, Sieben Bergen, Mt Hohe Tafel | 08.05.2011 | J. Kruse | GLM-F107667, MF924708, MH022832, MF939262, MF939328 |
| E. acris | R. repens | Germany, Bavaria Oberpfalz, national park Bavarian Wood, county Regen | Zwieseler Waldhaus, Mittelsteig cabin, mixed mountain-forest on granite | 24.08.2012 | J. Kruse | GLM-F107670, MF924712, MH022836 - – |
| E. acris | R. repens | Germany, Bavaria Oberfranken, between Horbach at the Steinach and Leutendorf, flood hollow | 10.05.2013 | J. Kruse | GLM-F107662, MF924705, MH022829, MF939261, MF939327 |
| R. repens | Germany, Bavaria Oberpfalz, national park Bavarian Wood, county Regen | Zwieseler Waldhaus, Watzlikhain, mixed mountain-forest on granite, MTB/Q: 6945/2, elev. c. 700 m a.s.l. | 24.08.2012 | J. Kruse | GLM-F107670, MF924712, MH022836 - – |
| R. repens | Germany, Bavaria Oberfranken, Bayreuth, Eremitage, W of river Red Main, mixed forest | 02.05.2013 | J. Kruse | GLM-F107671, MF924711, MH022835 - – |
| R. repens | Germany, Baden-Württemberg county Konstanz, Hegau, W of Singen, way up Mt Hohentwiel, wayside | 29.05.2013 | J. Kruse | GLM-F107672, MF924644, MH022764, MF939236, MF939320 |
| R. repens | Germany, Bavaria | county Rottal-Inn, Simbach, road St 2112, grasland at roundabout | 14.08.2014 | J. Kruse | GLM-F107661, MF924636, MH022760, MF939213, MF939282 |

Notes: ITS = internal transcribed spacer; atp2 = ATP synthase subunit 2; ssc1 = small subunit of the cytochrome c oxidase; map = nuclearlarge subunit of the cytochrome c oxidase.
### Table 1 (cont.)

| Species          | Host          | Location details                                                                 | Date       | Collector | DNA-ITS | Fungarium no. | GenBank no. |
|------------------|---------------|----------------------------------------------------------------------------------|------------|-----------|----------|---------------|-------------|
| **R. microsporum** | *R. repens*   | Austria, Upper Austria, Braunau am Inn, Hagenau in Inn County, Hagenauerstrasse,   | 18.08.2014 | J. Kruse  | 3036     | GLM-F10793   | MF909460    |
|                  |               | wayside, N48°16'23" E13°06'01", MTB/Q: 7744/2, elev. c. 340 m a.s.l.            |            |           |          |               |             |
| **R. repens**    | *R. repens*   | Germany, Baden-Württemberg, Gießen, Lahntal, county Gießen, c. 5.5 km SW of     | 21.05.2016 | J. Kruse  | 3642     | GLM-F107694  | MF924691    |
|                  |               | Gießen, Allendorf at river Kleebach, wayside, N50°33'18" E08°36'55", MTB/Q:     |            |           |          |               |             |
|                  |               | 5417/23, elev. c. 165 m a.s.l.                                                   |            |           |          |               |             |
| **R. polyanthemos** | *R. repens*  | Poland, Małopolska Province, Tatra Mts, between Hala Kalatówki glade and         | 17.07.2005 | J. & M. Piątek | 3646   | KRAM F-59039 | MF924685    |
|                  |               | Hala Kondratowa glade, elev. c. 1250 m a.s.l.                                    |            |           |          |               |             |
| **R. polyanthemos** | *R. repens*  | Slovakia, Tatra Mts, between Hala Kalatówki glade and Hala Kondratowa glade,     | 22.06.2008 | J. & M. Piątek | 3647   | KRAM F-59434 | MF924686    |
|                  |               | elev. c. 1250 m a.s.l.                                                            |            |           |          |               |             |
| **E. piepenbringiae** | *R. repens* | Germany, Bavaria, Oberfranken, county Bamberg, Mönchsweiher, mixed forest on     | 05.05.2012 | J. Kruse  | 103     | GLM-F107676  | MF924627    |
|                  |               | Keuper-Sandstone, MTB/Q: 6030/2, elev. c. 290 m a.s.l.                           |            |           |          |               |             |
| **R. polyanthemos** | *R. repens*  | Austria, Tyrol, Lechtal, N of Elbigenalp, wayside in mixed forest                | 26.08.2002 | U. Fischer & M. Lutz | ML523 | TUB-012566 | MF924716    |
|                  |               | TUB-012567, elev. c. 1460 m a.s.l.                                               |            |           |          |               |             |
| **R. polyanthemos** | *R. repens*  | Switzerland, Kanton Bern, north bottom slope of Sustenpass, c. 4 km to Hotel     | 12.06.2003 | U. Fischer & M. Lutz | ML230 | TUB-012566 | MF924717    |
|                  |               | Steingletscher, MTB/Q: 5533/43, elev. c. 970 m a.s.l.                            |            |           |          |               |             |
| **R. polyanthemos** | *R. repens*  | Slovenia, Triglav National Park, way to Siebenseen cabin, mixed forest           | 02.08.2005 | J. Kruse  | 103     | GLM-F107676  | MF924717    |
|                  |               | TUB-012568, elev. c. 970 m a.s.l.                                                |            |           |          |               |             |

**Table 1 (cont.)**
| Species | Host | Location | Location details | Date | Collector | DNA- Fungarium no. | GenBank no. |
|---------|------|----------|------------------|------|-----------|-------------------|-------------|
| E. ranunculosa | Germany, Bavaria | Oberpfaffnau, national park Bavarian Wood, county Regen, Zweateral Waldhaus, Mittelsteig cabin, mixed mountainous forest on granite, MTB/Q: 6945/2, elev. c. 700 m a.s.l. | 24.08.2012 | J. Kruse | GLM-F107677 | MP924628 | MH022752 | MF939208 |
| R. aspis | Germany, Saxony-Anhalt | Mittelsteig cabin, mixed mountainous forest on granite, MTB/Q: 6945/2, elev. c. 700 m a.s.l. | 13.11.2013 | J. Kruse | GLM-F107680 | MF924644 | MH022768 | MF939214 |
| R. aspis | Germany, Hesse | Hoher Meißner, Meißner eastern slope, Fulda-Werra-uplands, Werra-Meißner-county, Frau Holle lake, circular path, alpine meadow, MTB/Q: 4725/33, elev. c. 620 m a.s.l. | 09.06.2015 | J. Kruse | GLM-F107682 | MF924676 | MH022792 | MF939200 |
| E. ranunculosa | Poland Mazowieckie Province: Warszawa-Wesela | Wyrowian, county Kielbach, Lindau, Mt chain Rough Mt, wayside, MTB/Q: 4725/33, elev. c. 620 m a.s.l. | 17.05.2015 | P. Mędykowski | KRAM-F-59032 | – | – | – |
| R. auricomus | Germany, Bavaria | Oberpfaffnau, national park Bavarian Wood, county Regen, Zweateral Waldhaus, Mittelsteig cabin, mixed mountainous forest on granite, MTB/Q: 6945/2, elev. c. 700 m a.s.l. | 12.05.2012 | J. Kruse | GLM-F107686 | MF924688 | MH022793 | MF939207 |
| R. paludosus | Greece, Rhodes | eastcoast, c. 2.5 km N of Kalathos, street towards Masari, way side, olive grove, MTB/Q: 4635/12, elev. c. 40 m a.s.l. | 20.06.2005 | H. Jage | GLM-F086006 | MF924691 | MH022815 | MF939209 |
| E. savchenkoi | Greece, Rhodes | c. 1 km NW of Skala, way up Akramitis, open Phrygana, plateau, MTB/Q: 4635/12, elev. c. 40 m a.s.l. | 06.05.2005 | H. Jage | GLM-F076186 | MF924673 | MH022797 | MF939210 |
| E. thielii | Germany, Bavaria | Oberpfaffnau, national park Bavarian Wood, county Regen, Zweateral Waldhaus, Mittelsteig cabin, mixed mountainous forest on granite, MTB/Q: 6945/2, elev. c. 700 m a.s.l. | 19.04.1998 | H. Jage | GLM-F048093 | MF924659 | MH022783 | MF939231 |
| R. montanus | Germany, Saxony-Anhalt | Szklarska Poreba, Rapaanska cabin, near Rappanska, wayside, MTB/Q: 8726/21, elev. c. 2080 m a.s.l. | 29.07.2015 | J. Kruse | GLM-F107695 | MF924697 | MH022799 | MF939240 |
| R. montanus | Germany, Bavaria | Oberpfaffnau, national park Bavarian Wood, county Regen, Zweateral Waldhaus, Mittelsteig cabin, mixed mountainous forest on granite, MTB/Q: 6945/2, elev. c. 700 m a.s.l. | 11.07.2016 | J. Kruse | GLM-F107704 | MF924694 | MH022818 | MF939253 | MF939319 |
Considering the narrow host specificity for the species occurring on *Ficaria*, it is remarkable that these three *Entyloma* species are reported from about 46 mostly yellow flowered *Ranunculus* species, worldwide (Savchenko et al. 2012, Vánky 2012). *Entyloma microsorum* and *E. ranunculi-repentes* have the widest reported host range with 30 and 26 different *Ranunculus* host species, respectively (Vánky 2012). However, it is still to be demonstrated, whether these *Entyloma* species are indeed generalist species, like some biotrophic pathogens (Choi et al. 2009, Runge et al. 2011, Scholler et al. 2011, Morin et al. 2012), or represent complexes of specialised species that justify earlier attempts to split them into several species with narrow host spectra, specifically *Caenoma bulbosum* on *R. chaerophyllos* and *E. pygmaeum* on *R. pygmaeus* (Saccardo 1915, Ciferri 1928), *E. ranunculcearum* on *R. acris*, *E. ranunculi-scelerati* on *R. sceleratus*, *E. ranunculorum* on *R. auricomus*, and *E. wroblewskii* on *R. polyanthemos* (Kochman 1934, 1936, Liro 1938).

Only a small number of *Entyloma* spp. on *Ranunculus* species have been included in phylogenetic analyses (e.g., Begerow et al. 2000, 2002, 2006, Savchenko et al. 2014a, Savchenko & Carris 2017).

The aim of this study was to resolve the species boundaries of *Entyloma* species on *Ranunculus*, based on the combination of morphological, biological, and molecular markers, including four loci (ITS, atp2, ssc1, and map). For this, a broad set of host-fungus combinations was studied, including *Entyloma* specimens from eleven different *Ranunculus* species, mostly from Germany but also from the Mediterranean (Greece, Italy, Slovenia, Spain), and Central Europe (Austria, Poland, Slovakia).

### MATERIALS AND METHODS

#### Specimen sampling, documentation, and nomenclature

This study is based on morphological and/or phylogenetic analyses of 96 *Entyloma* specimens from eleven different *Ranunculus* species and one *Ficaria* species that were either collected in different regions of Europe or obtained from private herbaria (Table 1). They were deposited in the herbarium Senckenbergianum Götitz (GLM) and in the herbarium of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków (KRAM F). The nomenclature of the host plant species is according to Euro+Med PlantBase (Euro+Med 2006–onwards), the nomenclature of the fungi is according to Index Fungorum (http://www.indexfungorum.org/) and Vánky (2012). The *Entyloma microsorum* complex and the *E. ranunculi-repentes* complex are defined as species complexes having sori forming swollen pustules filled with spores with cracked surfaces and sori forming flat leaf spots with tissue-embedded smooth spores, respectively.

#### Morphological examination

The morphology of sori and spores was studied using dry herbarium specimens. For each of the host species of the two presumed complexes, up to five specimens were analysed in detail, using those specimens for which four loci (ITS, atp2, ssc1, and map) could be obtained, with four exceptions: for *Entyloma* sp. on *Ranunculus auricomus* specimens included in the two loci (ITS and atp2) dataset were used; for *Entyloma* sp. on *R. oreophilus*, one of two specimens had only two loci available; for *Entyloma* sp. on *R. sceleratus*, four of five specimens had only two loci available; and for *E. eburneum* one of six specimens had only two loci available. The specimens morphologically analysed are listed in the respective species descriptions.
DNA extraction, primer design, PCR, and sequencing

Genomic DNA was isolated from 96 Entyloma herbarium specimens (Table 1). For methods regarding isolation, homogenisation of fungal material, and DNA extraction see Lutz et al. (2004) as well as Kruse et al. (2017a). PCR amplification of the complete ITS nrDNA (internal transcribed spacers) was performed with the conditions outlined in White et al. (1990), using M-ITS1 (Stoll et al. 2003) as forward and ITS4 (White et al. 1990) as smITS-R2 (Kruse et al. 2017a) as reverse primers. Plant ITS was amplified using primer pair ITS1P/ITS4 (Ridgway et al. 2003) with an annealing temperature of 54 °C. The amplification of the atp2 (ATP synthase subunit 2) locus was done according to Kruse et al. (2017b), using the F8/R4 primer combination with an annealing temperature of 54 °C. For the ssc1 (member of the heat shock protein family) and map (methionine aminopeptidase) locus used in Kruse et al. (2017b) two new primer sets specific for the Exobasidiomycetes were designed in this study on the basis of unpublished genome sequences of Exobasidium vaccini and Pseudomicrostroma juglandis. The set of primers designed along the lines described in Kruse et al. (2017b) was tested on a variety of Exobasidiomycetes genera (Entyloma, Exobasidium, and Tilletia) and Ustilaginomycetes (Urocystis) with an annealing temperature of 53 °C. For the primer combinations providing best results gradient PCRs were conducted (50 °C to 60 °C and 60 °C to 72 °C) using Entyloma sp. samples and the optimal temperature was selected based on amplification strength and the absence of unspecific amplification. For the amplification of the ssc1 locus of Entyloma spp. this revealed the optimal primer pair to be ssc1_F3ex (5'GWGGWWGAAGCTTGTTCCTGT3') and ssc1_R5ex (5'ACACACGCTGRTGSGAGCG3') with an annealing temperature of 54 °C. For the amplification of the map locus of Entyloma spp. map_F3ex (5'AGYTGCTRTARCTGTCACAC3') and map_R3ex (5'CCAAYGCCAAYTGGCAGA3') with an annealing temperature of 60 °C gave the best results.

PCR conditions were according to Kruse et al. (2017b), but with 46 PCR cycles. The resulting amplicons were sequenced at the sequencing laboratory of the Senckenberg Biodiversity and Climate Research Centre (BfK-ExSenckenberg, Germany) using the primers used in PCR, except for the map_F3ex/map_R3ex amplicons which were sequenced with a shortened reverse primer: map_R3exShort (5'CCAAYTTGCGGCAAGAC3'). Sequences were deposited in GenBank (accession numbers are given in Table 1).

Molecular phylogenetic reconstruction

In total 91 ITS, 91 atp2, 64 ssc1, and 64 map sequences from Entyloma species affecting members of the genus Ranunculus were used for phylogenetic reconstructions in two different datasets. In addition to Entyloma on Ranunculus some Entyloma species on Ficaria verna were included because initial analyses suggested that Entyloma species on Ficaria verna might belong to the E. ranunculi-repentis complex. The first dataset comprised all four loci for 66 Entyloma specimens. The second comprised only ITS and atp2 sequences for 96 Entyloma specimens. Alignments were done for each locus independently using MAFFT (Katoh & Standley 2013) v. 7, employing the G-INS-i algorithm, and subsequently leading and trailing gaps were removed. After this and after checking for supported phylogenetic conflicts between the loci using Minimum Evolution analysis as outlined below, the aligned sequences of the individual loci were concatenated to obtain the datasets for phylogenetic analyses. For dataset 1 the resulting total alignment contained 1871 characters (ITS: 523, atp2: 480, ssc1: 394, map: 474) for dataset 2 the resulting total alignment contained 1003 characters (ITS: 523, atp2: 480). The methods for phylogenetic analyses were according to Kruse et al. (2018) for reconstructions using Minimum Evolution, Maximum Likelihood, and Bayesian Inference. To determine diagnostic bases for the different Entyloma species, alignments were checked manually for differences between the different host-fungus combinations. Host plant determination was verified comparing their ITS sequences to those deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) using BLASTN (Altschul et al. 1997).

RESULTS

Molecular phylogenetic reconstruction

There were no strongly supported conflicts between the topologies of the trees obtained from single loci. Minimum Evolution, Maximum Likelihood, and Bayesian Analyses yielded consistent topologies for both datasets. The results of the phylogenetic reconstructions based on four and two loci are given in Fig. 1 and Fig. 2, respectively.

All analyses revealed three strongly supported major lineages. The first lineage corresponded to the E. microsporum complex and included specimens from Ranunculus acris, R. paludosus, R. polyanthemos subsp. nemorosus, and R. repens, with gross morphology that matched E. microsporum. The second lineage corresponded to the E. ranunculi-repentis complex and included specimens from Ficaria verna, Ranunculus acris, R. auricomus, R. bulbosus, R. lanuginosus, R. marginatus, R. montanus, R. oreophilus, R. paludosus, R. polyanthemos subsp. nemorosus, R. repens, and R. scalaratus, with gross morphology that matched E. ranunculi-repentis. The third lineage was represented by E. verruculosum on R. lanuginosus. Within both the Entyloma microsporum complex and the E. ranunculi-repentis complex, specimens from the same host plant species grouped together, with few exceptions. Within the E. microsporum complex the majority of specimens from Ranunculus repens formed a clade together with two accessions on R. acris. Two specimens from R. repens (ML471, 102) clustered with specimens from R. polyanthemos subsp. nemorosus. Within the E. ranunculi-repentis complex, specimens from Ranunculus repens clustered together with specimens from R. bulbosus and R. polyanthemos subsp. nemorosus.

Comparing the results from both datasets, support values for the topology inferred from two loci (ITS and atp2) were mostly lower than from four loci (ITS, atp2, ssc1, and map), and the topology was generally more resolved in the latter. Within the E. microsporum complex, a group of specimens on both Ranunculus polyanthemos subsp. nemorosus and R. repens, were a sister lineage to specimens on R. acris and R. repens. The specimens on R. paludosus formed the sister group to all specimens mentioned so far. Within the E. ranunculi-repentis complex support values for the relationships of the well-supported host-specific clades were generally low. Diagnostic bases enable the molecular identification of species given on the basis of a defined alignment (Bennett et al. 2017, Kruse et al. 2018). Diagnostic bases for the different Entyloma species are given as an overview in Fig. 3 and detailed in Table 2.
Fig. 1 Phylogenetic relationships of *Entyloma* species on *Ranunculus* spp., rooted with the specimens of the *Entyloma microsporum* complex, based on Minimum Evolution analyses of four loci (ITS, *atp2*, *sscl*, and *map*). Numbers on branches denote bootstrap support in Minimum Evolution and Maximum Likelihood, as well as *a posteriori* probabilities from Bayesian Analyses, in the respective order. Values below 60% are not shown. The scale bar indicates the number of substitutions per site.
Fig. 2 Phylogenetic relationships of Entyloma species on Ranunculus spp., rooted with the specimens of the Entyloma microsporum complex, based on Minimum Evolution analyses of two loci (ITS and atp2). Numbers on branches denote bootstrap support in Minimum Evolution and Maximum Likelihood, as well as a posteriori probabilities from Bayesian Analyses. Values below 60 % are not shown. The scale bar indicates the number of substitutions per site.
Morphology

The three major phylogenetic lineages could be distinguished by teliospore surface ornamentation. Spores from species in the *E. microsorum* complex had a cracked surface; those from the *E. ranunculi-repentis* complex were smooth; and those from *E. verruculosum* were verrucose. Species in the *E. microsorum* complex always formed sori in hard, swollen galls. Most species-specific lineages of the *E. ranunculi-repentis* complex produced an asexual morph, which was not observed in the *E. microsorum* and the *E. verruculosum* complexes. Morphological differences within the two species complexes were generally low. The morphological characterisation of the species is included in species descriptions for *Entyloma* species on *Ranunculus* highlighted in bold type.

Sor in the leaves, rarely leaf petioles, forming distinct, rounded, hard, swollen pustules on leaves, 1–2 mm diam, markedly delineated from the healthy host tissue, at first yellow-greenish, later brownish, usually closed but sometimes old pustules cracked. Spores embedded in the leaf tissue, single, very densely crowded in the intercellular space between the mesophyll cells, which, in older pustules are destroyed; spores subhyaline (in young sori), pale yellow to yellow (in mature sori), very variable in shape and size, globose, subglobose, broadly ellipsoidal, rarely elongated, usually more or less polygonal, (11.5–)15.0–21.5(–26.5) µm (av. ± SD, 18.1 ± 2.9 × 14.9 ± 1.8 µm, n = 150/5), with smooth surface; teliospore wall 2-layered, 2.5–7.0(–8.0) µm thick (including inner layer c. 0.8–1.0 µm thick), layers well visible in LM, often with angles, outer layer unevenly thickened, outer layer unevenly thickened, spore surface rough or superficially cracked, rarely smooth. Asexual morph not found.

Diagnostic bases — Within the *E. microsorum* complex there are 19 diagnostic bases across all four loci (Fig. 3, Table 2). Host plant — Parasitic on *Ranunculus paludosus*.

Additional specimens examined. *Greec*, Rhodes, c. 2.8 km NW of Archangelos, Tsambika, way up to the monastery, northern slope, phrygana, *Ranunculus paludosus* var. alpestre, 9 Mar. 2016, J. Kruse (MLB-F216458) (ITS), M.H.22782 (atp2), M.F939296 (asc1), M.F939230 (map).

Notes — The smut specimens with swollen pustules on the leaves of *Ranunculus paludosus* are usually assigned to *Entyloma microsorum* (*Vánky* 2012), but the molecular analyses in the present study reveal that they form a distinct lineage, for which the name *Caemoa bulbosa* is available. This species was described by Saccardo (1915) from leaves of *Ranunculus*...
The sequenced specimens to fix the application of this name. *Entyloma bullosum* differs from the other currently recognized species in the *E. microsorum* complex by a larger mean spore size and thicker spore walls.

**Entyloma microsorum** (Unger) J. Schrötl., in Rabenhorst, Fungi Europ. no. 1872. 1874 — Fig. 5

Basionym. *Protomyces microsporus* Unger, *Die Exantheme der Pflanzen*, etc.: 343. 1833.

Synonym. *Entyloma ungerianum* de Bary, Bot. Zeitung (Berlin) 32: 101.

**Entyloma ranunculi-repentis** was identical with *E. microsorum* complex by a larger mean spore size and thicker spore walls.

**Entyloma microsorum** (Unger) J. Schrötl., in Rabenhorst, Fungi Europ. no. 1872. 1874 — Fig. 5

Basionym. *Protomyces microsporus* Unger, *Die Exantheme der Pflanzen*, etc.: 343. 1833.

Synonym. *Entyloma ungerianum* de Bary, Bot. Zeitung (Berlin) 32: 101. 1874, nom. nov. superfl. pro *P. microsporus*.

**Entyloma chaerophyllos** (Unger) J. Schrötl., in Rabenhorst, Fungi Europ. no. 1872. 1874 — Fig. 5

Basionym. *Protomyces chaerophyllos* Unger, *Die Exantheme der Pflanzen*, etc.: 343. 1833.

Synonym. *Entyloma ranunculi-repentis* de Bary, Bot. Zeitung (Berlin) 32: 101. 1874, nom. nov. superfl. pro *E. microsorum*.

**Entyloma bullosum** (Unger) J. Schrötl., in Rabenhorst, Fungi Europ. no. 1872. 1874 — Fig. 5

Basionym. *Protomyces bullosus* Unger, *Die Exantheme der Pflanzen*, etc.: 343. 1833.

Synonym. *Entyloma ranunculi-repentis* de Bary, Bot. Zeitung (Berlin) 32: 101. 1874, nom. nov. superfl. pro *E. microsorum*.

**Entyloma paludosus** (Unger) J. Schrötl., in Rabenhorst, Fungi Europ. no. 1872. 1874 — Fig. 5

Basionym. *Protomyces paludosus* Unger, *Die Exantheme der Pflanzen*, etc.: 343. 1833.

Synonym. *Entyloma ranunculi-repentis* de Bary, Bot. Zeitung (Berlin) 32: 101. 1874, nom. nov. superfl. pro *E. microsorum*.

**Entyloma lanuginosus** (Unger) J. Schrötl., in Rabenhorst, Fungi Europ. no. 1872. 1874 — Fig. 5

Basionym. *Protomyces lanuginosus* Unger, *Die Exantheme der Pflanzen*, etc.: 343. 1833.

Synonym. *Entyloma ranunculi-repentis* de Bary, Bot. Zeitung (Berlin) 32: 101. 1874, nom. nov. superfl. pro *E. microsorum*.

**Entyloma marginatus** (Unger) J. Schrötl., in Rabenhorst, Fungi Europ. no. 1872. 1874 — Fig. 5

Basionym. *Protomyces marginatus* Unger, *Die Exantheme der Pflanzen*, etc.: 343. 1833.

Synonym. *Entyloma ranunculi-repentis* de Bary, Bot. Zeitung (Berlin) 32: 101. 1874, nom. nov. superfl. pro *E. microsorum*.

**Entyloma montanus** (Unger) J. Schrötl., in Rabenhorst, Fungi Europ. no. 1872. 1874 — Fig. 5

Basionym. *Protomyces montanus* Unger, *Die Exantheme der Pflanzen*, etc.: 343. 1833.

Synonym. *Entyloma ranunculi-repentis* de Bary, Bot. Zeitung (Berlin) 32: 101. 1874, nom. nov. superfl. pro *E. microsorum*.

**Entyloma ungerianum** (Unger) J. Schrötl., in Rabenhorst, Fungi Europ. no. 1872. 1874 — Fig. 5

Basionym. *Protomyces ungerianum* Unger, *Die Exantheme der Pflanzen*, etc.: 343. 1833.

Synonym. *Entyloma ranunculi-repentis* de Bary, Bot. Zeitung (Berlin) 32: 101. 1874, nom. nov. superfl. pro *E. microsorum*.

**Entyloma ochroleucus** (Unger) J. Schrötl., in Rabenhorst, Fungi Europ. no. 1872. 1874 — Fig. 5

Basionym. *Protomyces ochroleucus* Unger, *Die Exantheme der Pflanzen*, etc.: 343. 1833.

Synonym. *Entyloma ranunculi-repentis* de Bary, Bot. Zeitung (Berlin) 32: 101. 1874, nom. nov. superfl. pro *E. microsorum*.
Sori in the leaves, rarely leaf petioles, on the leaves forming distinct, rounded or elongated, hard, swollen pustules, 1–6 mm diam, markedly delineated from the healthy host tissue, at first yellow-cream, later brownish, pustules at first closed but at the maturity cracked. Spores embedded in the leaf tissue, single, very densely crowded in the intercellular space between the mesophyll cells, which in mature pustules are totally destroyed; spores subhyaline or rarely pale yellow, variable in shape and size, globose, subglobose, broadly ellipsoidal, rarely elongated, often more or less irregular, 10.0–18.5(–24.0) × (9.5–)10.0–13.5(–17.5) µm (av. ± SD, 14.6 ± 2.8 × 12.2 ± 1.7 µm, n = 150/5), with smooth or granular context; wall 2-layered, (1.5–)2.0–4.5 µm, occasionally 7.0 µm thick (including inner layer c. 0.5–1.0 µm thick), sometimes with angles, layers well visible in LM, inner layer evenly thickened, outer layer evenly or unevenly thickened, spore surface rough or superficially cracked, rarely smooth. Asexual morph not found.

Host plants — Parasitic on Ranunculus acris and R. repens.

Additional specimens examined. Germany, Baden-Württemberg, county Konstanz, Hegau, W of Singen, way up to Mt Hohentwiel, wayside, elevation c. 600 m a.s.l., on Ranunculus repens, 29 May 2013, J. Kruse (GLM-F107672); Bavaria, county Rottal-Inn, Simbach, road St 2112, grassland at roundabout, N48°16'23" E13°00'53", elevation c. 370 m a.s.l., on Ranunculus acris, 14 Aug. 2014, J. Kruse (GLM-F107663); Lower Saxony, county Hildesheim, Brüggen, Kirschweg, Sieben Bergen, Mt Hohe Tafel, wayside, elevation c. 395 m a.s.l., on Ranunculus repens, 8 May 2011, J. Kruse (GLM-F107667). — Poland, Małopolska Province, Tatra Mts, Hala Gąsienicowa glade (near Murowaniec cabin), elevation c. 1510 m a.s.l., on Ranunculus repens, 24 Sept. 2005, J. Piątek & M. Piątek (KRAM F-59041).

Notes — This species has been first described as Protomyces microsporus. In the protologue, Unger (1833) contrasted it with Protomyces macrosporus (Ascomycota, Taphrinales) as a species forming pustules on stems and leaf veins of Ranunculus repens and having very small, rounded and pale sporidia (= spores). De Bary (1874) obtained spore germination of this species and concluded that it is not a member of Protomyces but a smut fungus, for which he described the distinct genus, Entyloma. He introduced the new name Entyloma ungerianum for this species. However, this was superfluous and Schröter (in Rabenhorst 1874) combined the species in Entyloma as E. microsporum. The original material probably does not exist anymore. Piepenbring (2003) could not locate it in BPI, GJO, M, and W. The current species concept of E. microsporum is based on a long tradition of application of this name to any specimen of Ranunculus displaying the characters reported by Unger (1833). However, spore sizes were not reported in the protologue (Unger 1833). Also De Bary (1874) did not provide spore sizes for material examined by him. Schröter (1887) finally measured the spores of this species, reporting the following values: spores 15–24 µm long and 12–17 µm wide, wall up to 7 µm thick. Similar counts were reported more recently, e.g., Vánky (1994, 2012: spores 11–23 × 10–16 µm, wall 1–9 µm thick), Scholz & Scholz (1988: spores 10–25 µm diam, wall 1–9 µm thick), but Kochman (1936) reported that spores were 10–20 µm diam (with mean 14 µm) and the wall thickness was reported as 1.5–5 µm. The latter observations are in agreement with our observations, and it seems possible that the larger spore sizes reported by other authors result from the presentation of extreme values without indicating which values predominated in the overall spore counts. In the phylogenetic analyses the specimens forming swollen pustules on Ranunculus repens clustered in two lineages: one
containing the majority of accessions on *R. repens* and two accessions on *R. acris*, and the other containing the minority of accessions on *R. repens* with predominance of accessions on *R. polyanthemos* subsp. *nemorosus*. The specimens in both lineages were morphologically similar, and it is not clear to which of the two lineages the name *E. microsporum* could be applied. Therefore, to stabilize this fungus name we designate a neotype from specimens from the lineage where most accessions on *R. repens* were placed. The specimens on *R. acris* were inseparable morphologically and only very weakly separated genetically, and are therefore currently remain in *E. microsporum*. The specimens forming the second lineage are accommodated in the novel species, *E. piepenbringiae*.

**Entyloma piepenbringiae** J. Kruse, M. Lutz, Piątek & Thines, *sp. nov.* — MycoBank MB824511; Fig. 6

**Etymology.** Named in honour of Prof. dr Meike Piepenbring (Frankfurt a. Main), for her contributions to the knowledge of temperate and tropical smut fungi.

**Type.** Germany, Bavaria, Oberallgäu, Eindöbsbach, Allgäu Alps, hiking path from Black cabin to Rappensee cabin, meadow W Rappensee cabin, N47°17’24” E10°14’40”, elevation c. 1900 m a.s.l., on *Ranunculus polyanthemos* subsp. *nemorosus*, 26 July 2015, J. Kruse (GLM-F107687 holotype; ex-type sequences available in GenBank: MF924664 (ITS), MH022788 (*atp*2), MF939302 (*ssc*1), MF939236 (*map*)).

**Sori** in the leaves, rarely leaf petioles, on the leaves forming distinct, rounded or elongated, hard, swollen pustules, 1–5 mm diam, markedly delineated from the healthy host tissue, at first creamy yellow, later brownish, usually closed but sometimes old pustules cracked. **Spores** embedded in the leaf tissue, single, very densely crowded in the intercellular space between the mesophyll cells, which in older pustules are totally destroyed; spores subhyaline or rarely pale yellow, variable in shape and size, globose, subglobose, broadly ellipsoidal, rarely elongated, often more or less irregular, (10.5–)12.0–17.5(–21.0) × (9.0–)10.0–15.5(–16.0) µm (av. ± SD, 14.5 ± 2.4 × 12.5 ± 1.4 µm, *n* = 150/5), with smooth context; wall 2-layered, (1.5–)2.5– 4.0(–6.0) µm thick (including inner layer c. 0.7–1.0 µm thick), sometimes with angles, layers well visible in LM, inner layer evenly thickened, outer layer evenly or unevenly thickened, spore surface rough or superficially cracked, rarely smooth. **Asexual morph** not found.

**Host plants** — Parasitic on *Ranunculus polyanthemos* subsp. *nemorosus* and *R. repens*.

**Additional specimens examined.** Germany, Baden-Württemberg, county Konstanz, communal Moos, S of Weiler, near Grey Reed, wayside, elevation c. 445 m a.s.l., on *Ranunculus repens*, 30 May 2013, J. Kruse (GLM-F107694); Bavaria, Upper Bavaria, county Garmisch-Partenkirchen, c. 2.8 km SE of Mittenwald, Karwendel mountains, meadow around Brunnstein cabin, N47°24’49” E11°16’41”, elevation c. 1475 m a.s.l., on *Ranunculus polyanthemos* subsp. *nemorosus*, 8 July 2016, J. Kruse (GLM-F107690); hiking path 291 from Brunnstein cabin towards Mt Brunnsteinspitze, scree, N47°24’33” E11°16’59”, elevation c. 1760 m a.s.l., on *Ranunculus polyanthemos* subsp. *nemorosus*, 7 July 2016, J. Kruse (GLM-F107691).
Notes — The specimens on *Ranunculus polyanthemos* subsp. *nemorosus* and *R. repens* were morphologically similar and clustered together, and are therefore considered as belonging to the same species.

**Entyloma ranunci-repentis complex**

*Entyloma eburneum* (J. Schröter) J. Kruse, M. Lutz, Piątek & Thines, *comb. nov.* — MycoBank MB824512; Fig. 7

*Basionym.* *Fusidium eburneum* J. Schröter., *Beitr. Biol. Pflanzen* 2 (3): 373. 1877.

*Type.* On *Ranunculus repens* (further details not included in the protologue, but probably the material was collected in Silesia, now in Poland, by J. Schröter, before 1877 (type could not be located, probably lost). – *Poland.* Małopolska Province, Kraków-Pleszów, at Suchy Jar street, on *Ranunculus repens*, 20 Nov. 2010, M. Piątek (KRAM F-59037 neotype designated here; MycoBank MBT380062; ex-type sequences available in GenBank: MF924689 (ITS), MH022813 (atp2)).

*Synonyms.* *Ramularia repentis* Oudem., *Beih. Bot. Centralbl.*: 15. 1902. *Type.* The Netherlands, Valkenberg, on *Ranunculus repens*, 1900, C.A.J.A. Oudemans (L, see Braun 1998).

*Entyloma ranunci-repentis* Sternon, *L’hétérogenité du genre Ramularia*, These, Nancy: 34, 45. 1925. *Type.* Belgium, Gembloux, Virton and Rochefort, on *Ranunculus repens*, 1917, F. Sternon (no type designated, see Vánky 2012).

*Entyloma wroblewskii* Kochman, *Acta Soc. Bot. Poloniae* 11 (Suppl.): 291. 1934. *Type.* Poland, Anin near Warszawa, on *Ranunculus polyanthemos*, 15 Sept. 1933, J. Kochman (KRAM F-2658 holotype; KRAM F-2656 and KRAM F-2657 isotypes).

*Sori* in the leaves, forming very distinct, flat, rounded, polyanlular or irregular spots, 0.5–4 mm long, 0.5–2 mm wide, usually partly delimited by the leaf veins of the host, at first whitish or cream-coloured due to the presence of the conidiophores and conidia of the asexual morph, later pale brown on both sides of the leaf. *Spores* embedded in the leaf tissue, single, loosely scattered or moderately densely crowded in the intercellular space between the mesophyll cells; spores pale yellow to yellow, globose, subglobose or rarely broadly ellipsoidal, regular in shape, (9.5–)11.0–13.5(–16.0) × (9.0–)9.5–13.5(–14.5) μm (av. ± SD, 12.3 ± 1.4 × 11.3 ± 1.3 μm, n = 200/6), with smooth context; wall 2-layered, 1.0–1.5(–2.0) μm thick (including inner layer c. 0.5–0.8 μm thick), without angles, layers well visible in LM, both layers well visible in LM, both layers evenly thickened, spore surface smooth. *Asexual morph* entylomella-like, very well developed. *Caespituli* both hypophyllous and epiphyllous, conidiophores in dense, agglutinated fascicles, emerging through stomata, hyaline, conidiogenous loci inconspicuous. *Conidia* solitary, hyaline, dimorphic, cylindrical, straight or somewhat curved, 15–22(–25) × 2.5–4.0 μm, and acicular, straight or somewhat curved, 30.0–45.0(–60.0) × (2.0–)2.5–3.5 μm, non-septate, hilum inconspicuous, not darkened.

*Host plants* — Parasitic on *Ranunculus bulbosus*, *R. polyanthemos*, and *R. repens*.

*Additional specimens examined.* Germany, Baden-Württemberg, Swabian Alps, county Sigmaringen, Leibertingen-Wildenstein, S of Beuron, ascent to castle Wildenstein, mixed forest, wayside, N48°02′49″ E08°58′17″, elevation c. 682 m a.s.l., on *Ranunculus repens*; 6 June 2014, J. Kruse (GLM-F107538); Bavaria, Upper Bavaria, county Garmisch-Partenkirchen, c. 4.9 km NE of Mittenwald, Kanwendel mountains, hiking path 266 from Rehbergalm to...
Hochland cabin, mixed mountainous forest, N47°27'37" E11°18'36", elevation c. 1575 m a.s.l., on Ranunculus polyanthemos subsp. nemorosus, 11 July 2016, J. Kruse (GLM-F107647); Hesse, Main-Taunus-county, Hattersheim on Main, grassland at Welschenstream, Kuckucksplad, wayside, N50°03'54" E08°30'03", elevation c. 90 m a.s.l., on Ranunculus bulbosus, 30 Apr. 2016, J. Kruse (GLM-F107644); Lower Saxony, county Northeim, at the bottom of the Katlencastle, wayside near river, elevation c. 110 m a.s.l., on Ranunculus repens, 23 Apr. 2010, J. Kruse (GLM-F107648).

Notes — The Entyloma species on Ranunculus repens causing flat spots is usually referred to as Entyloma ranunculi-repentis, which is the earliest available name for the teleomorph (Vánky 2012). Ramularia gibba (= Entylomella gibba) was considered to be the earliest name for the asexual morph (Braun 1998, Vánky 2012), which is an earlier name than Entyloma ranunculi-repentis, and following the ‘one fungus, one name rule’ (Hawksworth et al. 2011), Rossman & Castlebury (in Rossman et al. 2016) proposed the new combination Entyloma gibbum. However, they were apparently not aware that the original description and type material of Ramularia gibba were based on mixed elements of two fungi: the entylomella-like asexual morph of E. eburneum, and the sexual morph of E. microsporum. Due to the inseparable chimeric description and material, Kruse & Thines (2017) proposed the rejection of Ramularia gibba. The oldest available name for a flat-spotting Entyloma species on Ranunculus repens is Fusidium eburneum. This species has been described by Schröter (1877) for a conidial fungus on Ranunculus repens resembling the conidial state of Entyloma ranunculi (= Entyloma ficariae), producing whitish or yellowish spots, 1.5–2 mm diam and having hyaline, filamentous conidia 40–50 µm long and 2.5–3.0 µm wide. This morphological characterisation agrees well with the morphology of the asexual state in the holomorphic specimens analysed in the current study. Schröter (1877) did not observe corresponding Entyloma-like spores in the leaves. He thus might have analysed a young infection in which leaf spots and conidia are prominently developed, but teliospores are lacking. Fusidium eburneum is an earlier name than Entyloma ranunculi-repentis, and in line with the current International Code of Nomenclature for algae, fungi, and plants (McNeill et al. 2012) should be applied for the holomorph. In the protologue, Schröter (1877) did not provide a specific localization of the collected material, but in the monograph dealing with Silesian fungi (Schröter 1887), he enumerated several collections from Silesia. Authentic material of Fusidium eburneum is not preserved in the herbarium of J. Schröter deposited in WRSL (M. Halama, pers. comm.). Likewise, we could not locate any original material in other herbaria where some specimens of J. Schröter might have been deposited (e.g., in HBG; T. Feuerer, pers. comm.). Therefore, we are designating a neotype from among the specimens that were sequenced in this study. The neotype represents a holomorphic specimen with an asexual morph having characters that perfectly fit with the description in the protologue.

The present molecular and morphological analyses suggest that Entyloma specimens on Ranunculus bulbosus, R. polyanthemos subsp. nemorosus, and R. repens p.p. represent a single species. Entyloma on Ranunculus polyanthemos was...
previously described as a distinct species, *Entyloma wroblewskii* (Kochman 1934), which is considered as synonym with *Entyloma eburneum*, here. In the protologue of *E. wroblewskii*, Kochman (1934) reported one collection on *Ranunculus polyanthemos* collected in September 1933 in Anin near Warszawa (now within the borders of Warszawa) in Poland. In the herbarium KRAM F there are three specimens of *E. wroblewskii* having labels matching all information from the protologue, with the exception that the date of collection is given precisely as 15 September 1933 – these specimens apparently represent one original gathering. The label on one of these specimens is written in Latin and the species name is given as ‘*Entyloma Wróblewskii* n. sp. Kochman’ – this specimen should be considered as holotype. The labels on two remaining specimens are written in Polish and lack ‘n. sp.’ next to the species name – these specimens should be considered as isotypes. Vánky (2012) mentioned that type of *E. wroblewskii* is deposited in the herbarium WA. However, the corresponding herbarium specimen in WA was apparently collected in Anin a year later, on 15 September 1934 (M. Graniszewska, pers. comm.) and distributed in Kochman’s exsiccates, Ustilaginales Poloniae no. 28 – therefore, this specimen does not represent the original gathering.

*Entyloma eburneum* is morphologically distinct from most other *Entyloma* species infecting *Ranunculus* spp. in having prominently developed leaf spots, relatively large spores and dimorphic conidia (cylindrical and acicular). *Entyloma ranunculi-sclerati* is the most similar species, but differs in having somewhat smaller spores and longer, predominantly acicular conidia.

**Entyloma jolantae** J. Kruse, M. Lutz, Piątek & Thines, sp. nov. — MycoBank MB824513; Fig. 8

**Etymology.** Named after Jolanta Piątek (Kraków, Poland), Polish phycologist, who together with the second author of this work collected this smut and many other smut fungi during joint field trips in Europe and Africa.

**Type.** POLAND, Małopolska Province, Tatra Mts, Mała Dolinka valley – northern slopes of Giewont Mt, elevation c. 1230 m a.s.l., on *Ranunculus oreophilus*, 25 Aug. 2008, J. Piątek & M. Piątek (KRAM F-59030 holotype; ex-type sequences available in GenBank: MF924688 (ITS), MH022812 (atp2), MF939316 (ssc1), MF939250 (map)).

Sori in the leaves, forming distinct flat spots, 0.5–3 mm long, 0.5–2 mm wide, rounded or more or less polygonal – usually well delineated by the leaf veins of the host, at first cream-coloured, later brownish on both sides of the leaf, finally necrotic. Spores embedded in the leaf tissue, single, densely crowded in the intercellular space between the mesophyll cells; spores subhyaline to pale yellow, globose, subglobose or broadly ellipsoidal and often somewhat irregular due to mutual pressure, 10.5–15.5(–16.5) × 10.0–13.5(–14.5) µm (av. ± SD, 13.2 ± 1.4 × 11.6 ± 1.1 µm, n = 60/2), with smooth context; wall 2-layered, 1.5–2.0 µm thick (including inner layer c. 0.5–0.8 µm thick), layers well visible in LM, inner layer evenly thickened, outer layer unevenly thickened, spore surface smooth. *Asexual morph* not found.

Diagnostic bases — Within the *E. ranunculi-repentis* complex there are seven diagnostic bases distributed among all loci (Fig. 3, Table 2).

Host plant — Parasitic on *Ranunculus oreophilus*.

**Additional specimen examined.** POLAND, Małopolska Province, Tatra Mts, Mała Dolinka valley – northern slopes of Giewont Mt, elevation c. 1260 m a.s.l., on *Ranunculus oreophilus*, 25 Aug. 2008, J. Piątek & M. Piątek (KRAM F-59031).

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Fig. 8 *Entyloma jolantae* on *Ranunculus oreophilus*. a. Macroscopic symptoms of infection (two leaves to the left from holotype, one leaf to the right from paratype); b–d. spores as seen in light microscopy (from holotype). — Scale bars = 10 µm.
Notes — This species differs from most other species in the *E. ranunculi-repentis* complex by having larger spores with larger mean spore sizes, somewhat thicker spore walls, and lacking the asexual morph. *Entyloma savchenkoi* is the most similar species that differs in having an asexual morph.

*Entyloma klenkei* J. Kruse, M. Lutz, Piątek & Thines, sp. nov. — MycoBank MB824514; Fig. 9

**Etymology.** Named after Friedemann Klenke (Naundorf, Germany), for his eminent contributions as field mycologist to the knowledge on plant pathogenic fungi, e.g. as the lead author of the reference work *Pflanzen-parasitische Kleinpilze* (Klenke & Scholler 2015).

**Type.** GREECE, Rhodes, c. 0.7 km W of Archipoli, Eparchiaki Odos Pastidas-Mesanagrou, field beneath street, N36°15′58″E28°03′11″, elevation c. 185 m a.s.l., on *Ranunculus marginatus*, 13 Mar. 2016, J. Kruse & V. Kummer (GLM-F107659 holotype; ex-type sequences available in GenBank: MF924663 (ITS), MH022787 (*atp*2), MF939301 (*ssc*1), MF939235 (*map*)).

**Sori** in the leaves, forming indistinct, flat, polyangularly rounded spots, 1.5–2 mm diam, dirty yellow in colour. Spores embedded in the leaf tissue, single, loosely scattered in the intercellular space between the mesophyll cells; spores subhyaline to pale yellow, globose or subglobose, regular in shape, 10.5–13.0 × 10.0–12.5 μm (av. ± SD, 11.7 ± 0.9 × 11.1 ± 0.7 μm, n = 30/1), with smooth context; wall 2-layered, 0.5–1.5 μm thick (including inner layer c. 0.2–0.5 μm thick), without angles but sometimes with hyaline appen-

Fig. 9 *Entyloma klenkei* on *Ranunculus marginatus*. a. Macroscopic symptoms of infection; b–d. spores, as seen in light microscopy (from holotype). — Scale bars = 10 μm.
dage, layers hardly visible in LM, both layers evenly thickened, spore surface smooth. *Asexual morph* entylomella-like, weakly developed. *Caespituli* hypophyllous, conidiophores in dense fascicles, emerging through stomata, hyaline, conidiogenous loci inconspicuous. *Conidia* solitary, hyaline, cylindrical, 20–24 × 3.0–3.5(–4.0) µm, non-septate, hilum inconspicuous, not darkened.

**Diagnostic bases** — Within the *E. ranunculi-repentis* complex there are three diagnostic bases distributed among all loci, except *map* (Fig. 3, Table 2).

**Host plant** — Parasitic on *Ranunculus lanuginosus*.

**Notes** — This species differs from most other species in the *E. ranunculi-repentis* complex by the combination of small spores (with thin walls) and cylindrical conidia. *Entyloma ranunculacearum* differs in having shorter conidia, while *E. ranuncularum* differs in having longer conidia.

**Entyloma ranunculacearum** Kochman, Pl. Polon. 4: 105. 1936 — Fig. 11

**Type.** Ukraine, district Mościska, Krukienice, on *Ranunculus acris*, 17 Aug. 1935, J. Kochman (KRAM F-2606 lectotype indicated by Lindeberg 1959: 41, but precisely designated here; MycoBank MBT380645).

**Reference specimen.** Germany, Saxony-Anhalt, county Wittenberg, Kemberg, district Rotta-Gniest, Heidestreet, wayside, N51°45’04” E12°35’33”, elevation c. 105 m a.s.l., on *Ranunculus acris*, 13 Nov. 2013, J. Kruse (GLM-F107680 reference specimen designated here; ex-reference specimen sequences available in GenBank: MF924637 (ITS), MH022761 (atp2), MF939280 (ssc1), MF939214 (map)).

**Sori** in the leaves, forming distinct, flat, rounded or somewhat irregular spots, 0.5–4 mm diam, usually partly delineated by the leaf veins of the host, yellowish on the upper side of the leaf, whitish on the lower side of the leaf due to the presence of the conidiophores and conidia of the asexual morph. Spores embedded in the leaf tissue, single, loosely scattered in the intercellular space between the mesophyll cells; spores subhyaline to pale yellow, globose, subglobose or rarely broadly ellipsoidal, regular in shape, 10.0–13.5(–14.5) × (9.0–)10.0–12.5(–13.5) µm (av. ± SD, 11.8 ± 1.1 × 10.9 ± 0.8 µm, n = 150/5), with smooth context; wall 2-layered, 0.8–1.5 µm thick (including inner layer c. 0.3–0.5(–0.8) µm thick), without angles, layers hardly visible in LM, both layers evenly thickened, spore surface smooth.

**Asexual morph** entylomella-like, prominently developed. *Caespituli* hypophyllous, conidiophores in dense, agglutinated fascicles, emerging through stomata, hyaline, conidiogenous loci inconspicuous. *Conidia* solitary, hyaline, cylindrical, straight, sometimes slightly curved, (10–)15–19(–25) × 2.5–3.5(–4.0) µm, non-septate, hilum inconspicuous, not darkened.

**Diagnostic bases** — Within the *E. ranunculi-repentis* complex there are four diagnostic bases within the ssc1 locus (Fig. 3, Table 2).

**Host plant** — Parasitic on *Ranunculus acris*.

**Additional specimens examined.** Germany, Baden-Württemberg, county Konstanz, Lake Constance, Radolfzell, SE of Möggingen, Mindelsee, circular path around lake, littoral and wayside, elevation c. 420 m a.s.l., on *Ranunculus acris*, 30 May 2013, J. Kruse (GLM-F107678); Bavaria, Oberfranken, county Bamberg, SE of Sandhof, Mönchswilcher, mixed forest on Keuper-Sandstone, elevation c. 290 m a.s.l., on *Ranunculus acris*, 5 May 2012, J. Kruse (GLM-F107676); Hesse, Rheingau-Taunus-county, Ettville on Rhine, Rheinstein, direction to forest-restaurant Rausch, N50°02’46” E08°05’44”, elevation c. 160 m a.s.l., on *Ranunculus acris*, 8 Mar. 2014, J. Kruse (GLM-F107679); Saarland, Mettlach-Orscholz, county Merzig-Wadern, Cloef-Street, surrounds—

![Fig 10 Entyloma kochmanii on Ranunculus lanuginosus. a. Macroscopic symptoms of infection; b–d. spores, as seen in light microscopy; e. conidiophores emerging through the stoma, as seen in light microscopy; f. conidium, as seen in light microscopy (from holotype). — Scale bars = 10 µm.](image-url)
ings of Cloef-Atrium and Varadeser Park, N49°30'20" E06°32'06", elevation c. 395 m a.s.l., on Ranunculus acris, 29 Sept. 2014, J. Kruse (GLM-F107684).

Notes — When describing this species, Kochman (1936) reported three collections: two on R. acris and one on R. lanuginosus. In the Polish text he wrote that the typical form of this species infects R. acris and an additional host is R. lanuginosus. In the Latin diagnosis, Kochman (1936) reported only R. acris as type host without an indication of the specific collection. Lindeberg (1959) designated the lectotype from one of the two collections on R. acris (collected in 1935 in Krukienice, district Mościska, then in Poland but now in Ukraine), but she did not mention where the specimen was deposited. This specimen is currently preserved in the herbarium KRAM F. Kochman (1936) reported the date of collection as 1935, but on the lectotype specimen the exact date is given as 17 August 1935. Entyloma sp. on R. lanuginosus belongs to a distinct species, described here as E. kochmanii, which is phylogenetically closely related but distinct from E. ranunculacearum in having longer conidia.

Entyloma ranunculi-scelerati Kochman, Pl. Polon. 4: 104. 1936 — Fig. 12

Type. POLAND, Skierniewice-Glinianki, on Ranunculus sceletratus, 2 July 1927, W. Konopacka (BRIP: HUV 974 lectotype, isolecotypes in Kochman, Ust. Pol. no. 29; lectotype designated by Lindeberg 1959: 41, corrected and narrowed by Vánky 1985: 66).

Reference specimen. POLAND, Mazowieckie Province, Warszawa-Wesoła, on Ranunculus sceletratus, 17 July 2015, P. Mędykowski (KRAM F-59032 reference specimen designated here; ex-reference specimen sequences available in GenBank: MF924691 (ITS), MH022815 (atp2)).

Sori in the leaves, forming distinct, flat, rounded spots, 1–4 mm diam, yellow or light brown on the upper side of the leaf, whitish or cream coloured on the lower side of the leaf due to the presence of the conidiophores and conidia of the asexual morph, surrounded by brownish rim, finally necrotic – starting from the centre of the sori. Spores embedded in the leaf tissue, single, loosely scattered in the intercellular space between the mesophyll cells; spores subhyaline, pale yellow or yellow, globose or subglobose, regular in shape, (9.5–)10.0–12.5(–13.5) × (9.0–)10.0–12.5(–13.0) µm (av. ± SD, 11.7 ± 0.9 × 11.0 ± 0.9 µm, n = 150/5), with smooth context; wall 2-layered, 1.0–1.5 µm thick (including inner layer c. 0.3–0.8 µm thick), without angles, layers hardly visible in LM, without angles, layers partly visible in LM, both layers evenly thickened, spore surface smooth. Asexual morph entylomella-like, prominently developed. Caespituli both hypophyllous and epiphyllous, conidiophores in dense fascicles, emerging through stomata, hyaline, conidiogenous loci inconspicuous. Conidia solitary, acicular, rarely cylindrical, usually straight, 20–60 × (2.0–)2.5–3.5(–4.0) µm, non-septate, hilum inconspicuous, not darkened.

Diagnostic bases — Within the E. ranunculi-repentis complex there are 26 diagnostic bases distributed among all loci (Fig. 3, Table 2).

Host plant — Parasitic on Ranunculus sceletratus.

Additional specimens examined. GERMANY, Saxony-Anhalt, SE of Allstedt, Ziegelrodaer forest (N-part), airport Allstedt (NW-side), on Ranunculus sceletratus, 23 Oct. 2005, H. Jage (GLM-F076138); Loddersleben, near castle, in Querne, on Ranunculus sceletratus, 6 May 2005, H. John & H. Jage (GLM-F076186); Friedersdorf near Lohsa S, WSW Neuhof, near Ballackmühle, Maxsee (part of Ballacksee), on Ranunculus sceletratus, 26 May 2006.
Entyloma ranunculi-scelerati is most similar to *E. eburneum*, which differs in having somewhat larger spores and shorter conidia.

**Entyloma ranunculorum** Liro, Mycoth. Fennic. Die Etiketten. No. 301–600: 25. 1939 — Fig. 13

*Synonym.* *Entyloma ranunculorum* Liro, Ann. Acad. Sci. Fenn., Ser. A, 42 (1): 111. 1938, invalid name, no Latin description or diagnosis.

*Type.* SWEDEN, Härjedalen, Fjellnäs, on *Ranunculus auricomus*, 25 July 1897, G. Lagerheim (BRIP: HUV 894 lectotype, isolectotypes in Sydow, Ust. no. 233, as *Entyloma ranunculi*; lectotype designated by Vánky 1985: 66).

*Reference specimen.* GERMANY, Bavaria, Oberpfalz, county Kulmbach, Lindau, Mountain chain Rough Mt, wayside, elevation c. 410 m a.s.l., on *Ranunculus sceleratus* agg., 12 May 2012, J. Kruse (GLM-F107685 reference specimen designated here, ex-reference specimen sequences available in GenBank: MF924629 (ITS), MH022753 (*atp*2)).

*Sori.* In the leaves, forming distinct, flat, rounded or somewhat polygonal spots, 1–4 mm diam, whitish or cream coloured on both sides of the leaf. *Spores* embedded in the leaf tissue, single, loosely scattered or moderately densely crowded in the intercellular space between the mesophyll cells; spores subhyaline, pale yellow to yellow, globose, subglobose or broadly ellipsoidal, usually regular but sometimes somewhat irregular due to
to mutual pressure, 10.0–12.5(–14.5) × (9.0–)10.0–12.5(–13.0) µm (av. ± SD, 11.8 ± 0.9 × 10.9 ± 0.9 µm, n = 60/2), with smooth context; wall 2-layered, 1.0–1.5(–1.8) µm thick (including inner layer c. 0.5–0.8 µm thick), without angles, layers hardly visible in LM, both layers evenly thickened, spore surface smooth. Asexual morph entylomella-like, well-developed. Caespituli hypophyllous, conidiophores in densely agglutinated fascicles, emerging through stomata, hyaline, conidiogenous loci inconspicuous. Conidia solitary, hyaline, cylindrical, usually curved, rarely almost straight, 16–28 × 2.5–3.5(–4.0) µm, non-septate, hilum inconspicuous, not darkened.

Diagnostic bases — Within the *E. ranunculi-repentis* complex there are six diagnostic bases within ITS and the *atp2* locus (Fig. 3, Table 2).

Host plant — Parasitic on *Ranunculus auricomus*.

Additional specimen examined. **GERMANY**, Saxony-Anhalt, E of Dölkau, Burgholz (E-part) Jagen 29, alluvial forest, elevation c. 25 m a.s.l., on *Ranunculus auricomus*, 19 Apr. 1998, H. Jage (GLM-F048093).

Notes — The most similar species are *Entyloma kochmanii* and *E. ranunculacearum*, which differ in having shorter conidia.

**Entyloma savchenkoi** J. Kruse, M. Lutz, Piątek & Thines, sp. nov. — MycoBank MB824516; Fig. 14

Etymology. Named after Kyrylo G. Savchenko (Pullman, United States), Ukrainian mycologist, for his contributions to *Entyloma* phylogeny and taxonomy.

Type. **GREECE**, Rhodes, eastern coast, c. 2.5 km N of Kalathos, street towards Masari, wayside, olive grove, N36°08′47″ E28°03′33″, elevation c. 15 m a.s.l., on *Ranunculus paludosus*, 20 Mar. 2016, J. Kruse (GLM-F107699 holotype; ex-type sequences available in GenBank: MF924662 (ITS), MH022766 (*atp2*), MF939300 (*ssc1*), MF939234 (*map*)).

Sori in the leaves, forming rather indistinct, flat, rounded or somewhat polyangular spots, 1–3 mm long, 1–2 mm wide, yellow or light brown on the upper side of the leaf, whitish or cream coloured on the lower side of the leaf. Spores embedded in the leaf tissue, single, loosely scattered or moderately densely crowded in the intercellular space between the mesophyll cells; spores subhyaline, pale yellow to yellow, globule, subglobule or broadly ellipsoidal, usually regular but sometimes somewhat irregular due to mutual pressure, (10.0–)12.0–16.5(–18.0) × (9.0–)11.0–14.5(–15.0) µm (av. ± SD, 13.9 ± 1.4 × 12.3 ± 1.2 µm, n = 120/4), with smooth context; wall 2-layered, 1.5–2.5(–3.0) µm thick (including inner layer c. 0.5–1.0 µm thick), without angles, layers well visible in LM, both layers evenly thickened, spore surface smooth. Asexual morph entylomella-like, weakly developed. Caespituli hypophyllous, conidiophores in densely agglutinated fascicles, emerging through stomata, hyaline, conidiogenous loci inconspicuous. Conidia solitary, hyaline, acicular-cylindrical, straight, 25–40 × 2.5–3.0(–3.5) µm, non-septate, hilum inconspicuous, not darkened.

Diagnostic bases — Within the *E. ranunculi-repentis* complex there are four diagnostic bases distributed among all loci, except map (Fig. 3, Table 2).

Host plant — Parasitic on *Ranunculus paludosus*.

Additional specimens examined. **GREECE**, Rhodes, c. 1 km S of Salakos, way up to Mt Profitis Ilias, Quercus coccifera forest, N36°16′59″ E27°56′42″, elevation c. 320 m a.s.l., on *Ranunculus paludosus*, 13 Mar. 2016, J. Kruse (GLM-F107696); c. 1 km NW of Siana, way up to Akramitis, open Phrygana, plateau, N36°09′23″ E27°45′59″, elevation c. 650 m a.s.l., on *Ranunculus...
Entyloma thielii J. Kruse, M. Lutz, Piątek & Thines, sp. nov. — MycoBank MB824517; Fig. 15

Etymology. Named after Hjalmar Thiel from Jameln (Germany), for his contributions to the knowledge of phytopathogenic fungi and for enabling well-sampled phylogenetic investigations in various plant pathogen groups by his collections.

Type. Germany, Bavaria, Upper Bavaria, county Garmisch-Partenkirchen, c. 2.6 km SE of Mittenwald, Karwendel mountains, meadows around Brunnstein cabin, N47°24'49" E11°16'41", elevation c. 1475 m a.s.l., on Ranunculus montanus, 8 July 2016, J. Kruse (GLM-F107702 holotype; ex-type sequences available in GenBank: MF924694 (ITS), MH022818 (atpB), MF939319 (ssc1), MF939253 (map)).

Sori in the leaves, forming indistinct, flat, polyangular spots, 1–3 mm long, 0.5–2 mm wide, partly delineated by the leaf veins of the host, yellow or light brown on the upper side of the leaf, greyish or cream coloured on the lower side of the leaf. Spores embedded in the leaf tissue, single, densely crowded, often in compact groups, in the intercellular space between the mesophyll cells; spores hyaline, subhyaline to pale yellow, globose, subglobose or broadly ellipsoidal, often somewhat irregular due to mutual pressure, (9.5–)11.0–14.5(–16.5) × 9.0–12.5(–13.0) µm (av. ± SD, 12.5 ± 1.5 × 10.8 ± 1.0 µm, n = 150/5), with smooth context; wall 2-layered, 0.8–1.5 µm thick (including inner layer c. 0.3–0.5 µm thick), without angles, layers very hardly visible in LM, both layers evenly thickened, spore surface smooth. Asexual morph not found.

Diagnostic bases. — Within the E. ranunculi-repentis complex there are 68 diagnostic bases distributed among all loci (Fig. 3, Table 2).

Host plant — Parasitic on Ranunculus montanus.

Additional specimen examined. Germany, Bavaria, Upper Bavaria, county Garmisch-Partenkirchen, c. 2.6 km SE of Mittenwald, Karwendel mountains, hiking path 290 from Brunnstein cabin towards Mittenwald, serpentine, sparse mixed mountainous forest, N47°24'48" E11°16'33", elevation c. 1390 m a.s.l., on Ranunculus montanus, 8 July 2016, J. Kruse (GLM-F107700); meadows around Brunnstein cabin, N47°24'49" E11°16'41", elevation c. 1475 m a.s.l., on Ranunculus montanus, 8 July 2016, J. Kruse (GLM-F107701); c. 4.9 km NE of Mittenwald, Karwendel mountains, hiking path 266 from Rehberghalm to Hochland cabin, mixed mountainous forest, N47°27'37" E11°18'36", elevation c. 1575 m a.s.l., on Ranunculus montanus, 11 July 2016, J. Kruse (GLM-F107704); Oberallgäu, Einödsbach, Rappensee cabin, near Rappensee, wayside, N47°17'11" E10°15'19", elevation c. 2080 m a.s.l., on Ranunculus montanus, 29 July 2015, J. Kruse (GLM-F107705).

Notes — This species differs from the other species in the Entyloma ranunculi-repentis complex in having densely crowded spores, often in compact groups, in the intercellular space between the mesophyll cells.
Entyloma verruculosum

Entyloma verruculosum Pass., Nuovo Giorn. Bot. Ital. 9: 239. 1877; in Rabenhorst, Fungi Europ. no. 2253. 1877; in Fischer von Waldheim, Bull. Soc. Imp. Naturalistes Moscou 52: 310. 1877 — Fig. 16

Type. ITALY. Parma, on Ranunculus velutinus, May 1873, G. Passerini (BRIP: HUV 1307 lectotype, isolectotypes in Rabenhorst, Fungi Europ. no. 2253; lectotype designated by Vánky 1985: 80).

Sori in the leaves, forming indistinct, flat, polygonal spots, 1–5 mm long, 1–3 mm wide, partly delineated by the leaf veins of the host, yellow or light brown on the upper side of the leaf, cream colored on the lower side of the leaf. Spores embedded in the leaf tissue, single, densely crowded in the intercellular space between the mesophyll cells; spores subhyaline to pale yellow, globose or subglobose, regular in shape, (11.0–)12.0–14.5–16.0 µm × (10.5–)11.0–14.5–15.0 µm (av. ± SD, 13.4 ± 1.4 × 13.1 ± 1.4 µm, n = 30/1), with granular context; wall apparently 1-layered, 1.5–2.5 µm thick, without angles, spore surface distinctly tuberculate. Asexual morph not found.

Host plant — Parasitic on Ranunculus spp.

Specimen examined. ITALY. Apulia, Monte Sant’Angelo, Provinz Foggia, c. 12 km N of Monte Sant’Angelo, road SP52b, Foresta Umbra, beech forest, N41°47′52″E15°58′44″, elevation c. 720 m a.s.l., on Ranunculus lanuginosus, 19 Apr. 2016, J. Kruse (GLM-F107706).

Notes — The specimens of Entyloma verruculosum on the type host (Ranunculus velutinus) were not available for molecular analyses, and the morphological description is based on the sequenced specimen on R. lanuginosus. The smut species was additionally reported on Ranunculus acris, R. repens, and R. sceleratus (Vánky 2012), which indicates that E. verruculosum may represent a species complex, too, to be resolved in future studies.

DISCUSSION

The analyses of the morphology and molecular phylogenetics presented in this study indicate that most of the Entyloma species on Ranunculus spp. are specific at the host species level. This provides evidence for two more assumed broad-range biotrophic pathogens to be species complexes, rather than single species, similar to the situation observed in other pathogens (e.g., Lutz et al. 2005, Beenken et al. 2012, Choi et al. 2015, Scholler et al. 2016, Kruse et al. 2018, Ziegler et al. 2018). The three major lineages found within Entyloma (the E. microsporum complex, the E. ranunculi-repentis complex, and E. verruculosum) are readily distinguished by teliospore surface ornamentation. Species in the E. microsporum complex have cracked spore surfaces, those in the E. ranunculi-repentis complex are smooth, and spores of E. verruculosum are verrucose. In addition, species in the E. microsporum complex cause swollen galls readily distinguishing them from the other two lineages. Entyloma verruculosum, for which we examined only a single specimen, may represent yet another complex to be resolved in future studies, as it has been reported on five different Ranunculus species (Vánky 2012).

For the Entyloma ranunculi-repentis complex the four-gene dataset (with ITS, atp2, ssc1, and map sequences) recovered 11 mostly highly supported host-specific lineages (nine on...
Ranunculus spp. and two on Ficaria verna). These lineages are also correlated with (sometimes subtle) morphological characters. The most informative morphological and biological characters were the arrangement of spores within the leaf spot; size of spores; mean size of spores; spore wall thickness; presence of an asexual morph; and the shape and size of conidia (see Table 3).

For some of the lineages in the *E. ranunculi-repentis* complex validly published names are available, previously often listed as synonyms of *E. ranunculi-repentis* s.lat. (Vánky 2012). The results of this study support *E. ranunculacearum* (on *R. acris*), *E. ranunculi-scelerati* (on *R. sceleratus*), and *E. ranunculorum* (on *R. auricomus*) as distinct species (Kochman 1936, Liro 1938). For six other lineages, each associated with a single host plant species, new species were introduced. In addition to these host-specific Entyloma species, one additional clade with specimens from related species (Paun et al. 2005), *R. bulbosus*, *R. polyanthemos* subsp. *nemorosus*, and *R. repens*, has been assigned to a new combination in Entyloma for *Fusidium eburneum*. Further study is needed to determine if this clade represents a recently-differentiated species complex. If it contained distinct species, the name *Entyloma wroblewskii* (Kochman 1934) could be adopted for the Entyloma pathogen on *Ranunculus polyanthemos*. As even more loci or microsatellites would be needed to resolve this question, we have taken a conservative approach, considering the whole clade to represent *E. eburneum*.

The species *Ramularia gibba*, which was thought to be connected with the asexual morph-forming species of Entyloma on *Ranunculus repens* (Braun 1998), is a chimera that contains the diagnostic features of both the *E. microsporum* and the *E. ranunculi-repentis* species complexes (De Bary 1874). An inspection of the type specimen revealed a dual infection was present on the leaves, explaining the chimeric nature of the description. Consequently, the name cannot be applied to a species in either group and has been proposed for rejection (Kruse & Thines 2017).

There was less resolution of species in the *E. microsporum* complex than in the *E. ranunculi-repentis* complex with the four loci used in the present study. However, as specimens from *Ranunculus paludosus* were clearly distinct, the name *Caeoma bullosum* should be reinstated in its combination in Entyloma. The additional two clades found in the *E. microsporum* complex were each represented by specimens from different host species. Both lineages include morphologically similar specimens and both include specimens from *Ranunculus repens*, the type host of *Entyloma microsporum*. To fix the application of the name *E. microsporum*, a neotype was selected from among the specimens in the clade containing most accessions on *Ranunculus repens*, and a new species is introduced for the specimens of the other clade. Both clades with specimens from *Ranunculus repens* showed some internal differentiation according to the host species and thus might be revealed to be species complexes in future studies.

The relationships of the Entyloma species covered in this study do not correspond to the relationships of the respective hosts (Paun et al. 2005). It is, thus, conceivable that, similar to the situation in obligate biotrophic downy mildews (Choi & Thines 2015), species of Entyloma do not diversify by long-term co-evolution, but rather by host jumps, subsequent radiation, and...
Table 3  Main diagnostic ecological (host species) and morphological characters for *Entyloma* species on *Ranunculus*. *E* = *Entyloma*, *R* = *Ranunculus*.

| Species                      | Host plant     | Arrangement of spores in the sori (between the leaf mesophyll cells) | Spore sizes (µm) | Mean spore sizes and standard deviation (µm) | Spore wall thickness (µm) | Asexual morph     | Conidia                                      |
|------------------------------|----------------|------------------------------------------------------------------------|------------------|---------------------------------------------|--------------------------|-------------------|------------------------------------------------|
| *Entyloma microsporum* complex (sori forming swollen pustules and spores with cracked surface) |
| *E. bullosum*                | *R. paludosus* | very densely crowded                                                    | (11.5–)15.0–21.5(–25.5) x (10.5–)12.0–16.5(–19.5) | 18.1 ± 2.9 x 14.9 ± 1.8                      | 2.5–7.0(–8.0)             | absent        | absent |
| *E. microsporum*             | *R. acris, R. repens* (type host) | very densely crowded                                                    | 10.0–18.5(–24.0) x (9.5–)10.0–13.5(–17.5) | 14.6 ± 2.8 x 12.2 ± 1.7                      | (1.5–)2.0–4.5             | absent        | absent |
| *E. piepenbringiae*          | *R. polyanthemos subsp. nemorosus* (type host), *R. repens* | very densely crowded                                                    | (10.5–)12.0–17.5(–21.0) x (9.0–)10.0–15.5(–16.0) | 14.5 ± 2.4 x 12.5 ± 1.4                      | (1.5–)2.5–4.0(–6.0)       | absent        | absent |
| *Entyloma ranunculi-repentis* complex (sori forming flat leaf spots and smooth spores) |
| *E. eburneum*                | *R. bulbosus, R. polyanthemos, R. repens* | loosely scattered or moderately densely crowded                          | (9.5–)11.0–13.5(–16.0) x (9.0–)9.5–13.5(–14.5) | 12.3 ± 1.4 x 11.3 ± 1.3                      | 1.0–1.5(–2.0)             | present        | dimorphic, cylindrical, 15–22 x 2.5–4.0 µm, and acicular, 30.0–40.0(–60.0) x (2.0–)2.5–3.5 µm |
| *E. jolantae*                | *R. oreophilus* | densely crowded                                                          | 10.5–15.5(–16.5) x 10.0–13.5(–14.5) | 13.2 ± 1.4 x 11.6 ± 1.1                      | 1.5–2.0                   | absent        | absent |
| *E. kleinkei*                | *R. marginatus* | loosely scattered                                                         | 10.5–13.0 x 10.0–12.5 | 11.7 ± 0.9 x 11.1 ± 0.7                      | 1.0–1.8                   | absent        | absent |
| *E. koehmani*                | *R. lanuginosus* | loosely scattered                                                        | (9.0–)11.0–13.0 x (9.0–)10.0–12.5 | 11.7 ± 0.9 x 10.9 ± 0.8                      | 0.5–1.5                   | present        | cylindrical, 20–24 x 3.0–3.5(–4.0) µm |
| *E. ranunculuscearum*        | *R. acris*     | loosely scattered                                                         | 10.0–13.5(–14.5) x (9.0–)10.0–12.5(–13.5) | 11.8 ± 1.1 x 10.9 ± 0.8                      | 0.8–1.5                   | present        | cylindrical, (10–)15–19(–25) x 2.5–3.5(–4.0) µm |
| *E. ranunculi-scelerati*     | *S. scleratus* | loosely scattered                                                         | (9.5–)10.0–12.5(–13.5) x (9.0–)10.0–12.5(–13.0) | 11.7 ± 0.9 x 11.0 ± 0.9                      | 1.0–1.5                   | present        | dimorphic, acicular, rarely cylindrical, 20–60 x (2.0–)2.5–3.5(–4.0) µm |
| *E. ranunculorum*            | *R. auricomus* | loosely scattered or moderately densely crowded                          | 10.0–12.5(–14.5) x (9.0–)10.0–12.5(–13.0) | 11.8 ± 0.9 x 10.9 ± 0.9                      | 1.0–1.5(–1.8)             | present        | cylindrical, 16–28 x 2.5–3.5(–4.0) µm |
| *E. savchenkoi*              | *R. paludosus* | loosely scattered or moderately densely crowded                          | (10.0–)12.0–16.5(–18.0) x (9.0–)11.0–14.5(–15.0) | 13.9 ± 1.4 x 12.3 ± 1.2                      | 1.5–2.5(–3.0)             | present        | acicular-cylindrical, 25–40 x 2.5–3.0(–3.5) µm |
| *E. thiellii*                | *R. montanus*  | densely crowded, often in compact groups                                  | (9.5–)11.0–14.5(–16.5) x 9.0–12.5(–13.0) | 12.5 ± 1.5 x 10.8 ± 1.0                      | 0.8–1.5                   | absent        | absent |
| *Entyloma verruculosum* (indistinct sori and distinctly tuberculate spores) |
| *E. verruculosum*            | *Ranunculus spp.* | densely crowded in the intercellular spaces                              | (11.0–)12.0–14.5(–16.0) x (10.5–)11.0–14.5(–15.0) | 13.4 ± 1.4 x 13.1 ± 1.4                      | 1.5–2.5                   | absent        | absent |
finally specific adaptation, leading to diversification into distinct species. As there are numerous additional hosts for Entyloma in the genus Ranunculus (Ványk 2012) that could not be included in the current study, it seems likely that additional species await discovery and more detailed patterns regarding the evolution of Entyloma await revelation.

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