Persoonia 41, 2018: 175–201
www.ingentaconnect.com/content/nhn/pimj

RESEARCH ARTICLE

ISSN (Online) 1878-9080
https://doi.org/10.3767/persoonia.2018.41.09

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

J. Kruse[1,2#], M. Piątek[3#], M. Lutz[4], M. Thines[1,2,5]

Key words

Entyloma microsporum complex
Entyloma ranunculi-repentis complex
host specificity
multigene analyses
new primers
six new taxa
smut fungi

Abstract Plant pathogenic smut fungi in the broader sense can be divided into the *Ustilaginomycetes*, which cause classical smut symptoms with masses of blackish spores being produced in a variety of angiosperms, and the *Exobasidiomycetes*, which are often less conspicuous, as many do not shed large amounts of blackish spores. The leaf-spot causing members of the genus *Entyloma* (*Entylomatales*, *Exobasidiomycetes*) belong to the latter group. Currently, 172 species that all infect eudicots are included in the genus. Vánky (2012) recognised five *Entyloma* species on species of *Ranunculus* s.lat. Two have been reported only from *Ficaria verna* s.lat., while three, *E. microsporum*, *E. ranunculi-repentis*, *E. verruculosum*, have been reported to have a broad host range, encompassing 30, 26, and 5 species of *Ranunculus*, respectively. This broad host range is in contrast to the generally high host specificity assumed for species of *Entyloma*, indicating that they may represent complexes of specialised species. The aim of this study was to investigate *Entyloma* on *Ranunculus* s.lat. using multigene phylogenies and morphological comparisons. Phylogenetic analyses on the basis of up to four loci (ITS, *atp*2, *ssc*1, and *map*) showed a clustering of *Entyloma* specimens according to host species. For some of these *Entyloma* lineages, names not currently in use were available and reinstated. In addition, *Entyloma microsporum* s.str. is neotypified. Six novel species are described in this study, namely, *Entyloma jolantae* on *Ranunculus oreophilus*, *E. klenkei* on *R. marginatus*, *E. kochmanii* on *R. lanuginosus*, *E. piepenbringiae* on *R. polyanthemos* subsp. *nemorosus* (type host) and *R. repens*, *E. savchenkoi* on *R. paludosus*, and *E. thielii* on *R. montanus*. For all species diagnostic bases and morphological characteristics are provided. The results in this study once more highlight the importance of detailed re-investigation of broad host-range pathogens of otherwise specialised plant pathogen groups.

Article info Received: 4 January 2018; Accepted: 20 March 2018; Published: 11 July 2018.

INTRODUCTION

The smut fungi in a broad sense (*Ustilaginomycotina*) contain more than 1 600 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. 2014a, Rooney-Latham 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-repentis*, and *E. verruculosum*. Two species, *Entyloma ficariae* and *E. majewskii*, infect hosts in the genus *Ficaria* that is closely related to *Ranunculus* (Hörandl et al. 2005, Emadzade et al. 2010). Only three *Entyloma* species, *E. microsporum*, *E. ranunculi-repentis*, and *E. verruculosum*, were reported to infect species of the genus *Ranunculus* s.str. (Vánky 2012).

[1] Department of Biosciences, Institute of Ecology, Evolution and Diversity, Goethe University Frankfurt am Main, Max-von-Laue-Str. 9, D-60438 Frankfurt am Main, Germany;
corresponding author e-mail: m.thines@thines-lab.eu.
[2] Biodiversity and Climate Research Centre (BiK-F), Senckenberg Gesellschaft für Naturforschung, Senckenberganlage 25, D-60325 Frankfurt am Main, Germany.
[3] Department of Mycology, W. Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, PL-31-512 Kraków, Poland.
[4] Plant Evolutionary Ecology, Institute of Evolution and Ecology, University of Tübingen, Auf der Morgenstelle 5, D-72076 Tübingen, Germany.
[5] Integrative Fungal Research Cluster (IPF), Georg-Voigt-Str. 14-16, D-60325 Frankfurt am Main, Germany.
major contributors.

Table 1 Smut specimens used for phylogenetic analysis.

Species	Host	Location	Location details	Date	Collector	DNA-no.	Fungarium no.	GenBank no.			
								ITS	*atp2*	*ssc1*	*map*
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.	09.03.2016	J. Kruse	3471	GLM-F107632	MF924658	MH022782	MF939230	MF939296
	R. paludosus	Spain, Andalusia	Cazorla, Parque Natural Sierras de Cazorla, 2.2 km E of Burunchel, A-319, slip rocks at wayside, N37°56'50" W02°56'28", elev. c. 1200 m a.s.l	23.04.2015	J. Kruse	3211	GLM-F107633	MF924651	MH022775	MF939223	MF939289
	R. paludosus	Greece, Rhodes	c. 2.8 km NW of Lindos, Phrygana, way up to the mountain, hiking path, N36°05'48" W28°03'13", elev. c. 145 m a.s.l.	10.03.2016	J. Kruse	3467	GLM-F107634	MF924654	MH022778	MF939226	MF939292
	R. paludosus	Greece, Rhodes	eastcoast, c. 3.5 km NE of Archangelos: Tsambika, way up Kloster, northern slope, Phrygana, N36°14'16" E28°09'16", elev. c. 160 m a.s.l.	11.03.2016	J. Kruse	3468	GLM-F107635	MF924655	MH022779	MF939227	MF939293
	R. paludosus	Greece, Rhodes	c. 1 km S of Salakos, way up Mt Profitis Ilias, Phrygana, N36°17'03" E27°56'38", elev. c. 275 m a.s.l.	13.03.2016	J. Kruse	3469	GLM-F107636	MF924656	MH022780	MF939228	MF939294
	R. paludosus	Greece, Rhodes	c. 1 km NW of Siana, way up to Akramitis, open Phrygana, plateau, N36°09'23" E27°45'59", elev. c. 650 m a.s.l.	15.03.2016	J. Kruse	3470	GLM-F107637	MF924657	MH022781	MF939229	MF939295
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.	28.05.2013	J. Kruse	107	GLM-F107639	MF924630	MH022754	MF939209	MF939275
	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.	31.05.2013	J. Kruse	108	GLM-F107640	MF924631	MH022755	MF939210	MF939276
	R. bulbosus	Germany, Bavaria	Oberfranken, S of Bayreuth, Swedebridge in direction to Studentwood, wayside, MTB/Q: 6035/34, elev. c. 360 m a.s.l.	12.06.2013	J. Kruse	109	GLM-F107641	MF924632	MH022756	–	–
	R. bulbosus	Germany, Baden-Württemberg	Swabian Alps, county Sigmaringen, Beuron, Leibertingen-Wildenstein, castle Wildenstein, N48°03'21" E9°00'0", MTB/Q: 7919/13, elev. c. 760 m a.s.l.	07.06.2014	J. Kruse	3049	GLM-F107642	MF924649	MH022773	MF939221	MF939287
	R. bulbosus	Germany, Hesse	Taunus, Bad Nauheim, Bad Nauheimer street, wayside, N50°22'50" E08°44'45", MTB/Q: 5618/12, elev. c. 175 m a.s.l.	09.11.2015	J. Kruse	3496	GLM-F107643	MF924665	MH022789	MF939237	MF939303
	R. bulbosus	Germany, Hesse	Main-Taunus-county, Hattersheim at Main, grasland at Welschenstream, Kuckuckspfad, wayside, N50°03'54" E08°30'03", MTB/Q: 5917/13, elev. c. 90 m a.s.l.	30.04.2016	J. Kruse	3621	GLM-F107644	MF924666	MH022790	MF939238	MF939304
	R. bulbosus	Italy, Liguria	Lower Varavalley, c. 1.5 km SW of Tavarone, circular path, Monte Alpe from Agriturismo Giandriale, east slope, meadow, N44°18'28" E09°31'58", elev. c. 725 m a.s.l.	10.05.2016	J. Kruse	3622	GLM-F107645	MF924667	MH022791	MF939239	MF939305
	R. bulbosus	Germany, Saxony-Anhalt	Kyffhäuser-northern area, county Sangerhausen: SW of Kelbra, Großes Rabental, wayside, N51°25'33" E11°01'14", MTB/Q: 4532/33	13.05.2008	H. Jage	2317	GLM-F095089	MF924639	MH022763	–	–
	R. repens	Germany, Baden-Württemberg	Swabian Alps, county Sigmaringen, Leibertingen-Wildenstein, S of Beuron, ascent castle Wildenstein, mixed forest, wayside, N48°02'49" E08°58'17", MTB/Q: 7919/42, elev. c. 682 m a.s.l.	06.06.2014	J. Kruse	3045	GLM-F107638	MF924646	MH022770	MF939218	MF939284
	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.	23.04.2010	J. Kruse	110	GLM-F107648	MF924633	MH022757	MF939211	MF939277
	R. repens	Germany, Schleswig-Holstein	county Rendsburg-Eckernförde, Barkelsby, Schusterredder, wayside, MTB/Q: 1425/33, elev. c. 22 m a.s.l.	25.04.2011	J. Kruse	113	GLM-F107649	MF924634	MH022758	–	–
	R. repens	Germany, Hesse	Franfurt at Main, Sachsenhausen, Landwehrstreet,South-Cementery, N50°05'20" E08°41'43", MTB/Q: 5918/11, elev. c. 150 m a.s.l.	22.03.2014	J. Kruse	3048	GLM-F107654	MF924648	MH022772	MF939220	MF939286
	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.	17.08.2014	J. Kruse	3046	GLM-F107653	MF924647	MH022771	MF939219	MF939285
	R. repens	Germany, Bavaria	Upper Bavaria, Chiemgauer Alps, county Rosenheim, Priener cabin, climb down towards Berg, Via Alpina, firsforest, wayside, N47°41'41" E12°18'24", MTB/Q: 8339/22, elev. c. 1290 m a.s.l.	22.07.2014	J. Kruse	3044	GLM-F107652	MF924645	MH022769	–	–
	R. repens	Germany, Hesse	Rüsselsheim, county Groß-Gerau, Varkaustreet, forest cementary, wayside, N49°59'22" E08°26'10", MTB/Q: 6016/21, elev. c. 100 m a.s.l.	08.03.2015	J. Kruse	3641	GLM-F107651	MF924680	MH022804	MF939245	MF939311
	R. repens	Germany, Hesse	Wiesbaden, Stationstraße, Reisingeranlage, wayside, N50°04'21" E08°14'38", MTB/Q: 5915/12, elev. c. 110 m a.s.l.	21.03.2015	J. Kruse	3640	GLM-F107650	MF924679	MH022803	MF939244	MF939310
	R. repens	**Poland**	**Małopolska Province: Kraków-Pleszów, at Suchy Jar street**	**20.11.2010**	**M. Piątek**	3652	**KRAM F-59037**	**MF924689**	**MH022813**	–	–
	R. repens	Poland	Małopolska Province: near Bukowica Reserve, close to Wygiełzów	10.09.2014	J. & M. Piątek	3653	KRAM F-59038	MF924690	MH022814	–	–

Table 1 (cont.)

Species	Host	Location	Location details	Date	Collector	DNA-no.	Fungarium no.	GenBank no.			
								ITS	*atp2*	*ssc1*	*map*
E. eburneum (cont.)	*R. polyanthemos* subsp. *nemorosus*	Germany, Bavaria	Upper Bavaria, county Garmisch-Partenkirchen, c. 4.9 km NE of Mittenwald, Karwendel mountains, hiking path 266 from Rehbergalm to Hochland cabin, mixed mountain-forest, N47°27'37" E11°18'36", MTB/Q: 8533/24, elev. c. 1575 m a.s.l.	11.07.2016	J. Kruse	3659	GLM-F107647	MF924696	MH022820	MF939255	MF939321
	R. polyanthemos subsp. *nemorosus*	Austria, Salzburg	county Salzburg, Lungau, Prebersee, MTB/Q: 8849/1	14.08.2013	C. & F. Klenke	3631	GLM-F107646	MF924674	MH022798	–	–
E. ficariae	*Ficaria verna*	Germany, Schleswig-Holstein	Barkelsby, Schusterredder, wayside, MTB/Q: 1425/33, elev. c. 20 m a.s.l.	27.04.2008	J. Kruse	70	GLM-F107655	MF924702	MH022826	–	–
	Ficaria verna	Germany, Lower Saxony	Hannover, Misburg-North, Ludwig-Jahn-Street, country lane nearby the marlpit, wayside, MTB/Q: 3625/11, elev. c. 60 m a.s.l.	18.04.2011	J. Kruse	73	GLM-F107656	MF924703	MH022827	–	–
	Ficaria verna	Germany, Schleswig-Holstein	county Rendsburg-Eckernförde, Ascheffel, Old Station, near exit to the Asch Mt, mixed forest, wayside, MTB/Q: 1524/31, elev. c. 6 m a.s.l.	24.04.2011	J. Kruse	74	GLM-F107658	MF924704	MH022828	–	–
	Ficaria verna subsp. *chrysocephala*	Italy, Liguria	Varavalley, c. 2.5 km S of Varese Ligure, E of Stora, street from Sant Pietro Vare to Teviggo, shady wayside, N44°22'01" E09°37'39", elev. c. 530 m a.s.l.	08.05.2016	J. Kruse	3638	GLM-F107657	MF924677	MH022801	MF939242	MF939308
E. jolantae	***R. oreophilus***	**Poland**	**Małopolska Province: Tatra Mts, Mała Dolinka valley – northern slopes of Giewont Mt, elev. c. 1230 m a.s.l.**	**25.08.2008**	**J. & M. Piątek**	3650	**KRAM F-59030**	**MF924688**	**MH022812**	**MF939250**	**MF939316**
	R. oreophilus	Poland	Małopolska Province: Tatra Mts, Mała Dolinka valley – northern slopes of Giewont Mt, elev. c. 1260 m a.s.l.	25.08.2008	J. & M. Piątek	ML1535	KRAM F-59031	MF924714	MH022838	–	–
E. klenkei	***R. marginatus***	**Greece, Rhodes**	**c. 0.7 km W of Archipoli, Eparchiaki Odos Pastidas-Mesanagrou, field beneath street, N36°15'58" E28°03'11", elev. c. 185 m a.s.l.**	**13.03.2016**	**J. Kruse & V. Kummer**	3476	**GLM-F107659**	**MF924663**	**MH022787**	**MF939235**	**MF939301**
E. kochmanii	***R. lanuginosus***	**Italy, Liguria**	**Varavalley, c. 2 km NE of Caranza, Strada Provinciale from Caranza to passo della Cappelletta, canyon alluvial forest, N44°23'33" E09°38'44", elev. c. 840 m a.s.l.**	**09.05.2016**	**J. Kruse**	3639	**GLM-F107660**	**MF924678**	**MH022802**	**MF939243**	**MF939309**
E. majewskii	***Ficaria verna***	**Iran**	**Tehran Prov., 60 km E Tehran, Mts Elburz, 'Emamzadeh-Haskei', N35°50' E52°02', elev. c. 2610 m a.s.l.**	**17.05.1990**	**D. Ershad, T. Vánky & K. Vánky**	Efc34	**BRIP: HUV14888**	**MF924713**	**MH022837**	**MF939265**	**MF939331**
E. microsporum	*R. repens*	Germany, Lower Saxony	county Hildesheim, Brüggen, Kirschweg, Sieben Bergen, Mt Hohe Tafel, wayside, MTB/Q: 3924/42, elev. c. 395 m a.s.l.	08.05.2011	J. Kruse	95	GLM-F107667	MF924708	MH022832	MF939262	MF939328
	R. repens	Germany, Bavaria	Oberfranken, Bayreuth, cemetery Saas, Bärenleite, wayside, MTB/Q: 6035/3, elev. c. 360 m a.s.l.	24.05.2012	J. Kruse	96	GLM-F107668	MF924709	MH022833	MF939263	MF939329
	R. repens	Germany, Bavaria	Oberpfalz, national park Bavarian Wood, county Regen, W of Zwieseler Waldhaus, Watzlikhain, mixed mountain-forest on granite, MTB/Q: 6945/1, elev. c. 650 m a.s.l.	21.08.2012	J. Kruse	97	GLM-F107669	MF924710	MH022834	MF939264	MF939330
	R. repens	Germany, Bavaria	Oberpfalz, national park Bavarian Wood, county Regen, Zwieseler Waldhaus, Mittelsteig cabin, mixed mountain-forest on granite, MTB/Q: 6945/2, elev. c. 700 m a.s.l.	24.08.2012	J. Kruse	98	GLM-F107670	MF924711	MH022835	–	–
	R. repens	Germany, Bavaria	Oberfranken, Bayreuth, Eremitage, W of river Red Main, mixed forest, MTB/Q: 6035/42, elev. c. 375 m a.s.l.	02.05.2013	J. Kruse	99	GLM-F107671	MF924712	MH022836	–	–
	R. acris	Germany, Bavaria	Oberfranken, between Horbach at the Steinach and Leutendorf, flood hollow, MTB/Q: 5733/3, elev. c. 290 m a.s.l.	10.05.2013	J. Kruse	92	GLM-F107662	MF924705	MH022829	MF939261	MF939327
	R. repens	Germany, Baden-Württemberg	county Konstanz, Hegau, W of Singen, way up Mt Hohentwiel, wayside, MTB/Q: 8218/2, elev. c. 600 m a.s.l.	29.05.2013	J. Kruse	101	GLM-F107672	MF924624	MH022748	MF939205	MF939271
	R. repens	**Germany, Hesse**	**county Groß-Gerau, Ginsheim-Gustavsburg, Radweg zum Mainspitz-dreieck, wayside circular path, N49°59'37" E08°17'46", MTB/Q: 6015/22**	**17.11.2013**	**J. Kruse**	1631	**GLM-F107661, KRAM F-59043**	**MF924636**	**MH022760**	**MF939213**	**MF939279**
	R. repens	Austria, Tyrol	district Kufstein, county Walchsee, Kaiserwinkel, hiking track, Wandberg cabin towards Niederkaseralm, firsforest, slope, wayside, N47°41'16" E12°19'07", MTB/Q: 8339/22, elev. c. 1380 m a.s.l.	21.07.2014	J. Kruse	3040	GLM-F107675	MF924643	MH022767	MF939216	MF939282
	R. repens	Germany, Bavaria	Upper Bavaria, Chiemgauer Alps, county Rosenheim, way up Priener cabin, N47°42'00" E12°17'54", MTB/Q: 8239/44, elev. c. 1280 m a.s.l.	22.07.2014	J. Kruse	3038	GLM-F107674	MF924642	MH022766	–	–
	R. acris	Germany, Bavaria	county Rottal-Inn, Simbach, road St 2112, grasland at roundabout, N48°16'23" E13°00'53", elev. c. 370 m a.s.l.	14.08.2014	J. Kruse	3037	GLM-F107663	MF924641	MH022765	MF939215	MF939281

Table 1 (cont.)

Species	Host	Location	Location details	Date	Collector	DNA-no.	Fungarium no.	GenBank no.			
								ITS	*atp2*	*ssc1*	*map*
E. microsporum (cont.)	*R. repens*	Austria, Upper Austria	Braunau am Inn, Hagenau in Inncounty, Hagenauer street, grasland, sidearm of Inn, wayside, N48°16'23" E13°06'01", MTB/Q: 7744/2, elev. c. 340 m a.s.l.	18.08.2014	J. Kruse	3036	GLM-F107673	MF924640	MH022764	–	–
	R. repens	Germany, Baden-Württemberg	Eschberg, county Waldshut, Buckmattstraße, meadow and forest edge around Liederbach, N08°10'49" E08°10'49", MTB/Q: 8315/31, elev. c. 400 m a.s.l.	03.07.2015	J. Kruse	3643	GLM-F107665	MF924682	MH022806	MF939246	MF939312
	R. repens	Austria, Carinthia	Völkermarkt, SW of Bad Eisenkappel-Vellach, Koschuta, Trögener land road, Trögener Klamm, towards Trögern, wet slope at wayside, N46°27'53" E14°30'18", elev. c. 720 m a.s.l.	06.07.2015	J. Kruse	3644	GLM-F107666	MF924683	MH022807	MF939247	MF939313
	R. repens	Germany, Hesse	Gießen, Lahntal, county Gießen, c. 5.5 km SW of Gießen, Allendorf at river Lahn, above parking at TSV Allendorf, street „In der Lache“, Slopeforest at stream Kleebach, wayside, N50°33'18" E08°36'55", MTB/Q: 5417/23, elev. c. 165 m a.s.l.	21.05.2016	J. Kruse	3642	GLM-F107664	MF924681	MH022805	–	–
	R. repens	Poland	Małopolska Province: Tatra Mts, between Hala Kalatówki glade and Hala Kondratowa glade, elev. c. 1250 m a.s.l.	17.07.2005	J. & M. Piątek	3646	KRAM F-59039	MF924685	MH022809	–	–
	R. repens	Slovakia	on tourist track from Lucky to Choc Mt	28.06.2008	J. & M. Piątek	3647	KRAM F-59040	MF924686	MH022810	–	–
	R. repens	Poland	Małopolska Province, Tatra Mts, Hala Gąsienicowa glade (near Murowaniec mountain hut), elev. c. 1510 m a.s.l.	24.09.2005	J. & M. Piątek	3649	KRAM F-59041	MF924687	MH022811	MF939249	MF939315
E. piepenbringiae	*R. polyanthemos* agg.	Germany, Bavaria	Upper Bavaria, county Weilheim, N Pähl, E of Hartschimmelhof, ‘Goaslweide’, region F3, MTB/Q: 8033/31, elev. c. 730 m a.s.l.	14.05.2013	J. Kruse	93	GLM-F107688	MF924706	MH022830	–	–
	R. polyanthemos agg.	Germany, Bavaria	Upper Bavaria, county Weilheim, N Pähl, E Hartschimmelhof, ‘Goaslweide’, region F4, MTB/Q: 8033/31, elev. c. 730 m a.s.l.	13.05.2013	J. Kruse	94	GLM-F107689	MF924707	MH022831	–	–
	R. repens	Germany, Baden-Württemberg	county Konstanz, communal Moos, S of Weiler, nearby Grey Reed, wayside, MTB/Q: 8219/4, elev. c. 445 m a.s.l.	30.05.2013	J. Kruse	102	GLM-F107694	MF924625	MH022749	MF939206	MF939272
	R. sp.	Spain, Andalusia	Cazorla, Parque Natural Sierras de Cazorla, c. 2.2 km S of Cazorla, hicking track, ascent Gililllo, slip rock, N37°53'30" W02°59'49", elev. c. 1185 m a.s.l.	24.04.2015	J. Kruse	3210	GLM-F107695	MF924650	MH022774	MF939222	MF939288
	R. polyanthemos* subsp. *nemorosus	**Germany, Bavaria**	**Oberallgäu, Einödsbach, Allgäu Alps, hiking path from Black Cabin to Rappensee cabin, meadow W of Rappensee cabin, N47°17'24" E10°14'40", MTB/Q: 8727/12, elev. c. 1900 m a.s.l.**	**26.07.2015**	**J. Kruse**	3493	**GLM-F107687**	**MF924664**	**MH022788**	**MF939236**	**MF939302**
	R. polyanthemos subsp. *nemorosus*	Germany, Bavaria	Upper Bavaria, county Garmisch-Partenkirchen, c. 2.8 km SE of Mittenwald, Karwendel mountains, hiking path 290 towards Brunnstein cabin, serpentines, light mixed mountain-forest, N47°24'44" E11°16'23", MTB/Q: 8533/43, c. 1260 m a.s.l.	06.07.2016	J. Kruse	3664	GLM-F107693	MF924701	MH022825	MF939260	MF939326
	R. polyanthemos subsp. *nemorosus*	Germany, Bavaria	Upper Bavaria, county Garmisch-Partenkirchen, c. 2.8 km SE of Mittenwald, Karwendel mountains, hiking path 290 towards Brunnstein cabin, serpentines, light mixed mountain-forest, N47°24'48" E11°16'33", MTB/Q: 8533/43, elev. c. 1380 m a.s.l.	06.07.2016	J. Kruse	3663	GLM-F107692	MF924700	MH022824	MF939259	MF939325
	R. polyanthemos subsp. *nemorosus*	Germany, Bavaria	Upper Bavaria, county Garmisch-Partenkirchen, c. 3.2 km SE of Mittenwald, Karwendel mountains, hiking path 291 from Brunnstein cabin towards Mt Brunnsteinspitze, scree, N47°24'33", E11°16'59", MTB/Q: 8533/43, elev. c. 1760 m a.s.l.	07.07.2016	J. Kruse	3662	GLM-F107691	MF924699	MH022823	MF939258	MF939324
	R. polyanthemos subsp. *nemorosus*	Germany, 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", MTB/Q: 8533/43, elev. c. 1475 m a.s.l.	08.07.2016	J. Kruse	3661	GLM-F107690	MF924698	MH022822	MF939257	MF939323
	R. polyanthemos agg.	Austria, Tyrol	Lechtal, N of Elbigenalp, wayside in mixed forest	26.08.2002	U. Fischer & M. Lutz	ML523	TUB-012566	MF924716	MH022840	MF939267	MF939333
	R. sp.	Switzerland	Kanton Bern, north bottom slope of Sustenpass, c. 4 km to Hotel Steingletscher, meadow	12.06.2003	W. Maier & M. Lutz	ML614	TUB-012567	MF924717	MH022841	MF939268	MF939334
	R. polyanthemos subsp. *nemorosus*	Slovenia	Triglav National Park, Lopucnica, way to Siebenseen cabin, tall herbaceous vegetation	02.08.2005	M. Kemler	ML838	TUB-012568	MF924718	MH022842	MF939269	MF939335
	R. repens	Germany, Baden-Württemberg	Tübingen, Bebenhausen, Goldersbachtal, littoral of lake, N48°33'30" E09°02'48", elev. c. 370 m a.s.l.	14.06.2002	M. Lutz	ML471	TUB-012570 MF924626	MF924715 MH022750	MH022839 MF939207	MF939266 MF939273	MF939332
E. ranunculacearum	*R. acris*	Germany, Bavaria	Oberfranken, county Bamberg, SE of Sandhof, Mönchsweiher, mixed forest on Keuper-Sandstone, MTB/Q: 6030/2, elev. c. 290 m a.s.l.	05.05.2012	J. Kruse	103	GLM-F107676	MF924627	MH022751	–	–

Table 1 (cont.)

Species	Host	Location	Location details	Date	Collector	DNA-no.	Fungarium no.	GenBank no.			
								ITS	*atp2*	*ssc1*	*map*
E. ranunculacearum (cont.)	*R. acris*	Germany, Bavaria	Oberpfalz, national park Bavarian Wood, county Regen, Zwieseler Waldhaus, Mittelsteig cabin, mixed mountainous forest on granite, MTB/Q: 6945/2, elev. c. 700 m a.s.l.	24.08.2012	J. Kruse	104	GLM-F107677	MF924628	MH022752	MF939208	MF939274
	R. acris	Germany, Baden-Württemberg	county Konstanz, Lake Constance, Radolfzell, SE of Möggingen, Mindelsea, circular path around sea, littoral and wayside, MTB/Q: 8220/1, elev. c. 420 m a.s.l.	30.05.2013	J. Kruse	105	GLM-F107678	MF924637	MH022761	MF939214	MF939280
	R. acris	**Germany, Saxony-Anhalt**	**county Wittenberg, Kemberg, district Rotta-Gniest, Heidestreet, wayside, N51°45'4" E12°35'33", MTB/Q: 4241/23, elev. c. 105 m a.s.l.**	**13.11.2013**	**J. Kruse**	1632	**GLM-F107680**	**MF924635**	**MH022759**	**MF939212**	**MF939278**
	R. acris	Germany, Hesse	Rheingau-Taunus-county, Eltville at river Rhein, Rheinsteig direction forest-restaurant Rausch, N50°02'46", E08°05'44", MTB/Q: 5914/41, elev. c. 160 m a.s.l.	08.03.2014	J. Kruse	1373i	GLM-F107679	MF924644	MH022768	MF939217	MF939283
	R. acris	Germany, Bavaria	Kirchdorf at Inn, Lower Bavaria, county Rottal-Inn, Hitzenau, Eckener street, wayside, N48°15'56" E12°58'53", MTB/Q: 7743/24, elev. c. 400 m a.s.l.	17.08.2014	J. Kruse	3043	GLM-F107681	MF924668	MH022792	–	–
	R. acris	Germany, Saarland	Mettlach-Orscholz, county Merzig-Wadern, Cloef-Street, surroundings of Cloef-Atrium and Varadeser Park, N49°30'20" E06°32'06", MTB/Q: 6405/33, elev. c. 395 m a.s.l.	29.09.2014	J. Kruse	3623	GLM-F107684	MF924653	MH022777	MF939225	MF939291
	R. acris	Germany, Hesse	Hoher Meißner, Meißner eastern slope, Fulda-Werra-uplands, Werra-Meißner-county, Frau Holle lake, circular path, N51°13'09" E09°52'07", MTB/Q: 4725/33, elev. c. 640 m a.s.l.	09.06.2015	J. Kruse	3315	GLM-F107683	MF924652	MH022776	MF939224	MF939290
	R. acris	Germany, Hesse	Hoher Meißner, Meißner eastern slope, Fulda-Werra-uplands, Werra-Meißner-county, Frau Holle lake, circular path, alpine meadow, N51°13'06" E09°52'13", MTB/Q: 4725/33, elev. c. 620 m a.s.l.	09.06.2015	J. Kruse	3314	GLM-F107682	MF924676	MH022800	MF939241	MF939307
E. ranunculi-scelerati	*R. sceleratus*	Germany, Bavaria	Oberpfalz, county Grafenwöhr, E of Hütten, littoral of lake, N49°40'52" E11°58'42", MTB/Q: 6337/22, elev. c. 410 m a.s.l.	01.05.2016	G. Hübner	3637	GLM-F107685	MF924669	MH022793	–	–
	R. sceleratus	Germany, Saxony-Anhalt	SSE of Elsnigk, S of Würflauer Schachtlake, near road B 185, MTB/Q: 4238/12	03.11.2004	H. Jage	3624	GLM-F074573	MF924670	MH022794	–	–
	R. sceleratus	Germany, Saxony-Anhalt	SE of Allstedt, Ziegelrodaer forest (N-part), aiport Allstedt (NW edge), MTB/Q: 4634/21	23.10.2005	H. Jage	3625	GLM-F076138	MF924671	MH022795	–	–
	R. sceleratus	Germany, Saxony-Anhalt	SW Sülldorf, Sülzetal, wet ditch right next to the brook Sülze (nearby salty area), MTB/Q: 3935/34	04.11.2005	H. Jage	3626	GLM-F076159	MF924629	MH022753	–	–
	R. sceleratus	Germany, Saxony-Anhalt	Lodersleben, near castle, in the Querne, MTB/Q: 4635/12	06.05.2005	H. John & H. Jage	3627	GLM-F076186	MF924673	MH022797	–	–
	R. sceleratus	Germany, Saxony-Anhalt	Friedersdorf near Lohsa (South), WSW of Neuhof, near Ballackmill, Maxlake (part of Ballacklakes), surceased, MTB/Q: 4652/14	26.05.2006	H. Jage	3628	GLM-F086008	MF924691	MH022815	–	–
	R. sceleratus	**Poland**	**Mazowieckie Province: Warszawa-Wesoła**	**17.07.2015**	**P. Mędykowski**	3654	**KRAM F-59032**	**MF924672**	**MH022796**	–	–
E. ranunculorum	***R. auricomus***	**Germany, Bavaria**	**Oberfranken, county Kulmbach, Lindau, Mt chain Rough Mt, wayside, MTB/Q: 5934/2, elev. c. 410 m a.s.l.**	**12.05.2012**	**J. Kruse**	106	**GLM-F107686**	**MF924638**	**MH022762**	–	–
	R. auricomus	Germany, Saxony-Anhalt	E of Dölkau, Burgholz (E-part) Jagen 29, alluvial forest, MTB/Q: 4638/24, elev. c. 25 m a.s.l.	19.04.1998	H. Jage	1768	GLM-F048093	MF924659	MH022783	MF939231	MF939297
E. savchenkoi	*R. paludosus*	Greece, Rhodes	c. 1 km S of Salakos, way up to Mt Profitis Ilias, *Quercus coccifera* forest, N36°16'59" E27°56'42", elev. c. 320 m a.s.l.	13.03.2016	J. Kruse	3472	GLM-F107696	MF924660	MH022784	MF939232	MF939298
	R. paludosus	Greece, Rhodes	c. 1 km NW of Siana, way up Akramitis, open Phrygana, plateau, N36°09'23" E27°45'59", elev. c. 650 m a.s.l.	15.03.2016	J. Kruse	3473	GLM-F107697	MF924661	MH022785	MF939233	MF939299
	R. paludosus	Greece, Rhodes	c. 1.2 km SE of Theologos, olive grove, N36°22'00" E28°02'45", elev. c. 40 m a.s.l.	16.03.2016	J. Kruse	3474	GLM-F107698	MF924662	MH022786	MF939234	MF939300
	R. paludosus	**Greece, Rhodes**	**eastcoast, c. 2.5 km N of Kalathos, street towards Masari, wayside, olive grove, N36°08'47" E28°03'33", elev. c. 15 m a.s.l.**	**20.03.2016**	**J. Kruse**	3475	**GLM-F107699** MF924697	**MF924675** MH022821	**MH022799** MF939256	**MF939240** MF939322	**MF939306**
E. thielii	*R. montanus*	Germany, Bavaria	Oberallgäu, Einödsbach, Rappensee cabin, near Rappensea, wayside, N47°17'11" E10°15'19", MTB/Q: 8727/21, elev. c. 2080 m a.s.l.	29.07.2015	J. Kruse	3632	GLM-F107705	MF924695	MH022819	MF939254	MF939320
	R. montanus	Germany, Bavaria	Upper Bavaria, county Garmisch-Partenkirchen, c. 4.9 km NE Mittenwald, Karwendel mountains, hiking path 266 from Rehbergalm to Hochland cabin, mixed mountainous forest, N47°27'37" E11°18'36", MTB/Q: 8533/24, elev. c. 1575 m a.s.l.	11.07.2016	J. Kruse	3660	GLM-F107704	MF924694	MH022818	MF939253	MF939319

Table 1 (cont.)

Species	Host	Location	Location details	Date	Collector	DNA-no.	Fungarium no.	GenBank no.			
								ITS	*atp2*	*ssc1*	*map*
E. thielii (cont.)	*R. montanus*	Germany, Bavaria	Upper Bavaria, county Garmisch-Partenkirchen, c. 4.9 km NE of Mittenwald, Karwendel mountains, hiking path 266 from Rehbergalm to Hochland cabin, mixed mountain-forest, N47°27'37" E11°18'36", MTB/Q: 8533/24, elev. c. 1575 m a.s.l.	11.07.2016	J. Kruse	3658	GLM-F107703	MF924693	MH022817	MF939252	MF939318
	R. montanus	**Germany, Bavaria**	**Upper Bavaria, county Garmisch-Partenkirchen, c. 2.8 km SE of Mittenwald, Karwendel mountains, meadows around Brunnstein cabin, N47°24'49" E11°16'41", MTB/Q: 8533/43, elev. c. 1475 m a.s.l.**	**08.07.2016**	**J. Kruse**	3657	**GLM-F107702**	**MF924692**	**MH022816**	**MF939251**	**MF939317**
	R. montanus	Germany, Bavaria	Upper Bavaria, county Garmisch-Partenkirchen, c. 2.8 km SE of Mittenwald, Karwendel mountains, meadows around Brunnstein cabin, N47°24'49" E11°16'41", MTB/Q: 8533/43, elev. c. 1475 m a.s.l.	08.07.2016	J. Kruse	3656	GLM-F107701	MF924719	MH022843	MF939270	MF939336
	R. montanus	Germany, Bavaria	Upper Bavaria, county Garmisch-Partenkirchen, c. 2.8 km SE of Mittenwald, Karwendel mountains, hiking path 290 from Brunnstein cabin towards Mittenwald, serpentines, thin mixed mountainous forest, N47°24'48" E11°16'33", MTB/Q: 8533/43, elev. c. 1380 m a.s.l.	08.07.2016	J. Kruse	3655	GLM-F107700	MF924684	MH022808	MF939248	MF939314
	R. montanus	Germany, Bavaria	Oberjoch, Iseler, elev. c. 1500 m a.s.l.	29.09.1997	M. Piepenbring	rr502	TUB-012586	MF924658	MH022782	MF939230	MF939296
Entyloma verruculosum	*R. lanuginosus*	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", elev. c. 720 m a.s.l.	19.04.2016	J. Kruse	3645	GLM-F107706	MF924651	MH022775	MF939223	MF939289

Type specimens are printed in **bold** face.

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 microsporum* and *E. ranunculi-repentis* 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 *Caeoma bullosum* on *R. chaerophyllos* and *E. pygmaeum* on *R. pygmaeus* (Saccardo 1915, Ciferri 1928), *E. ranunculacearum* 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, *atp*2, *ssc*1, and *map*). For this, a broad set of host-fungus combinations was studied, including *Entyloma* specimen 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 Görlitz (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 microsporum* complex and the *E. ranunculi-repentis* 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, *atp*2, *ssc*1, and *map*) could be obtained, with four exceptions: for *Entyloma* sp. on *Ranunculus auricomus* specimens included in the two loci (ITS and *atp*2) 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.

Preparations for light microscopy (LM) were done as follows. Thin freehand sections of sori with spores and conidiophores and conidia (if present) were mounted in 80 % lactic acid, heated to the boiling point, and then immediately examined using a Nikon Eclipse 80i light microscope (Nikon) at ×1000. Thirty spores were measured using the Nikon NIS-Elements BR 3.0 imaging software (Nikon). Measurements were rounded to the nearest 0.5 µm. LM micrographs were taken with a Nikon DS-Fi1 camera (Nikon). The species descriptions include combined values from all analysed specimens of the respective species.

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) or 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 53 °C. The amplification of the *atp*2 (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 *ssc*1 (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 vaccinii* 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 *ssc*1 locus of *Entyloma* spp. this revealed the optimal primer pair to be *ssc*1_F3ex (5'GWGGWGAAGACTTYGACTTGT3') and *ssc*1_R5ex (5'ACACCACCYTGRATSGAAGC3') with an annealing temperature of 58 °C. For the amplification of the *map* locus of *Entyloma* spp. *map*_F3ex (5'AGYTGCTRATRTCGTTCCACCA3') and *map*_R3ex (5'CCAYGCCAAYTTGGCCAAGAC3') 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 (BiK-F, Senckenberg, 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'CCAAYTTGGCCAAGAC3'). Sequences were deposited in GenBank (accession numbers are given in Table 1).

Molecular phylogenetic reconstruction

In total 91 ITS, 91 *atp*2, 64 *ssc*1, 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 *atp*2 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 1 871 characters (ITS: 523, *atp*2: 480, *ssc*1: 394, *map*: 474) for dataset 2 the resulting total alignment contained 1 003 characters (ITS: 523, *atp*2: 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. sceleratus*, 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 *atp*2) were mostly lower than from four loci (ITS, *atp*2, *ssc*1, 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.

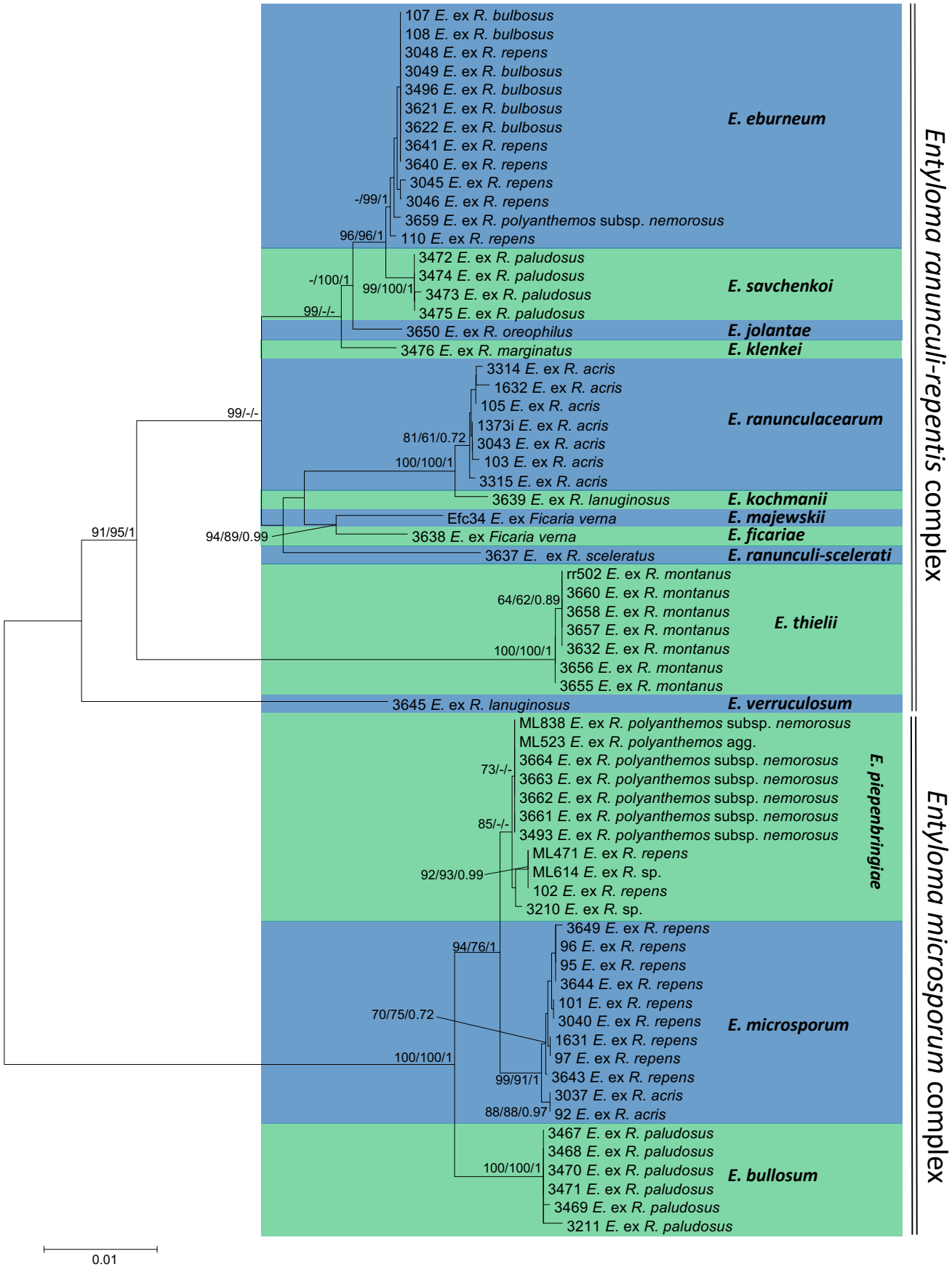

Fig. 1 Phylogenetic relationships of *Entyloma* species on *Ranunculus* spp., rooted with the specimens of the *Entyloma microsporum* complex, based on Minimum Evolution analyses of four loci (ITS, *atp*2, *ssc*1, and *map*). Numbers on branches denote bootstrap support in Minimum Evolution and Maximum 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.

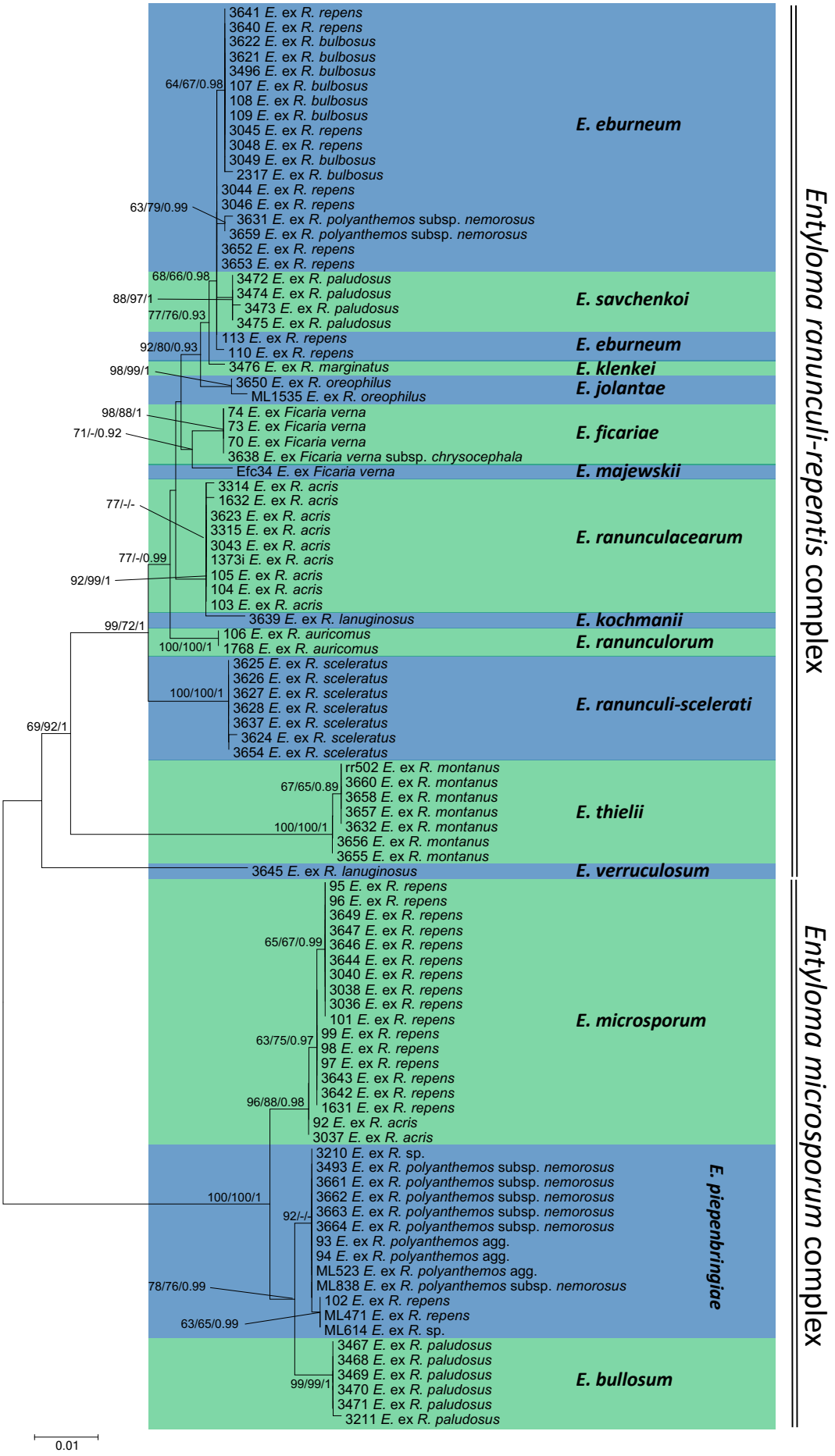

Fig. 2 Phylogenetic relationships of *Entyloma* species on *Ranunculus* spp., rooted with the specimens of the *Entyloma microsporum* complex, based on Minimum Evolution analyses of two loci (ITS and *atp*2). Numbers on branches denote bootstrap support in Minimum Evolution and Maximum Likelihood, as well as *a posteriori* probabilities from Bayesian Analyses. Values below 60 % are not shown. The scale bar indicates the number of substitutions per site.

*atp*2 – 480 bp

RGAGTCMARAGGGTCGAC**R**G C**R**GGGTA**R**ATRYC**B**AGSTCR GCAATACCACGRGCSAGCGA RGTGGTGGCGTCCAAGTGAG CGAAGGTSGTGGCKGGYGCA
GGRTCCGTCAAGTCATCRGC TGGYACGTATACGGCCTG**W**A C**S**GARGTRATGGAACCCTTC TTGGTCGT**R**GTAATGCGCTC TTGCATACCACCCATGTCGG
TGGASAGHGTRGGCTGGTA**R** CCTACGGCSGA**K**GGRATACG **R**CCGAGCAGGGCAGASGTCT CGGARCCRGCTTG**M**GTGAAR CGGAAAATGTTGTC**R**ATGAA
GAGCAGYACRTC**Y**TGTCCCC TRNN**N**R**HRTS**WR**N**KY**RH**RV**N NNNWRWGT**S**AG**YBBK**B**HD**DN N**BH**NRBBRWY**KH**RD**YRNYK**H** Y**RNN**ASM**RY**V**NNN**YTACTCA
CTCCTCGTCACGGAAGTACT C**R**GCAAT**V**GTCA**R**ACC**S**GT**R** AGGGCKACRCGGGCACG**V**GC **W**CCRGG**R**GGCTC**R**TTCATYT

ITS – 523 bp

TSAGACTGCAAAGAGTCTTT CGCCGRRTTAGAARCAGACR CW**NN**KG**Y**ACAAGAA**R**GTAAG GGCAGTTNNNNNRMCYGCCG CCTCAACGAATGACTTTATC
ACGTTGAGGACRCCTACRCA TN**YR**TRYAC**N**GGTCCAGCTA AT**R**MATTTGAGGTGAGC**B**GT **HN**NN**NNNNN**R**V**AACRGSAA **S**CACCCAAGGRCCAA**Y**CCCA
R**R**CAAC**R**C**R**A**B**AAAGCATTG AGGGT**K**GAGAATTCATGACA CTCAAACAGGCATGCTCCTC GGAATACCAAGGWGCGCAAG GTGCGTTCAAAGATTCGATG
ATTCACTGAATTCTGCAATT CACATTACTTATCGCATTTC GCTGCGTTCTTCATCGATGG GAGAACCAAGAGATCCGTTG TCAAAAGTTGT**N**TT**K**TGTTT
CTACTTGYGTAG**K**TACAGTG ACATTCAK**Y**TAYTTNNNNNN TGT**R**TG**WR**TAAA**N**GGN**K**TGC **YM**G**Y**R**R**CRRACRARATACT**K** GSGYATCRGBCGTCACTARC
GGTGCACAGGTGTGTGGATA TGG

map – 474 bp

VGA**B**ARTTCMGGGTTGGG**R**T G**R**TT**S**AG**D**GC**R**TCBGA**R**CT**D** CCGTGCTCGTGCARGTA**D**CC GGCRGGCGTRAGRACGTAGA A**S**GACTCYTT**R**TAVK**K**YTTG
GTGAARCGCTTCTT**R**CGCTC GAGAATGCCCTGGTGRACGC ARATRACSGAVGGRTC**Y**TCC TTGCC**K**GG**R**TA**R**TC**R**ATCGT YTCGGGGTTGCGCARRGGYG
TGTCWGG**R**TC**N**ARMAGGTG**R** TCCGA**R**CGMGCAGC**R**AARGC RATCCAYTCKGC**R**TT**B**GGCT GMAG**B**GCRCGCAT**Y**TGYTGA CCYARRCC**R**AGCCACGTGTC
YTGWACCTGDGCCGACATGC G**H**CCGCTCCACTCRTCRAAC GT**Y**TGCCAGGC**R**CTCTGRAT GCT**K**CGAACGATGCCCTCCT CRAAGTGAGCRCTGTTCTGC
TGCAT**R**ATRATGATRGACTT TTGYAG**B**GCRTTYTCCTCGT TDACTTGCTTTTGCARCTGA CGGAARACRGCRTT

*ssc*1 – 394 bp

RWGGAAGCRCCAATGGCAAC AGCYTC**R**TCGGGGTT**R**ACAC CCTTRGAYGG**R**TCGCG**Y**TTG AAGATGC**Y**CTT**R**AC**D**GTCTC **R**AGDACCTTKGGCAT**R**CGCG
ACATWCC**R**CC**D**ACCATGATG ACGTCCTGHACRTCGYTVGC C**Y**T**R**ATGCCTGCRTCRGCRA TGGCCTTCTK**R**CA**Y**GGCTC**R** ACSGTRCG**Y**TC**R**A**Y**GAGCTT
GCCAAC**V**AG**M**CCCTCRAGYT GCGMKCGGCTCATCTT**K**GTR TTGATGTGCTTGGG**Y**CC**R**GA **R**GC**R**TC**D**GCGGTRATGTAGG G**S**AGYGARATGTCSGTTTGC
GC**M**GTCGA**V**GARAGYTCRAT CTTGGCCTTCTCRGC**R**GCCT CGCG**R**ATRCGMTGAATGGCC ATGCGRTCYTGMRARAGGTC RATGCCAGA**R**TCCY

Fig. 3 Consensus sequences for *atp2*, ITS, *map*, and *ssc1*, with diagnostic positions for *Entyloma* species on *Ranunculus* highlighted in **bold** type.

Morphology

The three major phylogenetic lineages could be distinguished by teliospore surface ornamentation. Spores from species in the *E. microsporum* 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. microsporum* 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. microsporum* 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, depicted in Fig. 4–16, and summarised in Table 3.

TAXONOMY

In this section an overview on accepted *Entyloma* species on *Ficaria* and *Ranunculus* is given, and six new species are introduced. We have refrained from designating formal epitypes in the current study. The progress in sequencing technologies has already enabled the sequencing of the whole genome of specimens from the mid-19th century Irish Potato Famine (Yoshida et al. 2013, 2014). Thus, it seems to be only a matter of time until cheap and reliable whole genome sequencing from historic specimens will become routine. However, if the historic specimens turn out to be demonstrably devoid of DNA that can be used for sequencing, the reference specimens given in this section could be designated as epitypes.

Entyloma microsporum complex

Entyloma bullosum (Sacc.) J. Kruse, M. Lutz, Piątek & Thines, *comb. nov.* — MycoBank MB823957; Fig. 4

Basionym. *Caeoma bullosum* Sacc., Nuovo Giorn. Bot. Ital., n.s. 22: 32. 1915.

Type. Malta, Uied il Kleigha, on *Ranunculus* '*chaerophyllos*' (= *R. paludosus*), Mar. 1914, *A. Caruana-Gatto* (type could not be located, probably lost); – Greece, Rhodes, eastern coast, SE of Archangelos, c. 1.5 km S Stegna, Phrygana, northeast slope, N36°11'49" E28°08'06", elevation c. 70 m a.s.l., on *Ranunculus paludosus*, 9 Mar. 2016, *J. Kruse* (GLM-F107632 neotype designated here; MycoBank MBT380639; ex-type sequences available in GenBank: MF924658 (ITS), MH022782 (*atp2*), MF939296 (*ssc*1), MF939230 (*map*)).

Sori 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 polyangular, (11.5–)15.0–21.5(–26.5) × (10.5–)12.0–16.5(–19.5) 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, inner layer evenly thickened, outer layer unevenly thickened, spore surface rough or superficially cracked, rarely smooth. *Asexual morph* not found.

Diagnostic bases — Within the *E. microsporum* complex there are 19 diagnostic bases across all four loci (Fig. 3, Table 2).

Host plant — Parasitic on *Ranunculus paludosus*.

Additional specimens examined. Greece, Rhodes, c. 2.8 km NW of Lindos, Phrygana, way up Mountain, hiking path, N36°05'48" E28°03'13", elevation c. 145 m a.s.l., on *Ranunculus paludosus*, 10 Mar. 2016, *J. Kruse* (GLM-F107634); eastern coast, c. 3.5 km NE of Archangelos, Tsambika, way up to the monastery, northern slope, phrygana, N36°14'16" E28°09'16", elevation c. 160 m a.s.l., on *Ranunculus paludosus*, 11 Mar. 2016, *J. Kruse* (GLM-F107635); c. 1 km S of Salakos, way up to Mt Profitis Ilias, phrygana, N36°17'03" E27°56'38", elevation c. 275 m a.s.l., on *Ranunculus paludosus*, 13 Mar. 2016, *J. Kruse* (GLM-F107636); 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 paludosus*, 15 Mar. 2016, *J. Kruse* (GLM-F107637).

Notes — The smut specimens with swollen pustules on the leaves of *Ranunculus paludosus* are usually assigned to *Entyloma microsporum* (Vánky 2012), but the molecular analyses in the present study reveal that they form a distinct lineage, for which the name *Caeoma bullosum* is available. This species was described by Saccardo (1915) from leaves of *Ranunculus*

Table 2 The diagnostic bases within the *Entyloma microsporum* complex and the *Entyloma ranunculi-repentis* complex, apart from the type host of the respective complex.

	Gene Loci							
	atp2		*map*		*ssc1*		ITS	
	Position	Base	Position	Base	Position	Base	Position	Base
***E. microsporum* complex**								
on *Ranunculus paludosus*	232	T / G	28	G / A	36, 171	G / A	196	T / C
	364, 388, 440, 467	G / A	115, 289	A / G	68	T / C		
	375	C / T	256	T / G	237	T / G		
	382	A / G	322, 343	T / C	255	C / T		
					282	C / G		
***E. ranunculi-repentis* complex**								
on *Ranunculus acris*	x	x	x	x	27, 72, 96	A / G	x	x
					57	T / C		
on *Ranunculus auricomus*	142	C / G	x	x	x	x	168	G / A
	358	T / A o. G						
	437	G / C						
	440	G / A						
	473	A / G						
on *Ficaria verna* (*E. ficariae*)	x	x	22, 169	A / G	142	C / T	43	T / –
			364	T / G			44	G / –
							209	G / A
on *Ficaria verna* (*E. majewskii*)	328, 336, 428	A / G	4	T / G o. C	1, 261	G / A	226, 413	G / T
			19, 172	A / G	2	A / T		
					111	T / G o. A		
					258, 389	A / G		
on *Ranunculus lanuginosus*	389	T / C	x	x	144	A / G	462	C / A
on *Ranunculus marginatus*	458	C / G	226, 235	G / A	345	A / G	x	x
			253	A / G o. R				
			274	C / T				
on *Ranunculus montanus*	22, 28, 220, 241	G / A	1, 211	A / C o. G	75, 267	A / G o. T	130, 143, 171, 202, 207	A / G
	139	A / T	28, 58	G / T o. A	81, 108, 264, 267	A / G	47, 123,162, 429, 461	C / T
	274	C / A	37, 92, 175, 208, 220	A / G	189	T / C	55, 124, 169, 172, 444	G / A
	313, 327, 337, 352, 378	C / T	96	T / C	309	G / C	181	C / G
	325, 391	– /A o. G	166	G / T			457, 480	G / T
	354	T / G o. C	352	G / A			158	T / G
	339	C / A o. G	427	G / T o. C			161	A / T
	355	T / G					164	– / G
	362	G / T					211	G / T o. C
	372	C / T o. A					392	– / A
	380, 461	T / A						
	384	A / G o. C						
	392	– / T						
	422, 433	A / G						
on *Ranunculus oreophilus*	295, 374	A / G	31	A / G	192	A / G	447	A / T
	330	C / G			194	C / T		
on *Ranunculus paludosus*	333	T / G o. –	x	x	207	A / G o. C	395	G / T
							464	C / T
on *Ranunculus sceleratus*	1, 169	A / G	25, 82	C / G	51	A / G	448, 466	A / G
	34, 357	T / C	40	T / G	174	C / T	453	– / G
	349	G / C	94	A / G o. C	180, 336	G / A		
	354	C / G o. T	157	T / C	210, 303	C / A		
	358	G / A o. T	265	G / T o. C				
	371	T / G	406	A / G				
	384	C / A o. G						
	393	T / C o. –						

* / = instead of; x = no diagnostic bases; o. = or.

chaerophyllos collected in Malta. Sydow (1924) considered that *Caeoma bullosum* was identical with *E. microsporum*. In the protologue, Saccardo (1915) did not provide the author of the name *Ranunculus chaerophyllos*, and in the current usage this name can be applied to three species, namely *R. chaerophyllos*, *R. gracilis*, and *R. paludosus*, of which only the latter occurs in Malta (Euro+Med 2006–onwards). Thus, the host plant for *Caeoma bullosum* is assumed to have been *Ranunculus paludosus*. No authentic material of this species is currently preserved in the herbarium of P.A. Saccardo deposited in PAD (R. Marcucci, pers. comm.) or in the herbarium of H. Sydow in B (R. Lücking, pers. comm.). The morphological characters provided in the protologue (swollen sori, 1–2 mm diam, yellow, angular, globose, as well as ellipsoidal spores 20–23 × 18–20 µm, spore wall of 3–5 µm thickness with a more or less warty surface; – excerpt from the Latin description) agree well with the morphology of the specimens analysed in the current study. Therefore, a neotype was designated from among the sequenced specimens to fix the application of this name. *Entyloma bullosum* differs from the other currently recognized species in the *E. microsporum* complex by a larger mean spore size and thicker spore walls.

Entyloma microsporum (Unger) J. Schröt., 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*.

Type. Austria, Tirol, Kitzbühel, on *Ranunculus repens*, *F. Unger* (type could not be located, probably lost). – Germany, Hesse, county Groß-Gerau, Ginsheim-Gustavsburg, bikeway to Mainspitzdreieck, wayside, N49°59'37" E08°17'46", on *Ranunculus repens*, 17 Nov. 2013, *J. Kruse* (GLM-F107661 neotype designated here; KRAM F-59043 isoneotype; MycoBank MBT380061; ex-type sequences available in GenBank: MF924636 (ITS), MH022760 (*atp*2), MF939279 (*ssc*1), MF939213 (*map*)).

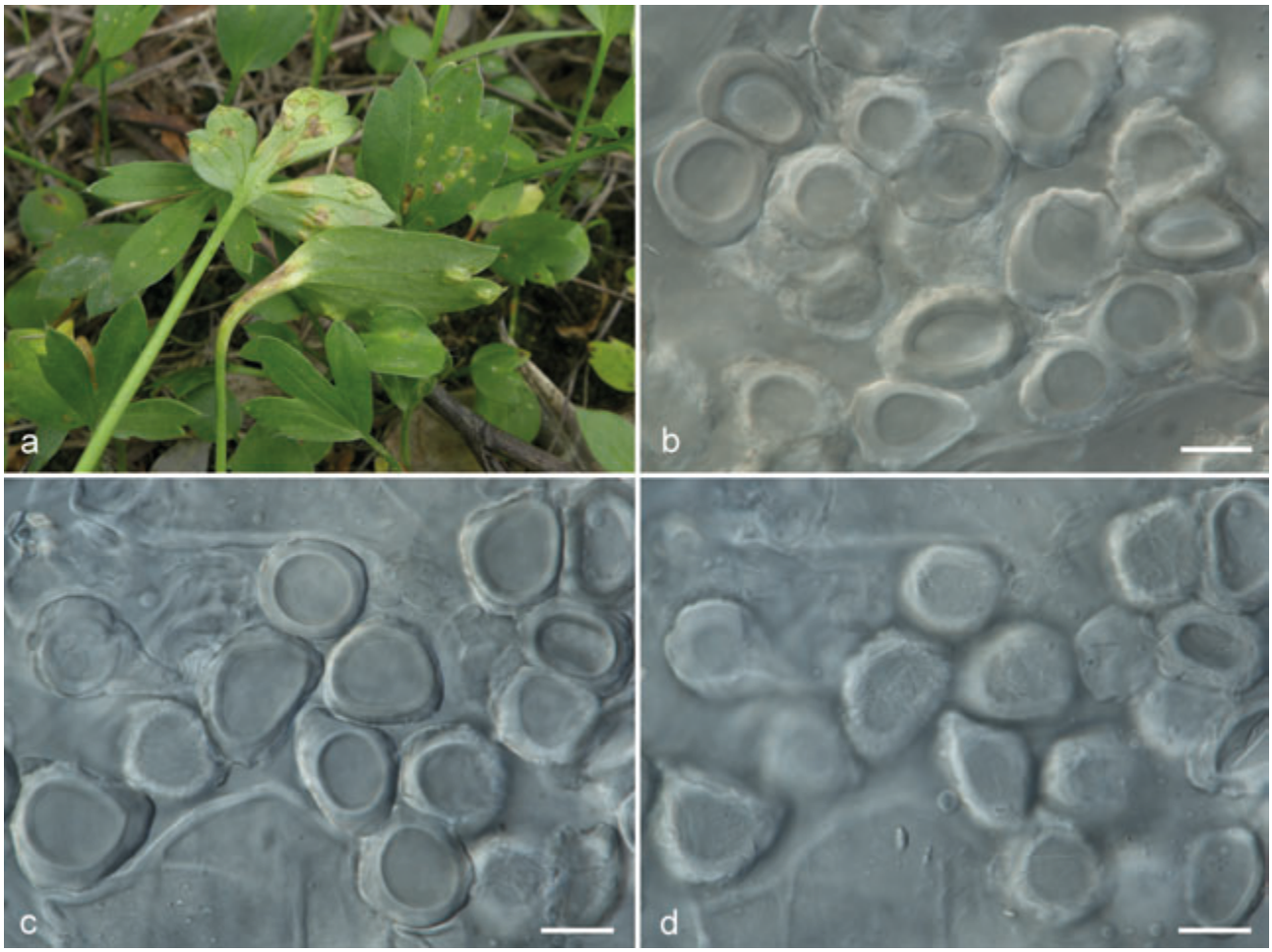

Fig. 4 *Entyloma bullosum* on *Ranunculus paludosus*. a. Macroscopic symptoms of infection; b–d. spores, as seen in light microscopy, median and superficial views (from neotype). — Scale bars = 10 µm.

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

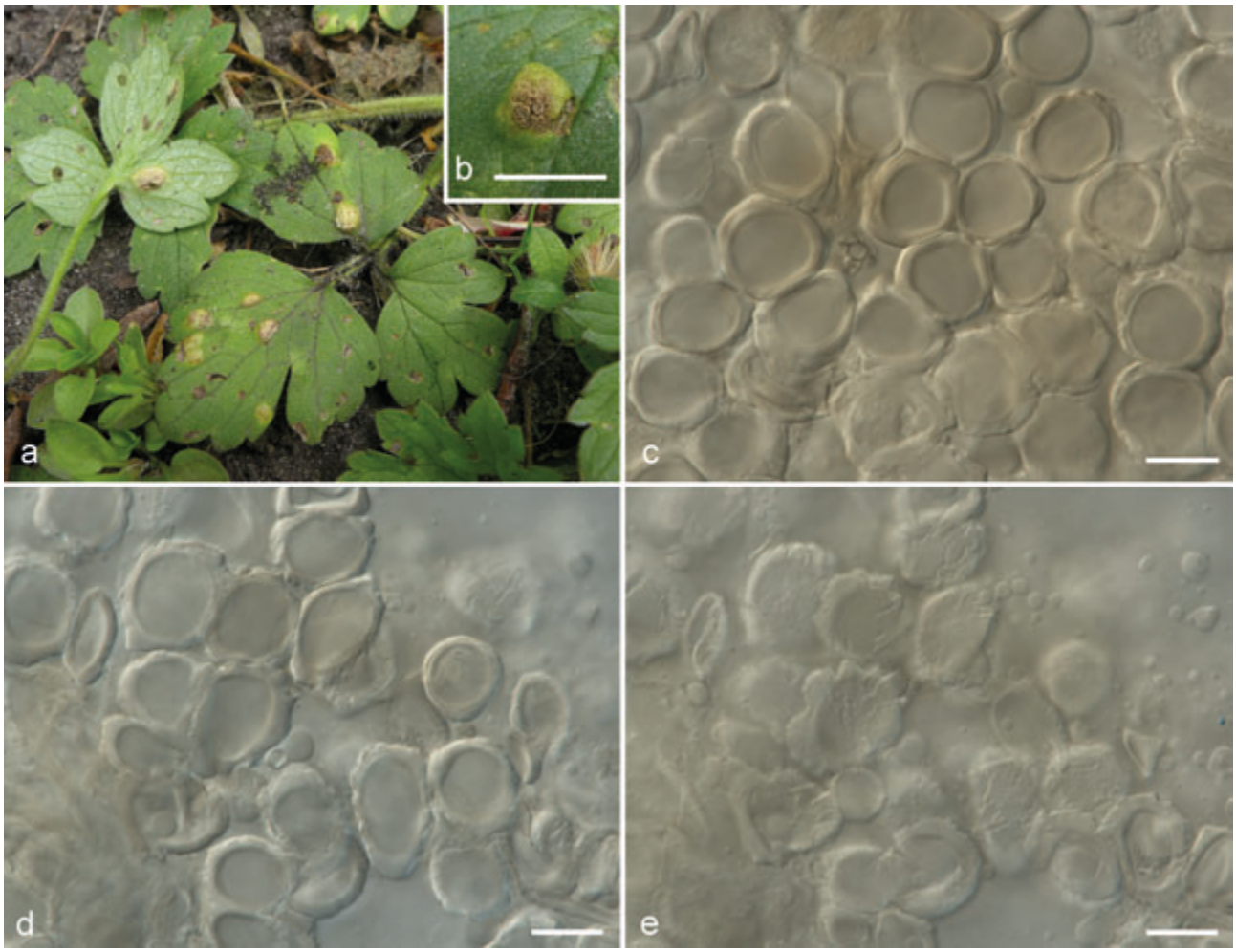

Fig. 5 *Entyloma microsporum* on *Ranunculus repens*. a–b. Macroscopic symptoms of infection; c–e. spores, as seen in light microscopy, median and superficial views (from neotype). — Scale bars: b = 5 mm; c–e = 10 µm.

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, Einödsbach, 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 290 towards Brunnstein cabin, serpentines, open mixed mountainous forest, N47°24'44" E11°16'23", elevation c. 1260 m a.s.l., on *Ranunculus polyanthemos* subsp. *nemorosus*, 6 July 2016, *J. Kruse* (GLM-F107693); c. 3.2 km SE of Mittenwald, Karwendel mountains, 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).

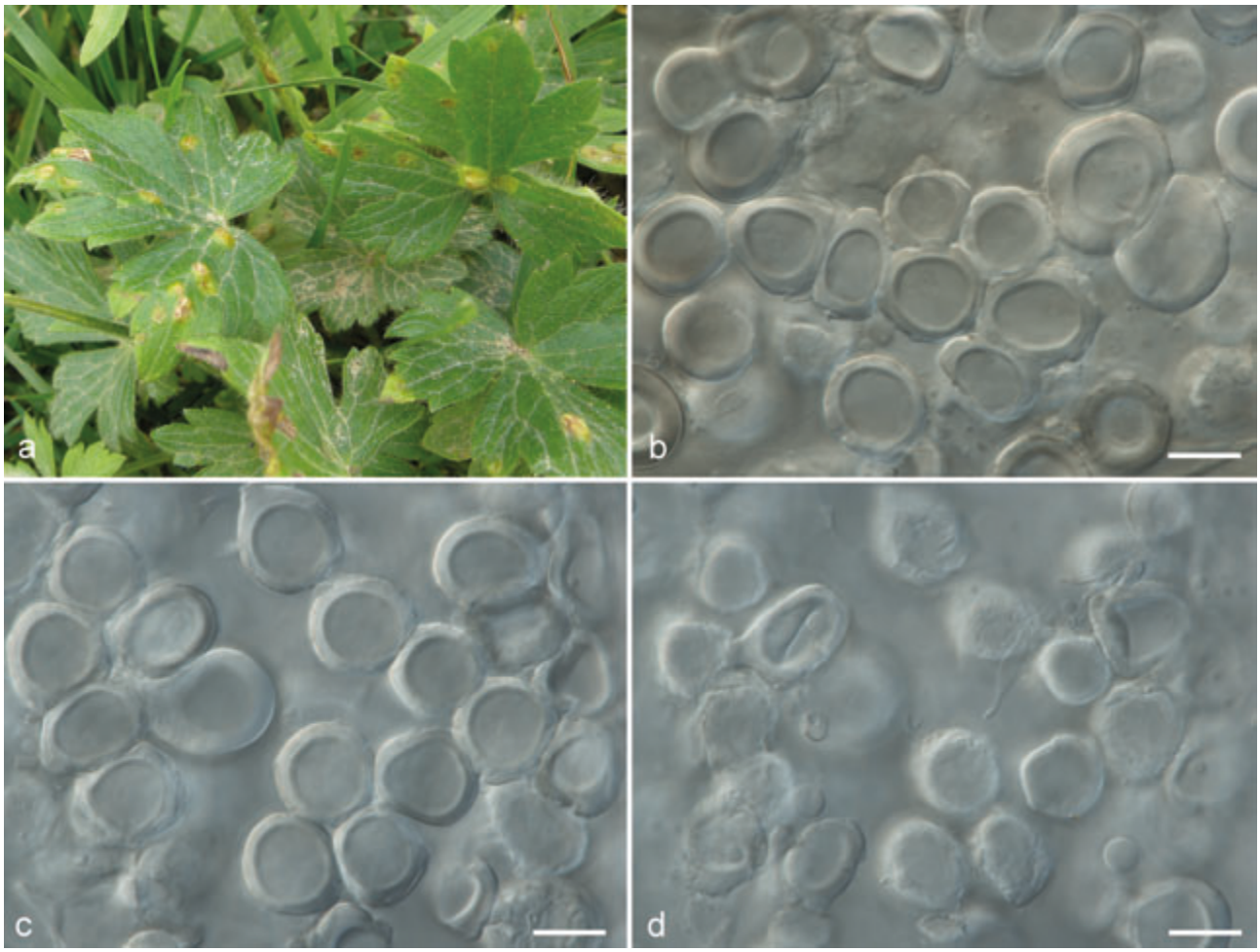

Fig. 6 *Entyloma piepenbringiae* on *Ranunculus polyanthemos* subsp. *nemorosus*. a. Macroscopic symptoms of infection; b–d. spores, as seen in light microscopy, median and superficial views (from holotype). — Scale bars = 10 µm.

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 ranunculi-repentis complex

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

Basionym. *Fusidium eburneum* J. Schröt., 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 (*atp*2)).

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 ranunculi-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, polyangular or irregular spots, 0.5–4 mm long, 0.5–2 mm wide, usually partly delineated 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 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 × 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-F107638); Bavaria, Upper Bavaria, county Garmisch-Partenkirchen, c. 4.9 km NE of Mittenwald, Karwendel mountains, hiking path 266 from Rehbergalm to

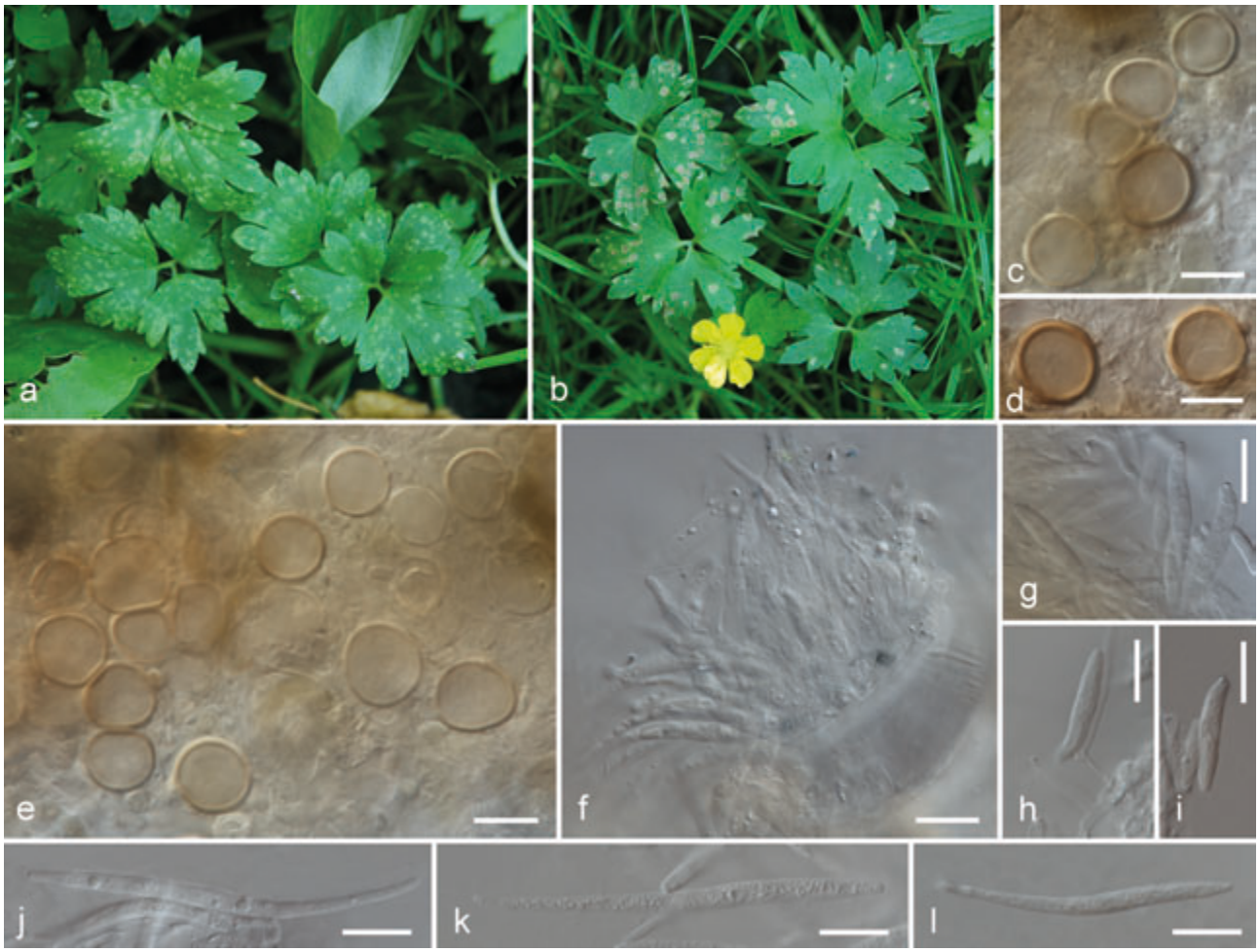

Fig. 7 *Entyloma eburneum* on *Ranunculus repens*. a–b. Macroscopic symptoms of infection; c–e. spores, as seen in light microscopy; f. conidiophores emerging through the stoma, as seen in light microscopy; g–i. cylindrical conidia (with one conidiophore on 'h'), as seen in light microscopy; j–l. acicular conidia, as seen in light microscopy (from neotype). — Scale bars = 10 µm.

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, Kuckuckspfad, 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). – Italy, Liguria, Lower Varavalley, c. 1.5 km SW Tavarone, circular path, Monte Alpe from Agriturismo Giandriale, east slope, meadow, N44°18'28" E09°31'58", elevation c. 725 m a.s.l., on *Ranunculus bulbosus*, 10 May 2016, *J. Kruse* (GLM-F107645).

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 (*atp*2), MF939316 (*ssc*1), 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 polyangular – 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).

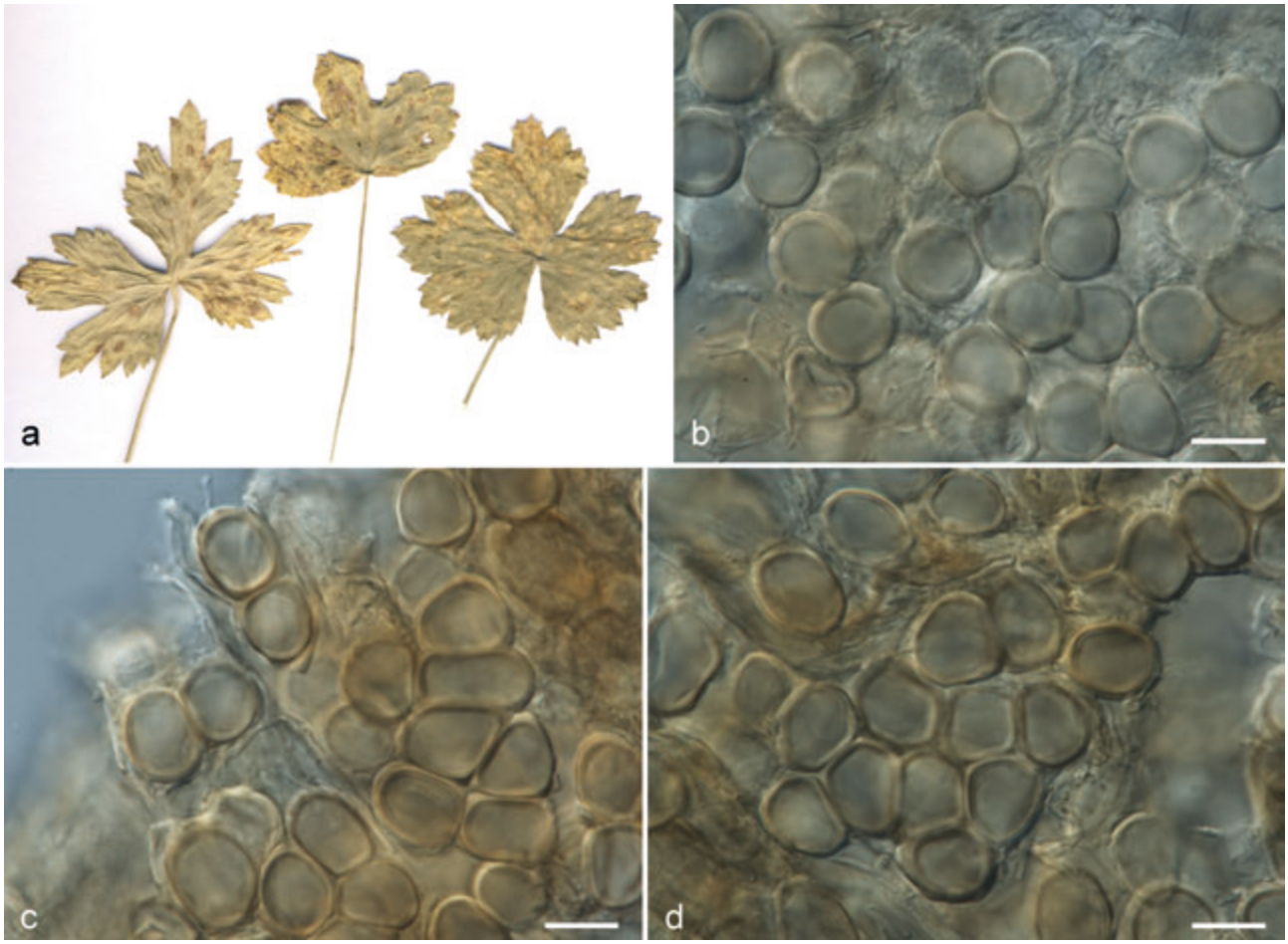

Fig. 8 *Entyloma jolantae* on *Ranunculus oreophilus*. a. Macroscopic symptoms of infection (two leaves to the left from holotype, one leaf to the right from paratype); b–d. spores as seen in light microscopy (from holotype). — Scale bars = 10 µm.

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 *Pflanzenparasitische 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, 1.0–1.8 µm thick (including inner layer c. 0.5 µm thick), without angles, layers 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 six diagnostic bases distributed among all loci except ITS (Fig. 3, Table 2).

Host plant — Parasitic on *Ranunculus marginatus*.

Notes — This species differs from most other species in the *E. ranunculi-repentis* complex by the combination of small spores (with thin walls), which are loosely scattered between leaf mesophyll cells, and lacking an asexual morph. The most similar species is *Entyloma thielii*, which differs in having densely crowded spores, often in compact groups, in the intercellular space between the mesophyll cells.

Entyloma kochmanii J. Kruse, M. Lutz, Piątek & Thines, *sp. nov.* — MycoBank MB824515; Fig. 10

Etymology. Dedicated to the memory of Józef Kochman (1903–1995), Polish smut taxonomist, who first challenged the view that *Entyloma* specimens on *Ranunculus* represent just three species.

Type. Italy, Liguria, Varavalley, c. 2 km NE of Caranza, Strada Provinciale from Caranza to Passo della Cappelletta, alluvial canyon forest, N44°23'33" E09°38'44", elevation c. 840 m a.s.l., on *Ranunculus lanuginosus*, 9 May 2016, *J. Kruse* (GLM-F107660 holotype; ex-type sequences available in GenBank: MF924678 (ITS), MH022802 (*atp*2), MF939309 (*ssc*1), MF939243 (*map*)).

Sori in the leaves, forming small, moderately distinct, flat, rounded or somewhat polyangular spots, 0.5–1 mm diam, usually delineated by the leaf veins of the host, yellow or cream-coloured 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 pale yellow, globose or subglobose, regular in shape, (9.0–)11.0–13.0 × (9.0–)10.0–12.5 µm (av. ± SD, 11.7 ± 0.9 × 10.9 ± 0.8 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-

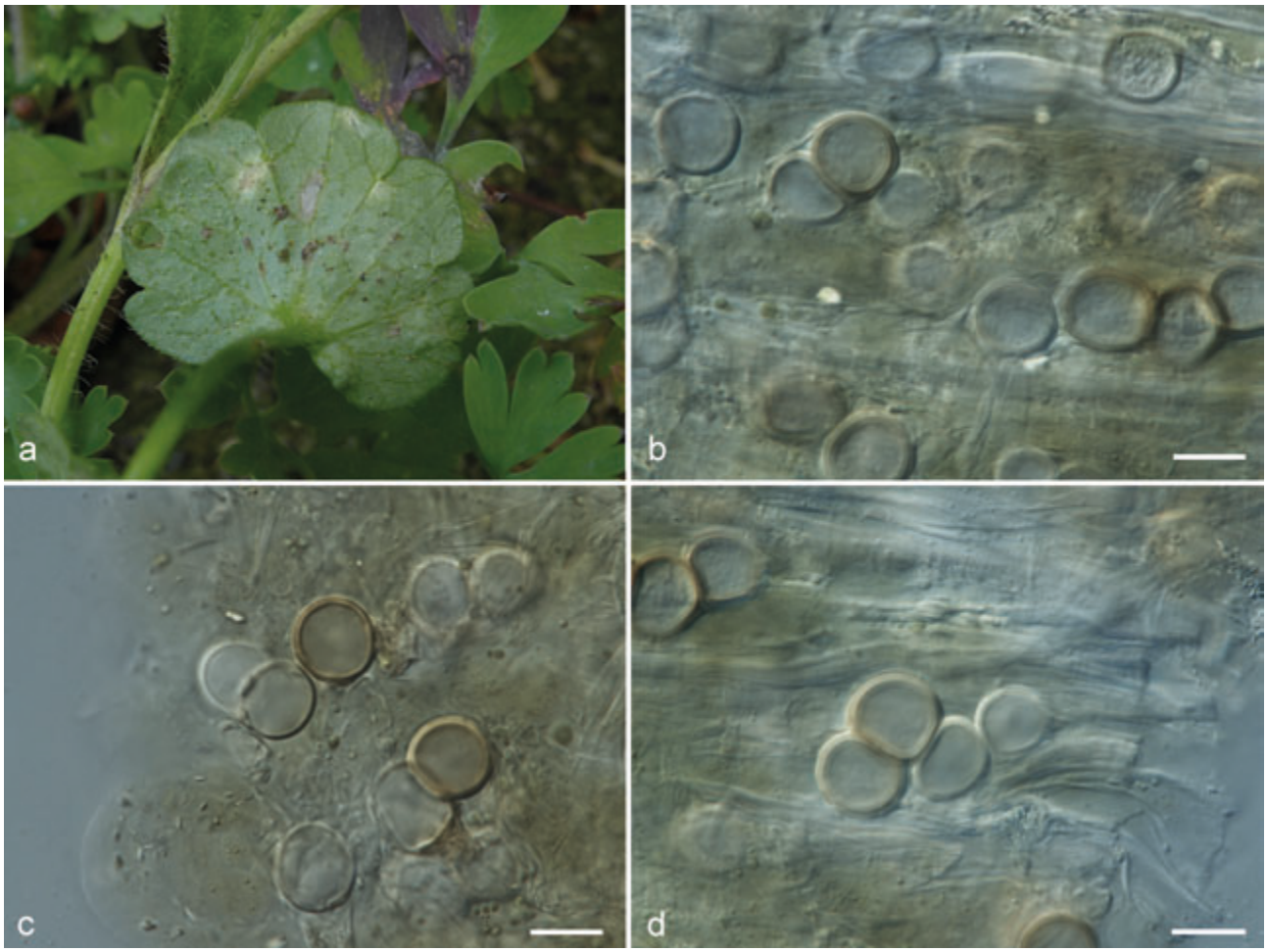

Fig. 9 *Entyloma klenkei* on *Ranunculus marginatus*. a. Macroscopic symptoms of infection; b–d. spores, as seen in light microscopy (from holotype). — Scale bars = 10 µm.

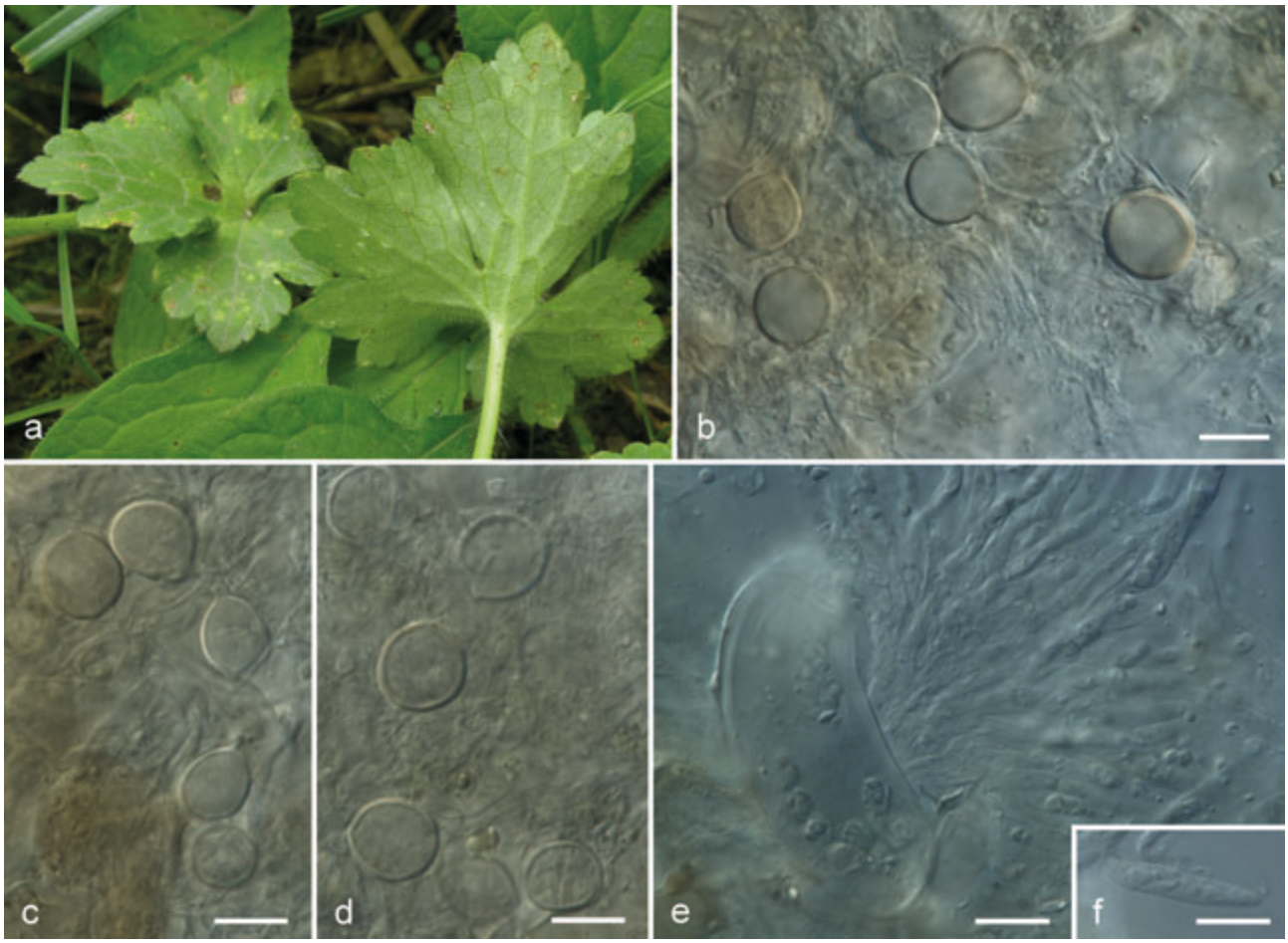

Fig. 10 *Entyloma kochmanii* on *Ranunculus lanuginosus*. a. Macroscopic symptoms of infection; b–d. spores, as seen in light microscopy; e. conidiophores emerging through the stoma, as seen in light microscopy; f. conidium, as seen in light microscopy (from holotype). — Scale bars = 10 µm.

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. ranunculorum* 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 (*atp*2), MF939280 (*ssc*1), 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 *ssc*1 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, Mönchsweiher, 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, Eltville on Rhine, Rheinsteig, 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, surround-

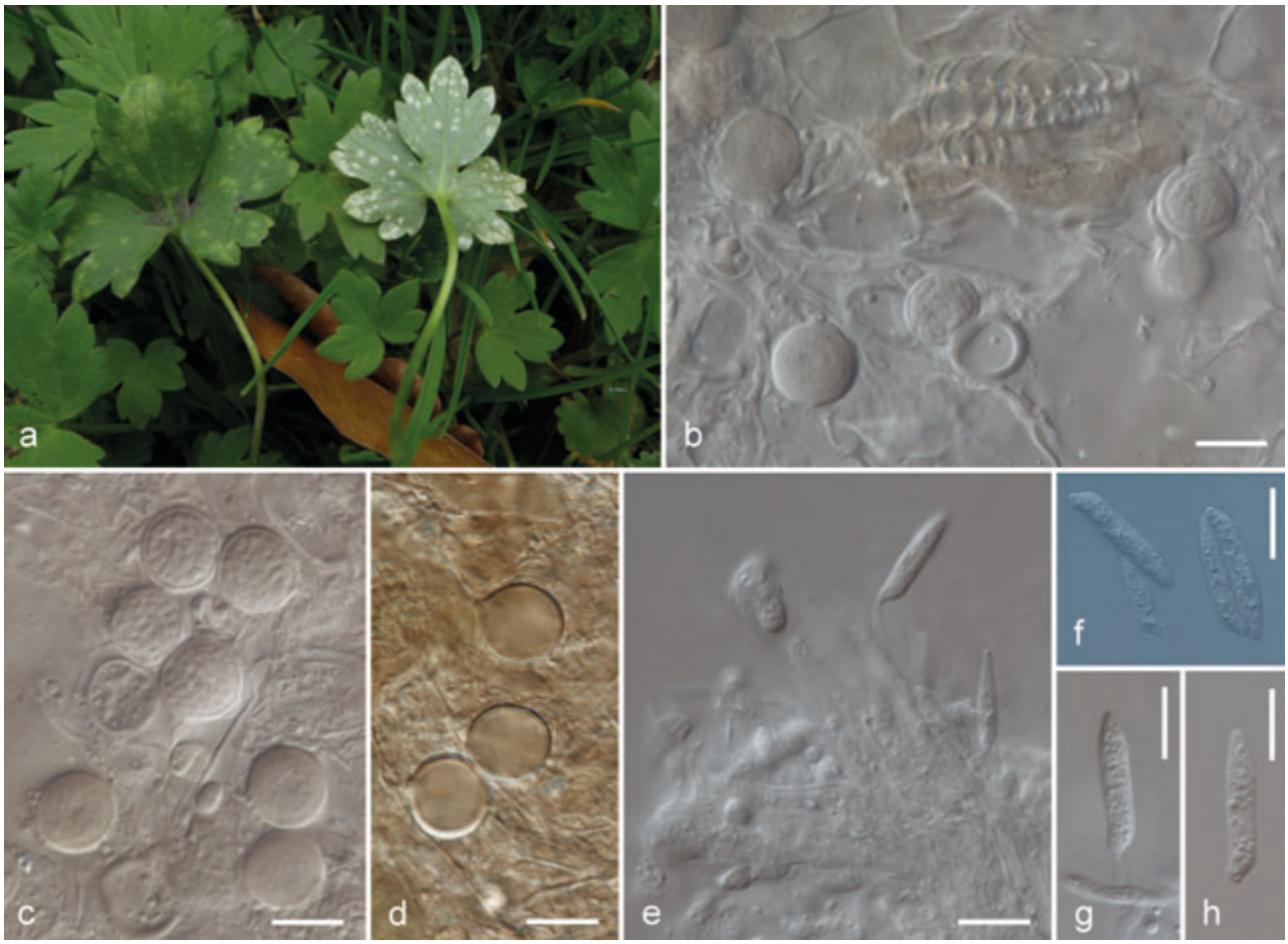

Fig. 11 *Entyloma ranunculacearum* on *Ranunculus acris*. a. Macroscopic symptoms of infection; b–d. spores, as seen in light microscopy; e. conidiophores emerging through the stoma (with one conidium attached to the conidiophore), as seen in light microscopy; f–h. conidia, as seen in light microscopy. Note conidium attached to the conidiophore seen on 'g' (from reference specimen). — Scale bars = 10 µm.

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 sceleratus*, 2 July 1927, *W. Konopacka* (BRIP: HUV 974 lectotype, isolectotypes 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 sceleratus*, 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, 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, hyaline, 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 sceleratus*.

Additional specimens examined. Germany, Saxony-Anhalt, SE of Allstedt, Ziegelrodaer forest (N-part), airport Allstedt (NW-side), on *Ranunculus sceleratus*, 23 Oct. 2005, *H. Jage* (GLM-F076138); Lodersleben, near castle, in Querne, on *Ranunculus sceleratus*, 6 May 2005, *H. John & H. Jage* (GLM-F076186); Friedersdorf near Lohsa S, WSW Neuhof, near Ballackmühle, Maxsee (part of Ballacksee), on *Ranunculus sceleratus*, 26 May 2006,

Fig. 12 *Entyloma ranunculi-scelerati* on *Ranunculus sceleratus*. a. Macroscopic symptoms of infection; b–c. spores, as seen in light microscopy; d. conidiophores emerging through the stoma, as seen in light microscopy; e–f. cylindrical conidia, as seen in light microscopy; g–i. acicular conidia, as seen in light microscopy (from reference specimen). — Scale bars = 10 µm.

H. Jage (GLM-F086008); Bavaria, Oberpfalz, county Grafenwöhr, E of Hütten, littoral of lake, N49°40'52" E11°58'42", elevation c. 410 m a.s.l., on *Ranunculus sceleratus*, 1 May 2016, *G. Hübner* (GLM-F107685).

Notes — In the protologue, Kochman (1936) reported two collections on *Ranunculus sceleratus*: one collected in Skierniewice-Glinianki in 1927 by W. Konopacka, and another, collected in Skierniewice-Zwierzyniec in 1925 by W. Siemaszko, both in Poland. Lindeberg (1959) designated the collection in Skierniewice-Glinianki as the lectotype, but erroneously wrote that the material was collected in 1925 by W. Siemaszko – apparently mixing data from both original collections. Also, Lindeberg (1959) did not mention where the specimen is deposited. Vánky (1985) corrected her mistake and narrowed the lectotype to the specimen in HUV. Kochman (1936) reported the date of collection as 1927 but on the lectotype specimen the exact date is given as 2 July 1927. KRAM F-2628 is a specimen labelled as *Entyloma ranunculi-scelerati* collected on *Ranunculus sceleratus* in Skierniewice-Glinianki by W. Konopacka on 28 May 1927. This specimen may represent authentic material but in the light of Vánky's (1985) lectotypification its status remains unclear.

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*, 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, Oberfranken, county Kulmbach, Lindau, Mountain chain Rough Mt, wayside, elevation c. 410 m a.s.l., on *Ranunculus auricomus* agg., 12 May 2012, *J. Kruse* (GLM-F107686 reference specimen designated here, ex-reference specimen sequences available in GenBank: MF924629 (ITS), MH022753 (*atp2*)).

Sori in the leaves, forming distinct, flat, rounded or somewhat polyangular 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

Fig. 13 *Entyloma ranunculorum* on *Ranunculus auricomus*. a. Macroscopic symptoms of infection; b–d. spores, as seen in light microscopy; e–g. conidia, as seen in light microscopy (from reference specimen). — Scale bars = 10 µm.

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 *atp*2 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), MH022786 (*atp*2), MF939300 (*ssc*1), 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, globose, subglobose 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*

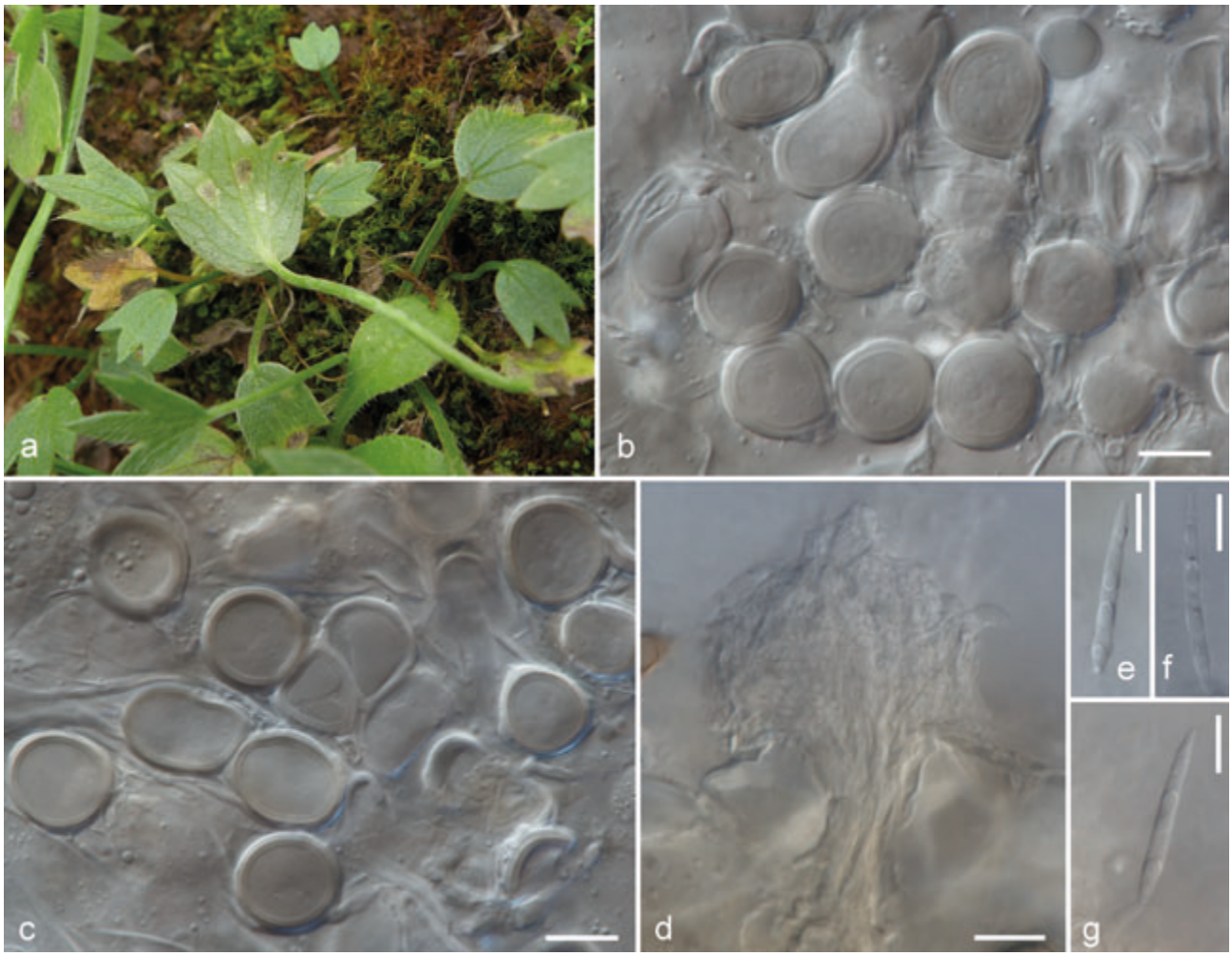

Fig. 14 *Entyloma savchenkoi* on *Ranunculus paludosus*. a. Macroscopic symptoms of infection; b–c. spores, as seen in light microscopy; d. conidiophores emerging through the stoma, as seen in light microscopy; e–g. conidia, as seen in light microscopy. Note conidium attached to the conidiophore seen on 'g' (from reference specimen). — Scale bars = 10 µm.

paludosus, 15 Mar. 2016, *J. Kruse* (GLM-F107697); c. 1.2 km SE of Theologos, olive grove, N36°22'00" E28°02'45", elevation c. 40 m a.s.l., on *Ranunculus paludosus*, 16 Mar. 2016, *J. Kruse* (GLM-F107698).

Notes — This species is most similar to *Entyloma jolantae*, which differs in lacking an asexual morph.

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. 2.8 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 (*atp2*), 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. 2.8 km SE of Mittenwald, Karwendel mountains, hiking path 290 from Brunnstein cabin towards Mittenwald, serpentines, sparse mixed mountainous forest, N47°24'48" E11°16'33", elevation c. 1380 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 Rehbergalm 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.

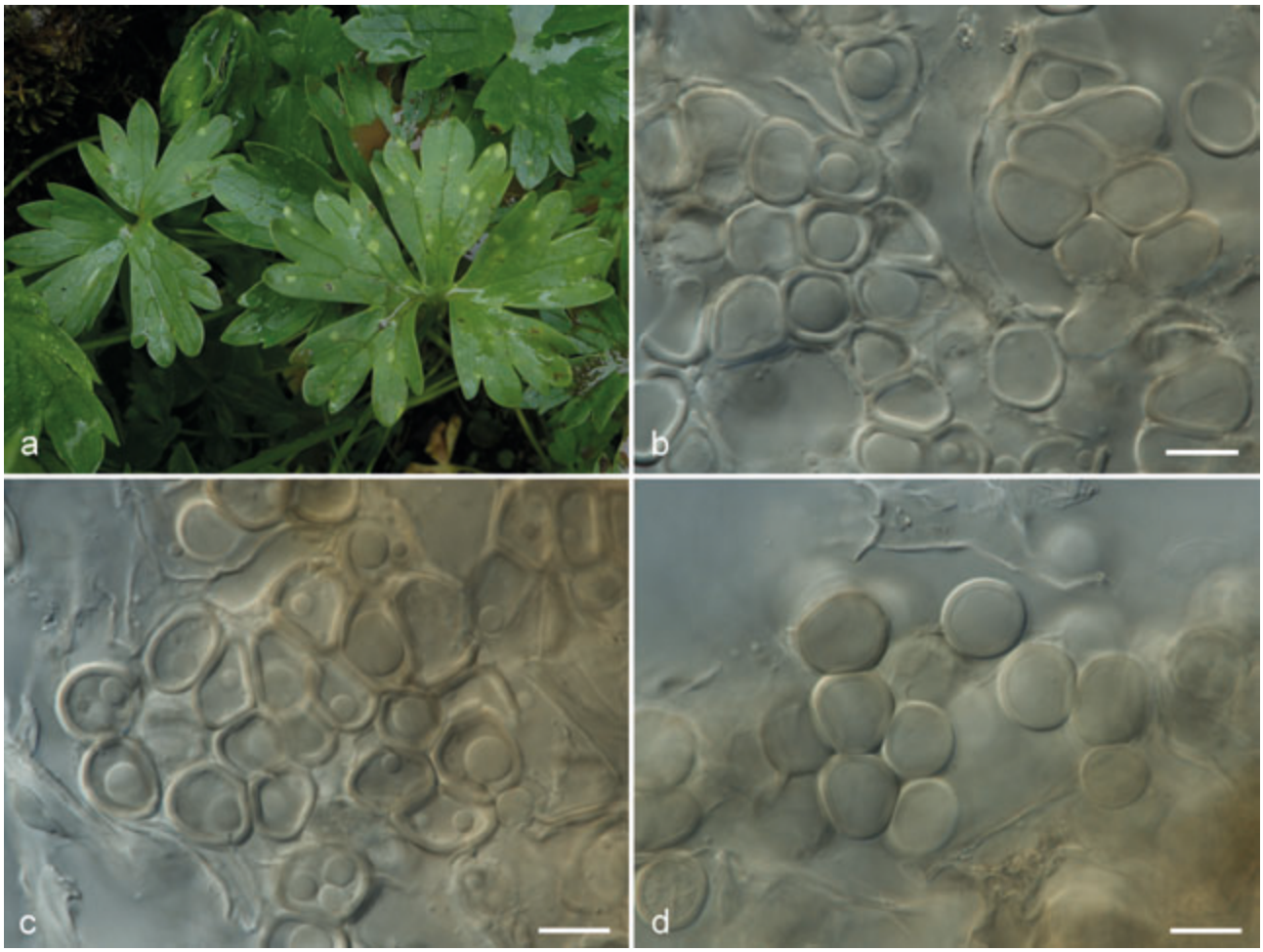

Fig. 15 *Entyloma thielii* on *Ranunculus montanus*. a. Macroscopic symptoms of infection; b–d. spores, as seen in light microscopy (from holotype). — Scale bars = 10 µm.

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, polyangular 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 coloured 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) × (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, *atp*2, *ssc*1, and *map* sequences) recovered 11 mostly highly supported host-specific lineages (nine on

Fig. 16 *Entyloma verruculosum* on *Ranunculus lanuginosus*. a. Macroscopic symptoms of infection; b–e. spores as seen in light microscopy, median and superficial views (from GLM-F107706). — Scale bars = 10 µm.

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(–26.5) × (10.5–)12.0–16.5(–19.5)	18.1 ± 2.9 × 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) × (9.5–)10.0–13.5(–17.5)	14.6 ± 2.8 × 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) × (9.0–)10.0–15.5(–16.0)	14.5 ± 2.4 × 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) × (9.0–)9.5–13.5(–14.5)	12.3 ± 1.4 × 11.3 ± 1.3	1.0–1.5(–2.0)	present	dimorphic, cylindrical, 15–22 × 2.5–4.0 µm, and acicular, 30.0–45.0(–60.0) × (2.0–)2.5–3.5 µm
E. jolantae	*R. oreophilus*	densely crowded	10.5–15.5(–16.5) × 10.0–13.5(–14.5)	13.2 ± 1.4 × 11.6 ± 1.1	1.5–2.0	absent	absent
E. klenkei	*R. marginatus*	loosely scattered	10.5–13.0 × 10.0–12.5	11.7 ± 0.9 × 11.1 ± 0.7	1.0–1.8	absent	absent
E. kochmanii	*R. lanuginosus*	loosely scattered	(9.0–)11.0–13.0 × (9.0–)10.0–12.5	11.7 ± 0.9 × 10.9 ± 0.8	0.5–1.5	present	cylindrical, 20–24 × 3.0–3.5(–4.0) µm
E. ranunculacearum	*R. acris*	loosely scattered	10.0–13.5(–14.5) × (9.0–)10.0–12.5(–13.5)	11.8 ± 1.1 × 10.9 ± 0.8	0.8–1.5	present	cylindrical, (10–)15–19(–25) × 2.5–3.5(–4.0) µm
E. ranunculi-scelerati	*R. sceleratus*	loosely scattered	(9.5–)10.0–12.5(–13.5) × (9.0–)10.0–12.5(–13.0)	11.7 ± 0.9 × 11.0 ± 0.9	1.0–1.5	present	dimorphic, acicular, rarely cylindrical, 20–60 × (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) × (9.0–)10.0–12.5(–13.0)	11.8 ± 0.9 × 10.9 ± 0.9	1.0–1.5(–1.8)	present	cylindrical, 16–28 × 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) × (9.0–)11.0–14.5(–15.0)	13.9 ± 1.4 × 12.3 ± 1.2	1.5–2.5(–3.0)	present	acicular-cylindrical, 25–40 × 2.5–3.0(–3.5) µm
E. thielii	*R. montanus*	densely crowded, often in compact groups	(9.5–)11.0–14.5(–16.5) × 9.0–12.5(–13.0)	12.5 ± 1.5 × 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) × (10.5–)11.0–14.5(–15.0)	13.4 ± 1.4 × 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* (Vánky 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.

Acknowledgements This study was supported by the LOEWE excellence initiative in the framework of the cluster for Integrative Fungal Research (IPF). The work of the second author was supported by the statutory funds of the W. Szafer Institute of Botany, Polish Academy of Sciences in Kraków. We thank T. Feuerer (HGB), M. Graniszewska (WA), M. Halama (WRSL), R. Lücking (B), and R. Marcucci (PAD) for searching for potential type specimens in their herbaria and U. Damm (GLM) for providing several herbarium numbers for private smut collections. We are grateful to the curators of GLM, BRIP, and TUB for allowing the investigation of specimens in their keeping, and the private collectors H. Jage, H. John, U. Richter, and H. Richter for submitting parts of their collections to GLM, and P. Mędykowski for submitting one specimen to KRAM F. We also thank the private collectors G. Hübner, F. Klenke, C. Klenke, and V. Kummer for allowing us to include some of their collections in this study. We also thank V. Kummer for allowing us to use his picture of macroscopic infection of *Ranunculus sceleratus*.

REFERENCES

Altschul SF, Madden TL, Schäffer AA, et al. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25: 3389–3402.

Beenken L, Zoller S, Berndt R. 2012. Rust fungi on Annonaceae II: the genus Dasyspora Berk. & M.A. Curtis. Mycologia 104: 659–681.

Begerow D, Bauer R, Boekhout T. 2000. Phylogenetic placements of ustilaginomycetous anamorphs as deduced from nuclear LSU rDNA sequences. Mycological Research 104: 53–60.

Begerow D, Bauer R, Oberwinkler F. 2002. The Exobasidiales: an evolutionary hypothesis. Mycological Progress 1: 187–199.

Begerow D, Stoll M, Bauer R. 2006. A phylogenetic hypothesis of Ustilaginomycotina based on multiple gene analyses and morphological data. Mycologia 98: 906–916.

Bennett RM, De Cock AW, Lévesque CA, et al. 2017. Calycofera gen. nov., an estuarine sister taxon to Phytopythium, Peronosporaceae. Mycological Progress 16: 947–954.

Braun U. 1998. A monograph of Cercosporella, Ramularia and allied genera (Phytopathogenic Hyphomycetes). Vol. 2. IHW-Verlag Eching, Germany.

Choi Y-J, Klosterman SJ, Kummer V, et al. 2015. Multi-locus tree and species tree approaches toward resolving a complex clade of downy mildews (Straminipila, Oomycota), including pathogens of beet and spinach. Molecular Phylogenetics and Evolution 86: 24–34.

Choi Y-J, Shin HD, Thines M. 2009. The host range of Albugo candida extends from Brassicaceae through Cleomaceae to Capparaceae. Mycological Progress 8: 329–335.

Choi Y-J, Thines M. 2015. Host jumps and radiation, not co-divergence drives diversification of obligate pathogens. A case study in downy mildews and Asteraceae. PloS One 10: e0133655.

Ciferri R. 1928. Quarta contribuzione allo studio degli Ustilaginales. Annales Mycologici 26: 1–68.

De Bary A. 1874. Protomyces microsporus und seine Verwandten. Botanische Zeitung (Berlin) 32: 97–108.

Denchev TT, Denchev CM, Shivas RG. 2013. Two new Entyloma species (Entylomatales, Ustilaginomycotina) from the USA. Mycobiota 3: 35–39.

Emadzade K, Lehnebach C, Lockhart P, et al. 2010. A molecular phylogeny, morphology and classification of genera of Ranunculeae (Ranunculaceae). Taxon 59: 809–828.

Euro+Med. 2006 onwards. Euro+Med PlantBase – the information resource for Euro-Mediterranean plant diversity. Published on the Internet: http://ww2.bgbm.org/EuroPlusMed/ (accessed 15 May 2017).

Hawksworth DL, Crous PW, Redhead SA, et al. 2011. The Amsterdam declaration on fungal nomenclature. IMA Fungus 2: 105–112.

Hörandl E, Paun O, Johansson JT, et al. 2005. Phylogenetic relationships and evolutionary traits in Ranunculus s.l. (Ranunculaceae) inferred from ITS sequence analysis. Molecular Phylogenetics and Evolution 36: 305–327.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780.

Klenke F, Scholler M. 2015. Pflanzenparasitische Kleinpilze. Bestimmungsbuch für Brand-, Rost-, Mehltau-, Flagellatenpilze und Wucherlingsverwandte in Deutschland, Österreich, der Schweiz und Südtirol. Springer Verlag Berlin Heidelberg, Germany.

Kochman J. 1934. Contribution to the knowledge of the Polish Ustilaginales. Acta Societatis Botanicorum Poloniae 11 (Suppl.): 285–303. [In Polish.]

Kochman J. 1936. Grzyby głowniowe Polski (Ustilaginales Poloniae). Planta Polonica 4: 1–161. [In Polish.]

Kruse J, Choi Y-J, Thines M. 2017a. New smut-specific primers for the ITS barcoding of smut fungi (Ustilaginomycotina). Mycological Progress 16: 213–221.

Kruse J, Dietrich W, Zimmermann H, et al. 2018. Ustilago species causing leaf-stripe smut revisited. IMA Fungus 9: 49–73.

Kruse J, Mishra B, Choi Y-J, et al. 2017b. New smut-specific primers for multilocus genotyping and phylogenetics of Ustilaginales. Mycological Progress 16: 917–925.

Kruse J, Thines M. 2017. Proposal to reject the name Ramularia gibba (Entylomatales, Ustilaginomycotina). Taxon 66: 515–516.

Li Y-M, Shivas RG, Cai L. 2017. Cryptic diversity in Tranzscheliella spp. (Ustilaginales) is driven by host switches. Scientific Reports 7: 43549.

Lindeberg B. 1959. Ustilaginales of Sweden (exclusive of the Cintractias on Caricoideae). Symbolae Botanicae Upsalienses 16: 1–175.

Liro JI. 1938. Die Ustilagineen Finnlands. II. Annales Academiæ Scientiarum Fennicæ Series A, 42: 1–720.

Lutz M, Bauer R, Begerow D, et al. 2004. Tuberculina, rust relatives attack rusts. Mycologia 96: 614–626.

Lutz M, Göker M, Piątek M, et al. 2005. Anther smuts of Caryophyllaceae: molecular characters indicate host-dependent species delimitation. Mycological Progress 4: 225–238.

Lutz M, Piątek M. 2016. Phylogenetic placement, DNA barcoding, morphology and evidence for the spreading of Entyloma cosmi, a species attacking Cosmos bipinnatus in temperate climate gardens. European Journal of Plant Pathology 145: 857–869.

McNeill J, Barrie FR, Buck WR, et al. 2012. International Code of Nomenclature for algae, fungi, and plants (Melbourne Code). Regnum Vegetabile 154. A.R.G. Ruggell, Gantner Verlag, Liechtenstein.

Mishra B, Choi Y-J, Thines M. 2018. Phylogenomics of Bartheletia paradoxa reveals its basal position in Agaricomycotina and that the early evolutionary history of basidiomycetes was rapid and probably not strictly bifurcating. Mycological Progress 17: 333–341.

Morin L, Aveyard R, Lidbetter JR, et al. 2012. Investigating the host range of Puccinia psidii sensu lato across tribes of the family Myrtaceae present in Australia. PLoS One 7: e35434.

Paun O, Lehnebach C, Johansson JT, et al. 2005. Phylogenetic relationships and biogeography of Ranunculus and allied genera (Ranunculaceae) in the Mediterranean region and in the European alpine system. Taxon 54: 911–932.

Piątek M, Lutz M, Chater AO. 2013. Cryptic diversity in the Antherospora vaillantii complex on Muscari species. IMA Fungus 4: 5–19.

Piątek M, Lutz M, Nobis M, et al. 2015a. Phylogeny and morphology of Anthracoidea pamiroalaica sp. nov. infecting the endemic sedge Carex koshewnikowii in the Pamir Alai Mts (Tajikistan). Mycological Progress 14: 120.

Piątek M, Lutz M, Smith PA, et al. 2011. A new species of Antherospora supports the systematic placement of its host plant. IMA Fungus 2: 135–142.

Piątek M, Lutz M, Yorou NS. 2015b. A molecular phylogenetic framework for Anthracocystis (Ustilaginales), including five new combinations (inter alia for the asexual Pseudozyma flocculosa), and description of Anthracocystis grodzinskae sp. nov. Mycological Progress 14: 88.

Piątek M, Riess K, Karasiński D, et al. 2016. Integrative analysis of the West African Ceraceosorus africanus sp. nov. provides insights into the diversity, biogeography, and evolution of the enigmatic Ceraceosorales (Fungi: Ustilaginomycotina). Organisms Diversity and Evolution 16: 743–760.

Piepenbring M. 2003. Smut fungi (Ustilaginomycetes p.p. and Microbotryales, Basidiomycota). Flora Neotropica 86: iv + 1–291.

Rabenhorst L. 1874. Fungi europaei exs. Cent. XIX. Nr. 1801–1900. Hedwigia 13: 174–175, 184–189.

Rastipishe S, Pakravan M, Tavassoli A. 2011. Phylogenetic relationships in Ranunculus species (Ranunculaceae) based on nrDNA ITS and cpDNA trnL-F sequences. Progress in Biological Sciences 1: 41–87.

Ridgway KP, Duck JM, Young JPW. 2003. Identification of roots from grass swards using PCR-RFLP and FFLP of the plastid trn L (UAA) intron. BMC Ecology 3: 8.

Rooney-Latham S, Lutz M, Blomquist CL, et al. 2017. Entyloma helianthi: identification and characterization of the causal agent of sunflower white leaf smut. Mycologia 109: 520–528.

Rossman AY, Allen WC, Castlebury LA. 2016. New combinations of plant-associated fungi resulting from the change to one name for fungi. IMA Fungus 7: 1–7.

Runge F, Choi YJ, Thines M. 2011. Phylogenetic investigations in the genus Pseudoperonospora reveal overlooked species and cryptic diversity in the P. cubensis species cluster. European Journal of Plant Pathology 129: 135–146.

Saccardo PA. 1915. Fungi ex insula Melita (Malta) lecti a Doct. A. Caruana-Gatto et Doct. G. Borg annis MCMXII et MCMXIV. Series III. Nuovo Giornale Botanico Italiano 22: 24–76.

Savchenko KG, Carris LM. 2017. Two new Entyloma (Entylomataceae, Basidiomycota) species on North American Sanicula. Phytotaxa 327: 191–195.

Savchenko KG, Carris LM, Castlebury LA, et al. 2014a. Revision of Entyloma (Entylomatales, Exobasidiomycetes) on Eryngium. Mycologia 106: 797–810.

Savchenko KG, Carris LM, Castlebury LA, et al. 2014b. Stripe smuts of grasses: one lineage or high levels of polyphyly? Persoonia 33: 169–181.

Savchenko KG, Heluta VP, Wasser SP, et al. 2012. A contribution to the study of smut fungi of Israel. Mycotaxon 118: 303–309.

Savchenko KG, Lutz M, Piątek M, et al. 2013. Anthracoidea caricis-meadii is a new North American smut fungus on Carex sect. Paniceae. Mycologia 105: 181–193.

Savile DBO. 1947. A study of the species of Entyloma on North American composites. Canadian Journal of Research 25: 105–120.

Scholler M, Lutz M, Wood AR, et al. 2011. Taxonomy and phylogeny of Puccinia lagenophorae: a study using rDNA sequence data, morphological and host range features. Mycological Progress 10: 175–187.

Scholler M, Schmidt A, Siahaan SAS, et al. 2016. A taxonomic and phylogenetic study of the Golovinomyces biocellatus complex (Erysiphales, Ascomycota) using asexual state morphology and rDNA sequence data. Mycological Progress 15: 56.

Scholz H, Scholz I. 1988. Die Brandpilze Deutschlands (Ustilaginales). Englera 8: 1–691.

Schröter J. 1877. Bemerkungen und Beobachtungen über einige Ustilagineen. Beiträge zur Biologie der Pflanzen 2 (3): 349–385.

Schröter J. 1887. Die Pilze Schlesiens: Ustilaginei. In: Cohn F (ed), Kryptogamen-Flora von Schlesien 3 (1): 261–290. J.U. Kern's Verlag, Breslau.

Stoll M, Piepenbring M, Begerow D, et al. 2003. Molecular phylogeny of Ustilago and Sporisorium species (Basidiomycota, Ustilaginales) based on internal transcribed spacer (ITS) sequences. Canadian Journal of Botany 81: 976–984.

Sydow H. 1924. Notizen über Ustilagineen. Annales Mycologici 22: 277–291.

Tamura M. 1995. Angiospermae. Ordnung Ranunculales. Fam. Ranunculaceae. II. Systematic Part. In: Hiepko P (ed), Natürliche Pflanzenfamilien. 2nd edn: 223–519. Duncker & Humblot Berlin, Germany.

Unger F. 1833. Die Exantheme Pflanzen, etc. K. Gerold, Wien, Austria.

Vánky K. 1985. Carpathian Ustilaginales. Symbolae Botanicae Upsalienses 24 (2): 1–309.

Vánky K. 1994. European smut fungi. Gustav Fischer Verlag, Stuttgart, Jena, New York.

Vánky K. 2012. Smut fungi of the world. APS Press, St. Paul, US.

Vánky K, Lutz M. 2007. Revision of some Thecaphora species (Ustilaginomycotina) on Caryophyllaceae. Mycological Research 111: 1207–1219.

Vánky K, Lutz M. 2010. Entyloma majewskii sp. nov. (Entylomataceae) on Ranunculus ficaria from Iran. Polish Botanical Journal 55: 271–279.

Vasighzadeh A, Zafari D, Selçuk F, et al. 2014. Discovery of Thecaphora schwarzmaniana on Rheum ribes in Iran and Turkey: implications for the diversity and phylogeny of leaf smuts on rhubarbs. Mycological Progress 13: 881–892.

Wang QM, Begerow D, Groenewald M, et al. 2015. Multigene phylogeny and taxonomic revision of yeasts and related fungi in the Ustilaginomycotina. Studies in Mycology 81: 55–83.

Wang QM, Theelen B, Groenewald M, et al. 2014. Moniliellomycetes and Malasseziomycetes, two new classes in Ustilaginomycotina. Persoonia 33: 41–47.

White TJ, Bruns T, Lee S, et al. 1990. Amplification and direct sequencing of fungal ribosomal RNA sequences for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, et al. (eds), PCR protocols: A guide to methods and applications: 315–322. Academic Press, Inc., US.

Yoshida K, Burbano HA, Krause J, et al. 2014. Mining herbaria for plant pathogen genomes: back to the future. PLoS Pathogens 10: e1004028.

Yoshida K, Schuenemann VJ, Cano LM, et al. 2013. The rise and fall of the Phytophthora infestans lineage that triggered the Irish potato famine. eLife 2: e00731.

Ziegler R, Lutz M, Piątek J, et al. 2018. Dismantling a complex of anther smuts (Microbotryum) on carnivorous plants in the genus Pinguicula. Mycologia in press. https://doi.org/10.1080/00275514.2018.1451697.