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Entyloma ratibidae (Entylomataceae), a new smut fungus on *Ratibida pinnata* (Asteraceae) from USA based on molecular analyses, comparative morphology, and host specificity

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Abstract

Entyloma species are highly specialized smut fungi. To clarify the taxonomic status of the *Entyloma* species on *Ratibida pinnata* from USA, we conducted a molecular study based on ITS and LSU rDNA sequences. The phylogenetic analyses indicate that this species differs from the *Entyloma* species with available molecular data. Based on molecular and morphological evidence, and host specialization, a new species, *Entyloma ratibidae*, is described and illustrated. *Entyloma ratibidae* differs from *E. davisii* on *Rudbeckia hirta* by having smaller spores and thinner spore walls, from *E. anceps* on *Rudbeckia laciniata* by having thicker spore walls, and from the other *Entyloma* species by specialization on *Ratibida pinnata*. The phylogenetic placement and affinities of the new species in *Entyloma* are analyzed. Additional collections and molecular study are required to clarify the taxonomic status of '*Entyloma polysporum*' on *Ratibida columnifera* from USA.

Key words: *Entyloma*, Iowa tallgrass prairies, new species, phylogeny, *Ratibida columnifera*, *Rudbeckia*, smut fungi, taxonomy

Introduction

Entyloma de Bary is a large, cosmopolitan genus in the Entylomataceae, with 192 recognized species on host plants belonging to 27 families (Denchev *et al.* 2021). *Entyloma* species develop sori in the vegetative organs of their hosts, mostly in leaves and petioles, rarely in stems or roots, usually forming few to numerous spots, sometimes swellings or galls formed by hypertrophic growth of host tissue. Spores are permanently embedded in the host tissue, singly or in irregular groups, hyaline, yellow or yellowish brown, and usually with a smooth, two-layered wall; very rarely the outer layer is tuberculate or torn apart into prismatic, pyramidal or coarse and irregular pieces (Denchev *et al.* 2013, 2021, He *et al.* 2024). In the case of *E. eranthidis* T. Denchev *et al.*, the spore wall is initially smooth, at maturity cracking and rupturing irregularly, some ruptures reaching inner layer (Denchev *et al.* 2021). Spores are often with a hyaline gelatinous sheath.

As part of an ongoing study of smut fungi diversity, we conducted a molecular study of specimens of *Entyloma* on *Ratibida pinnata* (Vent.) Barnhart (Asteraceae) obtained on loan from Ada Hayden Herbarium (ISC) at Iowa State University. *Ratibida* Raf. is a small genus with seven species, all native to North America (Richards 1968, Anderberg *et al.* 2007). *Ratibida* belongs to subtribe Rudbeckiinae H. Rob. (tribe Heliantheae Cass.). *Rudbeckia* L. is the second genus in the Rudbeckiinae subtribe, with 17 species, all native to North America (Urbatsch & Cox 2006a, b, Anderberg *et al.* 2007). The species in these genera are known as coneflowers due to the conical or columnar shape of the receptacle of their flower heads (capitula). *Ratibida pinnata* and *R. columnifera* (Nutt.) Wooton & Standl., and species of *Rudbeckia* are highly valued as ornamental species due to their brightly colored flowers, long bloom period, and ability to attract pollinators and other beneficial insects (Richards 1968, Kersten *et al.* 2022).

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The concept of distinguishing species in *Entyloma* has varied significantly during the last eight decades. Savile (1947) applied a morphological species concept, based on spore sizes and asexual morph. He synonymized species with similar morphology, parasitizing host species on different genera from the same family. As a result of this broad species concept, Savile accepted only eight species of *Entyloma* on North American asteraceous hosts (Denchev *et al.* 2021). Other authors (*e.g.* Liro 1938, Lindeberg 1959, Vánky 2011) applied narrower species concepts, considering *Entyloma* species as infecting one or more hosts from the same host genus or occasionally a few closely related host genera. During the last two decades with the application of molecular methods, it became evident that members of *Entyloma* exhibit a far higher host specificity, parasitizing a single or only a few closely related host species (*e.g.* Begerow *et al.* 2002, Vánky & Lutz 2010, Kruse *et al.* 2018, Denchev *et al.* 2021, Chaverri *et al.* 2025).

There are 54 species of *Entyloma* known from the USA (Denchev *et al.* 2013, Savchenko *et al.* 2014, Rooney-Latham *et al.* 2017, Savchenko & Carris 2017, Piątek *et al.* 2024). The aim of the present study was to clarify the taxonomic status of the *Entyloma* species on *Ratibida pinnata*. A combined approach, using molecular data, host specialization, and comparative morphology revealed a new species, *E. ratibidae*, which is described and illustrated herein.

Materials and methods

Materials

This study is based on phylogenetic and/or morphological analyses of eight '*Entyloma compositarum*' specimens on *Ratibida pinnata* obtained from Ada Hayden Herbarium (ISC) at Iowa State University (herbarium code according to Thiers 2025+).

DNA extraction, PCR amplification, and sequencing

For DNA extraction, sori of *Entyloma* were used. The sample was milled in the Bead Ruptor 12™ homogenizer (Omni International), using two steel beads. Genomic DNA was isolated using the my-Budget Plant DNA Kit™ (Bio-Budget Technologies GmbH, Germany), according to the manufacturer's protocol (protocol 1: "Isolation of DNA from plant material using lysis buffer SLS"). PCR using GoTaq™ Master Mix (Promega, USA) with the primer combinations M-ITS1/ITS4 (Stoll *et al.* 2003, White *et al.* 1990) and LR0R/NL4 (Moncalvo *et al.* 1995, O'Donnell 1992) was performed to amplify the ITS and LSU rDNA regions, respectively, which are the standard molecular markers for *Entyloma* (*e.g.*, Piątek *et al.* 2024). Standard thermal cycling conditions with an annealing temperature of 52 °C were used for amplification. Five µl of PCR products were purified using a modified ExoSAP protocol (1 : 5 diluted in ddH_2O; New England Biolabs, USA). Amplicons were sequenced in both directions at Macrogen Europe (Macrogen Inc.) using the same primers as in the respective PCRs. Subsequently, forward and reverse reads were quality checked and assembled in Geneious 10.2.6 (Biomatters Ltd, New Zealand). Sequences were deposited in the NCBI nucleotide database (see Table 1 for accession numbers).

Phylogenetic analysis

The newly generated *Entyloma* sequences and representative sequences downloaded from GenBank (Table 1) were aligned with the g-ins-i option in MAFFT using the online server version (Katoh *et al.* 2019). GBlocks (Castresana 2000), implemented in SeaView (Gouy *et al.* 2010), was used to remove ambigous bases, as well as leading and trailing gaps while allowing small final blocks and gaps within the alignment. The alignment was partitioned for both DNA regions and the best nucleotide substitution model was determined using ModelTest-NG (Darriba *et al.* 2020), implemented in raxmlGUI 2.0 (Edler *et al.* 2021) for both individually. RaxML-NG (Kozlov *et al.* 2019), also implemented in raxmlGUI, was subsequently used for inferring a maximum likelihood phylogeny with 1000 thorough bootstrap replicates. The phylogeny was visualized in iTOL v.5 (Letunic & Bork 2021).

Morphological examination

Dried specimens of '*Entyloma compositarum*' from ISC were examined using a light microscope (LM). For LM observations and measurements, spores were mounted in lactoglycerol solution (w : la : gl = 1 : 1 : 2) on glass slides, gently heated to boiling point to rehydrate the spores, and then cooled. The measurements of spores are given as min–max (extreme values) (mean ± 1 standard deviation). The description below is based on the specimens examined. The shapes of spores are arranged in descending order of frequency.

TABLE 1. Specimens and NCBI nucleotide database accession numbers used for phylogenetic analysis (newly generated sequences indicated in boldface).

Species	Host/substrate	Country	Voucher	ITS	LSU
Entyloma arnicale	*Arnica montana*	Germany	TUB 012093	AY854964	MT332407
E. arnoseridis	*Arnoseris minima*	France	TUB 012523	AY081017	MT332396
E. atlanticum	*Geranium tuberosum*	Iran	BRIP: HUV 14885	AY081018	AY081011
E. australe	*Physalis peruviana*	Zimbabwe	BRIP: HUV 18886	MT332212	MT332423
E. belangeri	*Malus domestica* cv. Elstar	Netherlands	CBS 111600	AY259074	AY272019
E. bidentis	*Bidens pilosa*	Costa Rica	TUB 012576	AY081020	KP668990
E. browalliae	*Browallia americana*	Costa Rica	TUB 012577	AY081021	KP668989
E. bullosum	*Ranunculus paludosus*	Greece	GLM-F 107632	MF924658	N/A
E. calceolariae	*Calceolaria chelidonioides*	Ecuador	BRIP: HUV 16396	AY081022	MT332397
E. carmeli	*Eryngium falcatum*	Israel	HAI 4521	KF310892	N/A
E. chrysosplenii	*Chrysosplenium alternifolium*	Germany	TUB 012537	MT332207	MT332418
E. comaclinii	*Comaclinium montanum*	El Salvador	M: M. Piepenbring 1762	AY081025	MT332398
E. corydalis	*Corydalis solida*	Germany	BRIP: HUV 13171	AY081027	AY860053
E. cosmi	*Cosmos bipinnatus*	Japan	BRIP: HUV 20935	KJ728759	NG059239
E. costaricense	*Viguiera* sp.	Panama	PMA: M. Piepenbring 2384	AY081028	MT332399
E. dahliae	*Dahlia* sp.	Switzerland	TUB 012574	AY081029	KP668992
E. davenportii	*Malus × domestica* cv. Elstar	Netherlands	CBS 111604	AY259064	AY272010
E. deliliae	*Delilia biflora*	Costa Rica	USJ: M. Piepenbring 1004	AY081030	MT332400
E. diastateae	*Diastatea micrantha*	El Salvador	TUB 012579	AY854974	KP668993
E. doebbeleri	*Dahlia imperialis*	Costa Rica	USJ: M. Piepenbring 584	AY854973	MT332411
E. eburneum	*Ranunculus repens*	Poland	KRAM F-59037	NR161036	N/A
E. elstari	*Malus × domestica* cv. Elstar	Netherlands	CBS 111593	DQ206984	AY745721
E. eranthidis	*Eranthis longistipitata*	Uzbekistan	TUR 109345	MT118137	N/A
E. eryngii	*Eryngium campestre*	Hungary	BRIP: HUV 15339	AY081033	MT332401
E. eryngii-cretici	*E. creticum*	Israel	HAI 4526	KF310894	N/A
E. eryngii-plani	*E. planum*	Romania	BRIP: HUV 11650	AY081034	MT332402
E. eschscholziae	*Eschscholzia californica*	Germany	GLM: J. Kruse B0858	MT332213	MT332424
E. fergussonii	*Myosotis arvensis*	Austria	TUB 012098	AY854970	MT332410
E. ficariae	*Ficaria verna*	Germany	TUB 019287	HM046471	HM046481
E. fuscum	*Papaver rhoeas*	Germany	B 70000601	MT332209	MT332420
E. gaillardianum	*Gaillardia aristata*	Germany	R.B. 2055	AY081037	AF133575
E. glaucii	*Glaucium flavum*	Greece	BRIP: HUV 7035	AY081036	AY081014
E. guaraniticum	*Bidens pilosa*	Cuba	HAJB: M. Piepenbring 2264	AY081038	MT332403

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TABLE 1. (Continued)

Species	Host/substrate	Country	Voucher	ITS	LSU
E. helianthi	*Helianthus annuus*	USA	KRAM F-58718	NR172814	N/A
E. hieracii	*Hieracium lachenalii*	Germany	TUB 012100	AY854967	MT332409
E. hieracii	*H. murorum*	Switzerland	TUB 012583	AY081039	N/A
E. hieracii	*H. murorum*	Switzerland	TUB 012557	EU233810	N/A
E. holwayi	*Cosmos caudatus*	El Salvador	USJ: M. Piepenbring 1769	AY081040	KP668991
E. jolantae	*Ranunculus oreophilus*	Poland	KRAM F59030	MF924688	N/A
E. klenkei	*R. marginatus*	Greece	GLM-F 107659	MF924663	N/A
E. kochmanii	*R. lanuginosus*	Italy	GLM-F 107660	MF924678	N/A
E. lagoeciae	*Lagoecia cuminoides*	Greece	GLM-F 111625	MH295126	MT332417
E. leontodontis	*Leontodon hispidus*	Switzerland	TUB 012559	EU233809	MT332413
E. linariae	*Linaria vulgaris*	Switzerland	BRIP: HUV 13939	AY081041	AY860054
E. lobeliae	*Lobelia laxiflora*	Costa Rica	BRIP: HUV 21049	AY081042	AY081015
E. madiae	*Madia gracilis*	USA	BRIP: HUV 15053	AY081043	MT332404
E. magnusii	*Gnaphalium uliginosum*	Sweden	B 136207	MT332211	MT332422
E. magocsyanum	*Tordylium cordatum*	Israel	HAI 4625	KF310891	N/A
E. majewskii	*Ficaria verna*	Iran	BRIP: HUV 14888	MF924713	HM046478
E. matricariae	*Tripleurospermum perforatum*	Sweden	TUB 012101	AY854979	MT332412
E. matricariae	*T. perforatum*	Germany	TUB 012565	AY081044	N/A
E. meridionale	*Eryngium planum*	Ecuador	BPI 936826	PQ835106	N/A
E. microsporum	*Ranunculus acris*	Germany	GLM-F 107663	MF924641	MT332414
E. parthenii	*Parthenium hysterophorus*	Mexico	XAL: A. Romero 563	AY854966	MT332408
E. piepenbringiae	*Ranunculus polyanthemos*	Germany	GLM-F 107690	MF924698	MT332416
E. polysporum	*Ambrosia artemisifolia*	Hungary	BRIP: HUV 12228 (ITS)/BRIP: HUV 2960 (LSU)	AY081046	AF007529
E. randwijkense	*Malus × domestica* cv. Elstar	Netherlands	CBS 111606	AY259080	AY272033
E. ranunculacearum	*Ranunculus acris*	Germany	GLM-F 107683	MF924653	MT332415
E. ranunculi-scelerati	*R. sceleratus*	Germany	GLM-F 076186	MF924672	N/A
E. ranunculorum	*R. auricomus*	Germany	GLM-F 048093	MF924638	N/A
E. ratibidae	***Ratibida pinnata***	**USA**	**ISC-f-0076127**	**PV661648**	**PV661653**
E. ratibidae	***R. pinnata***	**USA**	**ISC-f-0076136**	**PV661649**	**PV661654**
E. ratibidae	***R. pinnata***	**USA**	**ISC-f-0076147**	**PV661650**	**PV661655**
E. ratibidae	***R. pinnata***	**USA**	**ISC-f-0076152**	**PV661651**	**PV661656**
E. ratibidae	***R. pinnata***	**USA**	**ISC-f-0076584**	**PV661652**	**PV661657**
E. savchenkoi	*Ranunculus paludosus*	Greece	GLM-F 107699	MF924662	N/A
E. scandicis	*Scandix verna*	Israel	HAI 4799	KF447773	N/A
E. serotinum	*Symphytum officinale*	Germany	TUB 012605	AY081048	MT332405

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TABLE 1. (Continued)

Species	Host/substrate	Country	Voucher	ITS	LSU
E. thielii	*Ranunculus montanus*	Germany	GLM-F 107702	MF924694	N/A
E. verruculosum	*R. lanuginosus*	Italy	GLM-F 107706	MF924684	N/A
E. zinniae	*Zinnia peruviana*	Bolivia	LPB: M. Piepenbring 2627	AY081049	MT332406

Results

Phylogenetic analysis

ModelTest-NG determined SYM+I+G4m+B as the best substitution model for both partitions. All sequences of *Entyloma* on *Ratibida pinnata* clustered together and formed a well-supported clade with a bootstrap of 99 (Fig. 1). Within this clade there was a further subdivision, with ISC-f-0076584 and ISC-f-0076152 for instance forming a well-supported group. The phylogenetic analysis inferred similar species relationships as in previous studies, whereby statistical support was missing in the backbone of the phylogeny. *Entyloma ratibidae* was included within a statistically well-supported group of *Entyloma* species that were mostly also isolated from Asteraceae, except for *E. belangeri* which was isolated in its anamorphic state from apple.

Morphology

The morphology of *Entyloma* species is very simple. They have very few diagnostic morphological characteristics and the morphological differences between the species are consequently very few and vague (Lindeberg 1959). In this genus, the morphological features most commonly used for separating species are: sorus location and characteristics (*e.g.*, in the case of *Entyloma* spp. on *Ranunculus*, there is a species complex that has sori forming flat leaf spots, and another with sori forming swollen pustules on leaves; Kruse *et al.* 2018); shape, size, color, and location of the spots formed by the sori on the leaves; spore shape and sizes, and presence of a hyaline gelatinous sheath; wall characteristics; presence of an asexual morph (Vánky 2013, Denchev *et al.* 2021).

The morphological description of the studied smut fungus on *Ratibida pinnata* was based on examination of specimens from Iowa (USA). Its spores are subglobose, broadly ellipsoidal or irregular, (8.5–)9.5–13(–14) µm long, spore wall is slightly unevenly thickened, two-layered, 0.8–1.4(–1.6) µm thick.

Taxonomy

It was found that the fungus on *Ratibida pinnata* is specifically associated with the host. Based on the host specialization, phylogenetic data, and comparative morphology, we propose a new species of *Entyloma* on *R. pinnata*.

Entyloma ratibidae T. Denchev, Denchev & Kemler, *sp. nov.* Figs 2–5

Index Fungorum number: IF 903937

Type:—On *Ratibida pinnata* (Vent.) Barnhart (Asteraceae). USA. Iowa: Cherokee Co., Steele Prairie State Preserve, 11 August 1997, L.H. Tiffany, s.n. (ISC 430019, **holotype**).

ITS rDNA GenBank accession no.:—PV661648, **LSU rDNA** GenBank accession no.:— PV661653.

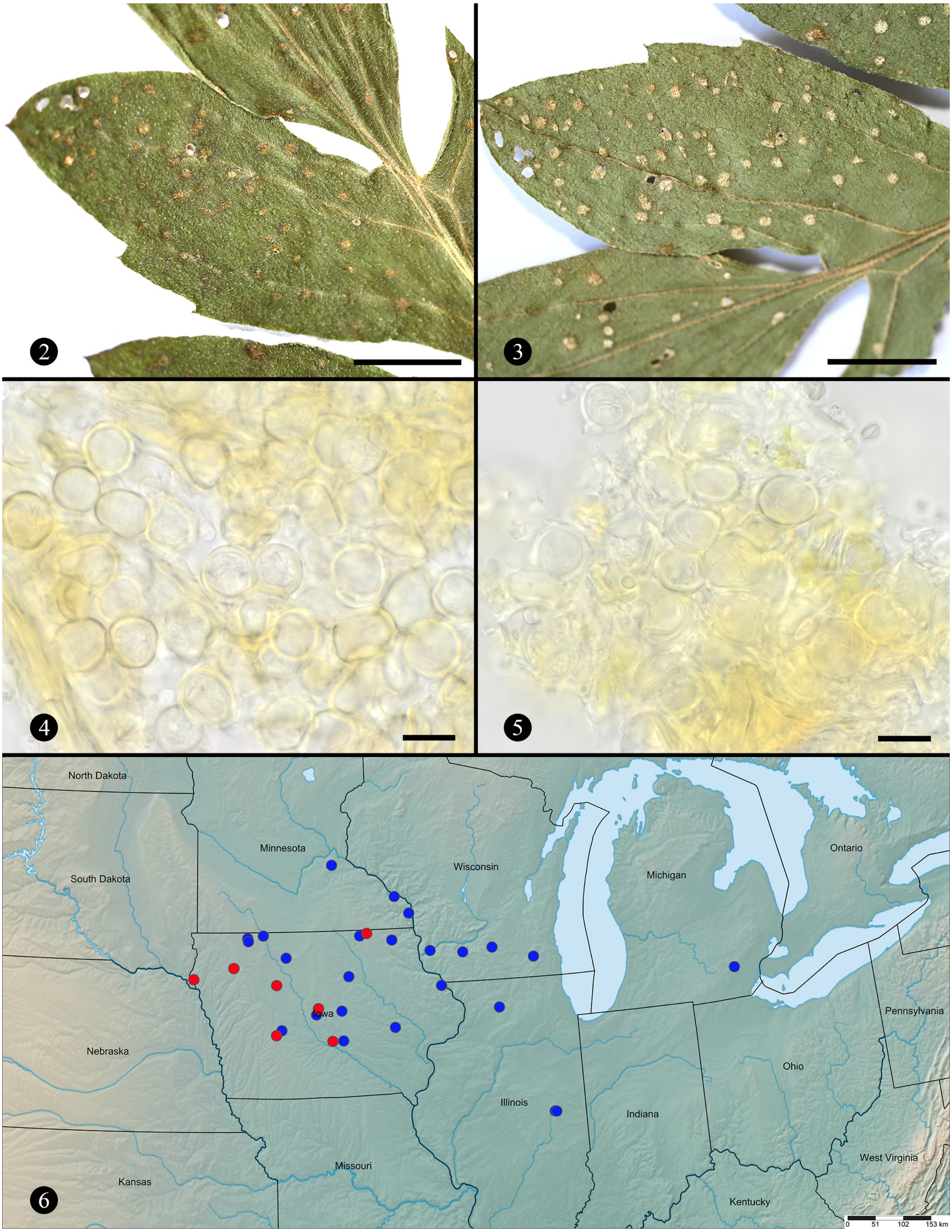
ISC barcode number linking the specimen to its digital record:—ISC-f-0076127.

Diagnosis:— Differs from *Entyloma davisii* by having smaller spores and thinner spore walls, from *E. anceps* by having thicker spore walls, and from the other *Entyloma* species by specialization on *Ratibida pinnata*.

Etymology:—The epithet is derived from the host genus, *Ratibida*.

Description:—*Sori* in leaves, forming irregularly rounded spots, 0.3–4.0(–5.0) mm in length, larger by fusion, amphigenous, not limited by veins, not protruding, clay buff to reddish brown, often peripherally greenish. *Spores* single or crowded in groups, embedded in the leaf tissue, subglobose, broadly ellipsoidal or irregular, (8.5–)9.5–13(–14) × (7.5–)8.5–10.5(–11.5) (11.0 ± 0.9 × 9.5 ± 0.8) µm (n_5 = 500), hyaline to subhyaline; spore wall slightly unevenly thickened, two-layered, 0.8–1.4(–1.6) µm thick.

FIGURE 1. RAxML phylogeny of species in the genus *Entyloma* based on a MAFFT alignment of ITS and LSU rDNA sequences. Bootstrap values ≥ 60 are shown above branches. The phylogenetic tree was rooted with *Entyloma leontodontis* and *E. magnusii*.



FIGURES 2–6. *Entyloma ratibidae* sp. nov. on *Ratibida pinnata*. 2–3. Habit—adaxial and abaxial leaf surfaces, respectively (from holotype). 4–5. Spores in LM (from holotype). 6. Geographic distribution of *E. ratibidae* (generated with Simple-Mappr, Shorthouse 2010): original specimens indicated by red circles, literature records—by blue circles. Scale bars: 2–3 = 1 mm, 4–5 = 10 µm.

Known host and distribution:—On *Ratibida pinnata*, North America (USA—IA, IL, IN, KS, MI, MN, WI) (Fig. 6).

Additional specimens examined:—On *Ratibida pinnata*. USA. Iowa: Howard Co., Hayden Prairie State Preserve, 17 June 1998, L.H. Tiffany, s.n. (ISC 432614/ISC-f-0076582, as '*E. compositarum*'); Plymouth Co., Five Ridges Prairie State Preserve, 11 August 1997, L.H. Tiffany, s.n. (ISC 430572/ISC-f-0076143, as '*E. compositarum*'); Pocahontas Co., Kalsow Prairie State Preserve, 30 June 1998, L.H. Tiffany, s.n. (ISC 431534/ISC-f-0076141, as '*E. compositarum*'); ditto, 14 July 1999, L.H. Tiffany, s.n. (ISC 431533/ISC-f-0076147, as '*E. compositarum*'); Story Co., Doolittle Prairie State Preserve, 13 July 1998, L.H. Tiffany, s.n. (ISC 433739/ISC-f-0076584, as '*E. compositarum*'); Guthrie Co., Sheeder Prairie State Preserve, 28 August 1999, L.H. Tiffany, s.n. (ISC 430876/ISC-f-0076136, as '*E. compositarum*'); Jasper Co., Walnut Creek National Wildlife Refuge, 20 June 1998, L.H. Tiffany, s.n. (ISC 428946/ISC-f-0076152, as '*E. compositarum*').

Based on literature sources (Fischer 1953, Greene 1954, Farr *et al.* 1989) and online specimen records (specimens not seen), *Entyloma* on *Ratibida pinnata* is also recorded from Illinois, Indiana, Kansas, Michigan, Minnesota, and Wisconsin. These records should be considered as belonging to *E. ratibidae*.

Comments:—The specimens of *Entyloma ratibidae* cited here were collected by Lois H. Tiffany (as '*E. compositarum*') in the 1990s during her studies of the phytoparasitic fungi of Iowa tallgrass prairies (Tiffany & Knaphus 1995, 2004). Based on the similarity of the spores, *E. compositarum* Farl. ex G.P. Clinton was reported in the past from many plant species and genera. For example, for this fungus Fischer (1953) listed 58 host species in 26 genera only from North America. Recent treatments of *Entyloma* on asteraceous hosts (Vánky 2011) recognize *E. compositarum* as associated only with *Aster* L., the type host genus of this fungus. There are no molecular data for *E. compositarum*, but *Aster* belongs to tribe Astereae Cass. while *Ratibida* and *Rudbeckia* are members of tribe Heliantheae (Anderberg *et al.* 2007). Thirteen *Entyloma* species are known on 11 host genera in the Heliantheae, as follows: on *Acmella* Rich. ex Pers.—*Entyloma spilanthis* Speg., on *Aldama* La Llave—*E. aldamae* Vánky, on *Ambrosia* L.—*E. polysporum* (Peck) Farl. and *E. ambrosiae-maritimae* Rayss, on *Delilia* Spreng.—*E. deliliae* Vánky *et al.*, on *Echinacea* Moench—*E. echinaceae* Vánky & McKenzie, on *Helianthus* L.—*E. helianthi* Piątek *et al.*, on *Parthenium* L.—*E. parthenii* Syd., on *Ratibida*—*E. polysporum*, on *Rudbeckia*—*E. davisii* Cif. and *E. anceps* Cif., on *Viguiera* Kunth—*E. costaricense* Cif., and on *Zinnia* L.—*E. zinniae* Syd. (Vánky 2011, Rooney-Latham *et al.* 2017). Phylogenetic analyses show that *Entyloma ratibidae* is a distinct species from *E. polysporum* on *Ambrosia*, and from *E. deliliae*, *E. helianthi*, *E. parthenii*, *E. costaricense*, and *E. zinniae* (Fig. 1). There are no molecular data for the *Entyloma* species on *Acmella*, *Aldama*, *Echinacea*, *Rudbeckia*, as well as for *Entyloma* on *Ratibida columnifera* and for *E. ambrosiae-maritimae*. *Entyloma ratibidae* differs from *E, spilanthis* on *Acmella*, *E. ambrosiae-maritimae* and *E. polysporum* on *Ambrosia*, *E. aldamae* on *Aldama*, and *E. echinaceae* on *Echinacea* by having different spore lenght and/or spore wall thickness. As it was already mentioned here, *Entyloma* species are highly specialized fungi, parasitizing a single or only a few closely related host species, and we consider *E. ratibidae* to be a distinct species.

Entyloma ratibidae can easily be differentiated from *E. davisii* on *Rudbeckia hirta* L. by having smaller spores and thinner spore walls (spores 10–23 µm long and wall 2–8 µm thick, for *E. davisii*, after Vánky 2011), and from *E. anceps* on *Rudbeckia laciniata* L. by having thicker spore walls (0.5–0.8 µm thick for *E. anceps*, after Vánky 2011).

On *Ratibida columnifera*, *Entyloma polysporum* is reported from South Dakota (Fischer 1953, Vánky 2011), however, the type host genus of *E. polysporum* is *Ambrosia*. Regarding the new species on *Ratibida pinnata*, there is molecular evidence that *E. ratibidae* is distinct from *E. polysporum* (Fig. 1). Thus additional collections and molecular study are required to clarify the taxonomic status of '*Entyloma polysporum*' on *Ratibida columnifera* from USA.

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