

## Two new *Entyloma* species (Ustilaginales) in Central America<sup>1</sup>

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Among the smut fungi (Ustilaginales) of Central America, especially inconspicuous species of the genus *Entyloma* are far from satisfactorily known. In the present paper two new Central American *Entyloma* species are described: *Entyloma diastatae* on *Diastatea micrantha* (Lobeliaceae) collected in El Salvador and Costa Rica has smaller spores with a thinner, more regular spore wall than the only *Entyloma* species known for this host family, *E. lobeliae* on *Lobelia* spp. *Entyloma comaclinii* on *Comaclinium montanum* (Asteraceae) from El Salvador is related to *E. polysporum* (collective species) but differs by larger spores and a very irregular thickness of its spore walls.

Keywords: smut fungi, *Entyloma diastatae*, *Entyloma comaclinii*, El Salvador, Costa Rica.

Among the far from satisfactorily known neotropical smut fungi (Ustilaginales, Basidiomycetes), especially inconspicuous genera like the genus *Entyloma* de Bary have until now been neglected. *Entyloma* species are characterized by light coloured spores embedded in intercellular spaces of host tissue, mainly in the foliage leaf lamina, causing more or less conspicuous, sometimes swollen spots. The shape of the spots is circular or angular, delimited by adjacent vascular strands of the leaf mesophyll. Typical spots are adaxially green-yellow coloured and abaxially white due to so-called anamorph hyphae with conidia growing out of stomata. It is not always easy to distinguish *Entyloma* spots from lesions caused by other pathogenic and abiotic agents without a microscope. In the field, examination of the spots against light can be helpful because areas with spores embedded in the host tissue appear more opaque than the surrounding tissue. Other lesions usually appear light coloured. Seen by light microscope, *Entyloma* spores measure about 10–20 µm in diameter, they are hyaline to yellowish or brownish, smooth (rarely verrucose), and mostly

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show a two-layered spore wall. The inner wall layer is of constant thickness while the thickness of the outer layer can be very variable. In humid conditions, the spores develop holobasidia with sessile basidiospores which conjugate and produce conidia.

From Central America seven different *Entyloma* species were known before 1992: *E. browalliae* H. Sydow, *E. costaricense* Cif. (= *E. compositarum* Farlow), *E. dactylidis* (Pass.) Cif., *E. dahliae* H. Sydow & Sydow, *E. galinsogae* H. Sydow & Sydow, *E. oryzae* H. Sydow & Sydow, and *E. tagetesium* Zundel. Recent smut collecting in Costa Rica (Piepenbring, 1994) showed the presence of *E. australe* Speg., *E. bidensis* Henn., *E. ecuadorensis* H. Sydow, *E. lobeliae* Farlow, *E. microsporium* (Unger) Schröter, and two new species: *E. delileae* Vánky, Döbb. & U. Braun (Vánky, 1992: 417) and *E. doebbeleri* M. Piepenbring (Piepenbring, 1995: 784). The majority of these *Entyloma* species infect Asteraceae, *E. australe* and *E. browalliae* Solanaceae, *E. dactylidis* and *E. oryzae* Poaceae, *E. lobeliae* Lobeliaceae, and *E. microsporium* Ranunculaceae. The two new Central American species of *Entyloma* presented in this paper have been collected in El Salvador and Costa Rica in 1995. To date, 16 different species of *Entyloma* are known for Central America.

The systematic position of *Entyloma vignae* Bat. & al. on Fabaceae (*Phaseolus* and *Vigna*) reported for Costa Rica by Vakili (1978) is unclear. It might be an ascomycete belonging to the Protomycetales (Ascomycetes), because germinating spores produce vesicles containing endospores (Haware & Pavgi, 1976; 1977; E. Vargas, pers. comm.). However, Reddy & Kramer (1975: 39) do not accept that the hitherto exclusively Asteraceae and Apiaceae infecting Protomycetales can be parasites of Fabaceae. A detailed study of this species is highly desirable.

### Material and methods

Fresh or herbarium dried pieces of leaf spots were squashed in lactophenol on slides and covered by a cover slip. Dried spores were briefly heated to boiling, and measured under the light microscope including the entire spore wall. The values given represent the mean minus one standard deviation (lower limit) and the mean plus one standard deviation (upper limit). Mean values were computed from the measurements of 50 spores for new species and 20 for known species. Extreme values are given in parentheses. Randomly encountered well preserved spores were drawn with a camera lucida.

Abbreviations: M = Botanische Staatssammlung in Munich, Germany; H. U. V. = private Herbarium Ustilaginales K. Vánky in Tübingen, Germany; H. U. P. = private herbarium of the author.

## Material examined for comparison

- Entyloma compositarum* Farlow on *Aster puniceus* L.: USA, "Woods Hall" [Mass., Wood's Hole], IX.1881, W. Telease, FH (BPI 195048, TYPE).
- Entyloma dahliae* H. Sydow & Sydow on *Dahlia* sp.: El Salvador, Dept. Ahuachapán, Apaneca, on the way up to the Laguna de las Ninfas, 1700 m, 26. 8. 1995, C. Ventura & M. Piepenbring 1755 (M, H.U.P.).
- Entyloma gaillardianum* Vánky on *Gaillardia aristata* Pursh (cult.): Germany, Baden-Württemberg, Tübingen, botanical garden, 24. 4. 1994, M. Piepenbring (M, H. U. P. 709).
- Entyloma lobeliae* Farlow on *Lobelia laxiflora* H. B. K.: Costa Rica, Prov. San José, 4 km SW Ciudad Colón, 800 m, 20. 1. 1991, P. Döbbeler (H.U.P. 328).
- Entyloma tagetesium* Zundel on *Tagetes* sp.: Guatemala, Chimaltenango, 28. VIII. 1942, A. S. Müller 188 (BPI 176678, TYPE).

## Results and discussions

*Entyloma* on *Diastatea micrantha* (Lobeliaceae)

Until now, a single *Entyloma* species is reported from Lobeliaceae, viz. *E. lobeliae* Farlow (1883: 275) on several different *Lobelia* species in North and Central America. *Diastatea micrantha* (H. B. K.) McVaugh, an inconspicuous herb of this family growing in moist, shaded places, has not yet been reported as a smut host. It was found infected by an *Entyloma* species in El Salvador and Costa Rica.

*Entyloma lobeliae* forms angular, abaxially reddish coloured spots on *Lobelia* leaves. Spores of *E. lobeliae* (Fig. 3) are ovoid and sometimes subangular, (9–)10–12 x 12–15(–17)  $\mu\text{m}$  (H.U.P. 328; Tab. 1). The inner layer of the spore wall is 0.7  $\mu\text{m}$ , the outer irregularly 0.8–2.5  $\mu\text{m}$ , and both are 1.5–3  $\mu\text{m}$  thick. The spores of the *Entyloma* on *Diastatea* (Figs. 2, 8) are smaller and the outer layer of the wall is of regular thickness and mostly thinner than in *E. lobeliae* spores. The *Entyloma* on *Diastatea* is described as a new species because its spores and its host genus are different to those of *E. lobeliae*.

Tab. 1. – Spore characteristics of *Entyloma* species on Lobeliaceae.

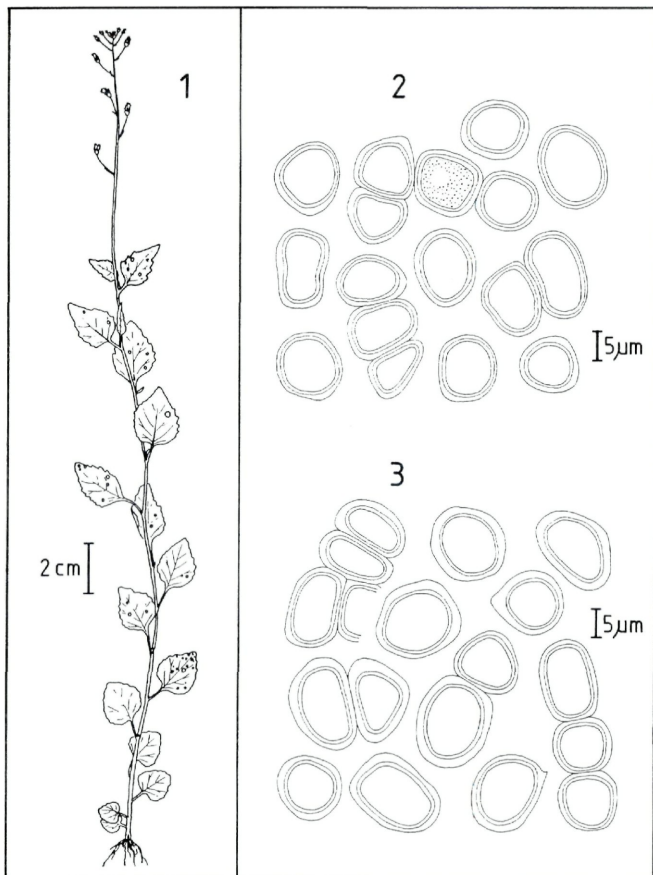
|                        | spore size                   | wall thickness      | thickness of outer wall layer |
|------------------------|------------------------------|---------------------|-------------------------------|
| <i>E. lobeliae</i>     | 12–15(–17) $\mu\text{m}$     | 1.5–3 $\mu\text{m}$ | irregular                     |
| <i>E. on Diastatea</i> | (9–)10–13(–16) $\mu\text{m}$ | 1.5 $\mu\text{m}$   | regular                       |

*Entyloma diastateae* M. Piepenbring, sp. nov. – Figs. 1, 2, 8.

Sori maculas in laminis foliorum formantes, quasi semper circulares, usque ad 1.5–2 mm diametro, non incrassati; maculae in statu vivo adaxialiter visae dilute virides vel luteolae, abaxialiter visae albiae. Sporae abaxialiter in lacunis intercellularibus mesophylli, globosae ad subglobosae, (8–)9–11(–12) x (9–)10–13(–16)  $\mu\text{m}$ , luteolae hyalinae. Parietis sporarum duobus stratis compositus, regulariter 1.5  $\mu\text{m}$  crassus, laevis.

In matrice *Diastateae micranthae* (H.B.K.) McVaugh (Lobeliaceae).

Sori (Fig. 1) as leaf spots, mostly circular, diam. 1.5–2 mm or smaller, sometimes larger by fusion, without swelling, fresh adaxially difficult to see, light green or yellowish, without clear limits, abaxially whitish, clearly delimited, dry brownish on both sides and difficult to distinguish from surrounding tissue. – Spores (Figs. 2, 3) in inter-



Figs. 1–3. – 1. Leaf spots of *Entyloma diastatae* on *Diastatea micrantha* (MP 1691). – 2. Teliospores of *Entyloma diastatae* (holotype). – 3. Teliospores of *Entyloma lobeliae* (H.U.P. 328).

cellular spaces of the abaxial mesophyll, spherical to ovoid, (8-)9-11(-12) x (9-)10-13(-16)  $\mu\text{m}$ , yellowish to hyaline. Spore wall two-layered, the inner layer ca. 0.7  $\mu\text{m}$ , the outer regularly ca. 0.8  $\mu\text{m}$ , both ca. 1.5  $\mu\text{m}$  thick, smooth. - Germination was not obtained, although repeatedly tried with fresh and dry spores. - Anamorph not observed.

Known from El Salvador and Costa Rica.

**Holotype.** - On *Diastatea micrantha* (H.B.K.) McVaugh, El Salvador, Dept. La Libertad, Ciudad Merliot, Finca El Espino, 850 m, 19.8.1995, C. Alemán, R. Esquivel & M. Piepenbring 1717 (in M; Isotypes in H.U.P. and distributed by D. Triebel: Microfungi Exsiccati).

**Paratypes.** - EL SALVADOR, Dept. La Libertad, Ciudad Merliot, Finca El Espino, 850 m, 12. 8. 1995, O. Molina & M. Piepenbring 1691 (in M and H.U.P.). - EL SALVADOR, Dept. Sonsonate, Juayua, La Calera, close to the waterfalls, 1030 m, 27. 8. 1995, C. Ventura & M. Piepenbring 1767 (in M and H.U.P.). - COSTA RICA, Prov. San José, San José, San Pedro, campo de la Universidad de Costa Rica, 1200 m, 29. 9. 1995, M. Piepenbring 1836 (in M and H.U.P.).

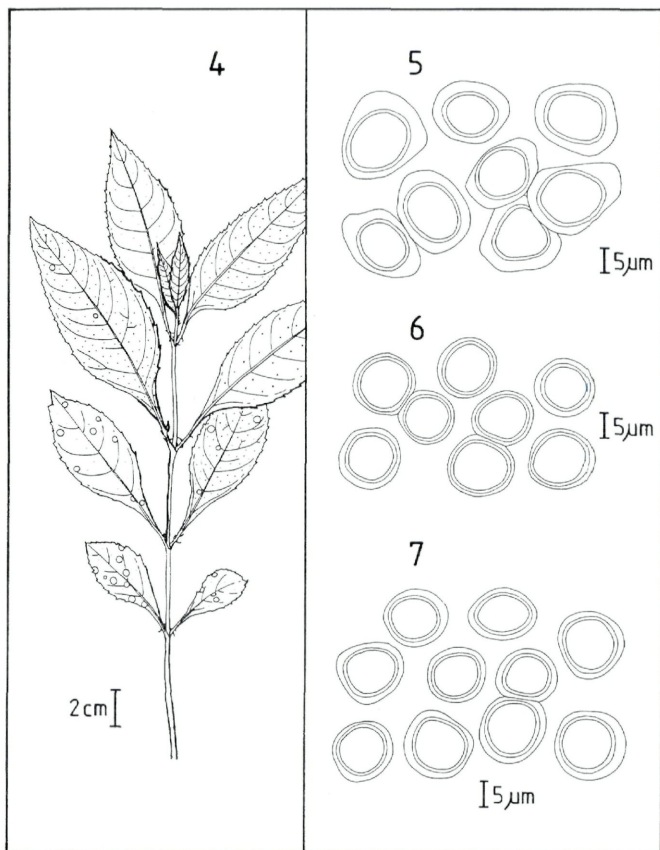
#### *Entyloma* on *Comaclinium montanum* (Asteraceae)

In El Salvador, an *Entyloma* species was found on *Comaclinium montanum* (Benth.) Strother [= *Dyssodia montana* (Benth.) Gray], Asteraceae, tribe Helenieae. Hitherto, no *Entyloma* species is known from the genus *Comaclinium* (nor *Dyssodia*; tribe Helenieae), but there are numerous *Entyloma* species on other asteraceous genera. In Tab. 2 the spore characteristics of the *Entyloma* species on *Comaclinium* are compared to those of *E. compositarum* on *Aster puniceus* (TY-PE), *E. gaillardianum* on *Gaillardia aristata* (Fig. 6; H.U.P. 709), and *E. tagetesium* Zundel (TYPE) on *Tagetes* sp., all reported from asteraceous hosts of the Helenieae tribe, and to *E. polysporum* (Peck) Farlow (data from Vánky, 1994: 102) and *E. dahliae* on hosts of the closely related tribe Heliantheae. The data for *E. dahliae* (Fig. 7) are obtained from a collection (MP 1755) made at about one kilometer away from the locality, where the *Entyloma* on *Comaclinium* was collected.

Tab. 2. - Spore characteristics of selected *Entyloma* species on Asteraceae (for references see text).

|                          | spore size                 | wall thickness            | thickness of outer wall layer |
|--------------------------|----------------------------|---------------------------|-------------------------------|
| <i>E. compositarum</i>   | 10-12(-14) $\mu\text{m}$   | 0.7-1(-2) $\mu\text{m}$   | regular                       |
| <i>E. gaillardianum</i>  | 9-14 $\mu\text{m}$         | 2(-3) $\mu\text{m}$       | rather regular                |
| <i>E. polysporum</i>     | 10-14.5(-17) $\mu\text{m}$ | 0.8-2(-2.5) $\mu\text{m}$ | rather regular                |
| <i>E. dahliae</i>        | 10-14(-15) $\mu\text{m}$   | 2-2.5 $\mu\text{m}$       | rather regular                |
| <i>E. tagetesium</i>     | 11-14(-16) $\mu\text{m}$   | 2(-3) $\mu\text{m}$       | rather regular                |
| <i>E. on Comaclinium</i> | 11-16(-18) $\mu\text{m}$   | 2-4 $\mu\text{m}$         | irregular                     |





Figs. 4-7. - 4. Leaf spots of *Entyloma comacolinii* on *Comaclinium montanum* (holotype). - 5. Teliospores of *Entyloma comacolinii* (holotype). - 6. Teliospores of *Entyloma gaillardianum* (H.U.P. 709). - 7. Teliospores of *Entyloma dahliae* (MP 1755).

Ványky (1982: 106) states that in comparison to the type of *Entyloma compositarum*, *E. gaillardianum* has more irregular and somewhat larger spores with a thicker, often two-layered wall seen by the light microscope (Fig. 6). Savile (1947: 110, 116), however, calls the *Entyloma* species on *Gaillardia* *E. compositarum*. Adopting a narrow species

concept, i. e. accepting *E. gaillardianum* as a distinct species, it is evident that the *Entyloma* on *Comaclinium* with its larger spores and a thicker spore wall (Figs. 5, 9) is distinct from *E. compositarum* and *E. gaillardianum*.

*E. polysporum*, which in the narrow species concept is restricted to *Ambrosia* as host genus (comp. Vánky, 1994: 102 and photo on p. 128), has spores that are almost as large as those of the *Entyloma* on *Comaclinium* but the spore wall of *E. polysporum* is of rather regular and minor thickness. The *Entyloma* on *Comaclinium* could be included in Savile's *E. polysporum* collective species [spore size 8.5–13.5 (–16) × 9–17(–21.5) µm, with a spore wall usually 1–3 µm thick; Savile 1947: 112]. This complex may also include *E. dahliae* (Savile, 1947: 115) and *E. tagetesium* from Guatemala. However, the spores of *E. dahliae* collected close to the infected *Comaclinium* are smaller and possess a thinner, more regular wall than the spores of *Entyloma* from *Comaclinium*. *E. tagetesium* differs from the *Entyloma* on *Comaclinium* by smaller spores and pustular sori (Zundel, 1945: 373).

I apply a narrow species concept using spore characteristics and the new host genus to describe a new species for *Entyloma* on *Comaclinium*.

***Entyloma comacolinii* M. Piepenbring, sp. nov. – Figs. 4, 5, 9.**

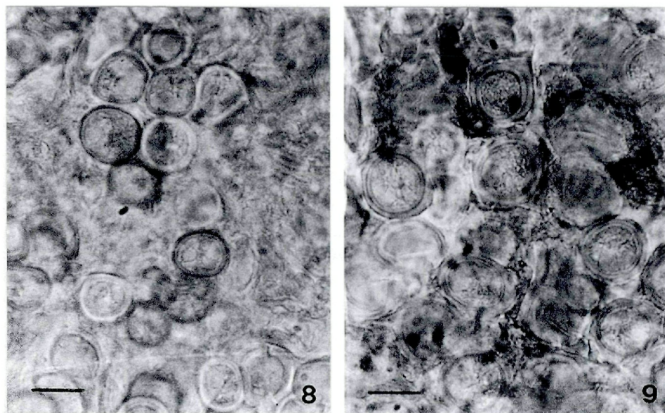
Sori maculas in laminis foliorum formantes, regulariter circulares, quasi semper 2–5 mm diametro, non incrassati; maculae in statu vivo adaxialiter visae dilute virides vel luteolae, abaxialiter visae albidae, maculae majores centro brunneo. Sporae dense aggregatae in lacunis intercellularibus mesophylli, globosae, subglobosae vel subangulares, 10–13(–14) × 11–16(–18) µm, luteolae hyalinae. Parietis sporarum duobus strati compositus, irregulariter 2–4 µm crassus, laevis.

In matrice *Comacolinii montani* (Benth.) Strother [= *Dyssodia montana* (Benth.) Gray] (Asteraceae, Helenieae).

Sori (Fig. 4) as leaf spots, regularly circular, diam. mostly 2–5 mm, sometimes larger by fusion, without swelling, fresh adaxially light green or yellowish, abaxially whitish, large spots with brown centre, dry brownish on both sides. – Spores (Figs. 5, 9) densely crowded in intercellular spaces of the mesophyll, irregularly spherical, ovoid, or subangular, 10–13(–14) × 11–16 (–18) µm, yellowish hyaline. Spore wall two-layered, the inner layer ca. 1 µm, the thickness of the outer very irregular up to 3 µm, both 2–4 µm thick, smooth. – Germination not obtained although repeatedly tried with fresh and dry spores. – Anamorph not observed.

Hitherto only once collected in El Salvador.

Holotype. – On *Comaclinium montanum* (Benth.) Strother [= *Dyssodia montana* (Benth.) Gray], El Salvador, Dept. Ahuachapán, Apaneca, ca. 200 m E of



Figs. 8–9. – Light microscopy photographs of the teliospores of *Entyloma diastataeae* (8) (holotype; bar = 10  $\mu\text{m}$ ) and *Entyloma comacolinii* (9) (holotype; bar = 10  $\mu\text{m}$ ).

the Laguna de las Ninfas, coffee plantation, 1750 m, 26. 8. 1995, C. Ventura & M. Piepenbring 1762 (in M; Iso types in H.U.V., H.U.P.).

*Comacolinium montanum* grows in coffee and corn plantations, as well as in secondary vegetation. The infected host specimens have neither flowers nor seeds, but are easily identified by the basal appendices of the leaves, by the laminar glands, and by the very characteristic shape of the leaf blade border.

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