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## First contribution to the study of *Cryptosphaeria* from Argentina

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A new species of *Cryptosphaeria*, from Tierra del Fuego, Argentina, *C. sulcata*, is described. The representation of this genus in Argentina is low; only *C. lignyota* and the present species are known. *Cryptosphaeria populicola*, described by Spegazzini, is a later synonym of *Eutypella scoparia*. *Cryptosphaerina heterospora* and *C. cumingii*, proposed by Spegazzini, are excluded from the *Diatrypaceae*. Observations of asci with fluorescence microscopy are presented.

**Key words:** ascomycete, *Diatrypaceae*, Diatrypales, new species.

### Introduction

*Cryptosphaeria* contains four species (Rappaz, 1987), *C. eunomia* (Fr.: Fr.) Fuckel, *C. lygniota* (Fr.: Fr.) Auersw., *C. subcutanea* (Wahl.: Fr.) Rappaz and *C. pullmanensis* Glawe and one variety *C. eunomia* (Fr.: Fr.) Fuckel var. *fraxini* (Richon) Rappaz. All taxa occur on bark and were described from the northern hemisphere (Rappaz, *op. cit.*). Ju *et al.* (1996) however, in a study of *Valsaria*, have mentioned that *C. eunomia* var. *fraxini* could be placed in *Endoxylina*. Rappaz (1987) considered *Endoxylina* to be a synonym of *Eutypa*. We consider that Ju *et al.* (1996) are more likely to be correct, according to their commentaries and description and to the drawings of Berlese (1900-1905: Vol. I Tab. XXXIV, 3, as *Thyridaria fraxini* Ellis & Everh.). It is however, necessary to study all holotypes before making a final decision. In addition Læssøe and Spooner (1993) proposed the synonym of the genus *Anthostoma* with *Cryptosphaeria* with the new combination *C. decipiens* (DC: Fr.) Læssøe & Spooner. We consider that this may be correct because of the similarity of the spore shape and colour (as it was mentioned by these authors), as well as the similar apical structure of the asci (see photographs of Rappaz, 1992 and Læssøe and Spooner, 1993). We also include *C. venusta* Vasilyeva (1986) within *Cryptosphaeria*.

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Our knowledge of *Cryptosphaeria* in South America is very poor, no records are known from Bolivia, Brazil, Colombia, Paraguay, Peru, Uruguay or Venezuela. Spegazzini (1910, 1925 respectively) described *Cryptosphaeria cumingii* Speg. (as *Cryptosphaeria ? cumingii*) and *Cryptosphaeria heterospora* Speg. from Chile. *Cryptosphaeria* Lambote & Fautrey was transferred to *Cryptosphaeria* by Rappaz (1987).

In Argentina, there has been little work on the Diatrypales in general. The only papers are those by Carmarán and Romero (1992) and more recently by Carmarán (2002). No work has been carried out on *Cryptosphaeria*. There is however, a previous report of *C. populicola* Spegazzini (1898) on *Populus monilifera* from Buenos Aires Province.

In the current work concerning the *Diatrypaceae* of Argentina 400 collections were studied from 9 Provinces, and of this material, two species belong in the genus *Cryptosphaeria*. One has been identified as *C. lignyota* and the other one as a new species, *C. sulcata*. In this paper we discuss the representation of *Cryptosphaeria* in Argentina and describe the new species.

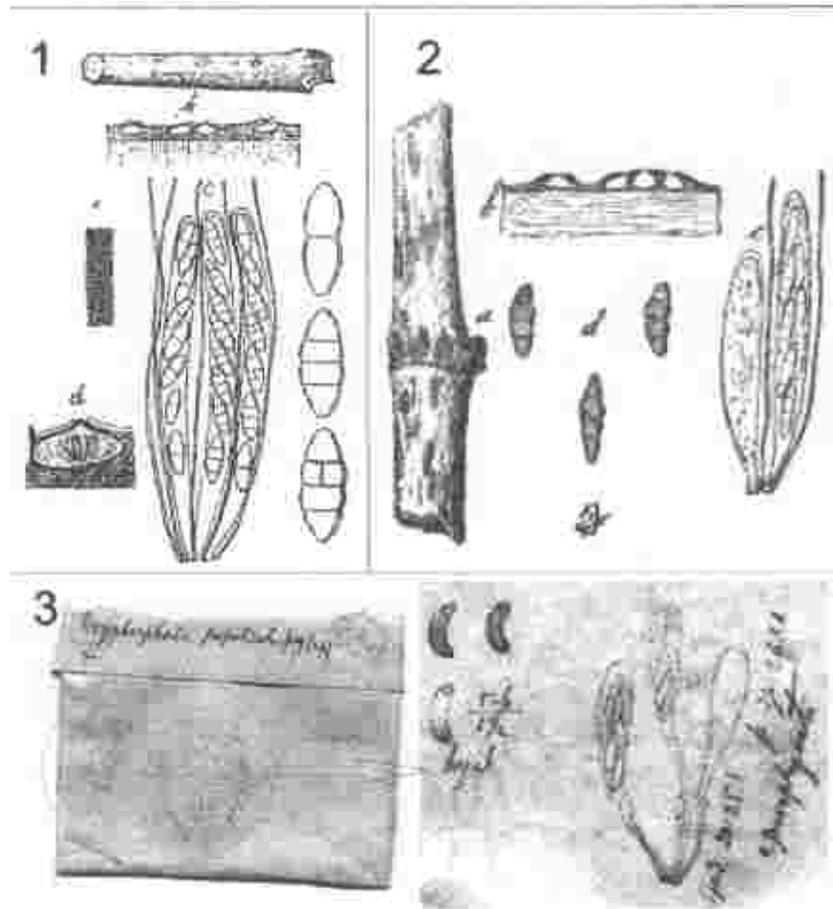
## Materials and methods

Collected material was air-dried. Specimens were preserved in BAFC. Holotypes from BR, WSP and LPS were also studied (herbarium abbreviations follow Holmgren *et al.*, 1990). Observations and measurements were taken from fresh material mounted in distilled water, 5% KOH and phloxine for optical microscopy (M) and in Melzer's reagent for the amyloid reaction (I). For epifluorescence light (EFM) the samples were mounted using 5% calcofluor (Romero and Minter, 1988). Drawings were made using a camera lucida and the photographs were taken with Tri X Pan 400 ASA film for fluorescence.

## Results and discussion

The holotypes of *Cryptosphaeria heterospora* Spegazzini (1925) and of *C. cumingii* Spegazzini (1910) were not found in LPS. After looking at Spegazzini's illustrations (Fig. 1) and description, Rappaz (1987) excluded *C. heterospora* from the *Diatrypaceae*, concluding it had bitunicate asci. We concur with his conclusion.

*Cryptosphaeria cumingii* was illustrated by Spegazzini (1910). According to his drawings (Fig. 2) it is possible to observe that this specimen is not a diatrypaceous. It has dark pigmented and 3-septate ascospores and ascus features are completely different to those of this family. It also is excluded from *Diatrypaceae*. It also appears to have bitunicate asci.



**Figs. 1-3.** Spegazzini's illustrations: **1.** *Cryptosphaerina heterospora*. **2.** *Cryptosphaerina cumingii*. **3.** *Eutypella scoparia*, Spegazzini's illustrations made on the envelope LPS 2136 (holotype of *Cryptosphaeria populicola*).

Following study of the holotype of *Cryptosphaeria populicola* Speg. (1898) we agree with its synonymy with *Eutypella scoparia* (Schwein.: Fr.) Ellis & Everh. (Fig. 3), as proposed by Rappaz (1987)

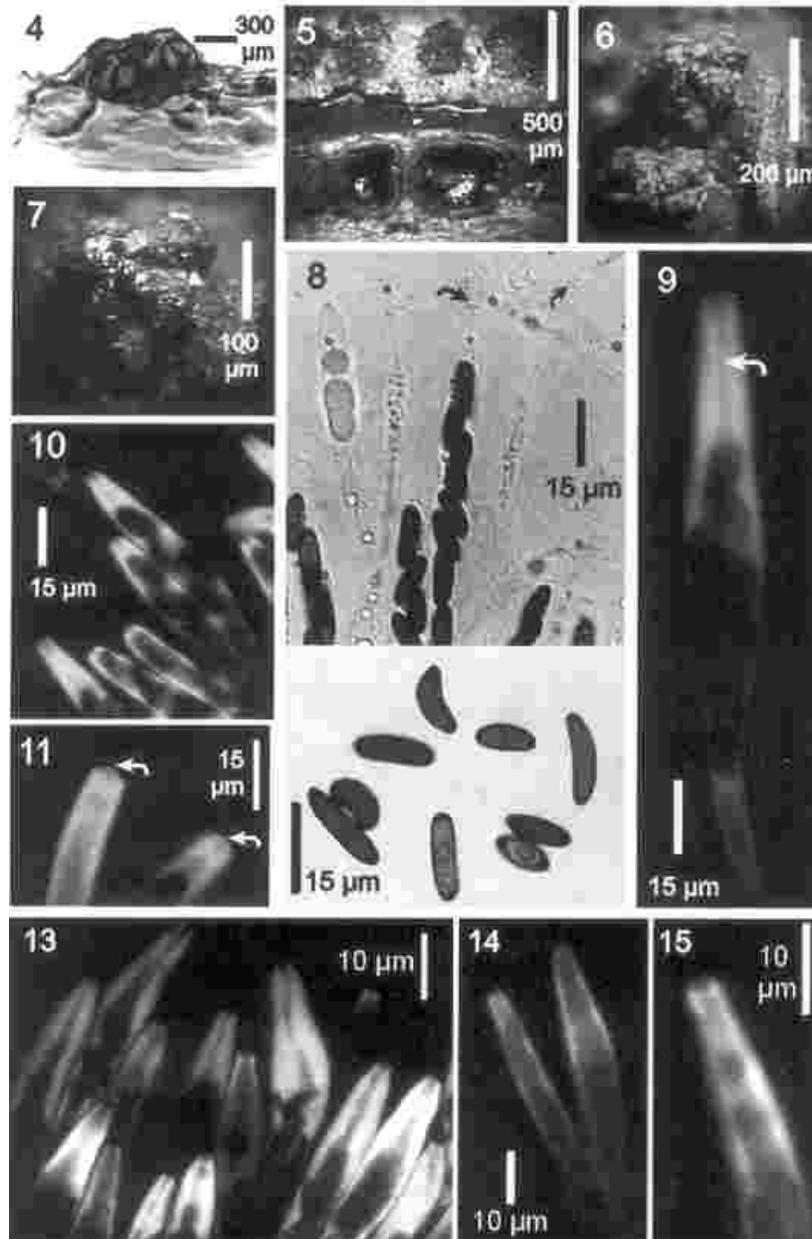
*Material examined:* ARGENTINA, Buenos Aires, La Plata, on *Populus monilifera*, July 1896, C. Spegazzini (LPS 2136, holotype of *C. populicola*).

***Cryptosphaeria sulcata* A.I. Romero & Carmarán, sp. nov.** (Figs. 4-12)

*Etymology:* *sulcata*, refers to ostioles with distinctive furrows.

A *C. pullmanensis* differt in ascosporis 12-15 × 4-6 μm, et in ostiola sulcata.

***Holotypus:*** ARGENTINA, Tierra del Fuego, Dpto. Ushuaia, Monte Olivia 54° 49'S; 068° 19'W, in ramo *Nothofagus* sp., 15 December 1997, C.C. Carmarán (BAFC 50.923).



**Figs. 4-12.** *Cryptosphaeria sulcata*. **4.** General aspect: scheme, showing the sulcate ostioles. **5.** Longitudinal section showing the immersed venter in bark. **6, 7.** Detail of the sulcate ostioles. **8.** Microscopic observations of asci, note apical inamyloid ring (arrows). **9.** Ascus observed with EFM, note the central channel (arrow). **10.** Several asci observed with EFM. **11.** A ring-like apical structure protruding in the upper part of the opened ascus (left arrow) and of an ascus still with ascospores (right arrow). **12.** Ascospores. **Figs. 13-15.** *Cryptosphaeria lignyota*. Observations of asci with fluorescence microscopy. **13.** BAFC 50.924. **14, 15.** Holotype.

*Stromata* poorly developed, immersed in bark. *Perithecia* single erumpent, ca. 500-600  $\mu\text{m}$ . *Ostioles* with 3-4 distinct sulcations. *Asci* spindle-shaped, long stipitate, with non amyloid apical ring, invaginated at the apex with cytoplasmic strands, with a narrow channel, 70-85  $\times$  4-7  $\mu\text{m}$ . *Ascospores* allantoid to cylindrical, brown, cell walls thickened at each end, 12-15  $\times$  4-6  $\mu\text{m}$ .

*Additional material examined:* USA, Washington, Whitman County, South Fairway, Pullman, 46° 44'N; 117° 06'W, on fallen branches of *Populus trichocarpa*, 11 December 1981 (WSP 67333, holotype of *Cryptosphaeria pullmanensis*).

*Notes:* Slide preparations of the asci in calcofluor show a thickened apical wall of unopened asci fluorescing strongly. A central channel could be observed in the middle of this strongly fluorescing wall. A ring-like apical structure fluoresced more strongly than surrounding wall material and sometimes protruded in the upper part of the opened asci (Fig. 11, arrowed). This new species is distinguished from *C. pullmanensis* Glawe (Glawe, 1984) by its distinct sulcate ostioles, slightly larger ascospores and the fagaceous host.

Glawe and Rogers (1986) mentioned the reduced stroma, the dark ascospores and salicaceous host of *C. vicinula* (Nyl.) Karst., now *C. subcutanea* (Wahl.: Fr.) Rappaz (Rappaz, 1987) indicate that this species is closely related to *C. pullmanensis* Glawe. These authors however, also said that the conidia are significantly shorter in *C. pullmanensis* than in *C. subcutanea*. Some collections of *C. subcutanea* (Berlese, 1900-1905; Glawe, 1984; Mathiassen, 1989, 1993) appear to have a well-developed pulvinate stroma, a feature not seen in *C. pullmanensis*. Although this new species has sulcate ostioles in common with *C. subcutanea* and *C. venusta* Vasilyeva (1986), it is referred to the species *C. pullmanensis* because it shares a similar type of stroma and dark ascospores with thick cell walls and darker ends. The close similarity among these three taxa: *C. subcutanea*, *C. pullmanensis* and *C. sulcata*, are supported by studies in numerical taxonomy (Carmarán, pers. comm.).

The asci of *C. sulcata* are considered to be type 1 b (Carmarán and Romero, 1992). The observations with fluorescent light microscopy are in agreement with Romero and Minter (1988) and indicate that the process of discharge of ascospores in *C. sulcata* are similar to *Diatrype disciformis* (Hoffm.) Fr.

Note that the two collection sites for this species (Argentina and USA) are similar principally in average precipitation, while other parameters have close values. In Pullman the average temperature is -1°C, with average precipitation of 40 mm and average relative humidity of 88% in December (collection month). In Ushuaia the average temperature is 8°C, with average

precipitation of 40 mm and with average relative humidity of 70% in December.

***Cryptosphaeria lignyota*** (Fr.:Fr.) Auersw in Rabenh., Fungi europaei exs., ed. nova ser. 2, cent. 13: 1269.1869 (under 'ligniota'). (Figs. 13-15)

*Description and synonym:* Rappaz (1987).

*Material examined:* ARGENTINA, Buenos Aires, Pdo. Gral. J. Madariaga, Villa Gesell, on fallen branch of *Populus* sp., on the sand, on the beach, 18 February 1998, A.I. Romero (BAFC 50.924); GERMANY, Arnstadt, on *Populus italica*, no date, Fleischhack. (BR 1269-neotype of *Cryptosphaeria lignyota*).

*Notes:* This species was previously only known from Europe and north America and this is the first record for Argentina. Observations of asci under fluorescence microscopy (Carmarán and Romero, 1992), indicate that the asci of this species are type 1 b. The apical wall of unopened asci fluoresced strongly, the central channel can be observed running up the middle of this strongly fluorescing wall. Similar results were observed in both material, BAFC (Fig. 13) and the neotype, except the older material is has collapsed (Figs. 14, 15).

The representation of the genus *Cryptosphaeria* seems to be poor in Argentina. We have studied 400 collections of *Diatrypaceae* from nine Provinces of Argentina and only *C. sulcata* and *C. lignyota* were found. The 3 species of *Cryptosphaeria* discussed by Spegazzini from Argentina and Chile are not *Cryptosphaeria* species.

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