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Dothiorina: taxonomic concepts and comments on its conidiogenesis

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Abstract — *Dothiorina tulasnei* was collected on bark of *Nothofagus pumilio*. It is cited and described for the first time in Argentina. This coelomycete was considered a peculiar species because of its unusual conidiogenesis; but reports on the process are contradictory and still under discussion. Here, the present circumscription of the genus and the status of all *Dothiorina* species are discussed. Also, its *Chalara*-like conidiogenesis is fully described and documented.

Keywords — mitosporic fungi, ring wall building, *Fagaceae, Nothofagaceae,* Patagonia

Introduction

In 1865, Tulasne & Tulasne described and illustrated a new stromatic coelomycete species as a variety of *Sphaeria moriformis* Tode but without giving it a formal name. Saccardo (1884) named this species as *Dothiorella tulasnei* and provided the formal description. In 1911, Höhnel created the new genus *Dothiorina* Höhn. and transferred this species there.

In addition to the type species, two more species were added later to the genus: *Dothiorina discoidea* (Höhnel 1925) and *D. subcarnea* (Riedl 1977).

The last contribution to the knowledge of the genus was that of Dixon (1975). Recently, *Dothiorina* was registered for the first time in Argentina (Sánchez et al. 2005). This fact gave us the opportunity to study fresh material. Also, we examined the available herbarium specimens of all the species described in the genus. In this paper, we discuss the present circumscription of the genus and provide a full description of its conidiogenesis in modern terms.

Material and methods

Recently collected materials were air-dried and are preserved in Bahía Blanca Biología Herbarium (BBB). Herbarium materials were rehydrated in tap water.

Sections were hand-made with a razor blade and were mounted in tap water or in 5% KOH with phloxine. All measurements were made in water. Herbaria are abbreviated according to Holmgren et al. (1990).

Results

Dothiorina Höhn. emend.

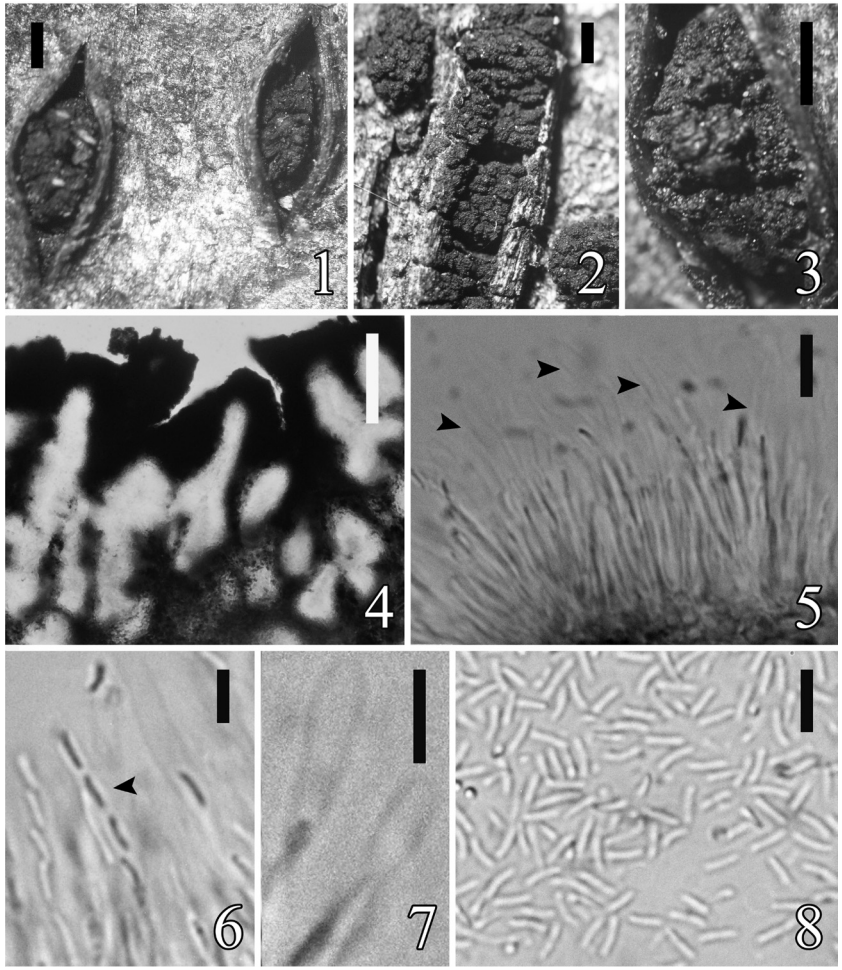
Conidiomata stromatic, plurilocular, superficial or immersed and then erumpent through the bark, subspherical to moriform, greenish to brownish and gelly when wet, becoming black and carbonaceous when dried. Locules ovoid to irregular, at different levels. Conidiophores branched, forming from the inner cells of the locular walls. Conidiogenous cells phialidic, integrated, determinate, smooth, hyaline, necks long, cylindrical, venters cylindrical to slightly ampulliform. Conidia in chains, unicellular, allantoid, smooth, hyaline, produced by **ring wall building** within phialides, forming basipetal chains of up to five conidia into the necks.

Accepted species

Dothiorina tulasnei (Sacc.) Höhn., Sitzungsber. K. Akad. Wiss., Math.-Naturwiss. Kl., Abt. 1, 120: 464 (1911) Figures **1–8**
≡ *Dothiorella tulasnei* Sacc., Sylloge Fungorum 3: 239 (1884)

Conidiomata stromatic, plurilocular, subspherical to moriform, superficial to erumpent through the bark, greenish to brownish and gelatinous when wet, becoming black and carbonaceous when dried, 0.26–7 × 0.2–2.5 mm. Locules ovoid to irregular, at different levels; separated by somewhat parallel, greenish to light brown textura oblita, individual cells small, hyaline to light brown in water, dark green stained in KOH, up to 2 µm diam. Conidiophores branched, hyaline to subhyaline, covering the interior of the locules, 5–31 × 1–3 µm. Conidiogenous cells phialidic, integrated, determinate, smooth, hyaline, necks long, cylindrical, 5–20 × 1-3 µm, venters cylindrical to slightly ampulliform, 5–21.5 × 1–2 µm. Conidia in chains, unicellular, allantoid, smooth, hyaline, 2–5 × 1 µm ($\overline{x}$ = 3.8 × 1 µm), produced by ring wall building within phialides, forming basipetal chains of up to five conidia into the necks.

specimens examined — **ARGENTINA**. **Chubut**: Huemules, (42°50'474"S 71°27'878"W, 1137m elevation), 20.XI.2003, on fallen branches of *Nothofagus pumilio*, leg. M. Rajchenberg *12131* (*BBB*). **Neuquén**: National Route 234 near Meliquina Lake (40°18'S 71°22'W), on log of *N. pumilio*, 16.V.2007, leg. MV Bianchinotti & RM Sánchez *569* (*BBB*). **AUSTRIA**: **Sonntagsberg**, "Auf *Pirus communis* Holz, *Dothiorina tulasnei* (Sacc.) v. Höhn.", Dec. 1910, leg. P. Strasser 3329, *FH 79620*, (isotype). **UNITED STATES**: **Connecticut**, West Haven, on alder, *Chlorosplenium aeruginosum* conidial stage, leg. R. Thaxter 194, *FH 79619*.



Figs. 1–8. *Dothiorina tulasnei* (all from MR 12131, BBB). 1–3. Conidiomata. 4. Vertical section of a conidioma. 5. Conidiophores and conidiogenous cells. Arrowheads point to empty necks. 6. Chain of conidia still into the neck of a conidiogenous cell (arrowhead). 7. Detail of conidiogenous cells. 8. Conidia. Bars: 1–3= 1 mm, 4= 100 µm, 5= 10 µm, 6–8= 5 µm.

ecology and distribution — Uncommon, on pieces of branches of *Alder* sp., *Pirus communis* Holz., and on fallen branches of *Nothofagus pumilio* (Poepp. & Endl.) Krasser. Known from Argentina, Austria, USA, Venezuela.

Comments — The specimens on *N. pumilio* differ from the North Hemisphere materials in several macro and microscopical features, as shown in Table 1, but conidiogenesis and conidial shape and size are the same. In all materials examined, necks of phialides are better seen in water mounts.

Table 1. Comparison among the examined collections of *Dothiorina tulasnei*

Dimensions	**FH** 79619	**FH** 79620	**BBB** MR 12131 MVB 569
Conidiomata (mm)	0.4–1.2 × 0.3–1	0.26–1.7 × 0.2–0.9	1–7 × 0.5–2.5
Locules (μm)	30–100 × 20–62.5	37.5–112.5 × 25–62.5	61–207.5 × 20–125
Conidiophores (μm)	5–14.5 × 1–2	5–8.5 × 1–2	6–31 × 1.5–3
Conidiogenous cells			
Necks (μm)	5–15.5 × 1–2	8–14.5 × 1–2	8–20 × 2–3
Venters (μm)	5–13.5 × 1–2	5–13.5 × 1–2	8–21.5 × 1–2
Conidia (μm)	2–5 × 1	3–5 × 1	3–5 × 1

Excluded species

Dothiorina discoidea (Berk. & Broome) Höhn.,
Mitt. Bot. Inst. Techn. Hochsch. Wien 2: 63 (1925) figures **9–15**

≡ *Psilonia discoidea* Berk. & Broome, Ann. Mag. Natur. Hist., 3 Ser., 18: 122 (1866)
≡ *Volutella discoidea* (Berk. & Broome) Sacc., Sylloge Fungorum 4: 687 (1886)

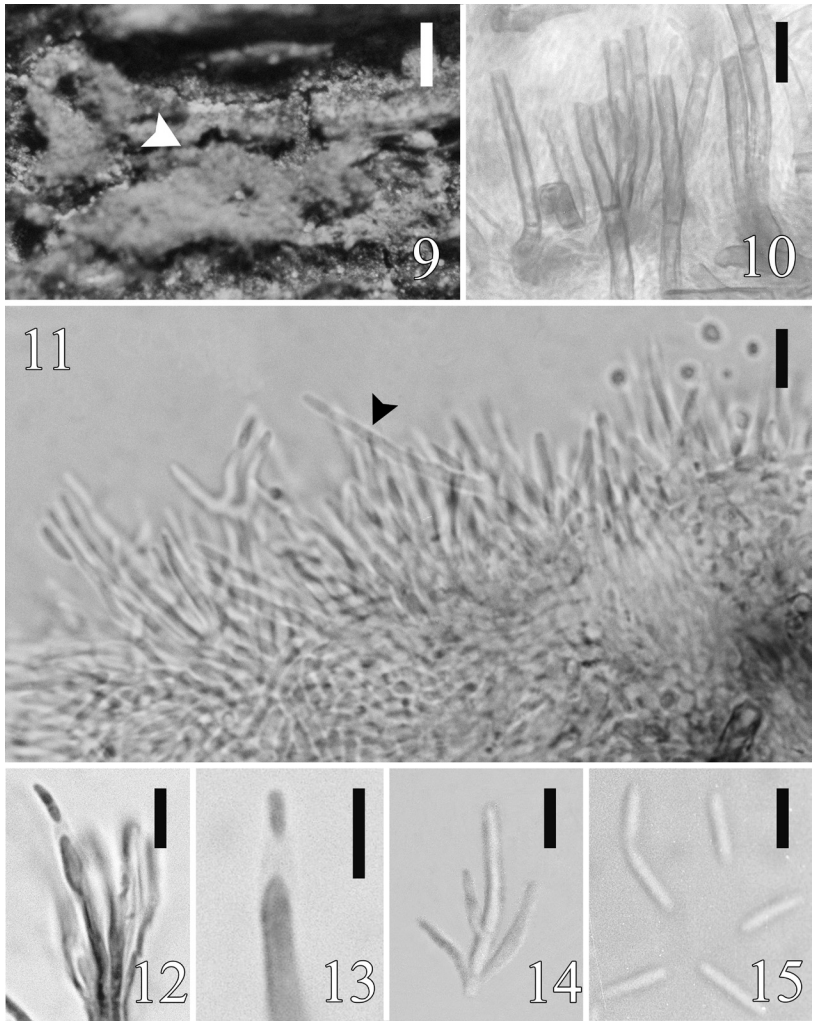
Conidiomata stromatic, multilocular, cup shaped, superficial, light brown to orange, setose, 0.26–2.04 × 0.2–1.2 mm; setae cylindrical, light brown to orange, often can produce conidiogenous cells, 11–21 × 3 μm. Locules irregularly disposed. Conidiophores covering the interior of the locules, branched, hyaline, 5–9.2 × 1–2 μm. Conidiogenous cells phialidic, integrated, determinate, rarely percurrent, smooth, hyaline, necks short, cylindrical, with periclinal thickening, 1–3 × 1–1.5 μm, venters cylindrical to slightly ampulliform, 4–20 × 1–2 μm. Conidia solitary, unicellular, allantoid, smooth, hyaline, 4–8 × 1–1.5 μm ($\overline{x}$ = 6 × 1.2 μm).

specimens examined– **United Kingdom:** Wiltshire, Langley, "in ligno putrido", January 1866, C. E. Broome (W 1075; isotype).

ecology and distribution– Rare, on rotten wood of a unknown tree species (probably oak). United Kingdom.

Comments — Unlike *D. tulasnei* when mounting *D. discoidea* on KOH no change on colouration was observed.

The combination of setose, cupuliform conidiomata, phialides with periclinal thickening and solitary conidia, led us to affirm, in agreement with Dixon (1975), that *D. discoidea* is not congeneric with *D. tulasnei*. It comes close to *Hainesia* Ellis & Sacc., but more material is needed in order to properly establish its generic disposition.



Figs. 9–15. *Dothiorina discoidea* (from W 1075). 9. Conidiomata (arrowhead). 10. Peripheral setae surrounding conidioma. 11–14. Conidiophores and conidiogenous cells. Arrowhead points to still unbranched conidiophore. 15. Conidia. Bars: 9= 250 μm, 10–11= 10 μm, 12–15= 5 μm.

Doubtful species

Dothiorina subcarnea Riedl, Sydowia 29: 151 (1977)

This species was described as follows:

> *Stromata semiglobosa vel irregulariter tuberculoso-convexa, carneo-brunnea, consistentia cerea, 300–350 μ in diam., rare confluentia; parietes exterior et interloculares e hyphis*

tenuibus, densissime intertextis, e cellulis brevibus, 0.5–0.7 μ fere longis, stratis extremis hypharum paulo obscurius coloratis; loculi irregulariter dispositi, aut poro ad superficiem superiorem stromatis, aperti aut profundius positi conidia in canalem stroma per altitudinem percurrentem diffundentes. Conidiophori 6–15 μ longi, usque ad 1 μ ad summum lati, fasciculati, parietes dense tegentes, interdum et in superficie stromatum evoluti, sed ibi plerumque steriles, rare conidia nonnula proferentes. Conidia apice conidiophori phialidei in catenis evoluta, mox loculos omnino complentia, minuta, oblonga vel oblongo-ellipsoidea, rare breviter bacillaria, 2–2.5 μ longa, 0.5 μ fere lata.

Comments– The holotype was deposited at Wien. It could not be located there and it is considered lost (Dr. Passauer, Curator of Cryptogams, Wien Herbarium, in lett.). Unfortunately, Riedl (1977) did not provide any drawings of the species so it is not possible to give a critical opinion about it. Until authenticated material can be studied, the identity of this species must remain in doubt.

Discussion

In 1865, Tulasne & Tulasne described a pulvinate and globose structure, with rugose but glabrous surface; comprised of several locules separated by light green parenchymatic tissue, covered internally with short, filiform and ramified conidiophores that originate very small, linear, straight and continuous conidia. The illustration they provided is considered the iconotype of *D. tulasnei* (Riedl 1977). Since its redescription (Riedl 1977) *Dothiorina* contained three species. We examined the existing types and we concluded that *D. discoidea* is not congeneric with *D. tulasnei* on the basis of the morphology of conidiomata and conidiogenesis. The third species, *D. subcarnea* must be considered a doubtful taxon as the type material is lost and no conclusion can be arrived through its protologue. So, taking into account the features of the conidiomata and the peculiar conidiogenesis, we consider that *Dothiorina* should be restricted to a single species, *D. tulasnei*.

Dothiorina tulasnei has been repeatedly considered the anamorphic state of *Chlorociboria aeruginascens* (Nyl.) Kanouse ex C.S. Ramamurthi et al. (Dixon 1975, Gamundí et al. 2004, Nag Raj 1977, Sutton 1980). The relationship was only based on the proximity of apothecia and conidiomata on natural substratum but it has never been demonstrated in culture. Saccardo (1884) doubted about the connection of the two fungi. However he was questioned by Höhnel (1911) who stated that Brefeld in 1891 obtained "rod conidia" in his cultures from *Chlorosplenium aeruginosum* (J. Koenig) De Not. ascospores. Dixon (1975) discredited Brefeld´s results because of the lack of sterile and pure culture techniques in the 19th century and also because of the "poor quality of his illustrations". Berthet (1964) also described a conidial stage obtained in culture from ascospores of an indeterminate species of *Chlorociboria*, but the phialides are very different from those found by us in *Dothiorina*. In their

recent monograph of *Chlorociboria*, Johnston & Park (2005) do not mention any connection to an imperfect state. Thus, we consider that the conidial stage obtained by Berthet does not represent a *Dothiorina* species. Until now, our attempts to obtain cultures have been unsuccessful. Future research will focus on obtaining isolates of both genera and to study them with molecular techniques.

The lack of detail regarding the conidiogenesis of *Dothiorina* has generated controversy about this process. Höhnel (1911) briefly described conidiophores as simple or branched, thread-shaped, densely covering the interior of locules. Dixon (1975: 205, fig. 27) illustrated conidiogenous cells as cylindrical to flask-shaped phialides bearing chains of three or four conidia in the long necks but he did not describe the process. Nag Raj (1977) described and illustrated blastic phialidic conidiogenous cells, with long necks, but with solitary conidia. Riedl (1977) mentioned long phialides and catenate conidia but did not provide illustrations. Sutton (1980) on the other hand, described "normal phialides" with minute channels and collaretes. He emphasized Berthet's (1964) description and pointed out the need to confirm Dixon's observations because of what he considered to be the scarcity of "*Chalara*-type" conidiogenesis among coelomycetes.

The differences between the conidiogenesis described by Berthet (1964) and our observations suggest there is no strong evidence that *Dothiorina* is related to *Chlorociboria*.

Our observations on type material and on the recently collected ones agree with the illustrations made by Dixon (1975). We have seen chains of three to five conidia in the long necks of the phialides. This conidiogenesis agrees with that described by Nag Raj & Kendrick (1993) as "*Chalara*-like". In this type of conidiogenesis there is no periclinal thickening in the phialides and the conidiogenous cells appear to undergo no modification between the production of successive conidia. "*Chalara*-like" conidiogenesis is widespread among unrelated genera of ascomycetes [viz. *Ceratocystis* s. str. (*Microascales*), some species of *Ceratocystiopsis* (*Ophiostomatales*), *Cryptendoxyla hypophloia* (*Sordariales*), *Quasiconcha reticulata* (*Hysteriales*) and *Pyxidiophora* (*Pyxidiophorales*)], all their mitosporic states being hyphomycetes (Nag Raj & Kendrick 1993). Our observations confirm the presence of true chains of basipetous conidia developed by a determinate conidiogenous cell in *D. tulasnei*. This is considered a strong evidence of ring wall-building activity (Nag Raj & Kendrick 1993).

We have also observed that the long necks of the phialides are easily visible in water but not in KOH. Care needs to be taken with the mounting medium used for studying conidiogenesis under light microscopy, a fact that is seldom mentioned in the literature.

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