

## A reinterpretation of the pseudo-bombardioid ascomal wall in taxa in the Lasiosphaeriaceae

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A coriaceous pseudo-bombardioid ascomal wall containing a central layer composed of thick-walled, gelatinized cells has been described in nine species in the Lasiosphaeriaceae (Sordariales). Three different methods of sectioning ascospores were employed to investigate the composition of this wall layer. Although this wall layer has previously been described as being composed of isodiametric cells, sections made using a freezing microtome revealed that it was actually composed of interwoven hyphae. Thus the term pseudo-bombardioid should be emended to describe a non-stromatic ascomal wall containing a gelatinized layer composed of interwoven, thin-walled hyphae.

Keywords: Ascomycetes, *Arnium*, *Bombardia*, *Bombardioidia*, *Cercophora*, *Podospora*, terminology.

A unique type of coriaceous ascomal wall referred to as a pseudo-bombardioid wall was defined by Lundqvist (1972: 17) in his seminal work on Nordic Sordariaceae as “a multi-layered, non-stromatic wall with a second pseudo-parenchymatous layer with very thick-walled, gelatinized cells”. Lundqvist (1972: 17) distinguished it from the bombardioid wall, which he described as “a multi-layered wall with at least two outer, stromatic layers, the second of which is fibrous and cartilaginous”. Thus, the pseudo-bombardioid wall is non-stromatic with a gelatinized cellular layer, whereas the bombardioid wall is composed of a stromatic gelatinized hyphal layer.

The pseudo-bombardioid wall has been described in nine species segregated into three genera in the Lasiosphaeriaceae (Sordariales) (Tab. 1). All but *Cercophora costaricensis* and *C. palmicola* were described by Lundqvist (1972) as having a pseudo-bombardioid wall. Although Carroll & Munk (1964) referred to the ascomal wall in *C. costaricensis* as bombardioid, they described the middle wall layer as being “composed of extremely thick-walled angular cells”.

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Tab. 1. – List of taxa in the Lasiosphaeriaceae possessing a pseudo-bombardioid ascomal wall and the specimens examined. Collectors names were abbreviated as follows: Roy Cain (Cain), George Carroll (GC), Sabine M. Huhndorf (SMH), Nils Lundqvist (Lqt), and Gary J. Samuels (GJS). Herbarium acronyms follow (Holmgren & al., 1990); GC refers to the private herbarium of George Carroll.

Genus and species	Specimen No.
<i>Arnium</i> Nitschke ex G. Winter emend. N. Lundq.	
<i>A. ontariense</i> (Cain) J. C. Krug & Cain	Cain 5343, Cain 32240 (TRTC)
<i>Cercophora</i> Fuckel	
<i>C. albicollis</i> N. Lundq.	Erb. Critt. Ital., ser. 1, 991 (Isotype, SIENA)
<i>C. costaricensis</i> (G. C. Carroll & Munk) O. Hilber & R. Hilber	GC 72, GC 73, GC 74, GC 75 (GC); SMH 2469, SMH 4021, SMH 4509, SMH 4537 (F)
<i>C. elephantina</i> (Henn.) N. Lundq.	TRTC 66.1093 (TRTC)
<i>C. palmicola</i> Hanlin & Tort.	Hanlin 12701 (Isotype, GAM)
<i>C. scortea</i> (Cain) N. Lundq.	GJS #556, SMH 4131, SMH 4441 (F)
<i>Podospora</i> Ces. emend. N. Lundq.	
<i>P. appendiculata</i> (Auersw.) Niessl	Lqt 5906-k, on the holotype specimen of <i>C. gossypina</i> (UPS)
<i>P. fimiseda</i> (Ces. & De Not.) Niessl	SMH 4452 (F); Rabenh., Kl. Herb. Viv. Mycol., ed. 2, 259, 1856 (Isolectotype, RO)
<i>P. perplexens</i> (Cain) Cain	Lqt 3347-k, Lqt 1664-d (TRTC)

Hilber & Hilber (1979) later described and illustrated the ascomal wall in *C. costaricensis* as pseudo-bombardioid. The ascomal wall was described as pseudo-bombardioid in *C. palmicola* (Hanlin & Tortolero, 1987; Hanlin, 1999), even though Hanlin (1999) noted "slender, dark-staining hyphae" traversing the middle wall layer. Although initially described as bombardioid (Munk, 1957), the ascomatal walls were later described as pseudo-bombardioid in *Podospora appendiculata* (Furuya & Udagawa, 1972; Lundqvist, 1972) and *P. fimiseda* (Lundqvist, 1972; Bell & Mahoney, 1997). Because workers have described the ascomatal walls differently in some taxa, the objective of this study was to verify previous interpretations of the composition of the pseudo-bombardioid wall morphology.

#### Materials and methods

Specimens representing all nine species reported to have a pseudo-bombardioid ascomal wall were obtained for this study (Tab. 1). All specimens used in this study were from dried herbarium material. Three different methods were employed to obtain longitudinal sections of the ascomata: 1) hand sections, 2) freezing microtome sections, and 3) plastic-embedded sections. All material

was rehydrated in water prior to sectioning. Hand sections of variable thickness were made by sectioning ascomata placed on a glass slide with a double-edged razor blade. Freezing microtome sections were made by freezing ascomata in Tissue-Tek<sup>®</sup> O.C.T. embedding compound followed by sectioning at 15, 30 and 45  $\mu\text{m}$  thickness on a Leica SM2000R freezing microtome. Plastic-embedded sections 5  $\mu\text{m}$  thick were made following the methods of Huhndorf (1991). Ascomatal sections were mounted in water (or Permount mounting medium for plastic-embedded sections) and images were captured using differential interference (DIC) microscopy and processed using Adobe Photoshop 3.0 and 5.5 (Adobe Systems Incorporated, Mountain View, California).

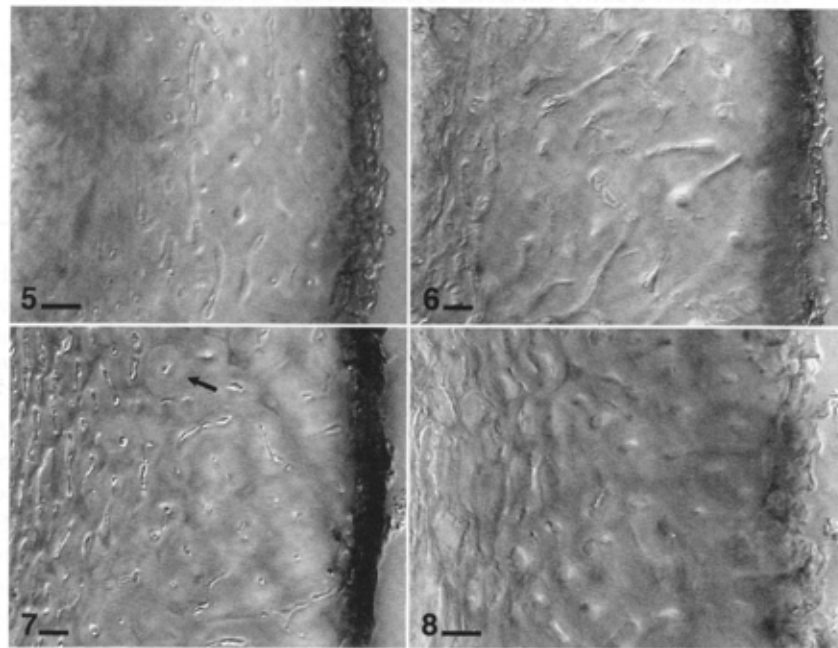
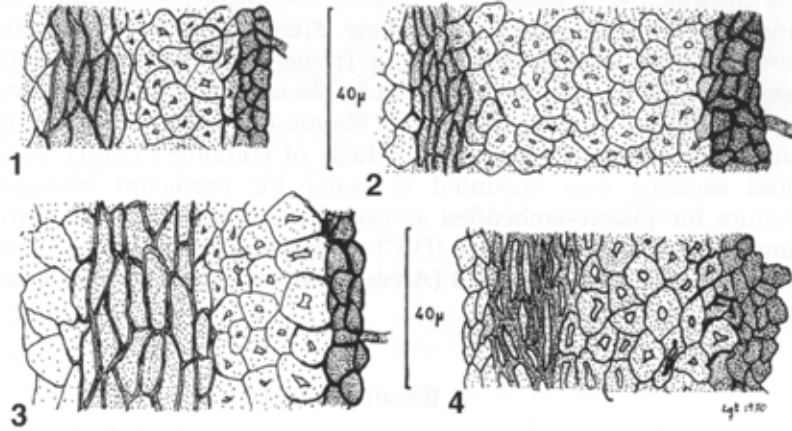
### Results

The highest quality sections to observe the actual anatomy of the gelatinized wall layer were produced using a freezing microtome. The composition of this wall layer was most clearly seen in sections produced at 30  $\mu\text{m}$ . Hand sections and plastic-embedded sections were either too thick or too thin, respectively, to properly interpret the anatomy of the gelatinized wall layer.

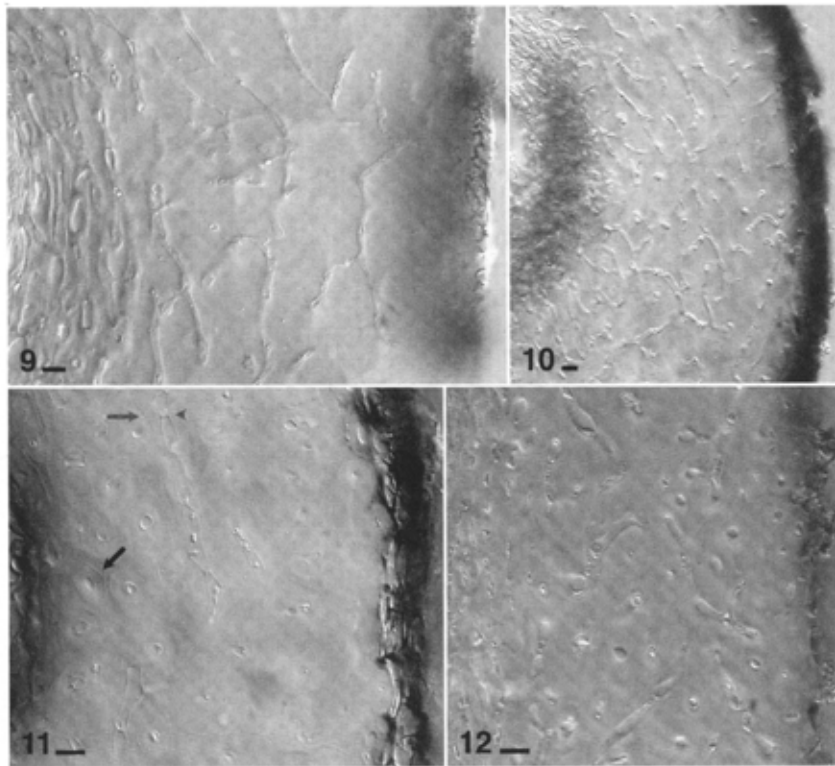
Nine species have previously been described as possessing an ascomal wall layer composed of thick-walled, gelatinized cells (Carroll & Munk, 1964; Lundqvist, 1972; Hilber & Hilber, 1979; Hanlin & Tortolero, 1987; Bell & Mahoney, 1997; Hanlin, 1999). Lundqvist (1972: Fig. 16) exquisitely illustrated the ascomatal walls in four of these species (*C. scorteae*, *P. appendiculata*, *P. fimiseda* and *P. perplexens*), which are reproduced here with permission in Figs. 1–4. Freezing microtome sections, however, revealed that the gelatinized wall layer is actually composed of interwoven hyphae embedded in a gelatinous matrix in all nine species (Figs. 5–12; *C. albicollis* not shown due to the poor condition of the isotype material). As clearly seen in several species (Figs. 5, 6, 9–11), hyphae in the gelatinized layer are thin-walled, septate, and 2–4  $\mu\text{m}$  diam.

### Discussion

The gelatinized wall layer occurring in species possessing a coriaceous pseudo-bombardioid wall was determined to be composed of interwoven, thin-walled hyphae rather than thick-walled, isodiametric cells as previously reported (Carroll & Munk, 1964; Lundqvist, 1972; Hilber & Hilber, 1979; Hanlin & Tortolero, 1987; Bell & Mahoney, 1997; Hanlin, 1999). The interpretation of the morphology of the gelatinized wall layer appears to be completely



Figs. 1-8. Median longitudinal sections of pseudo-bombardioid ascomatal walls, exterior to the right. - 1. *Cercophora scorteae*, Cain 12011 (UPS). - 2. *Podospora appendiculata*, Lqt 2371-e (UPS). - 3. *P. fimiseda*, Lqt 3165-a (UPS). - 4. *P. perplexens*, Lqt 3347-k (UPS). - 5. *C. scorteae*, SMH 4441 (F). - 6. *P. appendiculata*, with holotype of *C. gossypina* (UPS). - 7. *P. fimiseda*, SMH 4452 (F); note transverse section of a thin-walled hypha surrounded by a gelatinous sheath (arrow). - 8. *P. perplexens*, Lqt 3347-k (TRTC). - Figs. 1-4 adapted from Lundqvist (1972: Fig. 16); 5-8 = DIC. - Scale bars: 1-4 = 40  $\mu$ m; 5-8 = 10  $\mu$ m.



Figs. 9–12. Median longitudinal sections of pseudo-bombardioid ascomatal walls, exterior to the right. – 9. *Arnimium ontariensis*, Cain 5343 (TRTC). – 10. *Cercophora palmicola*, Hanlin 12701 (GAM). – 11. *C. costaricensis*, SMH 4021 (F); black arrow as in Fig. 7, note thin-walled hypha transversed by a septum (gray arrowhead) and outer limit of gelatinous sheath (gray arrow). – 12. *C. elephantina*, TRTC 66.1093 (TRTC). – Figs 9–12 = DIC. – Scale bars: 9–12 = 10  $\mu$ m.

dependent on the method chosen for sectioning ascomata. Carroll & Munk (1964) and Hilber & Hilber (1979) used a freehand method for sectioning ascomata, while Hanlin (1999) and Bell & Mahoney (1997) used a paraffin-embedded method. Although the methods for sectioning ascomata were not reported by Lundqvist (1972), he used a freezing microtome (in lit. 3/2003). The morphology of the gelatinized wall layer was accurately assessed in the present study only with the aid of a freezing microtome. Useful hand sections were difficult to produce due to the cartilaginous nature of the gelatinized wall layer, which tended to stretch and tear rather than cut cleanly in these taxa. Hand sections tore easily, were usually off-centered, and were either too thick or irregular to adequately observe the hyphae in the gelatinized wall layer. Although plastic-embedded sections are commonly used for studying the morphology of asco-

matal walls, they proved inadequate for properly interpreting the anatomy of the gelatinized wall layer, which appeared cellular since most hyphae were cut transversely due to the extremely thin sections produced using this method. Hanlin (1999), however, was able to observe "slender, dark-staining hyphae" traversing the gelatinized layer in *C. palmicola* (as shown in his Fig. 17) in 6–8  $\mu\text{m}$  thick paraffin-embedded sections.

The interpretation of the composition of this wall layer can be quite confusing and subjective. In fact, Lundqvist questioned the interpretation of this wall layer, but in the end, he finally decided that it was composed of more or less isodiametric cells with narrow lumens (in lit. 3/2003). In all three methods of sectioning, either some or most hyphae were sectioned transversely. As shown in Figs. 7 and 11 (black arrows), these hyphae appeared as angular cells with small lumens (small circle) surrounded by very thick-walled, gelatinized cell walls (larger circle). This is the most likely explanation for why previous workers interpreted this wall layer as cellular. However, it is now clear that the small circle refers to the actual cell wall of a thin-walled hypha (Figs. 5, 6, 9–11). Although the nature of the larger circle remains uncertain, it is interpreted at this time as the outer delimitation of some sort of gelatinous sheath which surrounds the hyphae. This is most clearly seen in Fig. 11 in which a hyphal filament that has been sectioned longitudinally reveals gelatinous material situated between a distinct inner cell wall (gray arrowhead), which is transversed by a septum, and a faint outer wall (gray arrow), which represents the outer limits of this gelatinous sheath.

The term pseudo-bombardioid has traditionally been used to describe a non-stromatic ascomal wall containing a layer composed of thick-walled, gelatinized cells (Lundqvist, 1972). However, this wall layer is composed of interwoven hyphae in all nine species observed in this study. Although the gelatinized wall layer in these taxa appears to be morphologically similar [but probably not homologous, see Lundqvist (1972: 60–61) for detailed discussion] to that found in species of *Bombardia* (Fr.) Karst. and *Bombardioidea* Moreau, the term "bombardioid" should not be applied here since this term describes ascomatal walls with two stromatic outer wall layers, which are so far only known to occur in members of *Bombardia* and *Bombardioidea*. Therefore, the term pseudo-bombardioid should be maintained, but emended to describe a non-stromatic ascomal wall containing a gelatinized layer composed of interwoven, thin-walled hyphae.

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