

***Bombardioidea bombardioides* (Auersw.) C. Moreau 1972 & its *Angulimaya sundara* Subram. & Lodha 1964 anamorph – AEB 1256 (= PDD 110498)**

**Substrate:** Moose dung

**Collection site:** The Border Route Trail (border between N. Minnesota and Ontario, Canada) near the south shore of Rat Lake

**Collection date:** September 9, 2013

**Collectors:** John Ipsen and Kris Wegerson; **Identifier:** Ann Bell

**Vouchers:** the dried herbarium specimen consists of a small cluster of mature perithecia from one pellet of moose dung and is accompanied by one semi-permanent Shear's mounting fluid (SMF) microscope slide; Dan Mahoney's digital photos of microscopic detail from AO PhaseStar and Olympus BX51 microscopes and in situ dissecting scope photos of the perithecia with an Olympus stereo microscope using Fujifilm 200 ASA film; Dan's comments.

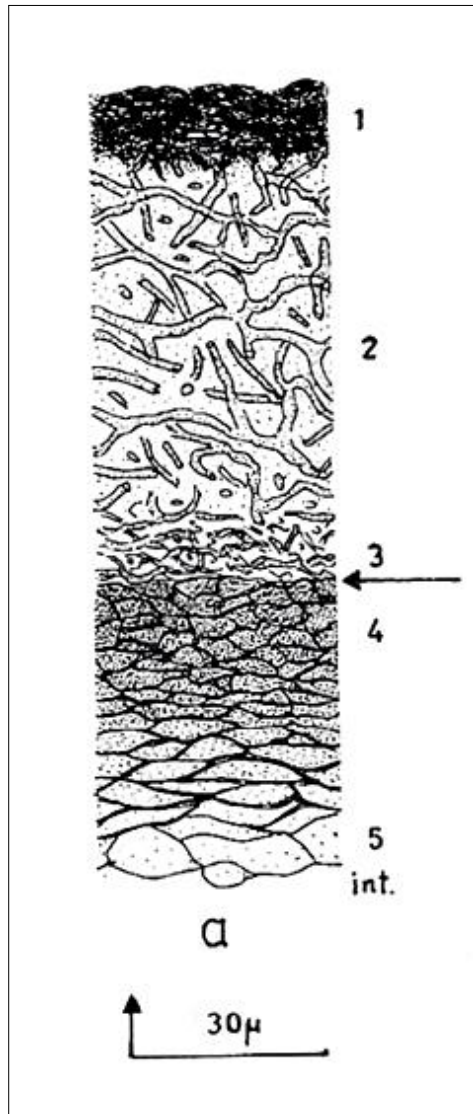
**Brief early comments:** Nine moose dung pellets were incubated and these had been frozen since the time of collection. Only 3 clusters of perithecia were seen (a cluster of 2 mature ascospore-bearing bodies, a cluster of 4 immature perithecia that lacked ascus development and a cluster of several mature perithecia).

Dung pellets were incubated in a moist chamber on 11 July 2015 and fertile bodies were first observed on 4 August. Earlier, in late July/early August, numerous mature and maturing perithecia of *Cercophora silvatica* Lundqvist were also observed on all 9 pellets.

**More comments:** Two frequently consulted references:

1. Lundqvist, N. 1972. Nordic Sordariaceae s. lat. – Symbolae Botanicae Upsalienses 20: 1–374.
2. Krug J.C. & Scott J.A. 1994. The genus *Bombardioidea*. Can. J. Bot. 72: 1302–1310.

*Bombardioidea bombardioides* is a coprophilous species, often reported as *Sordaria bombardioides* before Lundqvist 1972. According to Lundqvist, it is mostly confined to the droppings of leporine and cervine animals of Europe and N. America. The present collection is representative of descriptions given in the above references. The primary difference between their descriptions is provided by Krug & Scott in their discussion on the page after next. In short, Lundqvist treated the ascoma as a stroma while Krug & Scott recognized it as a perithecium. Most workers since Krug & Scott use the term perithecium. My brief summary of Lundqvist's peridium layer interpretation and that of Krug and Scott's is given on the next page. My own photographs only allow me good views of the outermost peridium layer.



### Peridium interpretations

Lundqvist 1972. page 276:

Fig. 64. a: *Bombardioidea bombardioides*, Rabenhorst, F. Eur. Exs. 1527 (UPS).

a: Median vertical section from middle part of the fruit-body; arrow points at border between stroma and perithecial wall; figs. 1–5 are outer crust, fibrous and cartilaginous layer, fibrous layer only, pseudoparenchymatous layer with subisodiametric cells, and ditto with tangentially flattened cells.

Peridium layers (Lundqvist: figs. 1-3 stroma, figs. 4-5 perithecium)

Most authors since Krug & Scott 1994 treat the ascoma as a perithecium, not a stroma (with all of Lundqvist's peridium layers – his figs. 1–5 – as layers of the perithecium peridium and then as only 3 layers rather than 5: layer 1 (Lundqvist's layer 1), layer 2 (Lundqvist's layers 2 & 3, layer 3 (Lundqvist's layers 4&5).

Paul Cannon, in his simplified peridium description for the Fungi of Great Britain and Ireland website, follows Krug & Scott :

“Peridium composed of an outer thin layer of heavily melanized rather amorphous textura angularis, a thick median layer of  $\pm$  hyaline textura intricata within a gelatinous matrix, and an inner layer of pale brown thin-walled flattened textura angularis.”

Krug J.C. & Scott J.A. 1994. The genus *Bombardioidea*. Can. J. Bot. 72: 1302–1310. Portions of pp. 1303, 1304 & 1308 are reproduced below

*Bombardioidea bombardioides* (Auersw. in Niessl) C. Moreau  
in Lundq., Symb. Bot. Ups. 20: 277. 1972 Type species of the genus *Bombardioidea*

#### Key to the species

- 1. Asci 4-spored.....2
- 1. Asci 8-spored.....3
- 2. Ascospores ellipsoidal, 35-43 x 16-22 µm, with two distinct germ pores .....*B. stercoris*
- 2. Ascospores ovoid, 25-31(-34) x 16-19(-20) µm, with a large apical germ pore and a less conspicuous basal pore.....*B. serignanensis*
- 3. Ascospores uniseriate, ellipsoidal, symmetrical, 19-29 x 10-14 µm, on leporid dung.....*B. bombardioides*
- 3. Ascospores frequently irregularly biseriate, oblong-ellipsoidal, asymmetrically flattened, (25-)26-35(-38) X 12-14(-15) µm, on cervid dung.....*B. anartia*

Perithecia aggregated in small clusters, confluent at the base or occasionally single, superficial, somewhat roughened, bare, ovoid to oblong or occasionally pyriform, 800-1500 x 500-800 µm, red-brown to blackish brown, with an indistinct, very dark brown ostiolar region with a small, very prominent ostiole at the apex; peridium dark brown by reflected light, thickened, very coriaceous, appearing in surface view of indistinct cellular structure, 125 µm thick, consisting of three layers, an outer layer 1-2 cells thick, of oblong, thick-walled, dark brown cells measuring 5-8 x 2-3 µm, a middle layer 80-100 µm thick, of noncellular gelatinous matrix sparsely interspersed with branching thin-walled, hyaline hyphae measuring 1.5-2 µm in diameter, and an inner layer 10-12 cells thick, of oblong to elongated, thin-walled, pale brownish cells measuring 8-15 x 2-3 µm, tapering towards the perithecial base where this layer is composed of angular, thick-walled, dark brown cells measuring 8-10 X 6-7 µm. Asci 8-spored, cylindrical, 240-350 X 15-20 µm, broadly rounded at the apices, tapering into a stipe measuring 90-200 µm long; apical ring indistinct. Paraphyses abundant, filiform, septate, hyaline, guttulate, longer than and mixed with the asci. Ascospores one-celled, obliquely uniseriate, surrounded by a hyaline gelatinous sheath reaching a width of 15 µm, ellipsoidal, rounded towards the ends, (17-)19-29 x 10-14 µm, ranging from hyaline when young to yellowish-brown, finally dark brown and opaque at maturity, containing at each opposing end of the spore a germ pore measuring 1.5-2 µm in diameter; apical pore often slightly smaller.

The anamorph of *B. bombardioides* is consistent with *Angulimaya* Subram. & Lodha, which was erected by Subramanian and Lodha (1964) based on a single species *A. sundara* Subram. & Lodha from cow dung. They described this fungus as having "annellophores bearing many distinct annellations." Ellis (1971) noted the spore-bearing structures were phialides and that the interpretation of the former authors was incorrect. Our examinations of the anamorph of *B. bombardioides* show that this fungus is exclusively phialidic in young cultures, but with age (> 12 weeks) some phialides show progression of the conidiogenous locus beyond the collarette. This annellidic tendency is supported by the occasional presence of distinctly apiculate conidia. A similar anamorph also has been noted for *B. anartia* by G. White (personal communication).

Anamorph on WSHA at 4 weeks with conidiophores 2-2.5 µm in diameter, brown to dark brown, becoming hyaline towards apex, thick-walled, septate, branched or unbranched, with short, 1-, 2-, or 3-celled, swollen, 3-5 µm in diameter lateral metulae bearing clusters of 2-7 phialides. Phialides numerous, subhyaline to dark brown, subglobose to ampulliform, 4-5.5 x 2.5-3.5 µm, broadly attached to subtending cell, with a prominent, dark collarette 2-2.5(-3) µm in diameter; phialides produced terminally and laterally on metulae, singly or more commonly in irregular whorls of 3-7; clusters of phialides occurring at intervals of 10-30 µm along fertile hyphae, causing a nodal appearance. Conidia numerous, one-celled, thin-walled, hyaline to light brown, globose to subglobose, 2-3 µm in diameter, often with a distinct, truncated apiculus, produced in dry, readily disarticulating chains; conidia from aging colonies (> 12 weeks) thick-walled, 4-6.5 µm in diameter. Except for our cultural observations for *B. bombardioides*, reported herein, *B. stercoris* is the only other species for which cultural observations have been published.

Fertile ascomata produced on WSHA after 12-16 weeks.

HABITAT: On hare and rabbit dung, occasionally recorded on cow, horse, sheep, moose, and porcupine dung

Krug J.C. & Scott J.A. 1994. The genus *Bombardioidea*. Can. J. Bot. 72: 1302–1310.  
Portions of pp. 1309 & 1310 are reproduced below

### Discussion

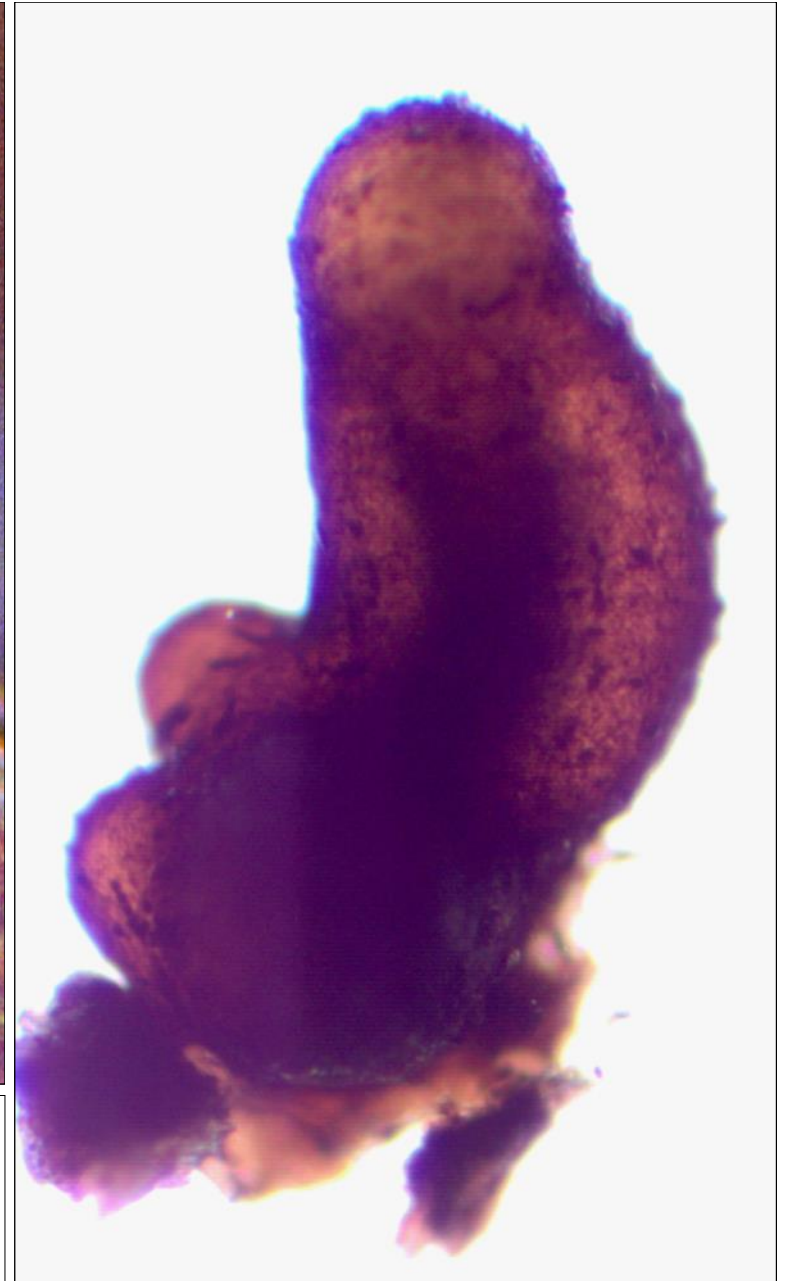
We consider that morphologically the peridium consists of three continuous layers. The outer melanized rind together with the partially gelatinous, hyphal-interspersed central layer are responsible for the coriaceous nature of the peridium. It is the opinion of Lundqvist (1972) that these two layers constitute stromatic tissue while only the innermost region of compact, thin-walled hyphae represents the true peridium. In addition, he suggested that this morphology represented an archaic feature. On the contrary, we feel that hyphal continuity throughout all three peridial layers clearly shows that they constitute a single tissue. Although this tissue appears to be specialized, we believe that it is of common ontogenetic origin and thus nonstromatic. As Wehmeyer (1926) points out, a stroma is an aggregation of vegetative mycelium not resulting from sexual stimulus. In discussing stromata he states that the term should be applied in a broad sense to distinguish a type of mycelial differentiation rather than any particular morphological structure. He goes on to say that the definition excludes tissues arising as the result of sexual stimulus, such as the wall of the perithecium. This appears to exclude Lundqvist's (1972) interpretation that these outer layers of the peridium can be considered stromatic. A number of authors (Bell 1983; Muroi and Udagawa 1984; Lorenzo 1992) have followed Lundqvist's interpretation, which may not be supportable in the absence of developmental evidence.

In discussing the evolution of the Xylariaceae, Rogers (1979) emphasized that upright stromata appear to be adaptations to minimize desiccation of perithecia while providing for more efficient spore dispersal. Similarly, the elevated, thick-walled ascomata of *Bombardioidea* function in the same way by enhancing spore discharge and slowing ascomatal desiccation.

Lundqvist (1972) suggested that the carbonaceous, functionally stromatic, three-layered peridium represents an archaic feature. Whether this structural morphology is derived as in the Xylariaceae or archaic, we conclude that it is correlated to the superficial growth habit of these taxa as an adaptation to xerophytic environments. The coriaceous outer rind and the hygroscopic middle layer of the peridium function together to retain moisture and provide a buffer to desiccation while concomitantly affording a physical and possibly chemical deterrent to insect grazing. We have observed in dung cultures of *Bombardioidea* that ascospore liberation occurs primarily upon desiccation of the ascomata rather than upon hydration. Additionally, the asci of species of *Bombardioidea* are characterized by staggered maturation. Since *Bombardioidea* is a late successional stage dung-inhabiting fungus, most prevalent on very old, dry dung, ascospore ripening and discharge is likely accomplished by repeated cycles of hydration and drying as in many Xylariaceae (Rogers 1979). Similar observations have been made for other lignicolous pyrenomycetes inhabiting old, exposed, decorticated wood (W.A. Untereiner, personal communication).

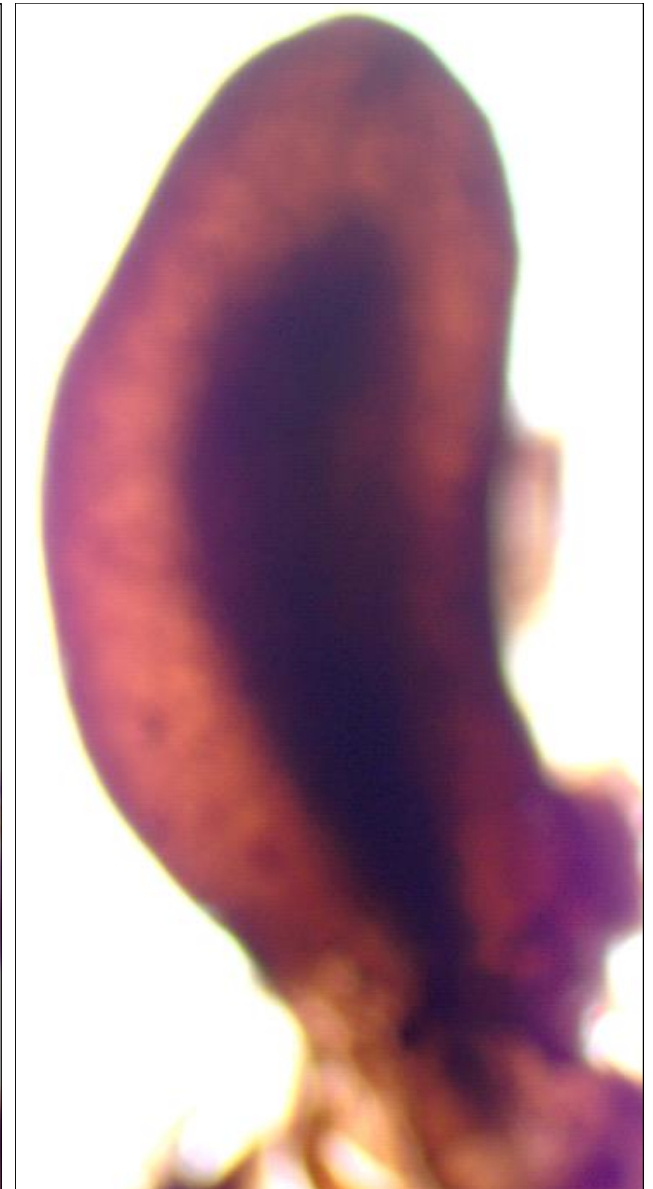
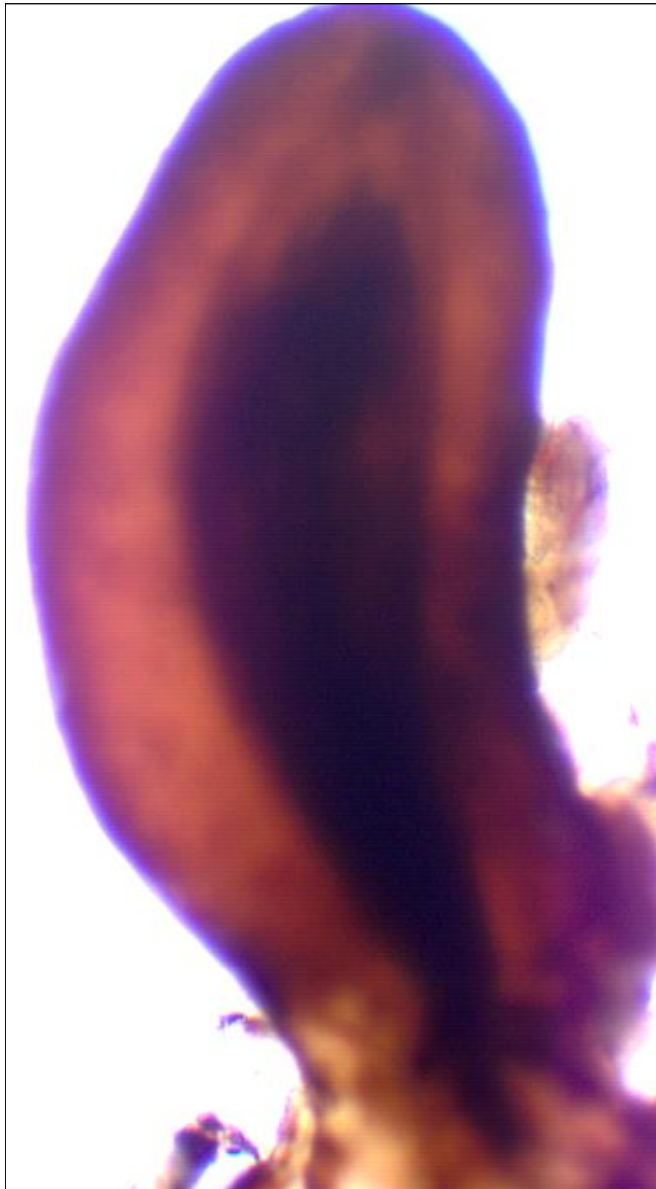
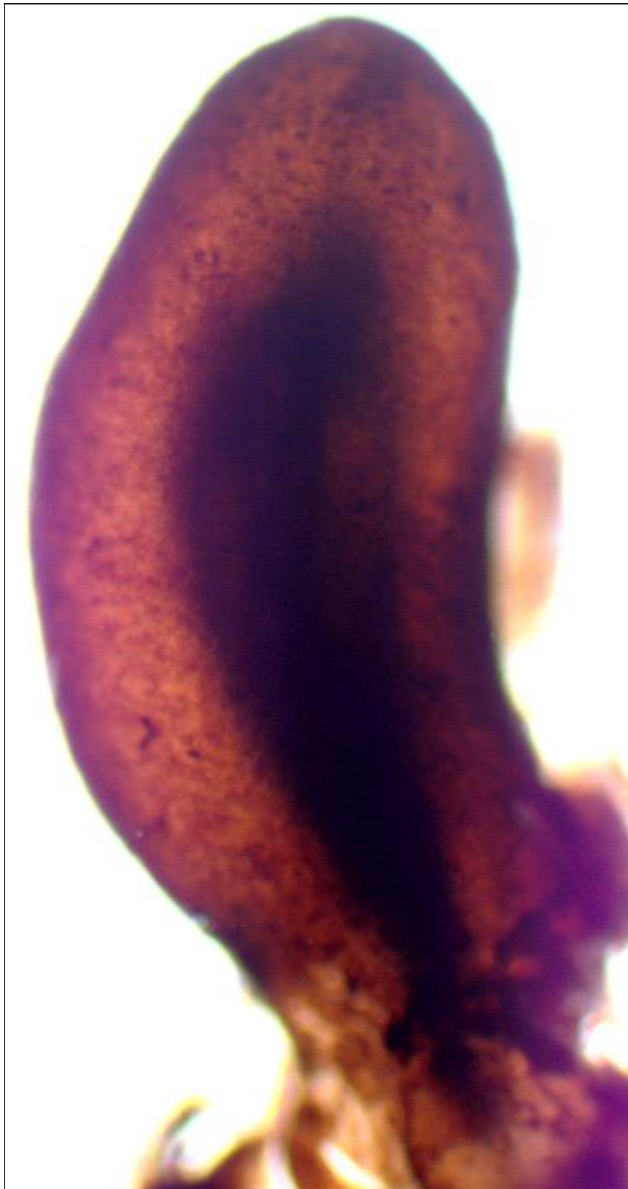
Spore dispersal in *Bombardioidea* is likely accomplished through gut passage. This hypothesis is supported by the fact that these taxa have darkly pigmented, forcibly discharged ascospores with sticky gelatinous sheaths. Furthermore, the genus is not known to occur on decaying wood.





Both photos the same cluster of immature perithecia (no asci) from moose dung. Left: in-situ on the dung. Right: that cluster mounted in water without a coverslip and photographed using a compound microscope, X4 objective. The largest ascoma shown here was  $575 \times 300 \mu\text{m}$  while the large fertile ascoma photographed on the page after next was  $1500 \times 575 \mu\text{m}$ .

All photos are the perithecium from the previous page after applying a coverslip & irrigating with SMF. Images from left to right are perithecium surface focus, deeper focus & deepest focus, resp. using the X4 objective & brightfield microscopy.







This page sets the stage for mature teleomorph & anamorph photos.

As noted on the first page of this pdf, only 3 clusters of perithecia were seen on the 9 incubating moose dung pellets.

The first cluster with immature perithecia, shown on the previous 2 pages, was sacrificed in preparing those photos. The second cluster, shown on this page, was photographed in situ to show both a mature perithecium (solid arrow) and its anamorph (dashed arrows) before sacrificing both to provide the remainder of teleomorph and anamorph photos in this pdf. The third cluster with mature perithecia was removed from its pellet to serve as the dried herbarium specimen.



Whole perithecium at the right and fragmented portions of another at the left, in a water mount without a coverslip. All from the same cluster of perithecia. Olympus dissecting scope with PM-6 camera.

Whole perithecium, 1500 X 575  $\mu\text{m}$  (black arrow). Dark body within (white arrow) is the inner-most wall layer plus the hymenial portion of the perithecium.

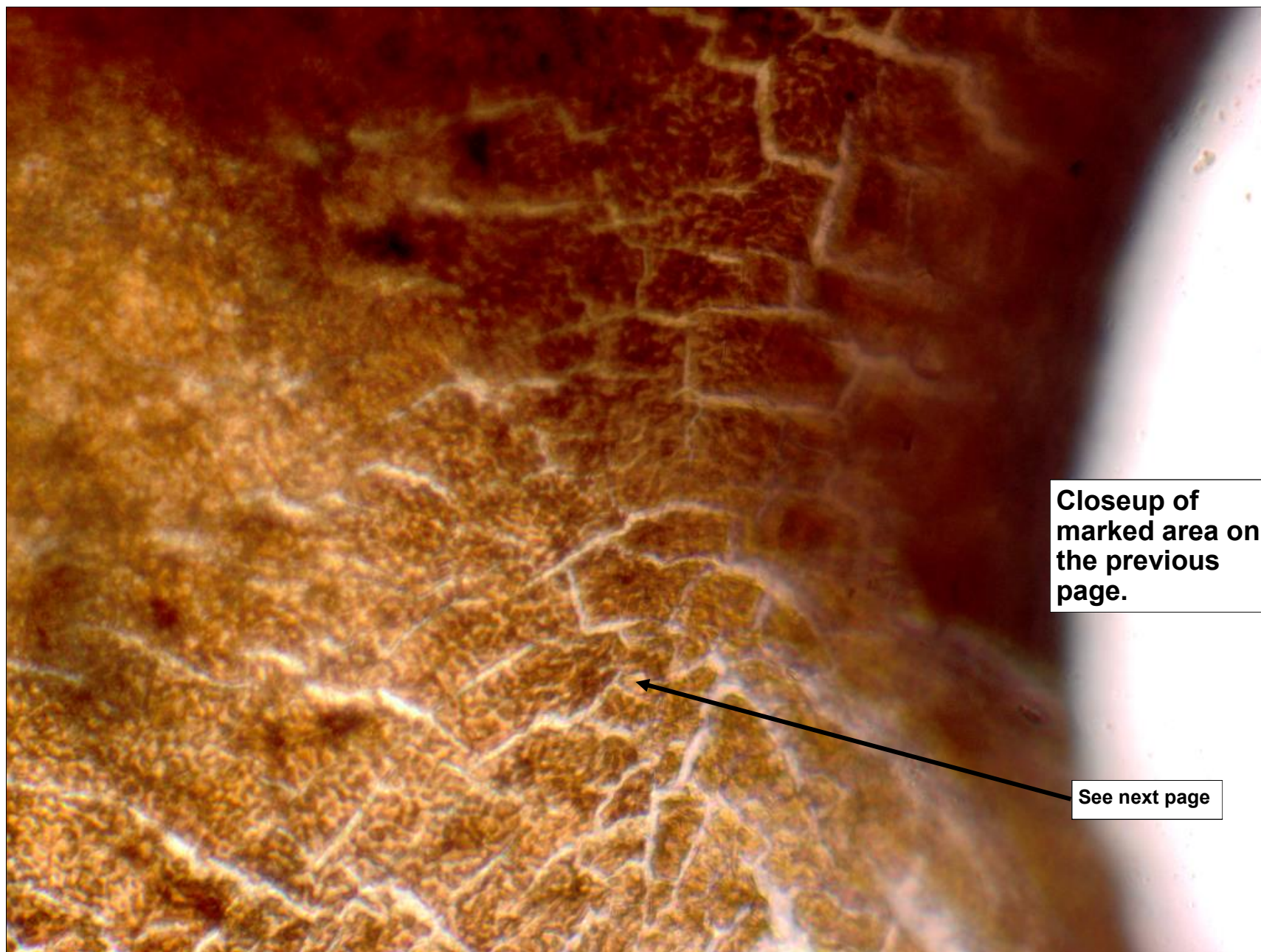


Empty 'pouch', consisting of the outer wall layers of the perithecium.

Middle (labelled 'X') and inner wall layers of the perithecium plus hymenial portion of the perithecium that 'popped out' of the outer-walled portion to the left (the 'pouch').



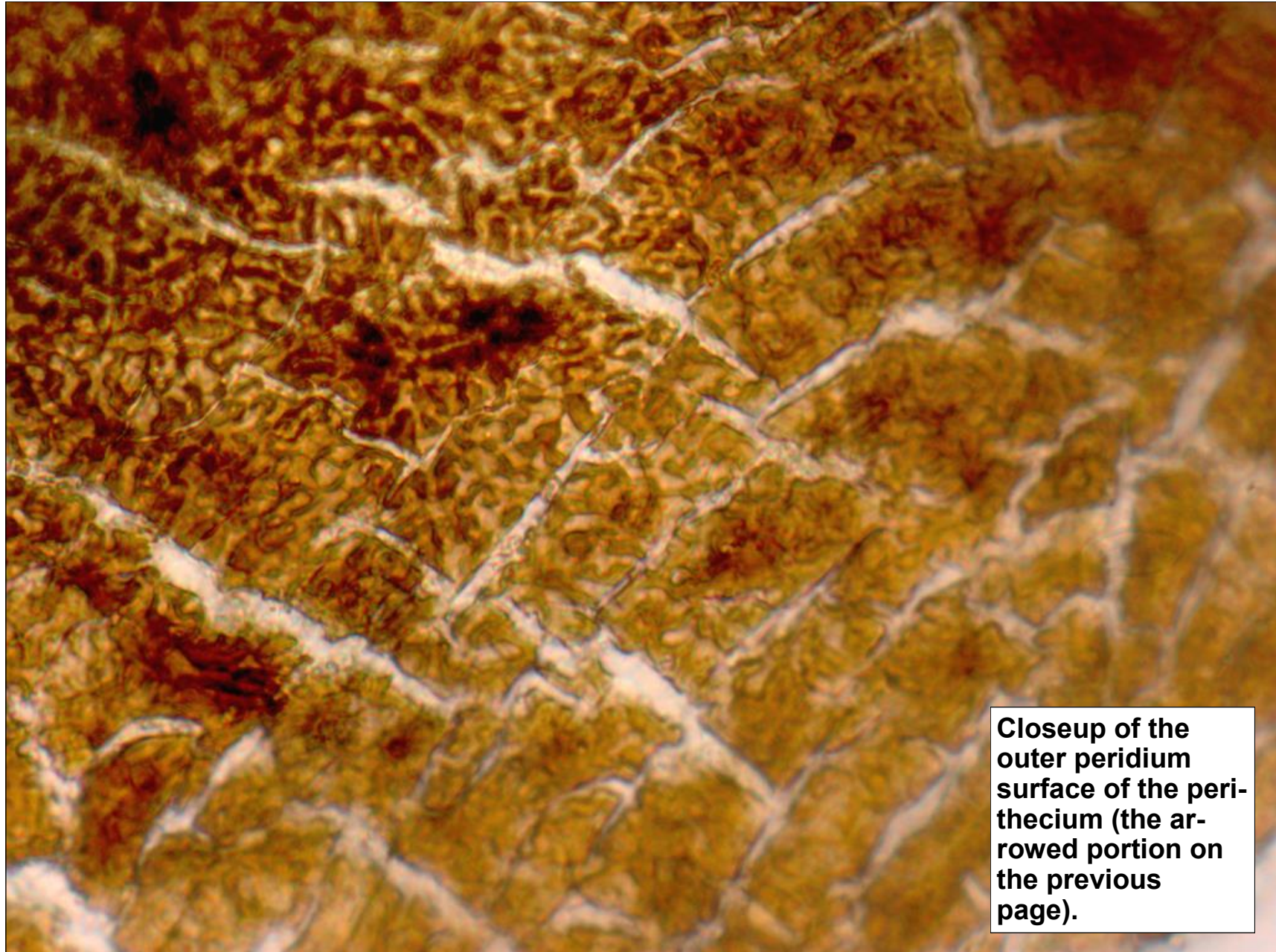




**Closeup of  
marked area on  
the previous  
page.**

**See next page**



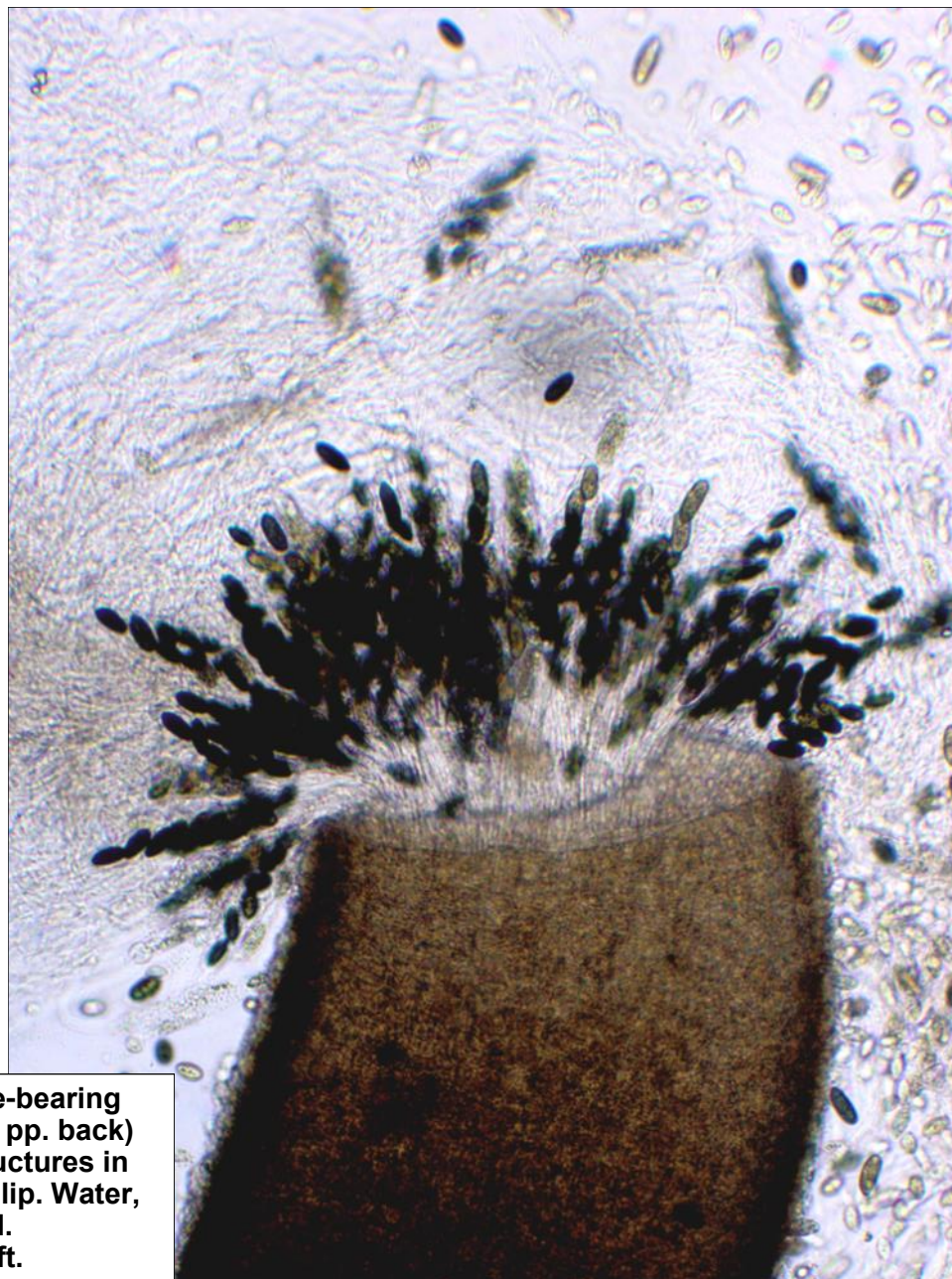


**Closeup of the  
outer peridium  
surface of the peri-  
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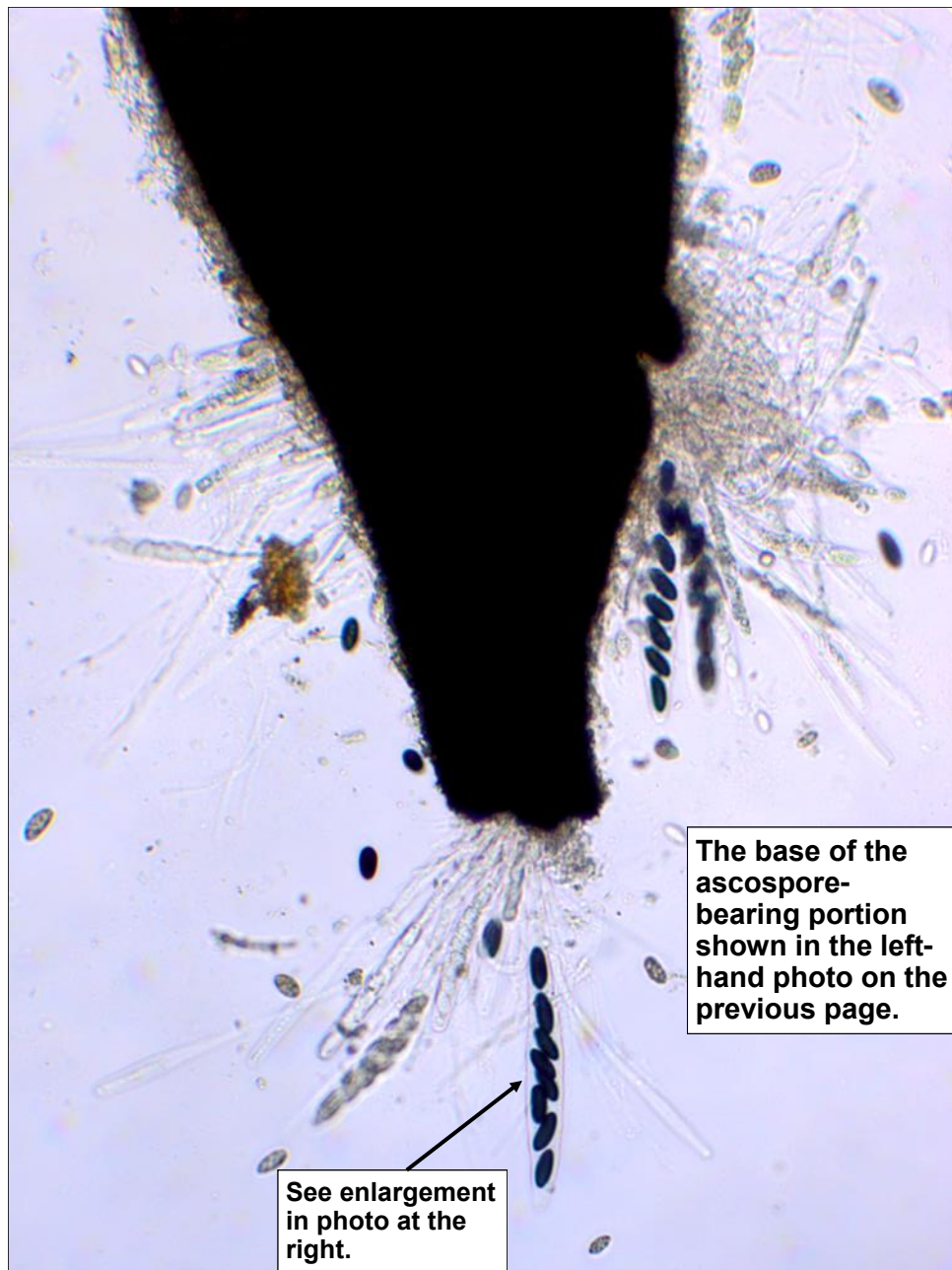




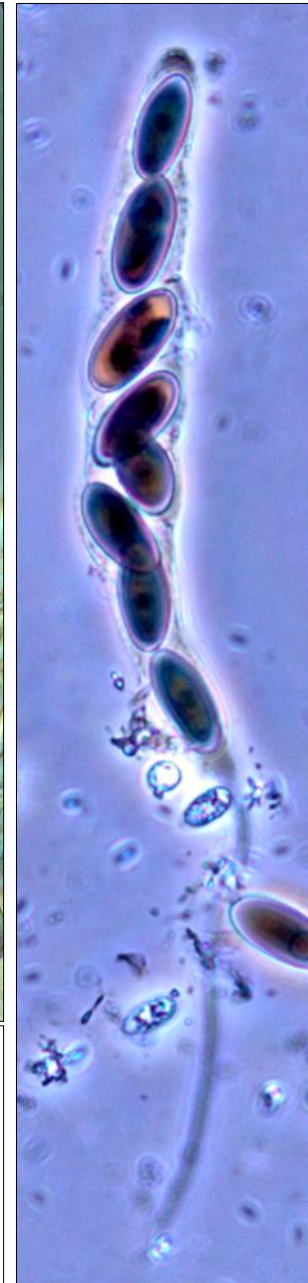
Left: The 'popped out' ascospore-bearing portion of the perithecium (see 4 pp. back) after separating it from other structures in the mount and applying a coverslip. Water, X4 objective enlarged, brightfield.  
Right: Closeup of photo at the left.





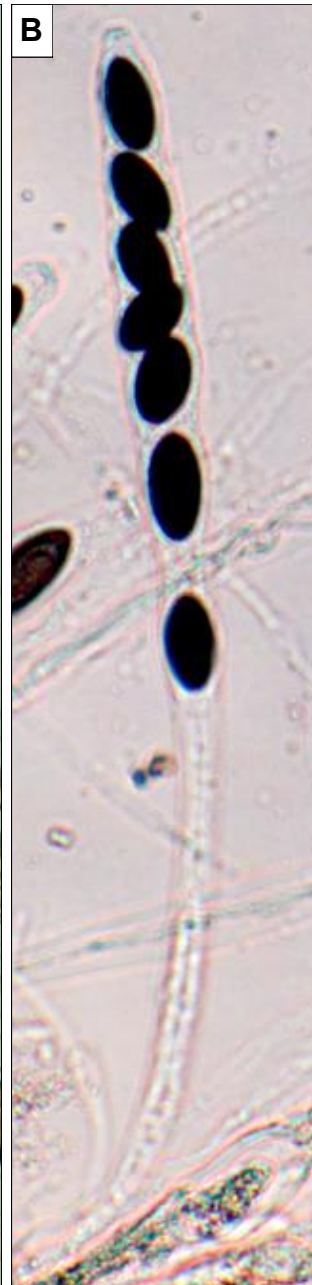




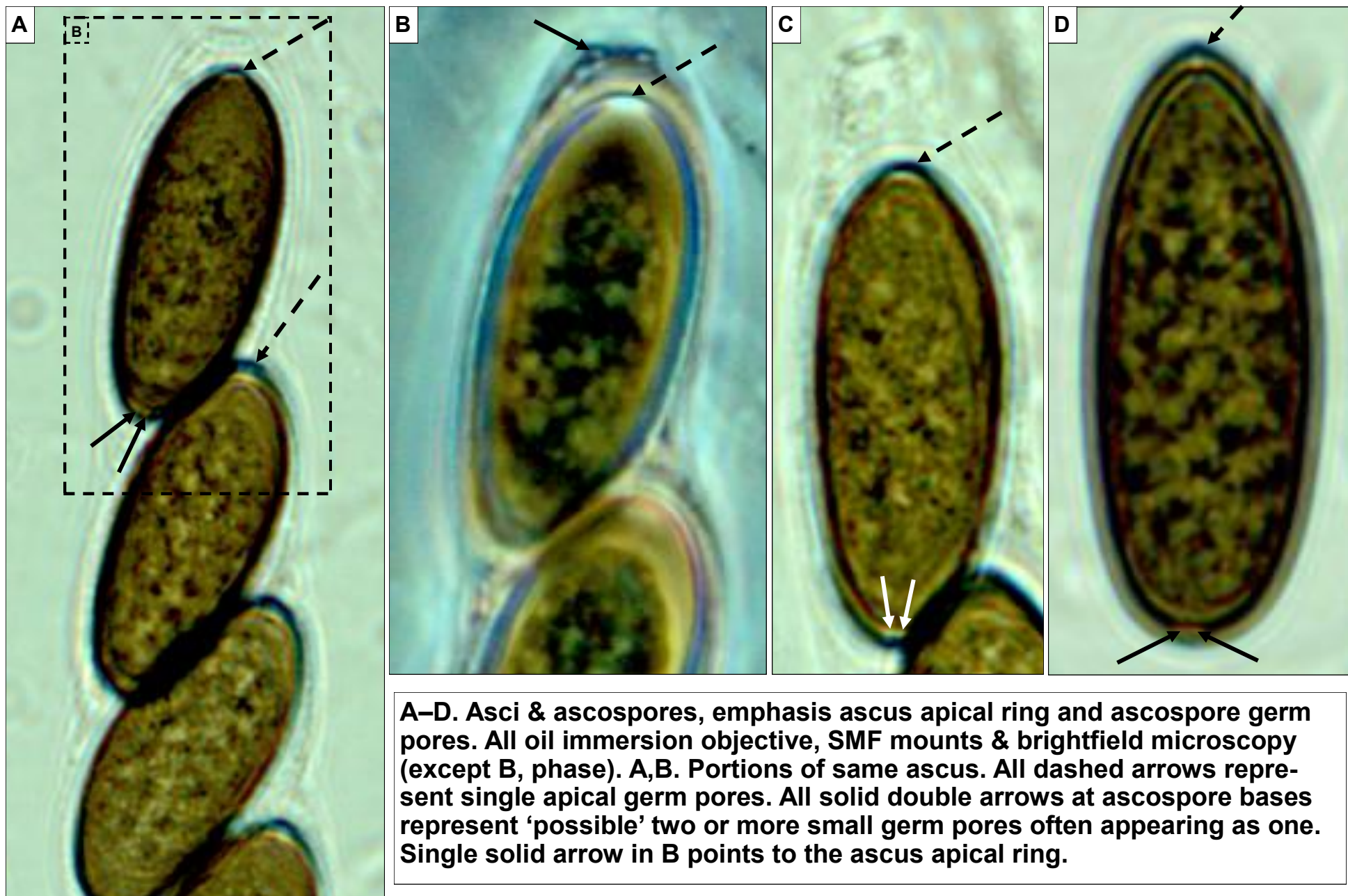


**Left:** Asci & ascospores observed 11 Aug. 2015 in SMF using the X40 objective and phase microscopy. Note variations in # of maturing ascospores per ascus and their stages of maturity. Also note the ascus apical rings (white arrows). **Right:** 2 photos, same ascus (different focus & lighting) from the original SMF microscope slide mount using X40 obj. & phase (2015) – photographed in 2022. Ascus  $275 \times 25 \mu\text{m}$  (stipe portion  $112.5 \times 3$ ). Far right ascus: note gelatinous sheaths (black arrows).

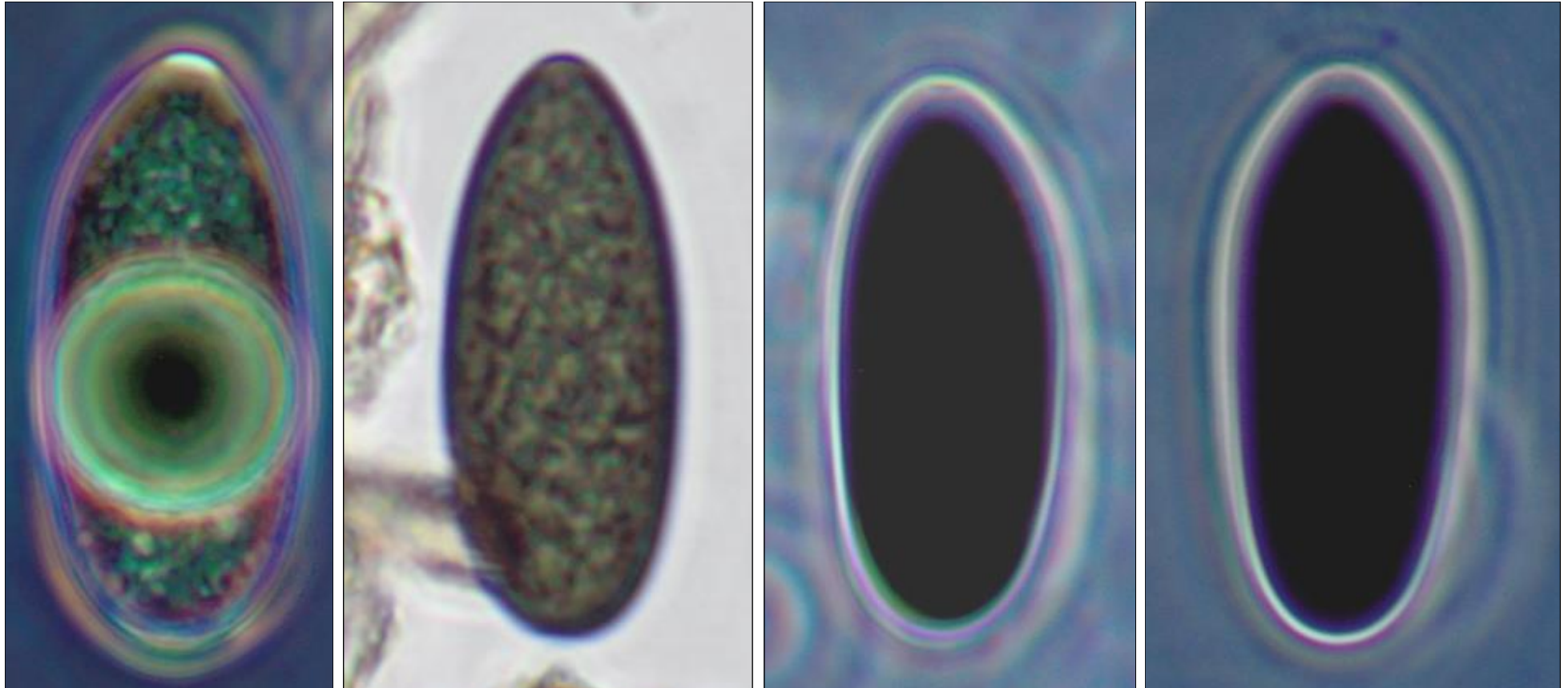




**A.** Asci & ascospores observed 11 August 2015. SMF, X40 obj., brightfield – a portion of the left-hand photo on the previous page. Note variations in # of maturing ascospores per ascus and their stages of maturity. **B,C.** Fertile asci, also observed 11 Aug. 2015. **B.** Water mount, X20 objective enlarged, brightfield. **C.** SMF mount, X40 objective enlarged, phase. Ascospore portion  $150 \times 20 \mu\text{m}$ , stipe portion broken with  $115+ \mu\text{m}$  seen.







**Ascospores.** All photographed under oil immersion in water mts. under phase microscopy (except the 2nd from left – under brightfield). Apical germ pores are faintly visible in those ascospores shown. Those viewed under phase appear to have a faint gelatinous sheath. Most ascospores are somewhat narrower at one extremity (apical or basal, usually apical). The most typical shapes are the 3 at the left. The 2 on the left are slightly immature with one exhibiting a large de Bary bubble. Ascospores measured were  $24\text{--}31 \times 11\text{--}14 \mu\text{m}$  ( $n = 20$ ). According to Lundqvist (1972, p. 277), this species has “an apical germ pore and 3–5 small pores at the base imitating a germ pore”. Krug & Scott (1994, p. 1304), however, report ascospores “containing at each opposing end of the spore a germ pore measuring  $1.5\text{--}2 \mu\text{m}$  in diameter; apical pore often slightly smaller” (with no mention of 3–5 small pores at the base imitating a germ pore). So far I’m unable to tell which of these reports is representative of this particular specimen.



Previous pages emphasized the teleomorph (see upper right picture insert.) Pages that follow emphasize the anamorph. The main photo on this page is an in-situ photo of the upper right insert but focuses on the anamorph. The beginning of ascospore discharge is represented by solid arrows while dotted arrows point to the *Angulimaya sundara* anamorph.





Ellis MB (1971). *Dematiaceous hyphomycetes*. Commonwealth Mycological Institute, U.K. pp. 532, 533

## 267. ANGULIMAYA

**Angulimaya** Subramanian & Lodha, 1964, *Antonie van Leeuwenhoek*, 30: 329.

*Mycelium* mostly immersed. *Stroma* none. Separate setae absent but ends of conidiophores often sterile and setiform. *Hyphopodia* absent. *Conidiophores* macronematous, mononematous, with a main simple or furcate, brown, septate, stipe on which are borne a number of short, lateral, phialide-bearing branches. *Conidiogenous cells* discrete, monophialidic; phialides flask-shaped, pale with dark collarettes. *Conidia* produced in basipetal succession, often hanging together in chains, spherical or subspherical, O-septate, colourless or very pale.

*Type species*: *Angulimaya sundara* Subram. & Lodha.

**Angulimaya sundara** Subram. & Lodha, 1964, *Antonie van Leeuwenhoek*, 30: 29. Fig. 385

*Mycelium* mostly immersed. *Conidiophores* erect, with a main, simple or furcate, brown, septate stipe up to 500 $\mu$  long, 4-7 $\mu$  thick, which bears a number of short lateral branches 4-5 $\mu$  thick; the stipe is sometimes sterile and acute at the apex. *Phialides* numerous, formed terminally and laterally on the branches and sometimes at the end of the stipe, flask-shaped, 5-14 $\mu$  long, 2.5-4 $\mu$  thick in the broadest part, colourless or very pale, each with a dark collarette. These phialides were incorrectly described as annellophores by the authors of the genus. *Conidia* produced in basipetal succession, emerging through the open ends of the phialides and often hanging together in quite long chains, spherical or subspherical, colourless or very pale, 2-2.5 $\mu$  diam.

On cow dung; India.

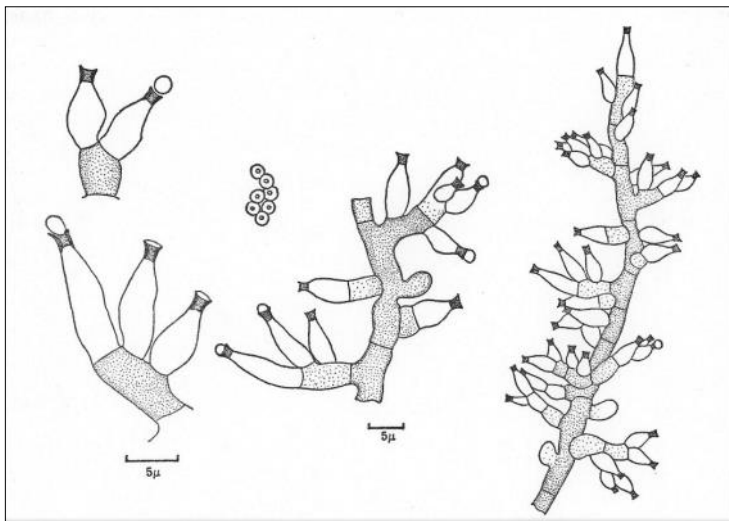


FIG. 385. *Angulimaya sundara* (x 650 except where indicated by scales).

Seifert K, Morgan-Jones G, Gams W, Kendrick B. 2011. *The Genera of Hyphomycetes*. CBS Biodiversity Series no. 9: 1– 997. CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands. pp. 81, 535

**ANGULIMAYA** Subram. & Lodha 1964-*Antonie van Leeuwenhoek* 30: 329 (327-329, Fig. 5) / *A. sundara* Subram. & Lodha 1964

CDM: none. SET: none or unbranched, brown extensions of cph. CPH: branched, trichodermoid, brown. CGC: phialides, dark collarettes, brown. CDA: amero, hyaline, in basipetal chains, schizo.

**Fig. 12C, Pl. 53A.** On dung: Asia. One species.

**Notes:** Anamorphic *Ascomycota* (*Lasiochaeriaceae*, *Sordariales*). Compare with *Phialophora*, *Cladorrhinum*.

**Ref.:** Ellis, *Demat. Hyphom.*, pp. 532-533, 1971 (illus.).

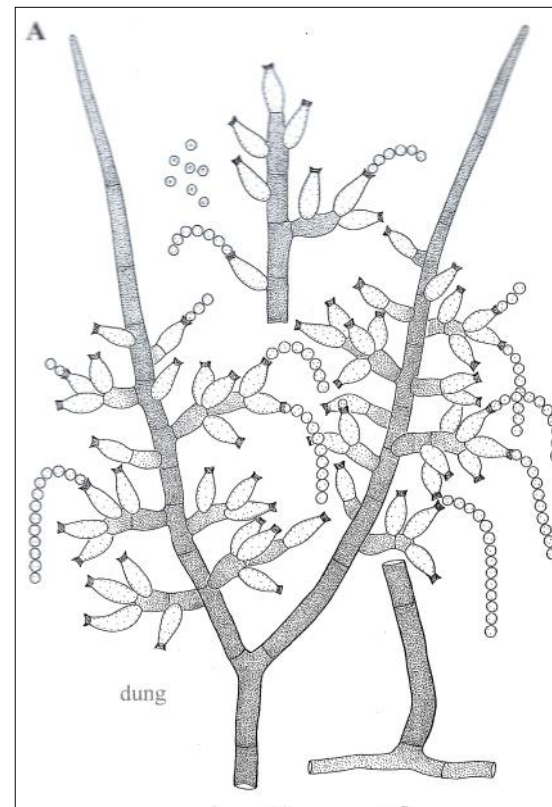
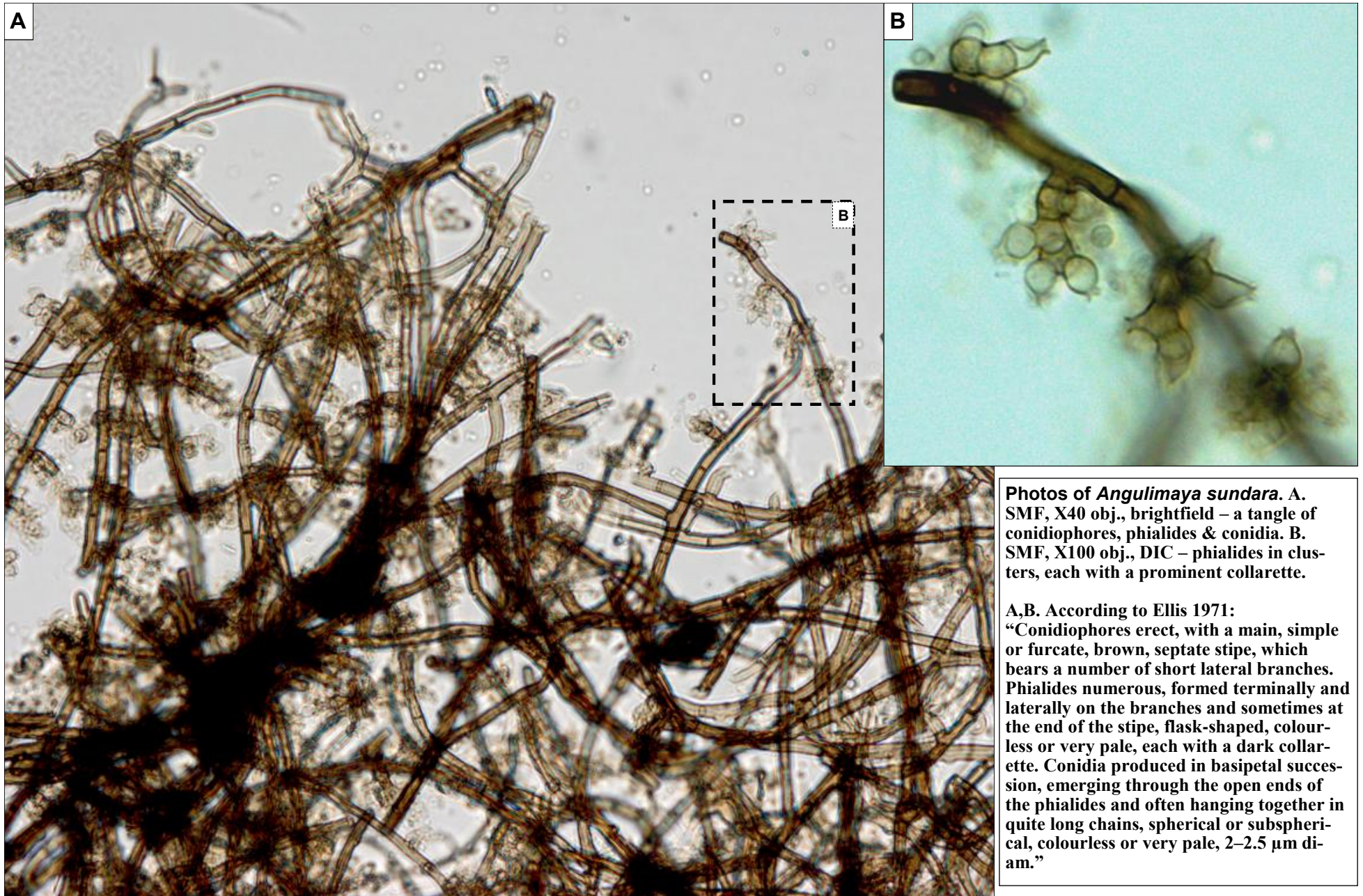


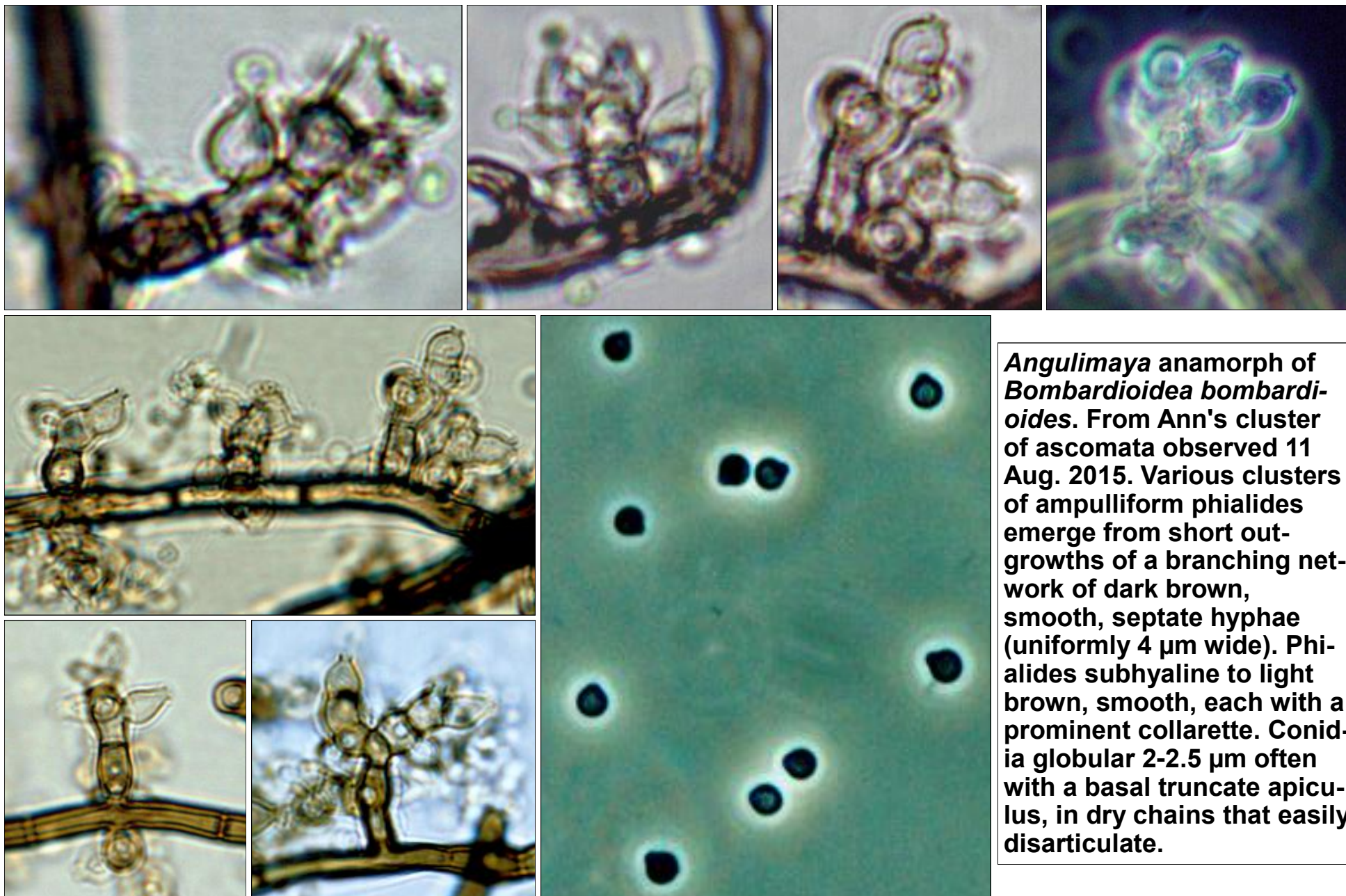
Plate 53. A. *Angulimaya sundara*



**Photos of *Angulimaya sundara*.** A. SMF, X40 obj., brightfield – a tangle of conidiophores, phialides & conidia. B. SMF, X100 obj., DIC – phialides in clusters, each with a prominent collarete.

A,B. According to Ellis 1971:  
 “Conidiophores erect, with a main, simple or furcate, brown, septate stipe, which bears a number of short lateral branches. Phialides numerous, formed terminally and laterally on the branches and sometimes at the end of the stipe, flask-shaped, colourless or very pale, each with a dark collar-ette. Conidia produced in basipetal succession, emerging through the open ends of the phialides and often hanging together in quite long chains, spherical or subspherical, colourless or very pale, 2–2.5  $\mu$ m diam.”





***Angulimaya* anamorph of *Bombardioidea bombardioides*. From Ann's cluster of ascomata observed 11 Aug. 2015. Various clusters of ampulliform phialides emerge from short outgrowths of a branching network of dark brown, smooth, septate hyphae (uniformly 4  $\mu$ m wide). Phialides subhyaline to light brown, smooth, each with a prominent collarette. Conidia globular 2-2.5  $\mu$ m often with a basal truncate apiculus, in dry chains that easily disarticulate.**