Aspergillus flavus **Link AEB 1341 (= PDD 117257) – axenic culture = ICMP 24137** **However, see the next page for an update and correction concerning the axenic culture.**

Collection date: 14 April 2021

Collection site: Remutaka Forest Park – Orongorongo Track

Substrate: small dead twig in shallow ground debris

Collector and Identifier: Dan Mahoney

Voucher material: dried field specimen AEB 1341 (= PDD 117257) accompanied by 1) an aniline blue lactic acid semi-permanent slide mount prepared from the fresh field specimen, 2) a lacto-fuchsin slide mount prepared from a 5-day-old colony on Difco cornmeal agar (CMA) that had been inoculated by streaking spores from the early-sporulating colony on the dead twig field collection and incubated at 25°C and 3) a dried 18-day-old axenic potato carrot agar (PCA) Petri dish culture; an axenic CMA tube slant was deposited at ICMP (ICMP accession # 24137) ; Dan's in-situ dissecting scope digitized photos and his compound scope digital photos of microscopic detail; Dan's brief description and comments.

Brief description and comments: Initial observations of the field specimen are illustrated in the first 7 photo plates of this pdf while those from its cultures on Difco CMA and PCA are found on plates 8–13. The difference between the two is seemingly characteristic of this highly variable species. Most striking is the conidial head difference. Vesicles on the decaying twig had biseriate sterigmata with early conidia borne radially while those on CMA and PCA were only uniseriate with conidia in loosely columnar to columnar heads. Although both conditions are described in the 1965 book "The Genus Aspergillus" by Raper and Fennell, the situation here seems more extreme. It is unclear whether *A. flavus* can accommodate strains with either or both biseriate and uniseriate heads or whether the same strain can be one or the other depending on its environment. The latter seems to be the case here. Unfortunately, my observations are based on a young field specimen grown under much cooler field conditions and its CMA and PCA cultures at 25°C. Also, its earlier cultured descriptions are based primarily on Czapek's solution agar and malt extract agar and I had neither available at the time. My measurements of vesicles, sterigmata and conidia fall in the lowermost portion of the *A. flavus* range whether viewed on the original twig or in culture. Conidia were faintly (and inconspicuously) echinulate on both, with those first formed on the twig globular and only 2 µm in diam and those in culture 3 µm and more ellipsoid. Conidiophores in cultures were roughened but less conspicuously so than described. Sclerotia were only observed in cultures and then were numerous, always <400 µm and usually more conspicuous than conidia.

Since Raper and Fennell's earlier work, considerable research has been done on strains of *A. flavus* producing aflatoxin on agricultural crops, those degrading commercial woods and those of concern to human pathology. These strains have also been divided on the basis of producing smaller (< 400 µm diam) or larger (> 400 µm) sclerotia. In fact, the species is now usually treated as a complex but, so far, has not been subdivided into separate species. It is my hope that my CMA culture, recently submitted to ICMP in Auckland, will be sequenced and its position within the *A. flavus* complex clarified. **See the next page for the results of that sequencing.**

Sequencing results and comments on ICMP 24137

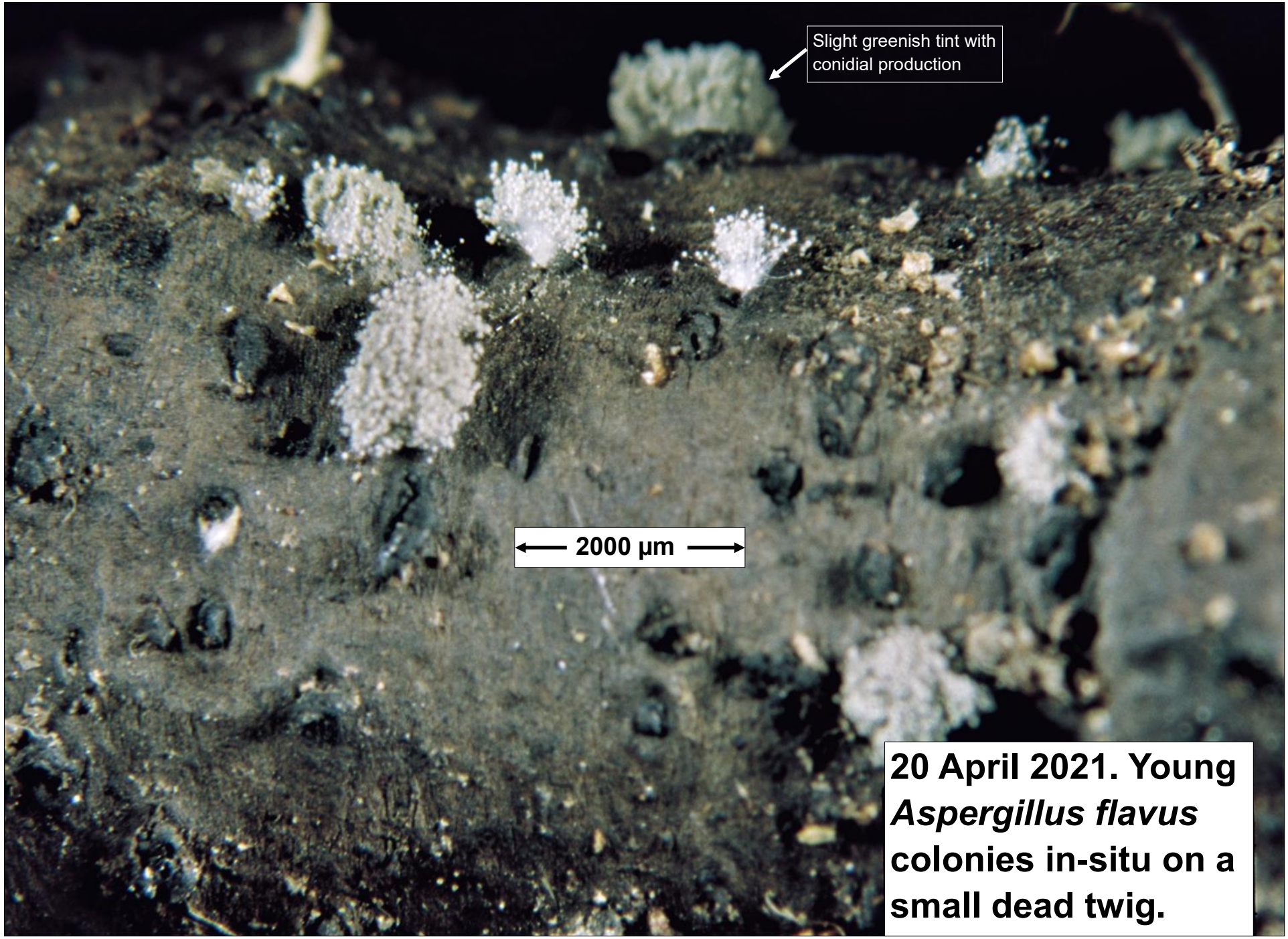
Further culturing and sequencing were done by Diana Lee (Technician - Mycology/Bacteriology - at Landcare Research ICMP) and Bevin Weir (Senior Researcher - Mycologist/Bacteriologist Systematics - at Landcare Research PDD). Their efforts have clarified and corrected the identity of the axenic culture that accompanied the dried herbarium specimen *Aspergillus flavus* Link AEB 1341 (= PDD 117257) – see the new online entries for *Penicillium jejuense* under ICMP 24137 and PDD 119684 (collection # AEB 1341a).

My earlier attempts to justify the culture identity as a variant of *A. flavus* were in error. The fungus I cultured was instead a *Penicillium* whose beta tubulin sequence matched closest to a new species described in 2015 from South Korea, *Penicillium jejuense*: Citation = "Myung Soo Park, Jonathan J. Fong, Seung-Yoon Oh, Jos Houbraken, Jae Hak Sohn, Seung-Beom Hong & Young Woon Lim (2015) *Penicillium jejuense* sp. nov., isolated from the marine environments of Jeju Island, Korea, Mycologia, 107:1, 209-216, DOI: 10.3852/14-180". I saw no *Penicillium* on the fresh collection but its spores must have been present and their colonies were those I found on my culture plates. No colonies (among the many on my culture plates) exhibited bi-sterigmatic heads characteristic of the *A. flavus* on the moist dead wood.

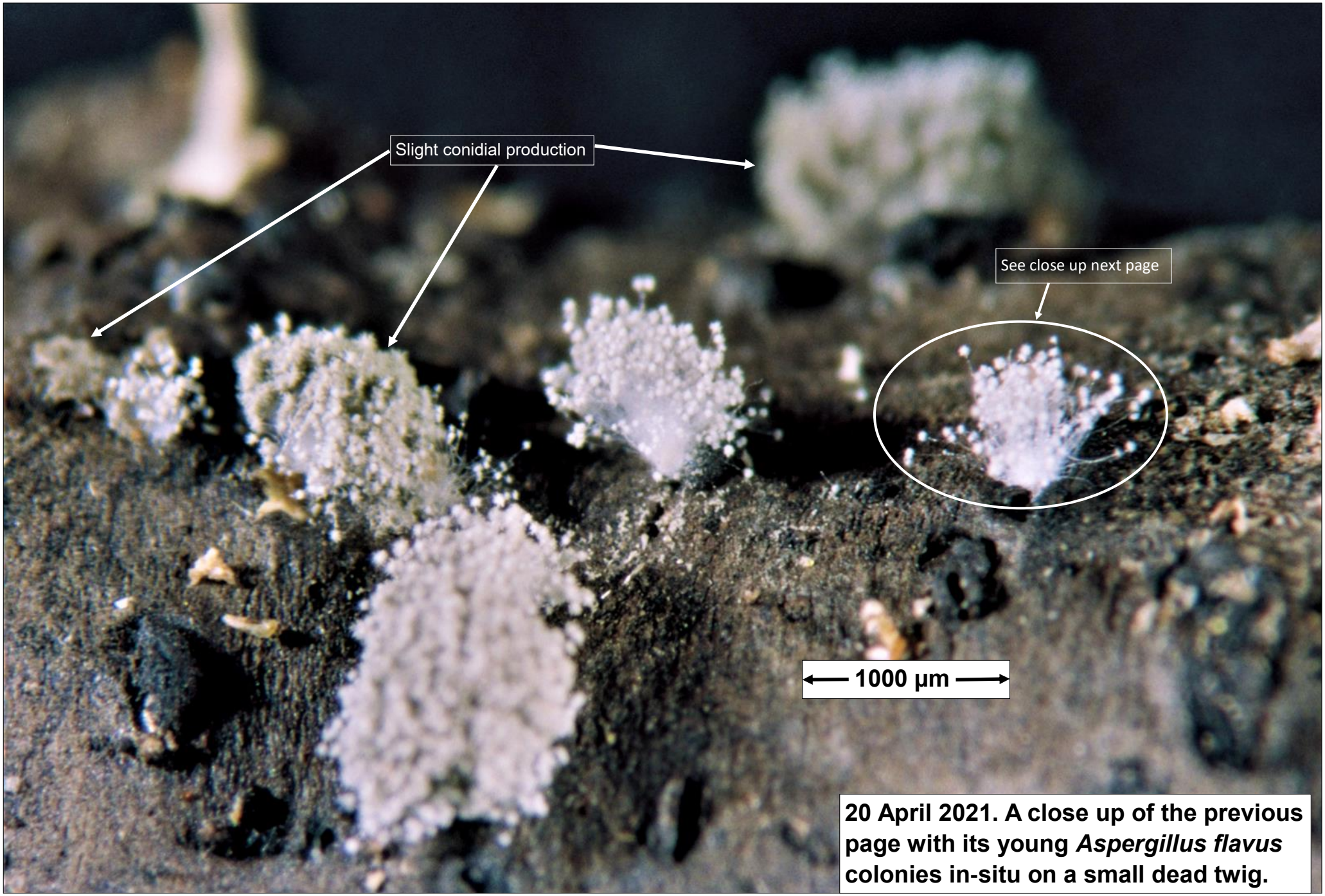
Photograph labels on the following pdf pages have been corrected where necessary. Pages 3–9 illustrate *Aspergillus flavus* while pages 9–15 illustrate the cultured *Penicillium jejuense*. This is the first record of *P. jejuense* in New Zealand.

A more recent paper by Park and others "MS Park, JW Lee, SH Kim, J.-H. Park, Y.-H. You, and YW Lim. 2020. Penicillium from Rhizosphere Soil in Terrestrial and Coastal Environments in South Korea. Mycobiology 48(6): 431–442." (Doi:10.1080/12298093.2020.1823611) records additional sequenced collections of *P. jejuense*.

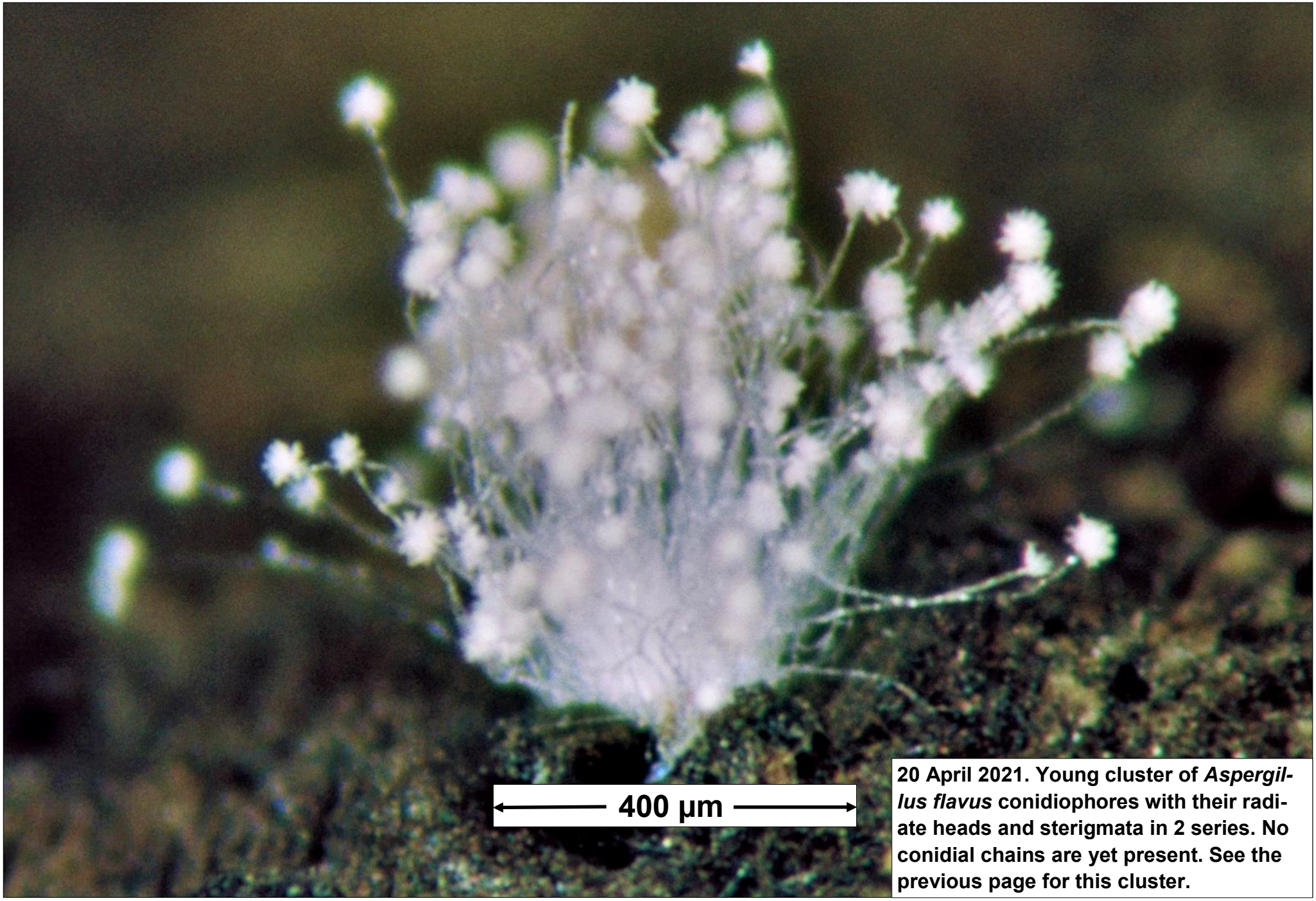
Of interest also is the placement of *P. jejuense* in the *Penicillium* section *Aspergilloides*, *P. thomii* clade. See "Houbraken J., Visagie C.M., Meijer M., Frisvad J.C., Busby P.E., Pitt J.I., Seifert K.A., Louis-Seize G., Demirel R., Yilmaz N., Jacobs K., Christensen M. & Samson R.A. 2014. A taxonomic and phylogenetic revision of *Penicillium* section *Aspergilloides*. Studies in Mycology 78: 373-451". Here sequenced collections of *P. jejuense* are also reported from Australia and South Africa.



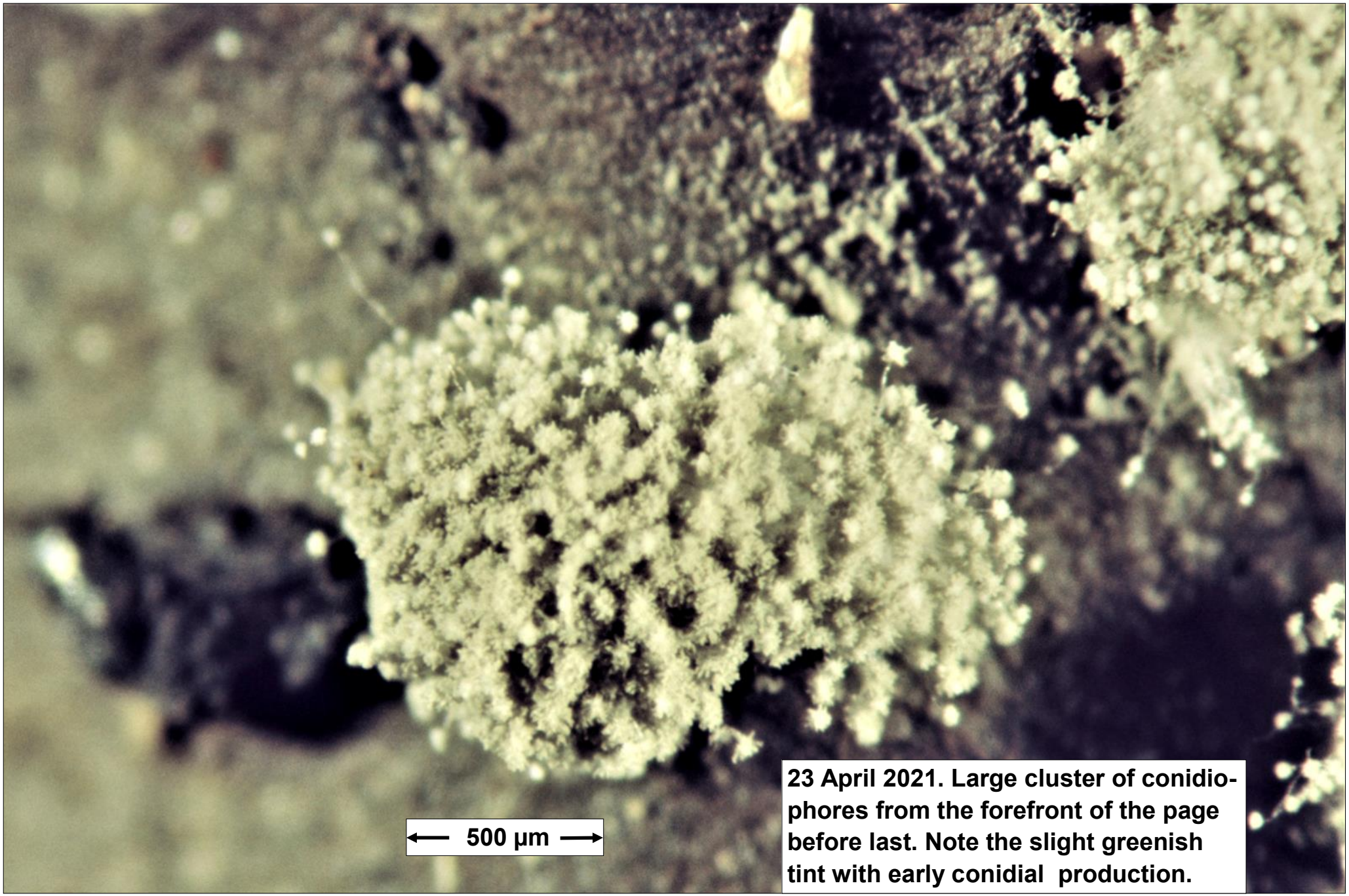
20 April 2021. Young *Aspergillus flavus* colonies in-situ on a small dead twig.



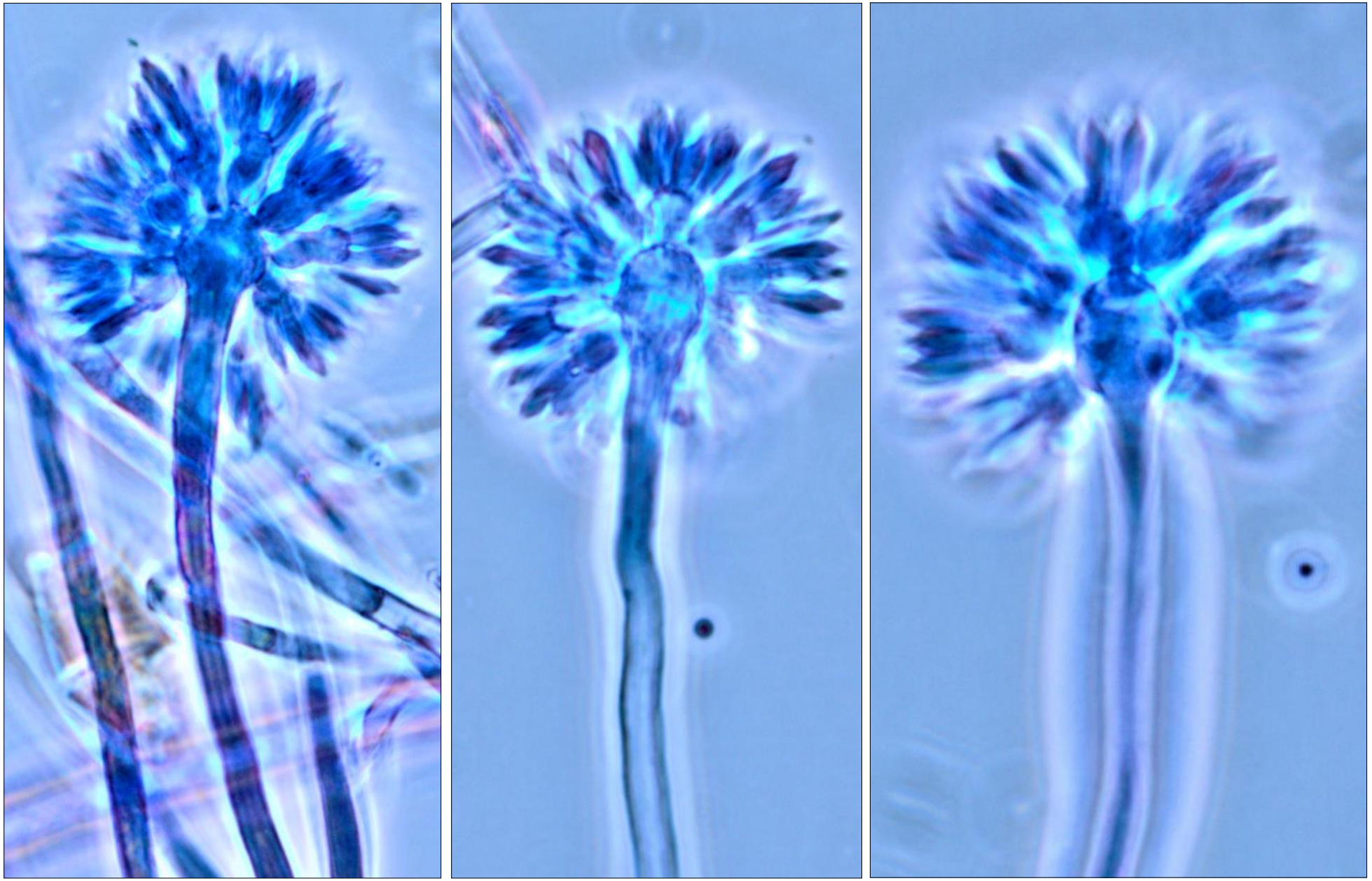
20 April 2021. A close up of the previous page with its young *Aspergillus flavus* colonies in-situ on a small dead twig.



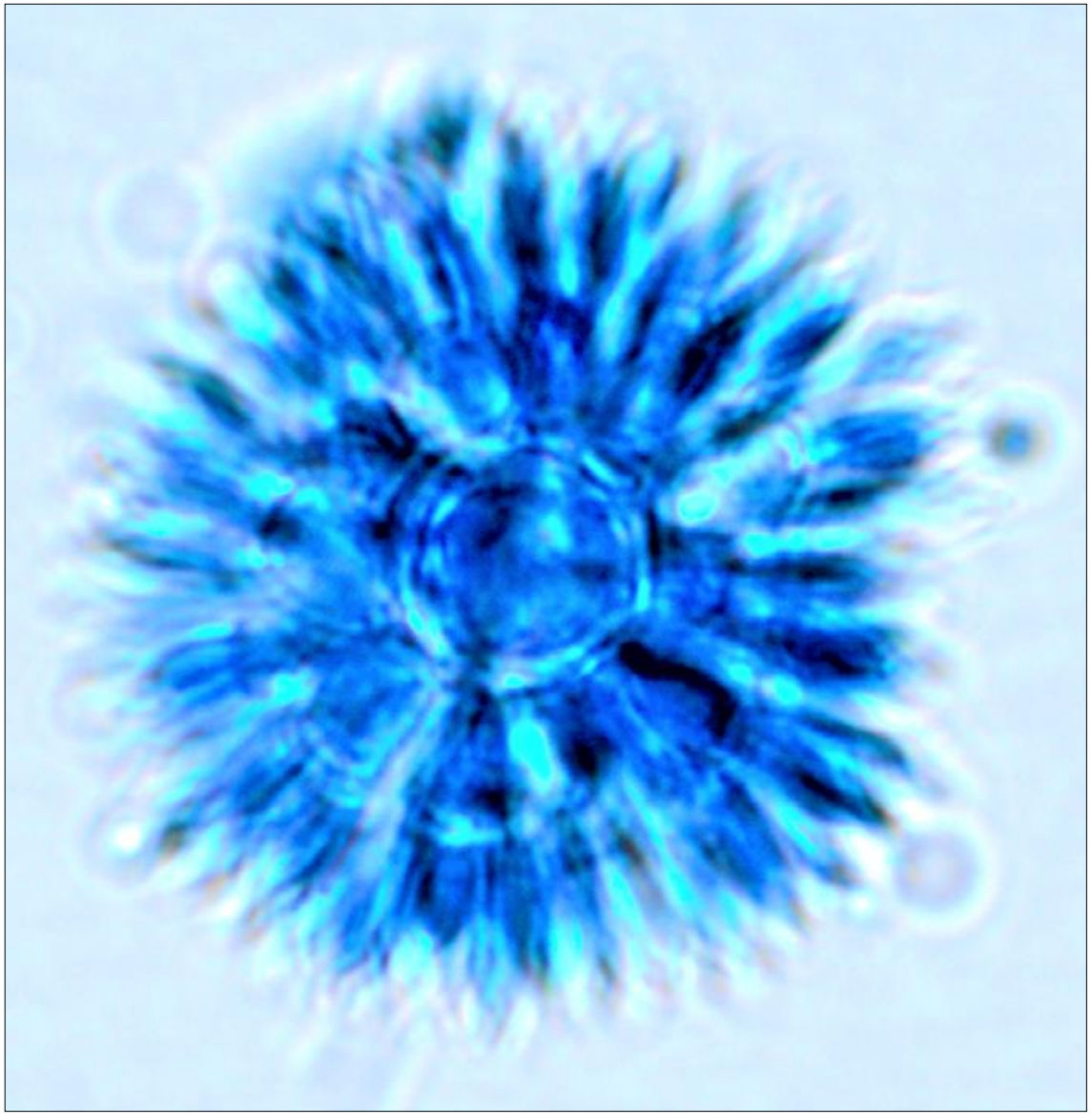
20 April 2021. Young cluster of *Aspergillus flavus* conidiophores with their radiate heads and sterigmata in 2 series. No conidial chains are yet present. See the previous page for this cluster.

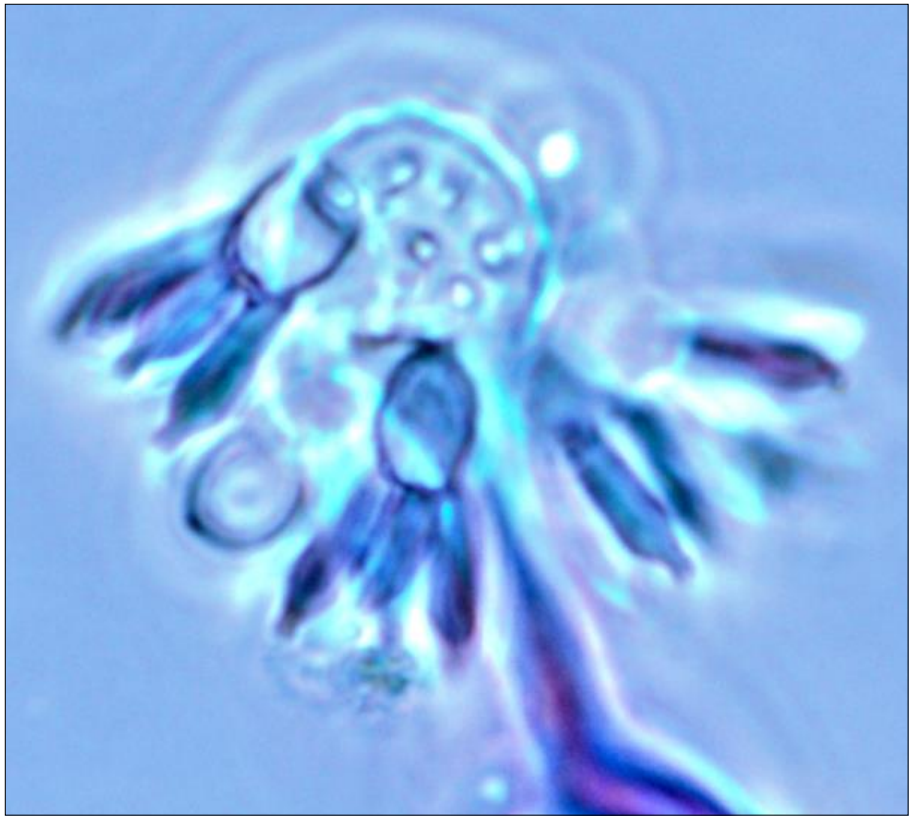


23 April 2021. Large cluster of conidiophores from the forefront of the page before last. Note the slight greenish tint with early conidial production.

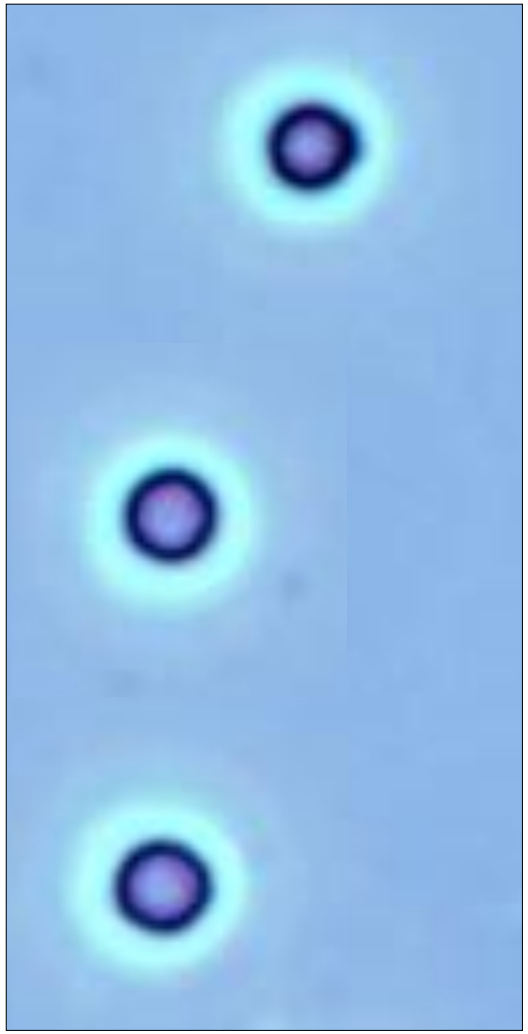


Aspergillus flavus **radiate heads showing sterigmata in two series. These photos taken from mounts of non-sporulating heads, like those shown on photo page 2 (circled) and p. 3 close up. Aniline blue lactic acid mounts, X100 objectives and brightfield microscopy.**



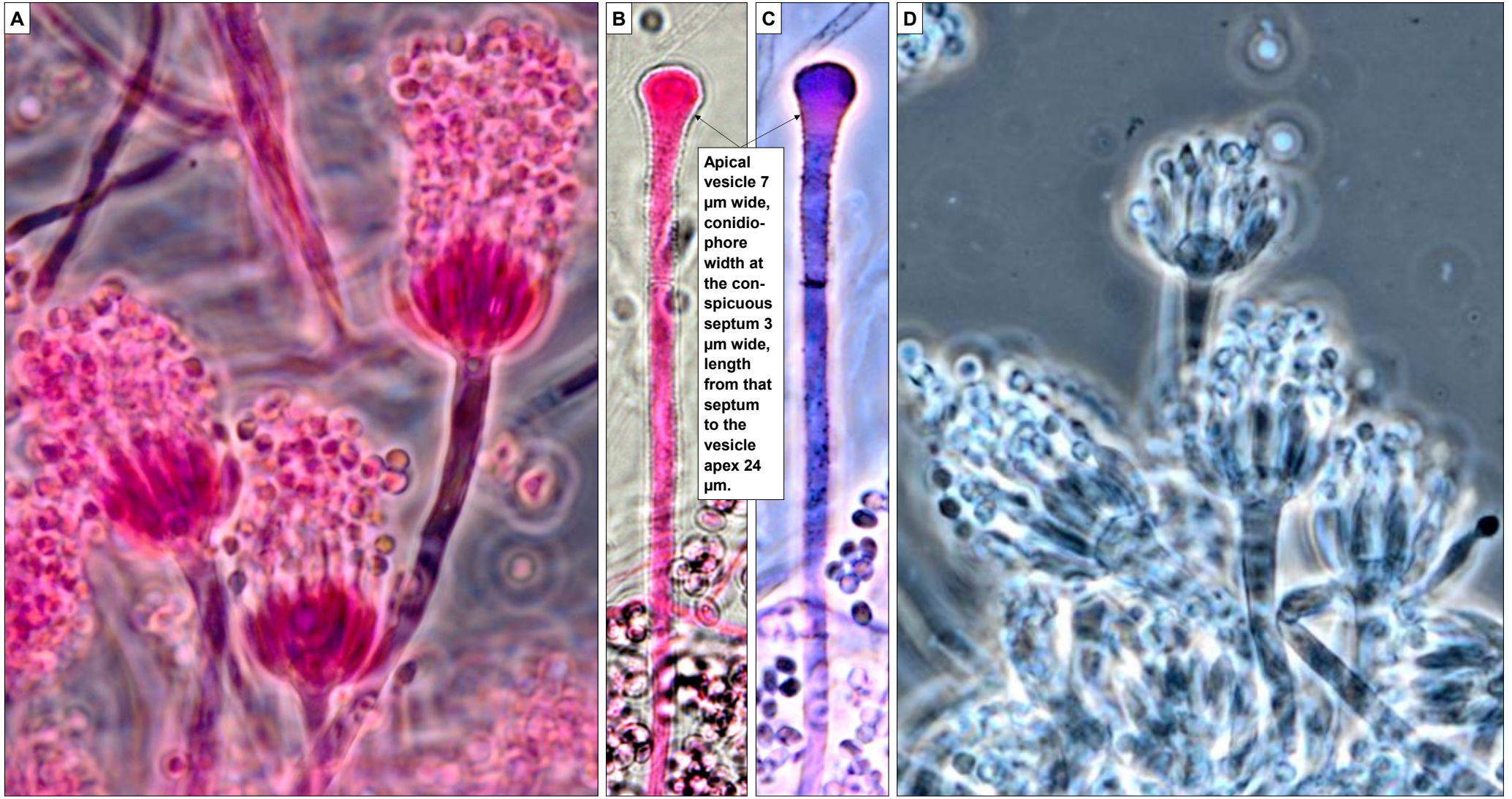


Aspergillus flavus **showing different views of the non-sporulating heads on the previous page. All aniline blue lactic acid mounts, X100 objectives and brightfield microscopy.Left photo: 'overhead view—no conidiophore' showing a globose vesicle and sterigmata in two series. Right 2 photos: head fragments showing close up views of primary and secondary sterigmata.**



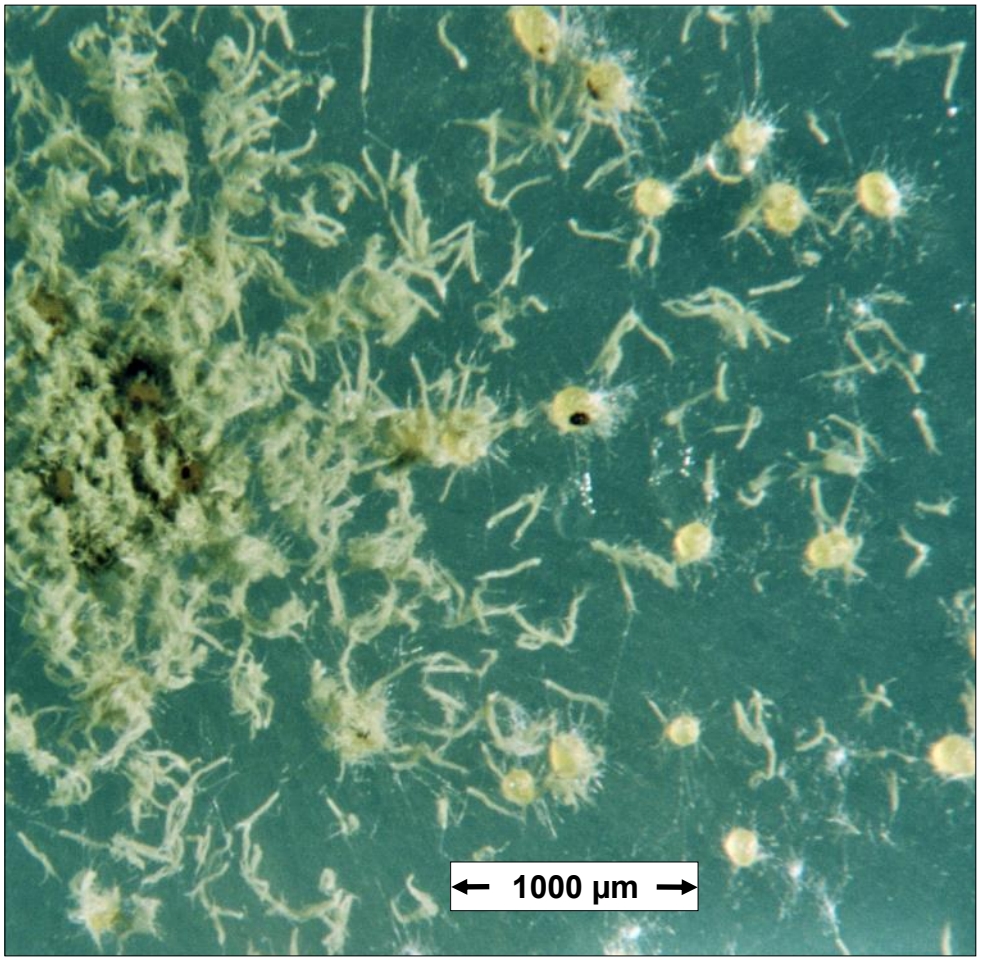
Aspergillus flavus **first-formed tiny (approx. 2 µm diam.) finely echinulate (arrowed) globose conidia with very slightly truncate bases. Aniline blue lactic acid mounts, X100 objectives enlarged) and bright-field microscopy.**

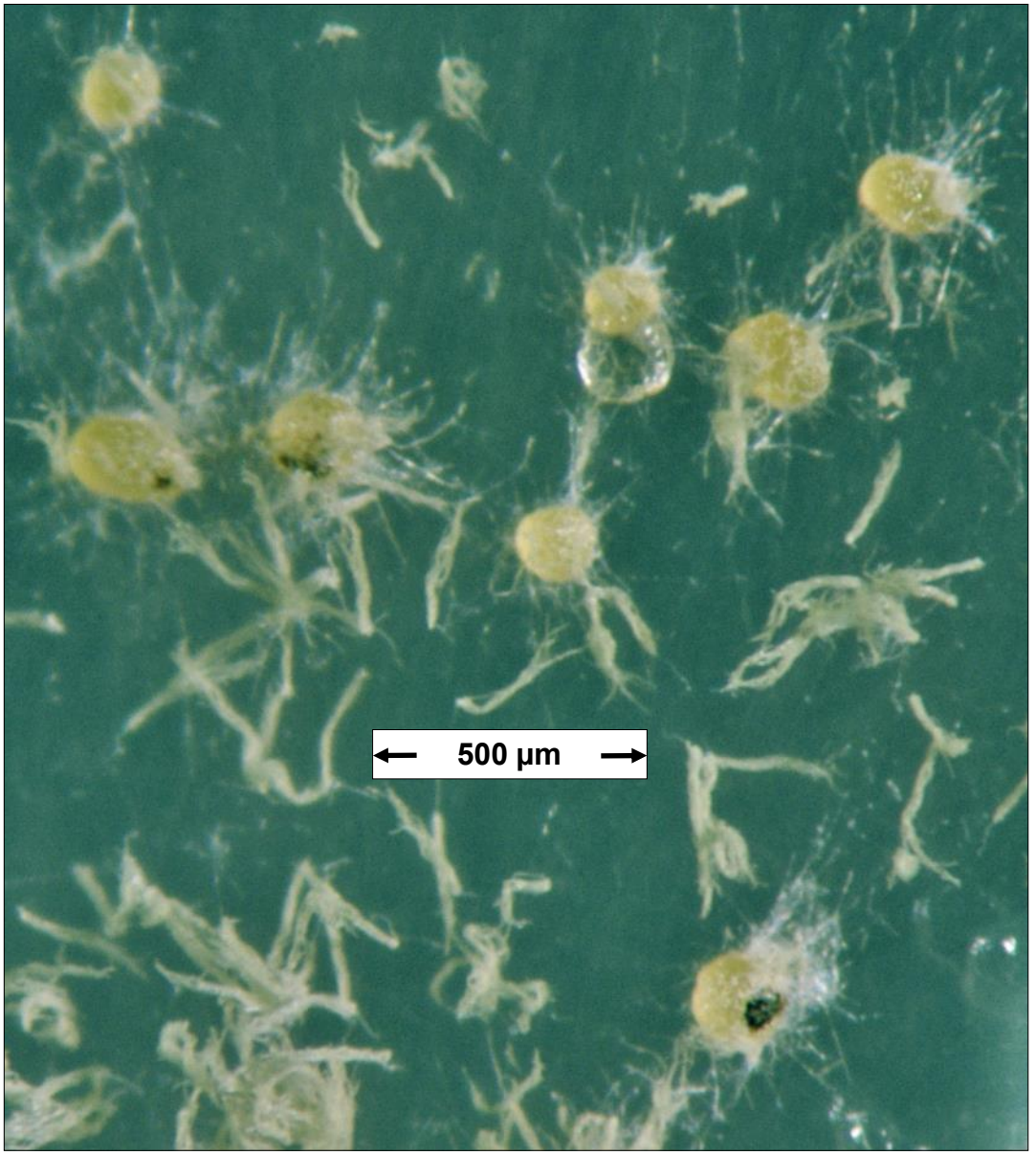
~~Aspergillus flavus~~ **=** ***Penicillium jejuense*****. Small heads showing a single series of sterigmata. The sterigmata more apically oriented on the vesicle and producing moderately columnar conidial heads. Lacto-fuchsin mount, X40 objective and brightfield microscopy. From a 5-day-old colony on Difco cornmeal agar (CMA) that had been inoculated by streaking spores from an early-sporulating colony on the dead twig field collection and incubated at 25°C. See the next page for a photo of the outlined area using the X100 objective.**



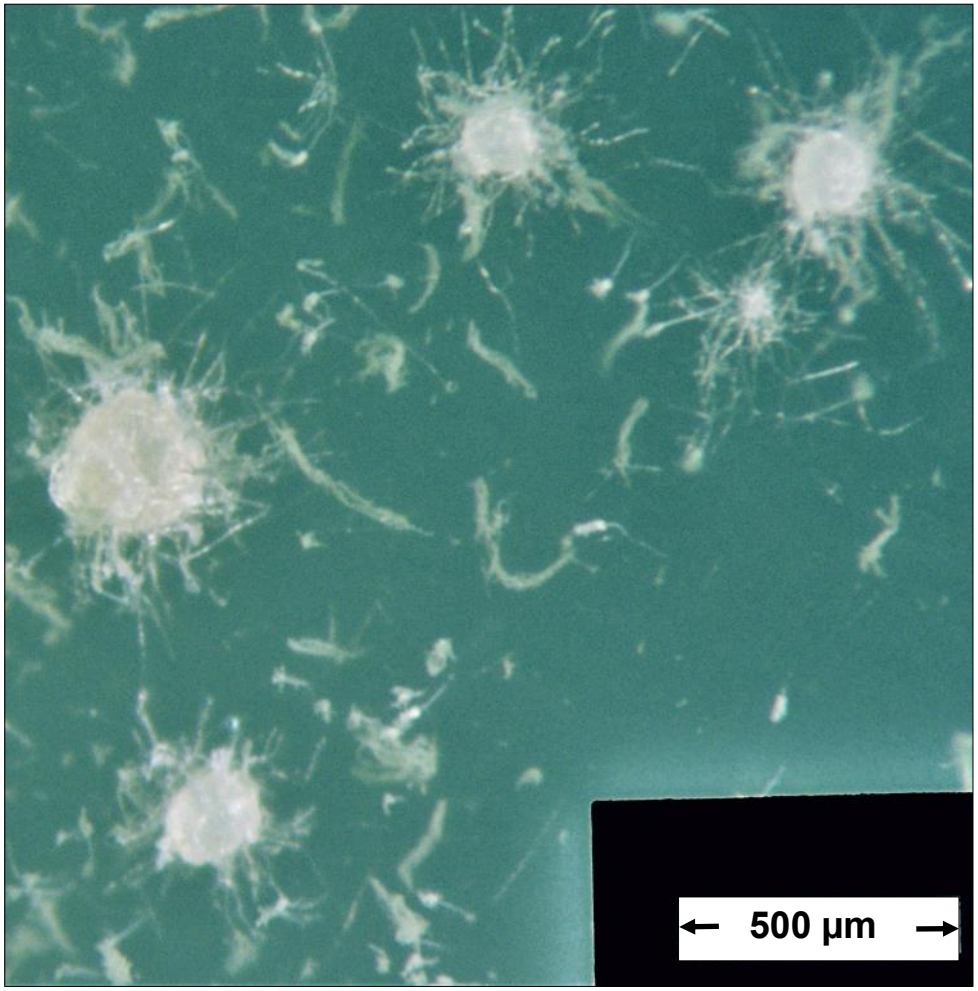
A–D. Photos of conidial heads from cultures of *Penicillium jejuense* (formerly thought to be *Aspergillus flavus*) using the X100 objective and phase microscopy (except B, brightfield). A,B. From a lacto-fuchsin slide mount. C,D. From an aniline blue lactic acid slide mount. A. See its X40 objective photo outlined on the previous page; both A and D photos were prepared from the same 5-day-old CMA colony. B,C. The same roughened conidio-phore and apical vesicle from the same (but older) axenic 3-spot PCA culture seen on the next page.

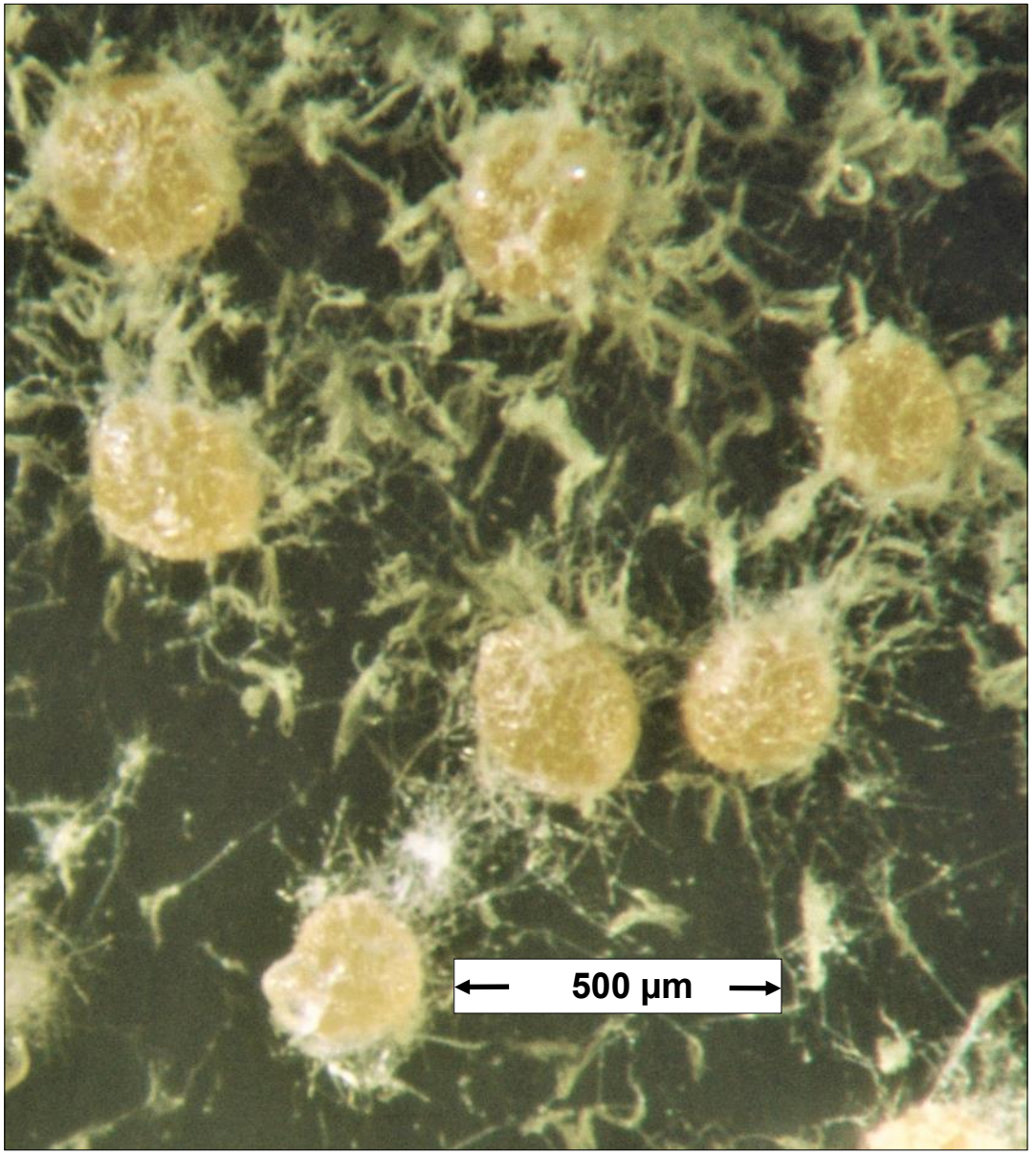
Samsung Galaxy A70 camera photos of a 6-day-old axenic PCA culture of *Penicillium jejuense* in a 9 cm Petri dish. Photos B and C on the previous page were taken from the same Petri dish after 18 days growth. That 18-day dish was dried and fumigated as a herbarium record. Most obvious are the numerous sclerotia with greenish conidia less so.



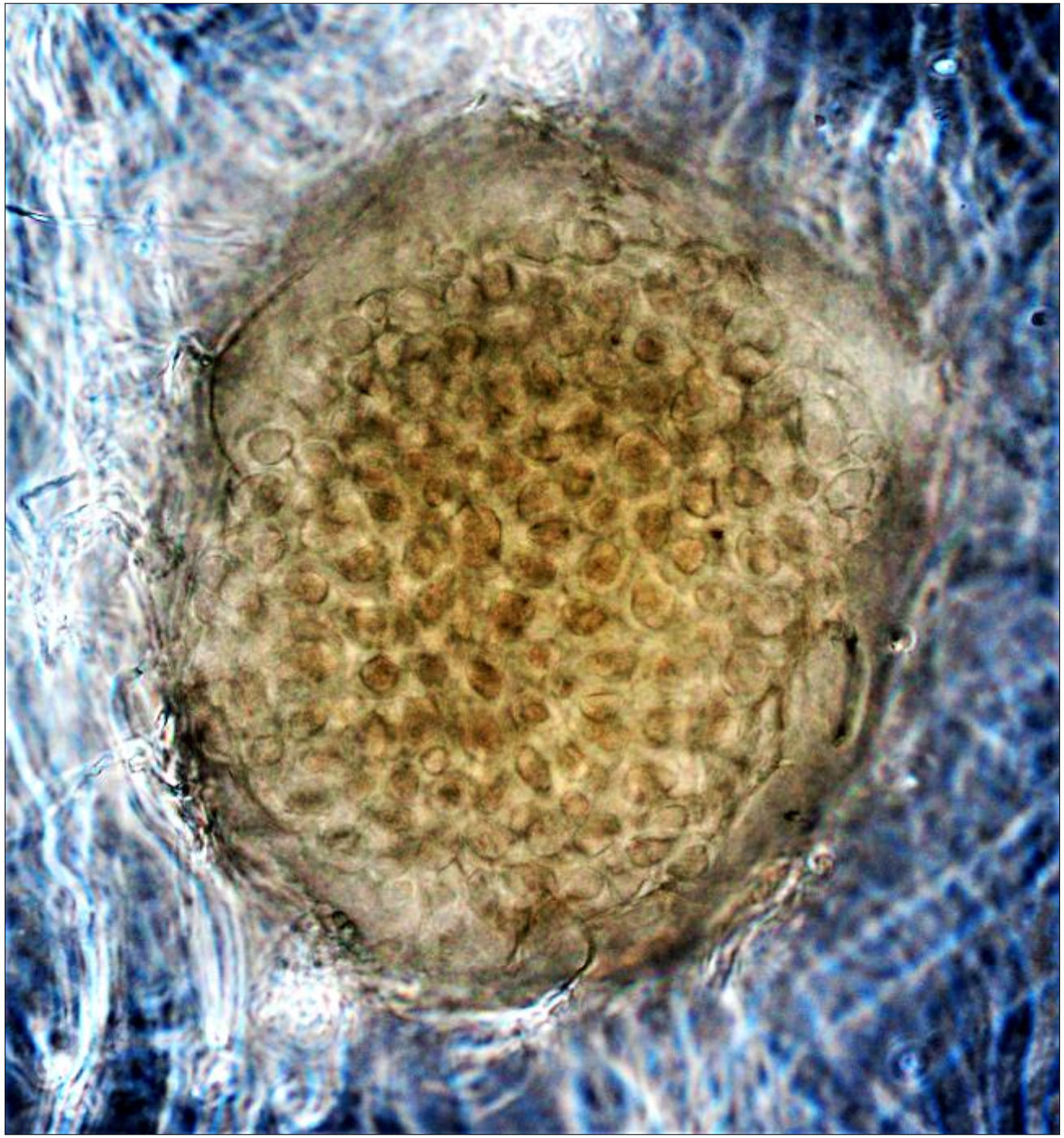


In-situ photos of a small peripheral 19-day-old colony on Difco cornmeal agar (CMA) that had been inoculated by streaking spores from an early-sporulating colony on the dead twig field collection and incubated at 25°C. Note the small yellowish sclerotia with occasional black areas and the greenish columnar to loosely columnar conidial heads.





In-situ photos of a 16-day-old colony on PCA. Left photo with younger, smaller, whitish sclerotia. Right photo with older, larger, yellowish sclerotia. In both, note the greenish, loosely columnar conidial heads.



Young, white to faintly yellow sclerotium (187.5 µm in diam). Note its thick-walled angular cells. The sclerotium was placed in a small drop of Tween 20 to eliminate bubbles and then washed with water to remove debris before applying a coverslip. The photo was taken under a X40 objective using phase microscopy.

As seen on previous pages, sclerotia become darker – whitish then yellow orange and occasionally with small blackish areas.