

***Piptocephalis freseniana* de Bary – PDD 121665 (= AEB 1375)**

Collection sites:

- 1) Raukumara Conservation Park, NZTM = E2014912, N5801777
- 2) residential property, Moonshine Hill Rd., Upper Hutt
- 3) Callaghan rabbit 2017, also *Sporormiella* and others were found there

Collection dates: 1) 5 April 2024, 2) 3 March 2023, 3) 12 November 2017

Substrates: 1) red deer (*Cervus elaphus*) dung, 2) European rabbit (*Oryctolagus cuniculus*) dung, 3) European rabbit (*Oryctolagus cuniculus*) dung

Collectors: 1) Ian Flux, 2) Ian Flux & Merryl Park, 3) Dan Mahoney

Identifier: Dan Mahoney (all 3)

Voucher materials: no dried herbarium specimens but two SMF (Shear's mounting fluid) semi-permanent glass slides from collection site 1; no herbarium dried material or slides from collection site 2 (but numerous photos), and no dried herbarium material but 1 SMF slide from collection site 3; in-situ dissecting scope photos and numerous photos of microscopic detail; pertinent references and Dan's comments.

Pertinent references:

1. Benjamin R.K. 1959. The merosporangiferous Mucorales. *Aliso* 4: 321–433. **A thorough introduction to this group of fungi.**
2. Gräfenhan T. 1998. Taxonomic revision of the genus *Piptocephalis* (Fungi) [MS thesis]. Greifswald, Germany: University of Greifswald. 102 p. **Unfortunately, I was unable to attain this reference.**
3. Benny, G.L. 2008. Methods Used by Dr. R.K. Benjamin, and Other Mycologists, to Isolate Zygomycetes. *Aliso: A Journal of Systematic and Evolutionary Botany*. Vol. 26(1): 37–61. **Page 37: "He (Benjamin) made drawings of most of the *Piptocephalis* species that he considered valid and these were included in a study of the genus (Gräfenhan 1998)."**
4. Reynolds N.K., Benny G.L. et al. 2019. Phylogenetic & morphological analyses of the mycoparasitic genus *Piptocephalis*. *Mycologia* 111: 54–68. **p. 64 (suggested synonymies of species with *P. freseniana*): "The synonymization of *P. corymbifera* and *P. repens* with *P. freseniana* further demonstrates the degree of infraspecific variation within *Piptocephalis*. Gräfenhan (1998) noted that all three show inconsistencies in the number of spores per merosporangium, branching patterns, and presence or absence of stolon-like growth, as well as head cells that are sometimes not deciduous."**
5. Hou Y-H, Chuang S-C & Ho H-M. 2023. *Piptocephalis chienii* sp. nov., a new species found in Taiwan and a key to the known *Piptocephalis* species. *Fung. Sci.* 38(1): 31–36.
6. Cruz et al. 2023. An overview on the mycoparasitic *Piptocephalis* (Zoopagomycota): taxonomic notes and geographic distribution with new occurrences for South America. *Acta Botanica Brasilica* 37: 1–12. **A key & brief descriptions are provided for 24 *Piptocephalis* species that they accept as valid. The key is reproduced on the next 2 pages and their brief description of *P. freseniana* on the page after that.**

Cruz et al. 2023. An overview on the mycoparasitic *Piptocephalis* (Zoopagomycota): taxonomic notes and geographic distribution with new occurrences for South America. *Acta Botanica Brasilica* 37: 1–12. **Pages 9 & 10 are reproduced below and on the next page.**

Key for species of genus *Piptocephalis*

1. Head cell not formed	2
1. Head cell formed	5
2. Merosporangia unbranched	3
2. Merosporangia regularly branched or occasionally branched	4
3. Merosporangiophores with terminal enlargements on the ultimate branches	<i>P. pseudocephala</i>
3. Merosporangiophores without terminal enlargements on the ultimate branches.....	<i>P. benjaminii</i>
4. Merospores moniliform	<i>P. moniliformis</i>
4. Merospores cylindrical	<i>P. minuta</i>
5. Merospores fusiform	<i>P. fuispora</i>
5. Merospores not fusiform	6
6. Merospores globose	<i>P. sphaerospora</i>
6. Merospores not globose	7
7. Spore head forming a liquid droplet at maturity	8
7. Spore head remaining dry at maturity	16
8. Head cells with multiple lobes	9
8. Head cells without multiple lobes	15
9. Ultimate branches of merosporangiophores curvate	<i>P. curvata</i>
9. Ultimate branches of merosporangiophores not curvate.....	10
10. Head cells falling at maturity leaving a truncate tip on the merosporangiophore ultimate branch	<i>P. fimbriata</i>
10. Head cells collapsing at maturity but not leaving a truncate tip on the ultimate branch of the merosporangiophore	11
11. Merosporangia may contain more than six merospores each	12
11. Merosporangia never containing more than six merospores each	14

Key for species of genus *Piptocephalis* continued

12. Ultimate branches often > 40 µm long *P. cruciata*
12. Ultimate branches usually < 40 µm long 13
13. Fertile branch system consisting of up to 4 successive dichotomies *P. macrocephala*
13. Fertile branch system consisting of more than 4 successive dichotomies *P. freseniana*
14. Merospores up to 4 µm long *P. formosana*
14. Merospores up to 9 µm long *P. debaryana*
15. Head cells globose *P. lemonnieriana*
15. Head cells not globose *P. microcephala*
16. Head cells heart-shaped 17
16. Head cells not heart-shaped 18
17. Primary branches of merosporangiophores in whorls of 2–4 *P. indica*
17. Primary branches of merosporangiophores never in whorls *P. xenophila*
18. Merospores ellipsoidal 19
18. Merospores never ellipsoidal 20
19. Merosporangiophores with longitudinal striations *P. lepidula*
19. Merosporangiophores lacking longitudinal striations *P. graefenhanii*
20. Merosporangia unispored *P. unispora*
20. Merosporangia multispored 21
21. Primary branches of merosporangiophores less than 500 µm in length 22
21. Primary branches of merosporangiophores more than 500 µm in length 23
22. Fertile branch system consisting of up to 6 successive dichotomies, merospores doliform to cylindrical *P. brijmohanii*
22. Fertile branch system consisting of more than 6 successive dichotomies, merospores cylindrical *P. cylindrospora*
23. Penultimate branches of branch system with 7–20 µm in length, merosporangia 6–11 µm in length *P. tieghemiana*
23. Penultimate branches of branch system with 15–40 µm in length, merosporangia 13–25 µm in length *P. arrhiza*

Cruz et al. 2023. An overview on the mycoparasitic *Piptocephalis* (Zoopagomycota): taxonomic notes and geographic distribution with new occurrences for South America. Acta Botanica Brasilica 37: 1–12. Page 7 featuring *Piptocephalis freseniana* is reproduced below.

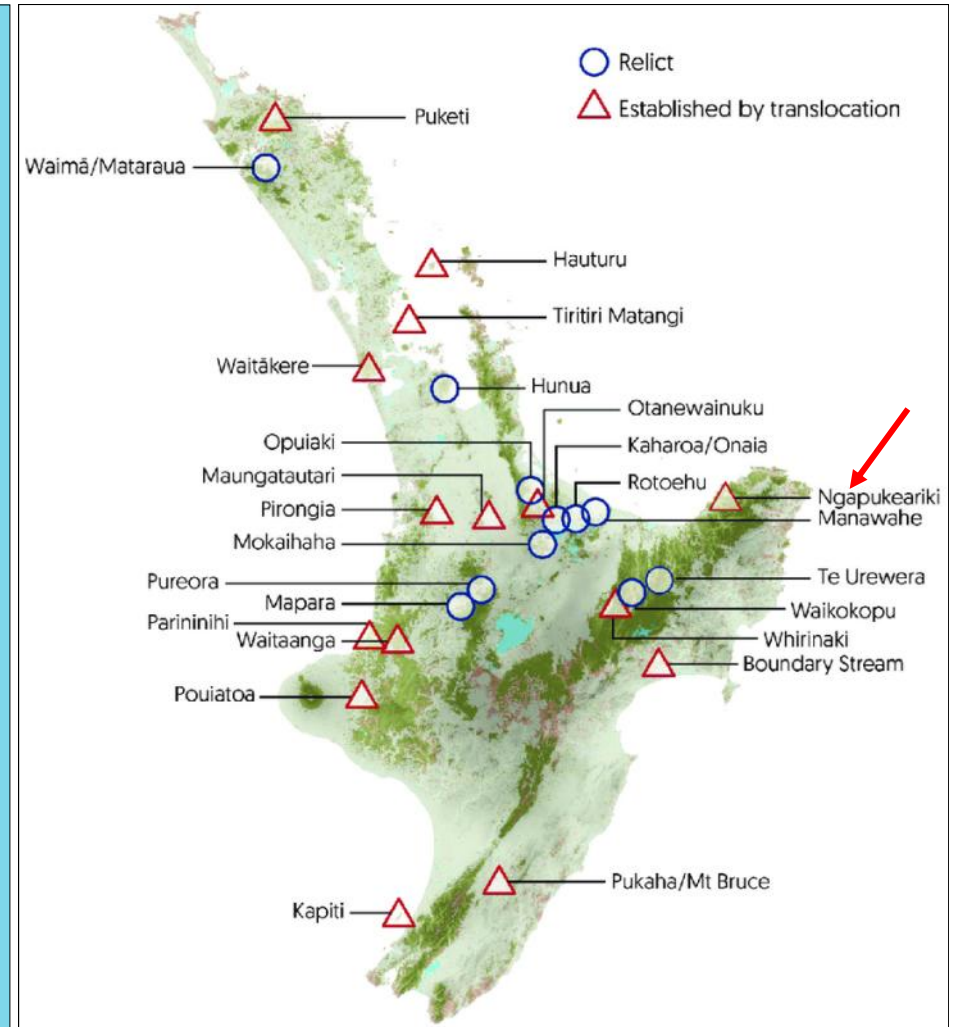
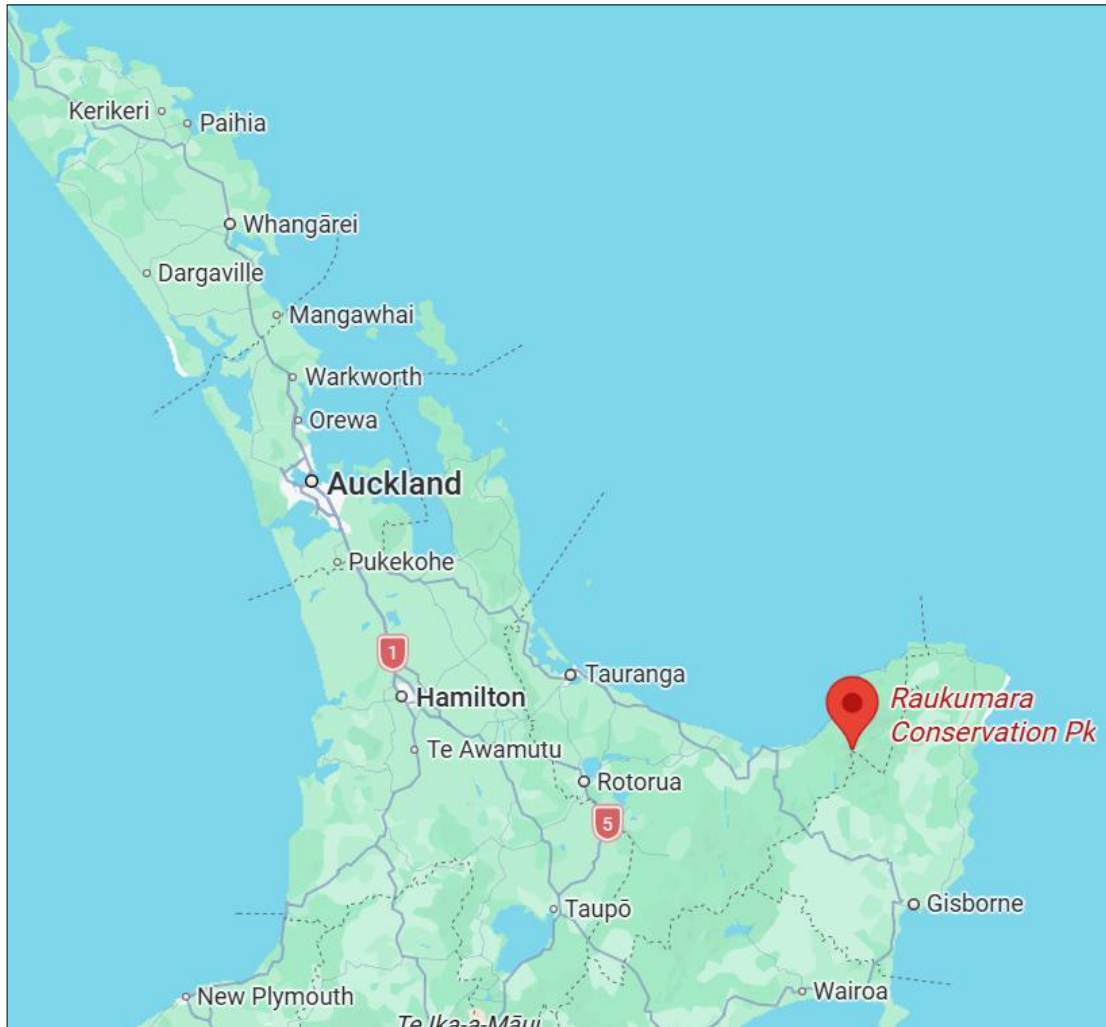
Piptocephalis freseniana de Bary, Abh. senckenb. naturforsch. Ges. 5: 356 (1865)

MycoBank number: 202311

Notes: According to Zycha *et al.* (1969) and Gräfenhan (1998), this homothallic species is characterized by the formation of striate merosporangiohores with 4–9 successive dichotomies, lobed head cells, merosporangia in a feather-like arrangement, sustaining 3–7 cylindrical to doliform merospores, which are 4–9 × 2.5–4 µm. Sporeheads form a liquid droplet at maturity. Zygosporangia are yellowish-brown to reddish-orange, 20–37 µm in diam., with a rough-walled exospore.

Habitat and geographic distribution: This species was previously reported on animal dung from Brazil, the United Kingdom and the United States of America; soil from the United States of America (Gräfenhan 1998; GBIF 2022; Genbank 2022). It has also been reported in Germany and Morocco without substrate indication (GBIF 2022).

Piptocephalis freseniana can parasitize *C. recurvatus* and *Mucor* sp. (Gräfenhan 1998).

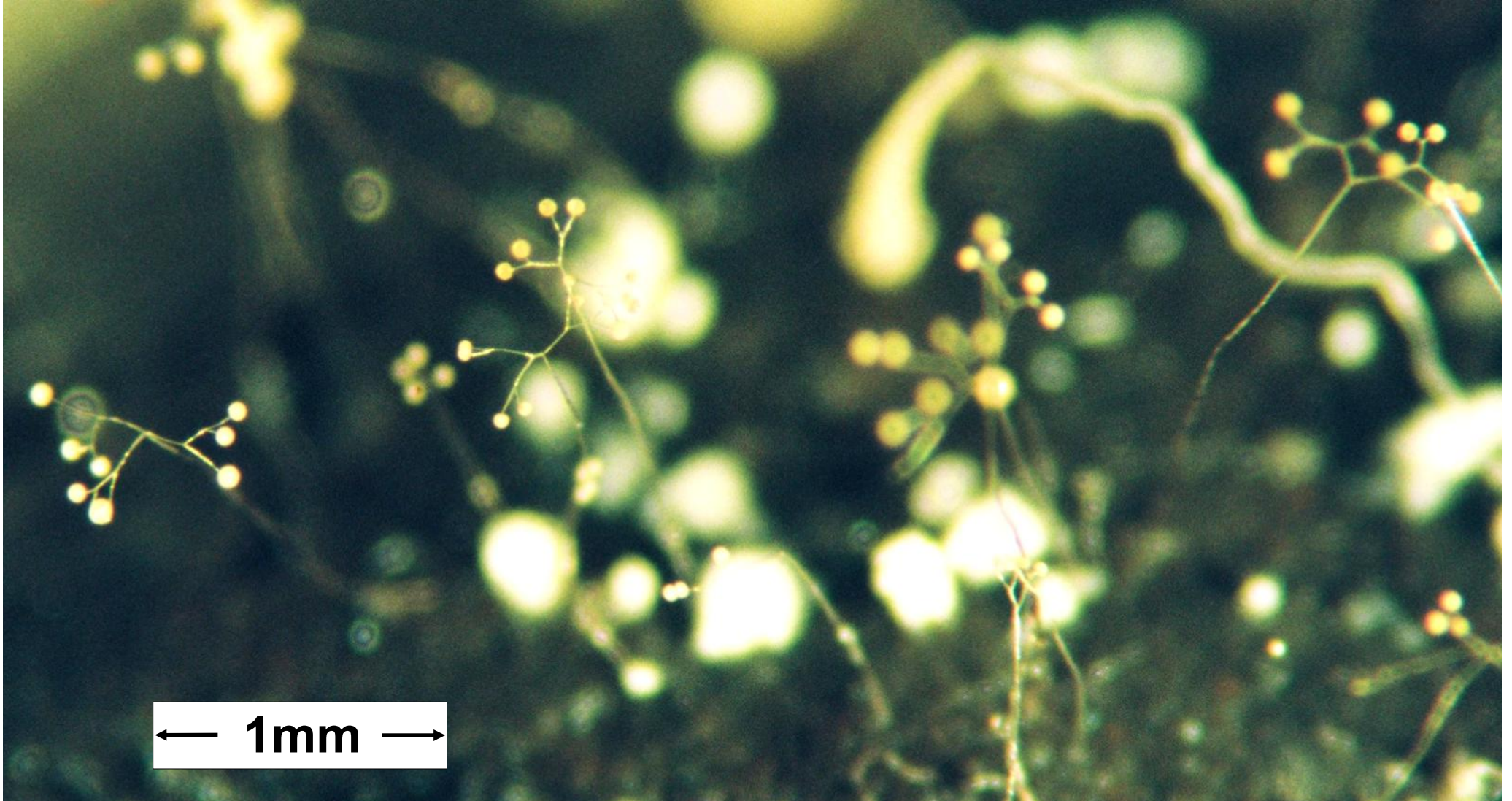


Collection site: Raukumara Conservation Park, NZTM = E2014912, N5801777; – NGAPUKEARIKI MAIN RIDGE; site – on ridge under forest; Vegetation: rewarewa, tawa, hinau, miro, hard-beech = mixed forest

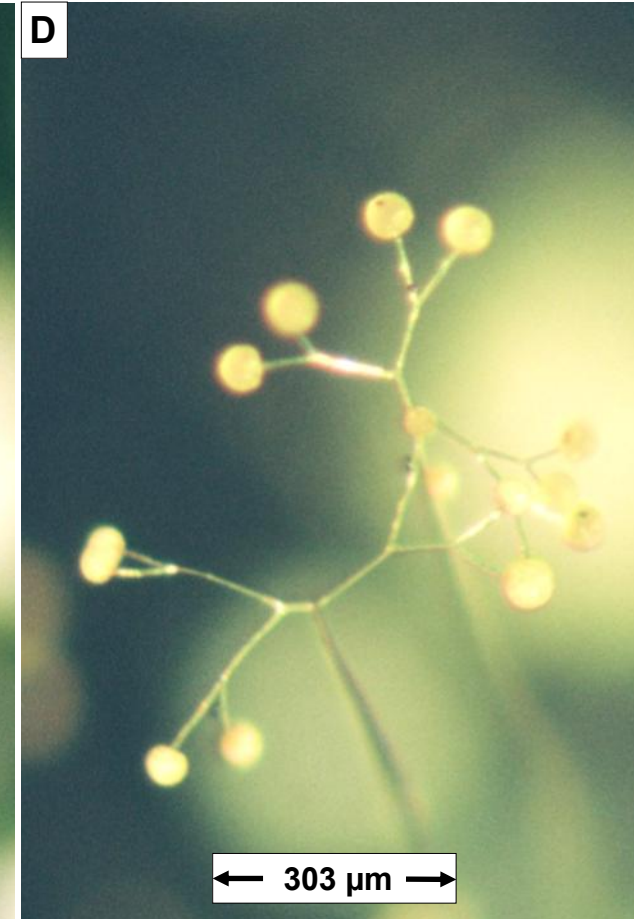
Collection date: 5 April 2024

Substrate: red deer (*Cervus elaphus*) dung

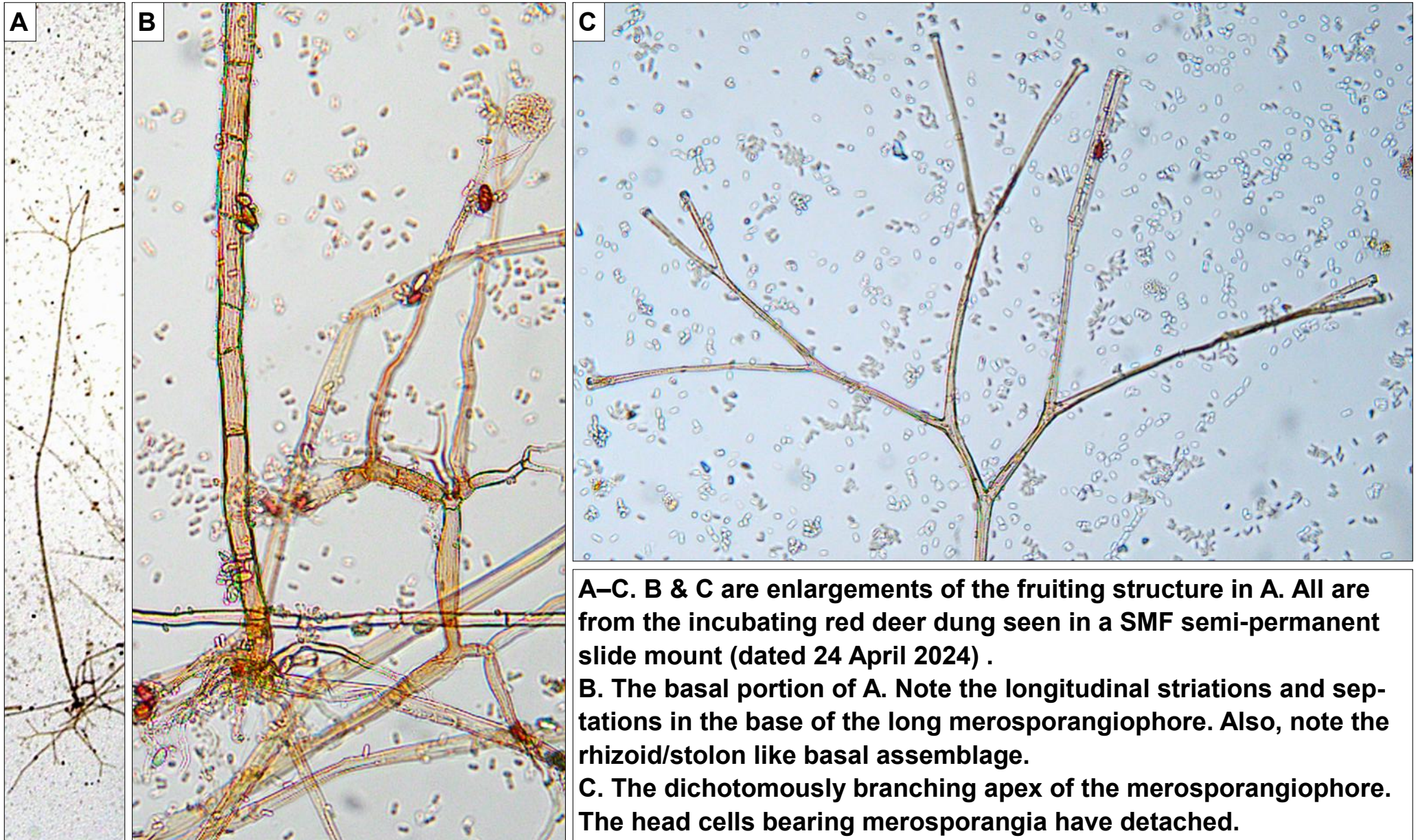
Collector: Ian Flux; **Identifier:** Dan Mahoney

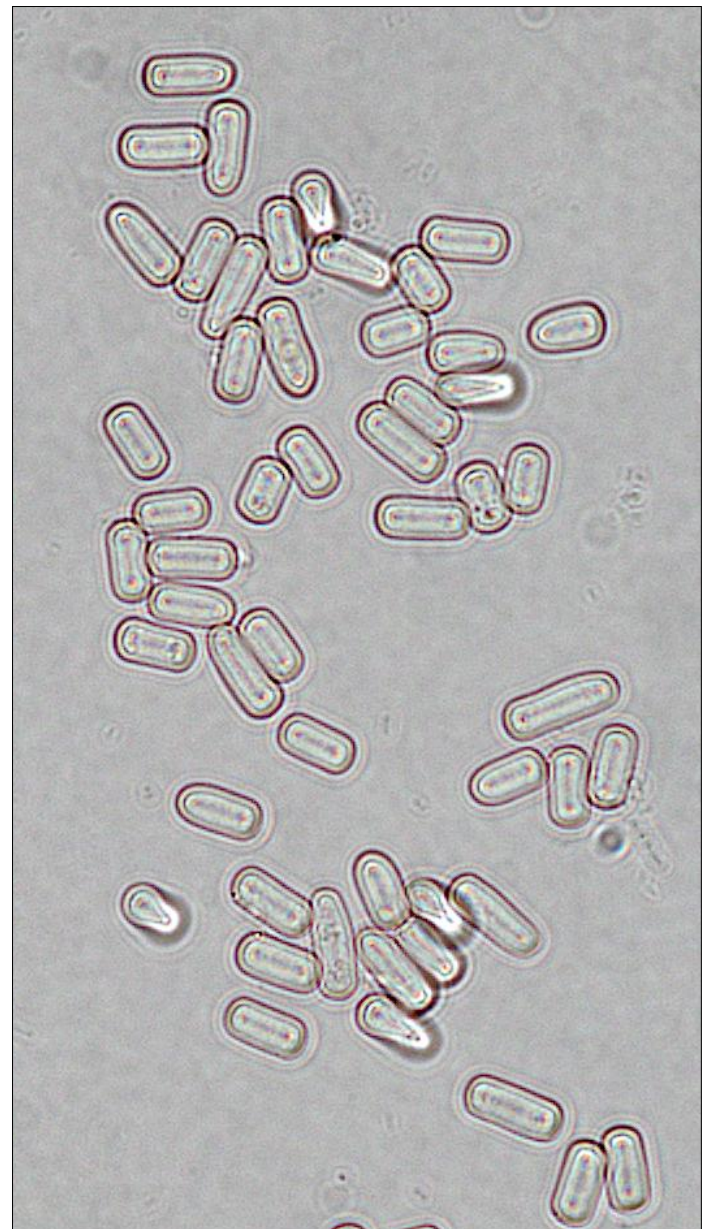
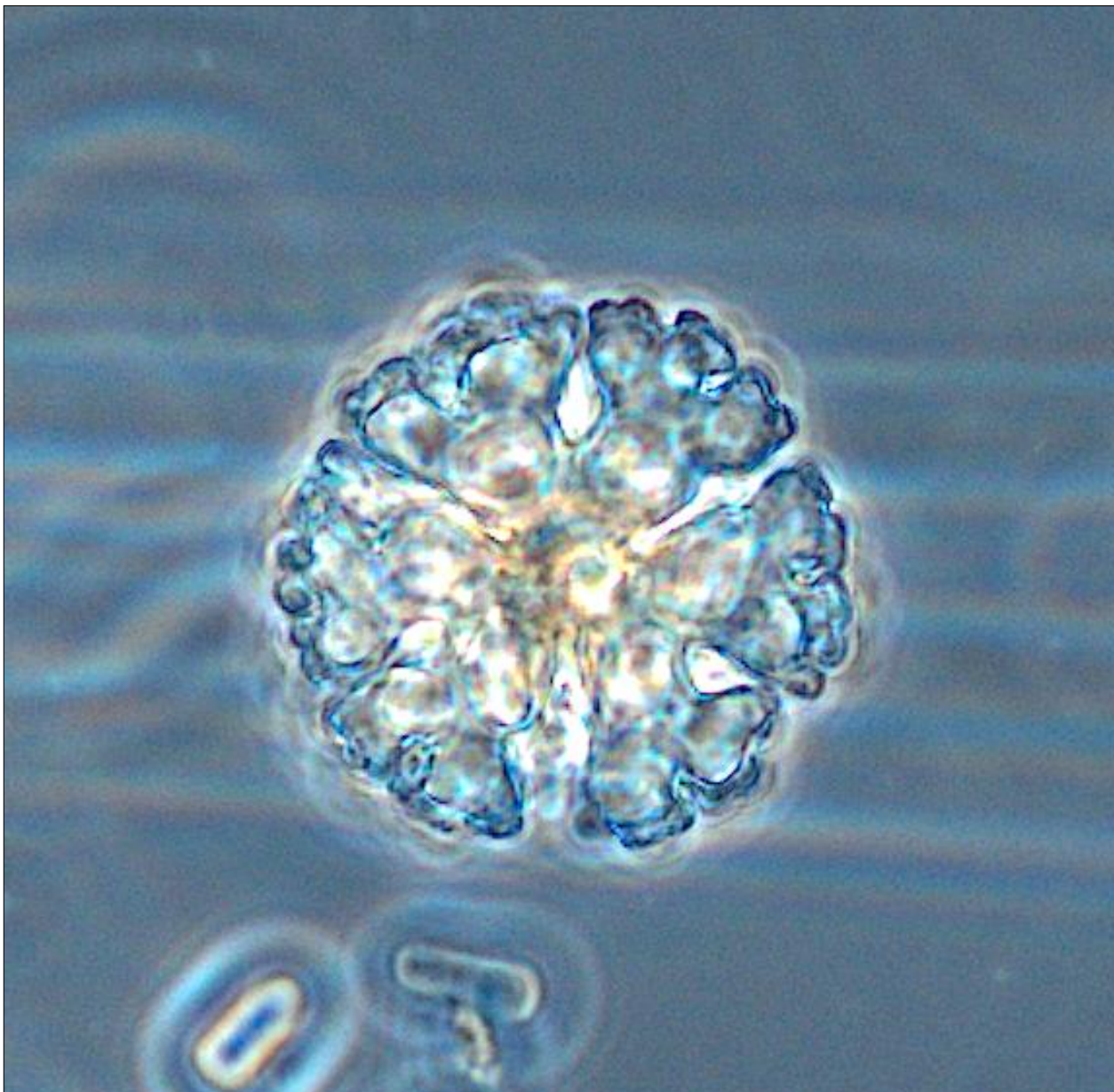


Spore heads forming a yellow liquid droplet at maturity. Seen in-situ on fresh incubating red deer dung.



A–D. Spore heads forming a yellow liquid droplet at maturity. Seen in-situ on fresh incubating red deer dung.





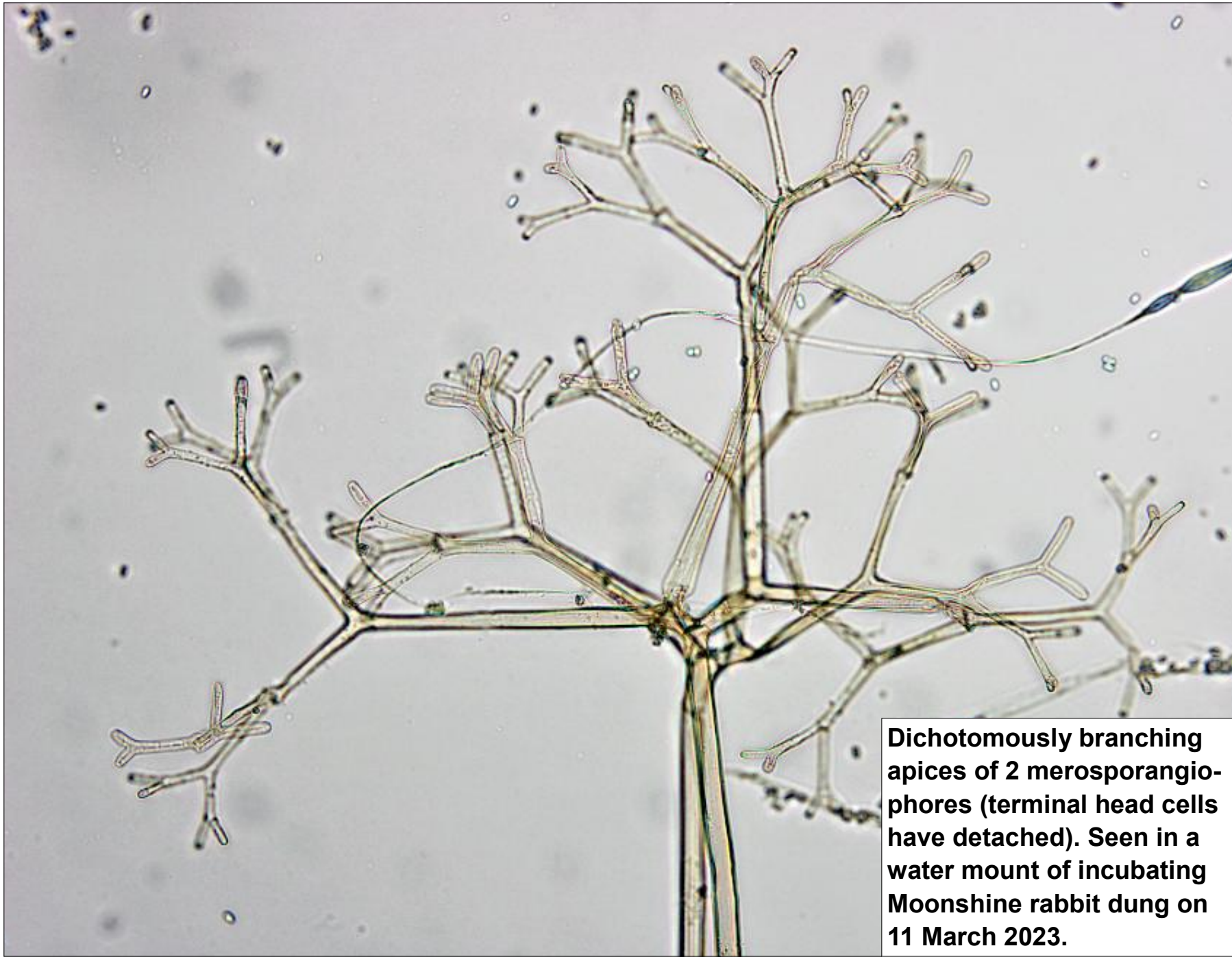
Incubating red deer dung photos taken 23 April 2024 from a SMF slide using the X100 objective on an Olympus BX51 microscope. Left photo: lobed head cell (32.5 μm diam.) with the many merosporangia detached. Right photo: merospores (mostly 5–10 \times 3–3.5 μm).



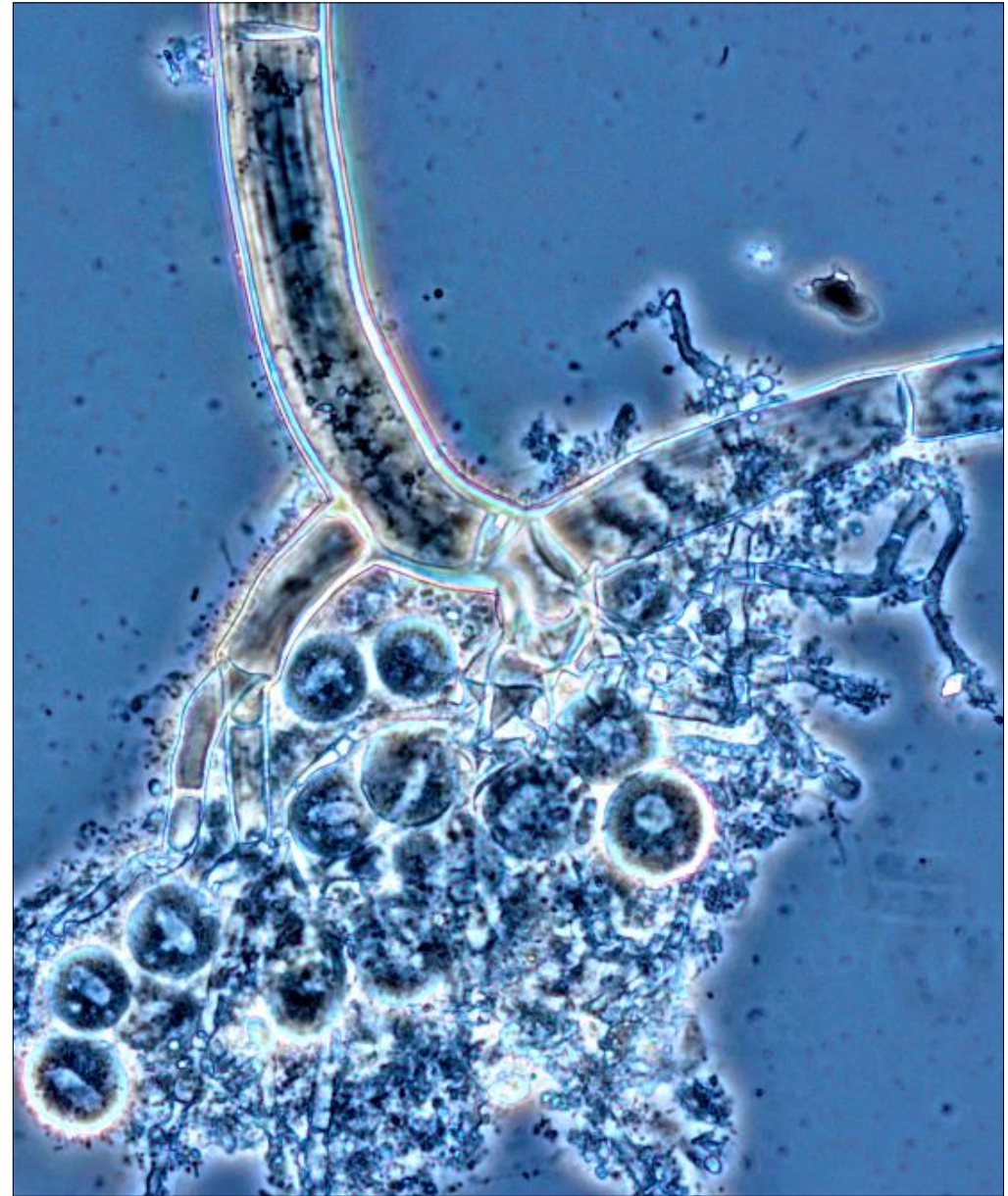
Spore heads forming a yellow liquid droplet at maturity. Seen in-situ on fresh incubating Moonshine Hill Road residential property rabbit dung on 11 March 2023.



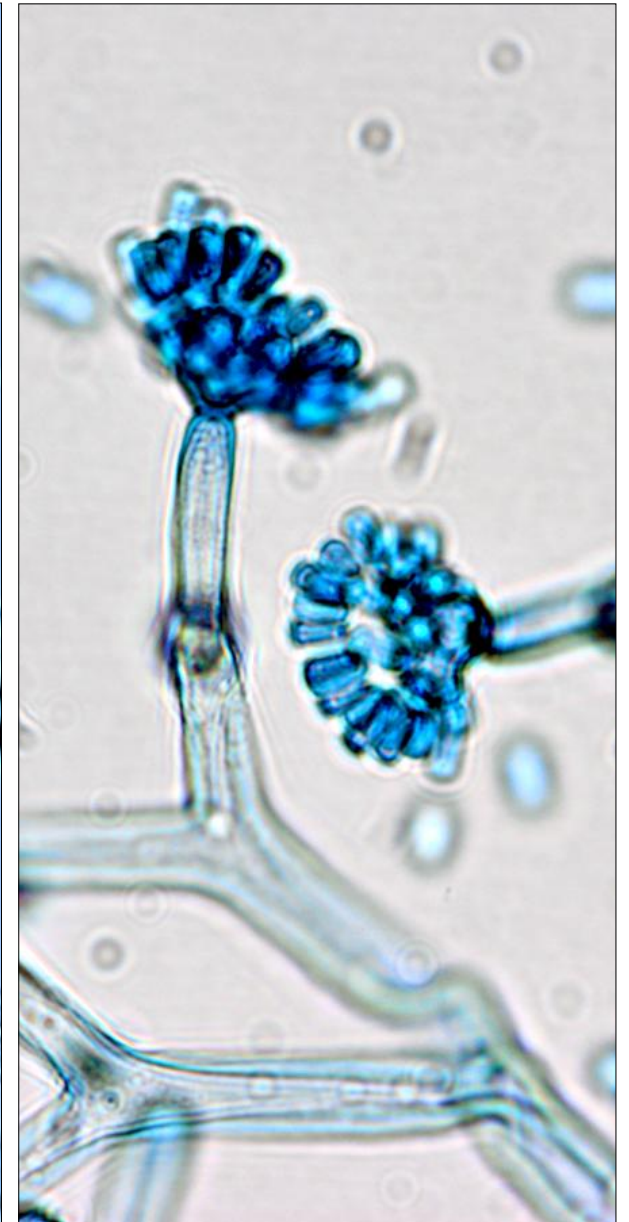
Spore heads forming a yellow liquid droplet at maturity. Seen in-situ on fresh incubating Moonshine Hill Road residential property rabbit dung on 11 March 2023.



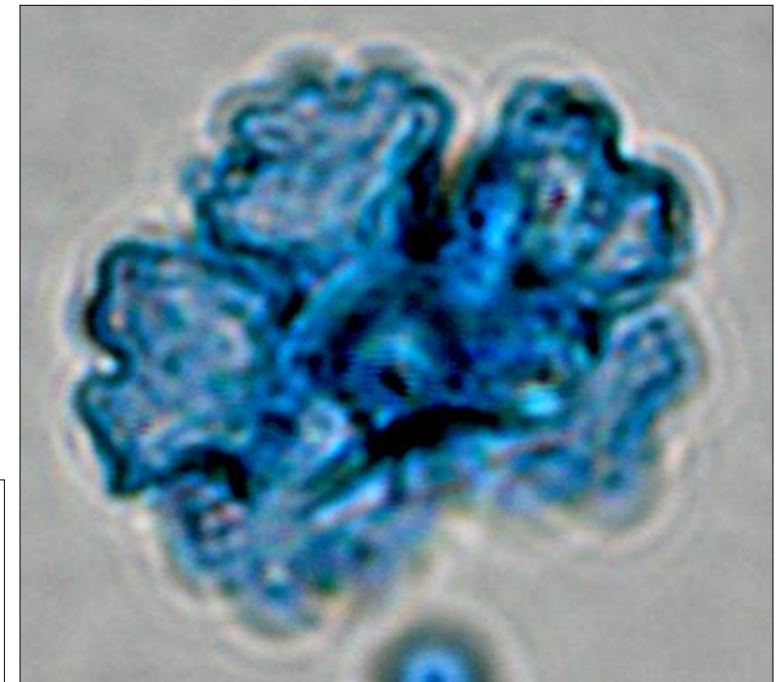
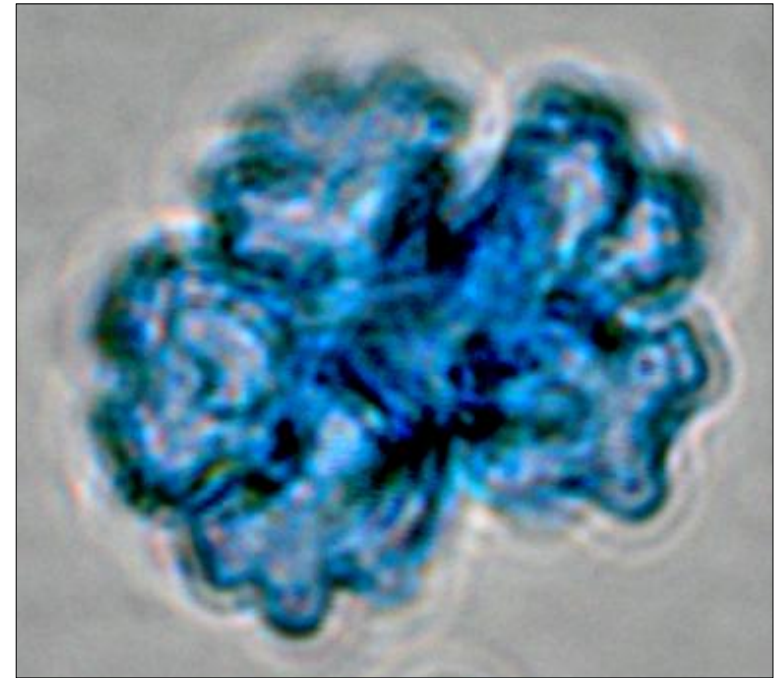
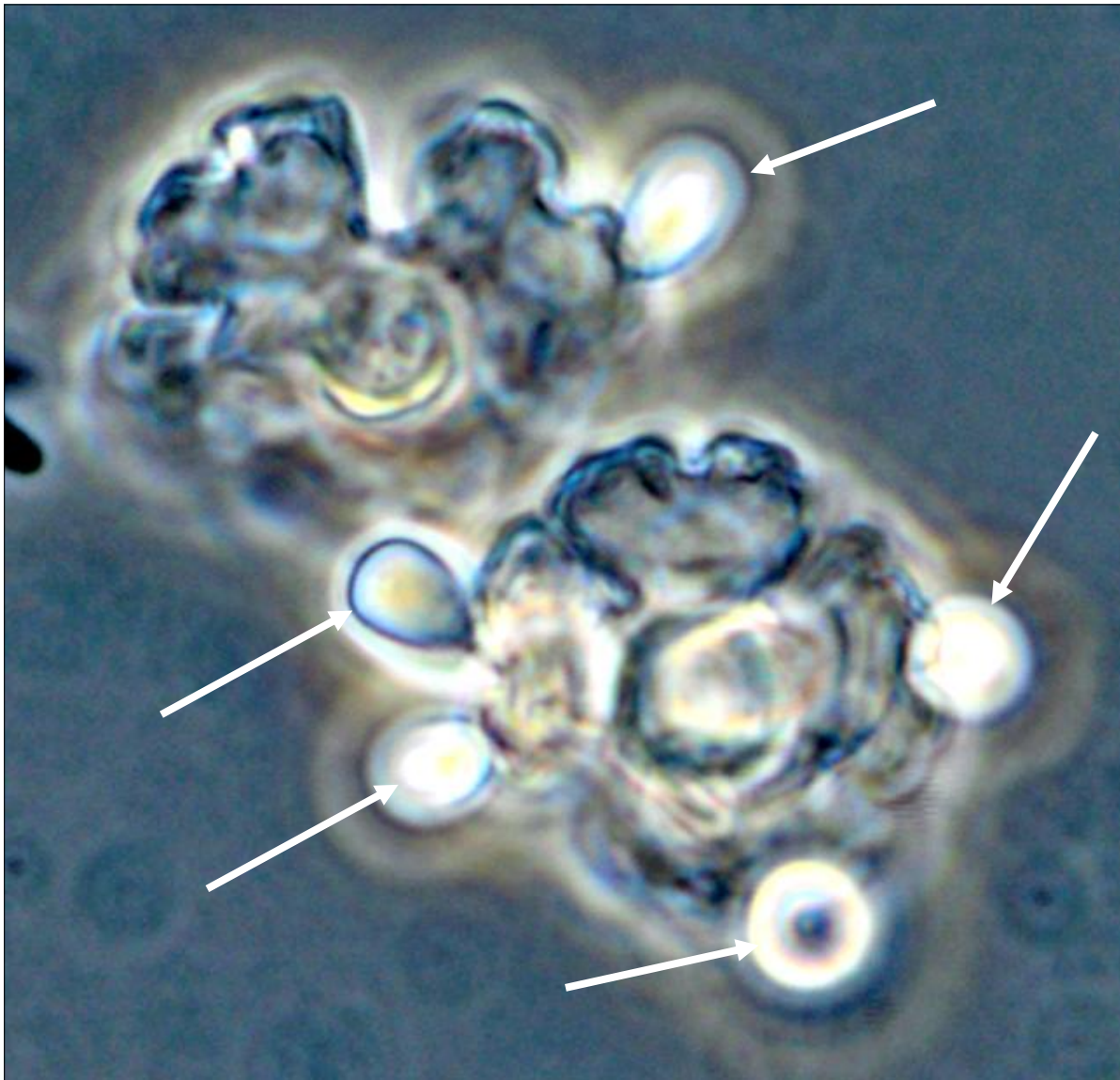
Dichotomously branching apices of 2 merosporangio-phores (terminal head cells have detached). Seen in a water mount of incubating Moonshine rabbit dung on 11 March 2023.



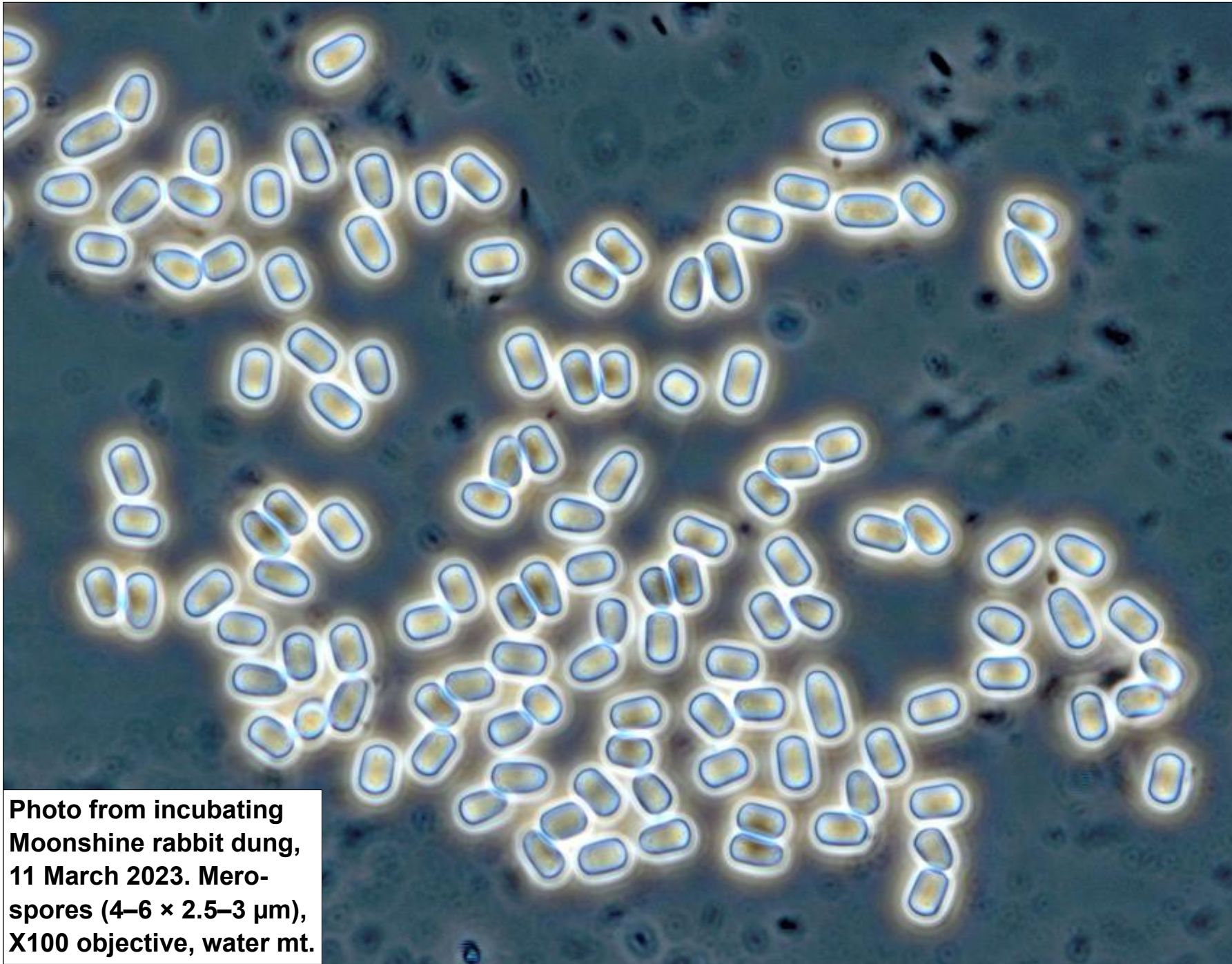
Both photos from incubating Moonshine rabbit dung on 11 March 2023. Left photo: rhizoidal, longitudinally striate base of a merosporangiophore in an aniline blue lactic acid slide mount. Right photo: two longitudinally striate merosporangiophore bases (one clearly septate) and a shared rhizoidal assemblage in a water slide mount.



Both photos in the same field of view (an aniline blue lactic acid slide mount) from incubating Moonshine rabbit dung on 11 March 2023. Left photo: X40 objective (the red-outlined portion is seen enlarged in the right photo). Note dichotomous branching with several 'young' head cells still attached & bearing young merosporangia.



Photos from incubating Moonshine rabbit dung on 11 March 2023. Left photo: X100 objective, water mount with 2 head cells & a few young merosporangia (arrowed). Right 2 photos: the same head cell but different focuses, X100 obj. in aniline blue lactic acid.



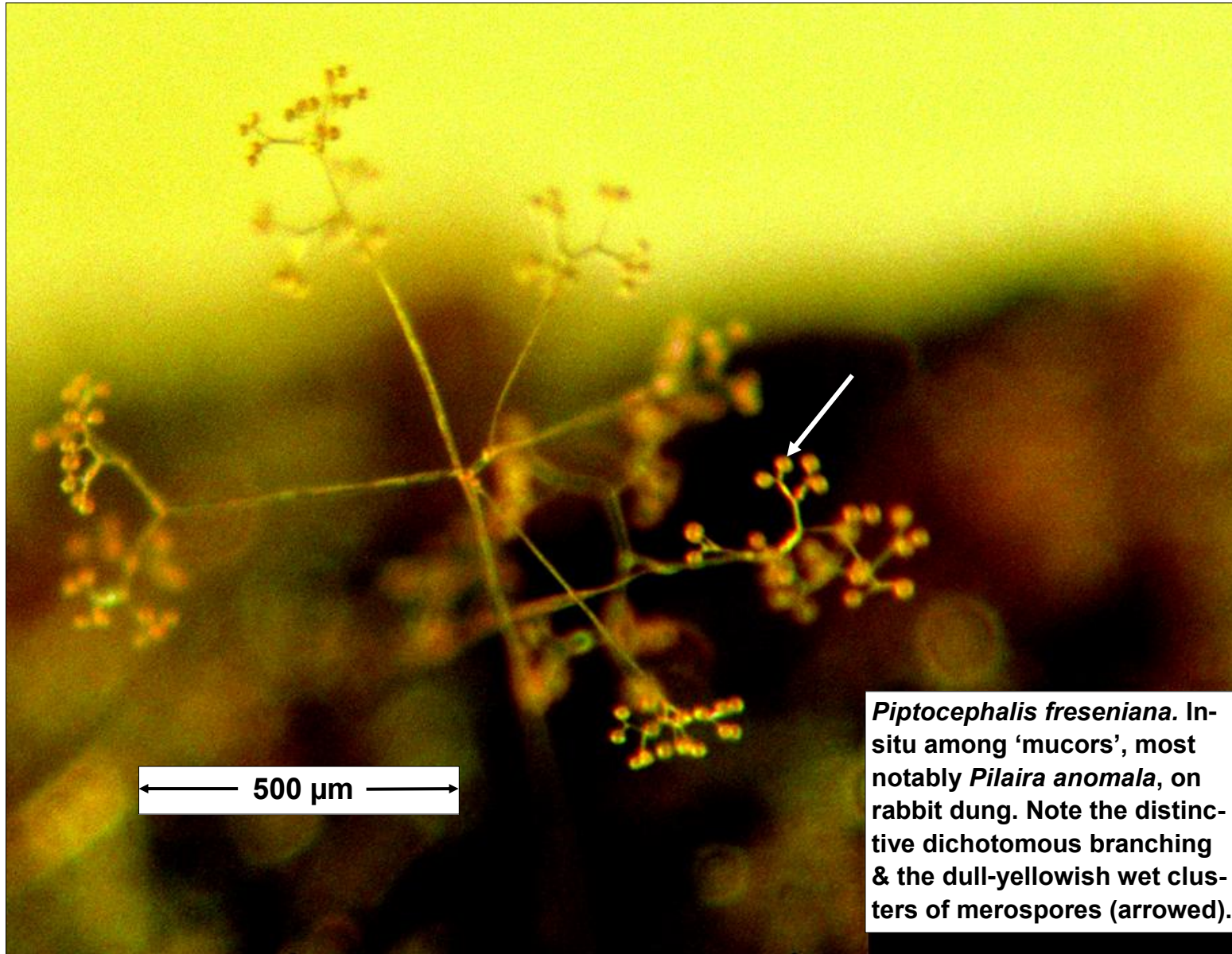
**Photo from incubating
Moonshine rabbit dung,
11 March 2023. Mero-
spores ($4-6 \times 2.5-3 \mu\text{m}$),
X100 objective, water mt.**

Additional information for the 2017 Callaghan rabbit collection, not presented on page 1 of this pdf.
Following pdf pages: In-situ photos on incubating dung & compound scope water mount details.

Collection site: Lower Hutt, New Zealand. Along the Waiwhetu Stream grassy areas that border Callaghan Innovation (base of the Wainuiomata Hill Road)

Other fungi also present on rabbit pellets from this collection include the following: *Ascobolus minutus*, *A. stictoideus*, *Collariella bostrychodes* (syn. *Chaetomium bostrychodes*), *Delitschia* (*patigonica?*), *Iodophanus carneus*, *Mucor* spp., *Pilaira anomala*, *Podospora curvicolla*, *Saccobolus caesariatus*, *Sporormiella australis*

Brief description: *Piptocephalis freseniana* merosporangiophores numerous among the many long sporangiophores of *Pilaira anomala*. It was unclear whether *P. freseniana* was parasitizing the *Pilaira* and/or one of the smaller less obvious *Mucor* spp. *P. freseniana* merosporangiophores were even observed arising from 'mucoraceous hyphae' on the glass edges of the incubation bowl. Merosporangiophore sizes varied considerably but all were characterized by their 3-dimensional, repeatedly dichotomous branching and their dull yellow-brown to light brown wet balls of merosporangia & merospores (surrounding a head cell) at the tips of the ultimate dichotomies. The main merosporangiophores & their increasingly smaller repeating dichotomies were also yellow-brown to light brown, with obvious septa & longitudinal striations (septae and striations most obvious and numerous on the wider more basal portions of the merosporangiophore). **Head cells** easily detached in water mounts where they were observed with merosporangia still attached, partially detached or completely detached. The latter were often seen as conspicuously lobed globular structures (17–18 µm in diameter) with the circular point of detachment at their center. Scars where the merosporangia had detached were obvious on the head-cell lobes. **Merosporangia** contained 3–5 uniseriately arranged merospores, the terminal spore apically rounded and those beneath it rectangular. **Merospores** small, nearly hyaline under transmitted light but dull yellow brown in mass, with bipolar inclusions in water mounts. Rectangular merospores 5–5.5 × 2.5 µm, apically rounded merospores slightly longer.



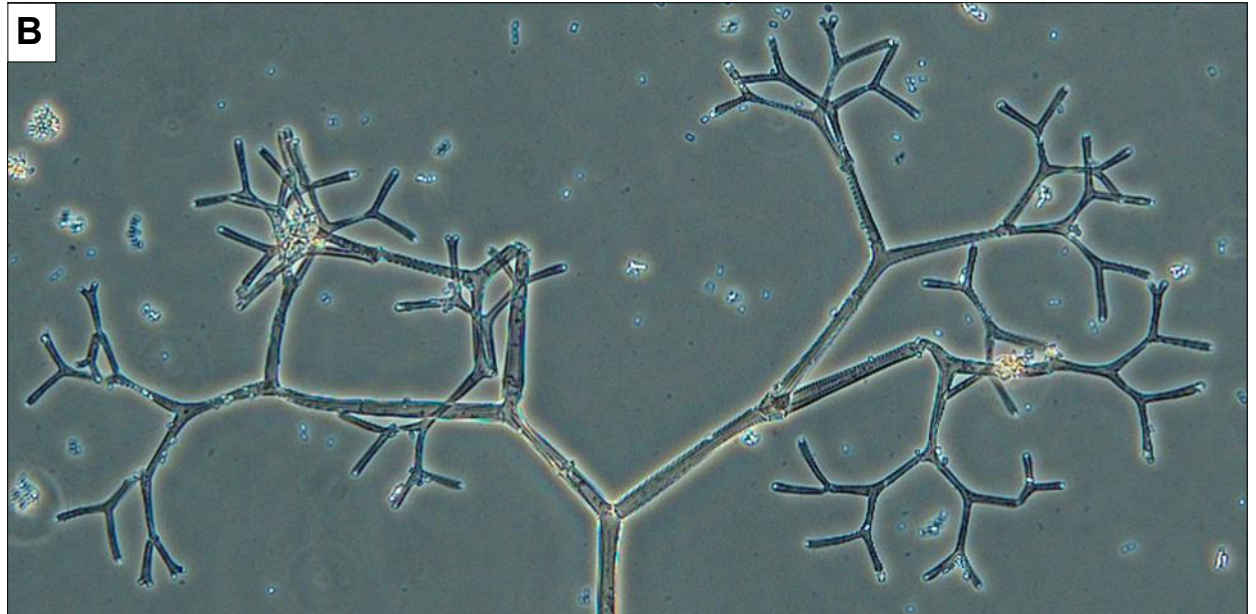
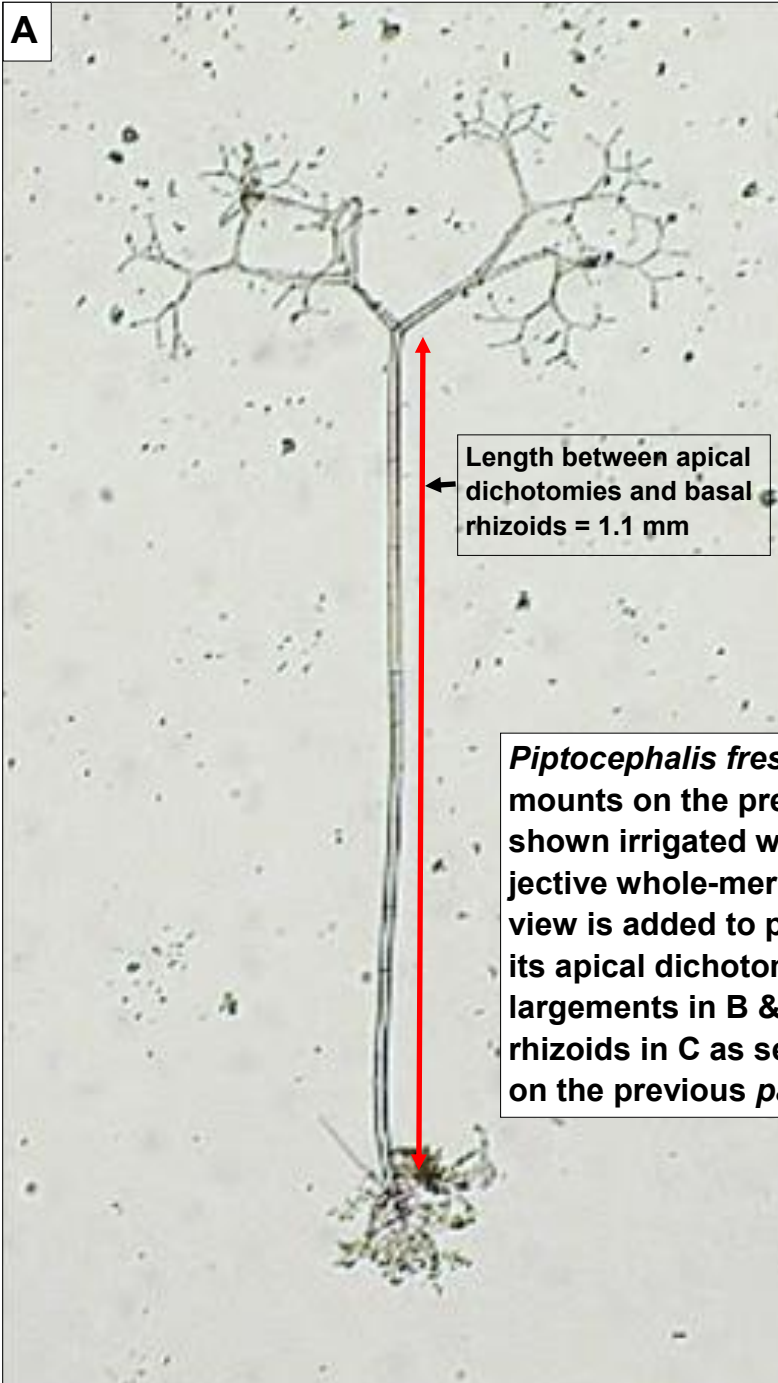
500 μm

Piptocephalis freseniana. In-situ among 'mucors', most notably *Pilaira anomala*, on rabbit dung. Note the distinctive dichotomous branching & the dull-yellowish wet clusters of merospores (arrowed).

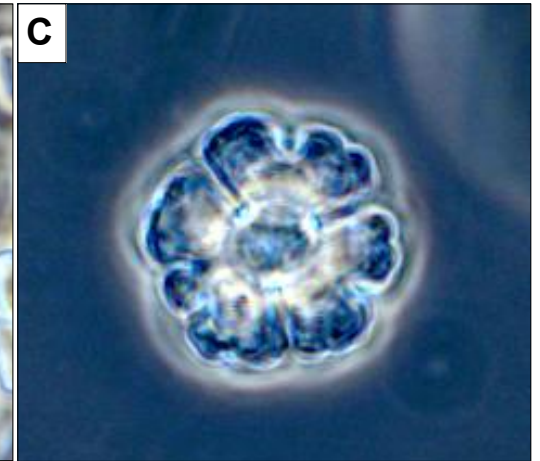
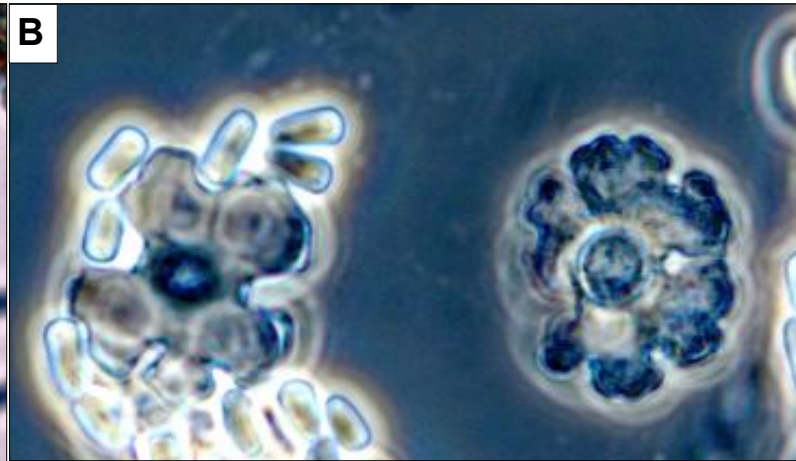
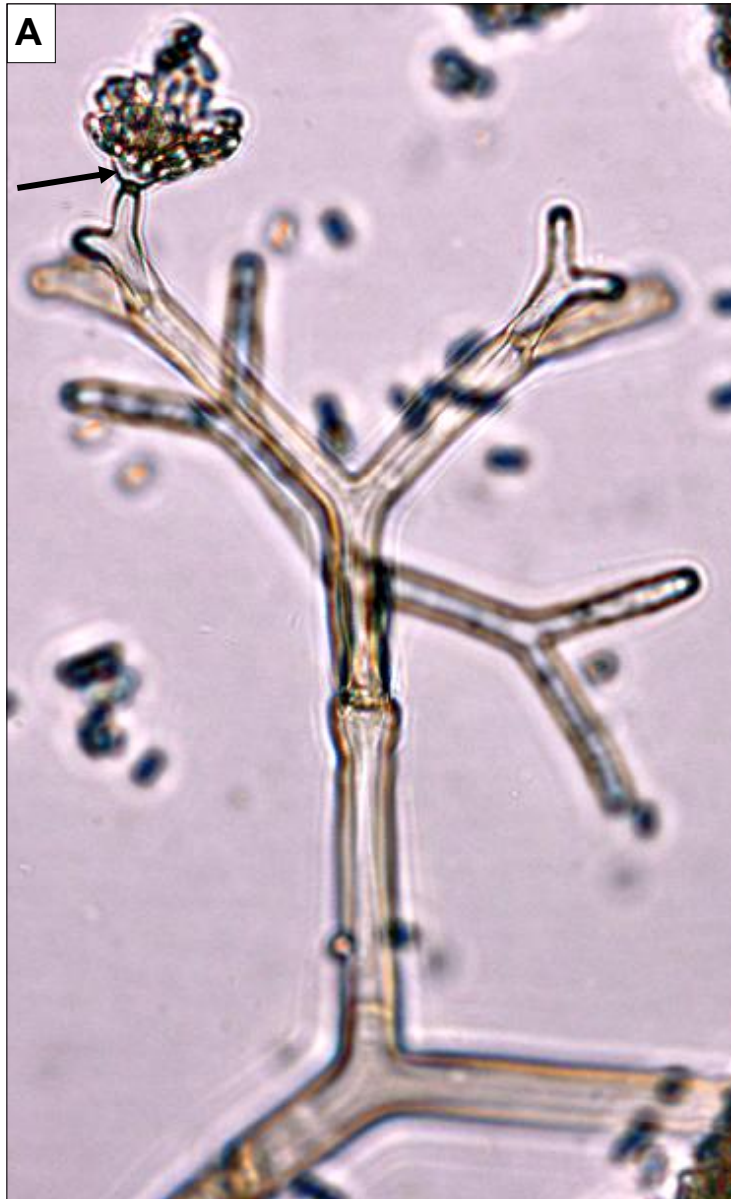




Piptocephalis freseniana. Left photo: Dichotomous branching at the merosporangiophore apex. Water, X10 obj., brightfield. Right photo: Basal rhizoidal attachment of a merosporangiophore. Water mt., X40 obj., brightfield.



Piptocephalis freseniana. A–C: Water mounts on the previous page are here shown irrigated with SMF. A. a X4 objective whole-merosporangiophore view is added to provide meaning to its apical dichotomies & near-basal enlargements in B & C. Note the basal rhizoids in C as seen in the right photo on the previous page.



Piptocephalis freseniana, emphasis attached and unattached head cells. All water mounts. A,D. X40 objective, brightfield. B,C. X100 objective, phase. A. Attached head cell (arrowed) with merosporangia. B–D. Detached lobed head cells. The head cells easily detach in slide mounts. B,C. Head cells 17–18 μm in diameter. B. Free merospores near left head cell. D. Merosporangia still attached to head cell. Note scar points on the head cell (arrowed), from which merosporangia have detached.



Merospores:
5–5.5 × 2.5 μm

Piptocephalis freseniana merospores. All photos water mounts & brightfield. Left photo: X40 objective with a few merosporangia still intact (arrowed), end merospore apically rounded, others rectangular. Right photos: X100 objective, resized for presentation here.