***Podospora bifida* N. Lundq. AEB 466 (= PDD 73636). Including information on AEB 515 (= PDD 73686)**

Collection site: near State Highway 1 at Kekerengu Beach and sand dunes on the Kaikoura Coast, Kaikoura District. The dung was collected in mixed vegetation near the ocean.

Collection date: 2 April 1988

Substrate: European rabbit (*Oryctolagus cuniculus*) dung

Dung collection number: 141

Collector & identifier: Dan Mahoney

Voucher materials: dried herbarium specimen AEB 466 (= PDD 73636) – one dung pellet with numerous perithecia; 2 semipermanent lactophenol slides and several ascospore photos using 35 mm Kodak TMAX 100 black & white film; Dan's composite description of this species based on 9 New Zealand collections, 2 of which are represented by dried herbarium material and the others by descriptions, illustrations and culture work (see below and the photos that follow in this pdf).

Nine New Zealand collections on rabbit and other herbivore dung, only two of which are represented by herbarium material (AEB 466 and AEB 515). Salient details of these are presented below in sequence by dung collection number and collection date:

Collection number **120**, goat dung collected 4 January 1988 at Kaiteriteri Health Farm near Abel Tasman National Park and minutes from Kaiteriteri Beach, Collectors & identifiers: Dan Mahoney & Ann Bell

Collection number **121**, European rabbit dung collected 4 January 1988 among sedges on the Westport dunes bordering the ocean, Collectors & identifiers: Dan Mahoney & Ann Bell

Collection number **141 (AEB 466, = PDD 73636)**, European rabbit dung collected 2 April 1988 in the Kaikoura District near the ocean – see AEB 466 details above

Collection number **188**, European rabbit dung collected March 1990 at Matata, Collectors & identifiers: Dan Mahoney & Ann Bell

Collection number **194,** horse dung collected 7 May 1990 in a very wet field west of Murchison near Lyell, Collectors & identifiers: Dan Mahoney & Ann Bell. Although not deposited as an herbarium specimen, it is present on the same horse dung deposited for the slime mold *Didymium anellus*, see PDD 110417 (= AEB SM40).

Collection number **200**, sheep/possum? dung collected 4 July 1990 among grasses and remnants of native shoreline plants along the coastal strand at Makara Beach, collectors Ann Bell & Winifred Long, identifiers Dan Mahoney & Ann Bell.

Continued on the next page

Collection number **251**, European rabbit dung collected 15 February 1994 along the Orongorongo Track in Remutaka Forest Park, Collectors & identifiers: Dan Mahoney & Ann Bell

Collection number **253 (AEB 515, = PDD 73686)**, European rabbit dung collected 14 January 1995 at Waikawa Beach, collectors Ann Bell, Dan Mahoney & Harold Keena, identifier Dan Mahoney. Dried herbarium specimen AEB 515 (one dung pellet with numerous perithecia). Unfortunately, most of the dung is covered with a white mycelial overgrowth – except for visible ascospore discharges above each perithecium.

Collection number **256**, horse dung collected 6 June 1995 near the Taupo Swamp (Ara Harakeke) a nationally significant flax wetland located between the seaside villages of Plimmerton and Pukerua Bay north of Wellington, Collector & identifier: Ann Bell

Dan's composite description of this species based on 9 New Zealand collections:

Perithecia: Upper portions emergent from the dung, overall broadly obpyriform with rounded apex and setose neck, venter lightly pigmented (asci clearly visible within) – peridium a small-celled textura angularis with light brown, branching, septate, flexuous hairs, neck dark with evenly spaced, non-agglutinated, moderately thick-walled (stiff), brown, unbranched, 1–2 septate setae that taper to a narrowly rounded tip (setae mostly 25–50 µm long – rarely to 85 µm, and approx. 2.5 µm wide near their base). Perithecia 450–750(–825) × 270–570 µm.

Paraphyses: Closely packed among the asci, as seen in water mounts. Consisting of simple longitudinal elements with swollen cells invaginated at their septa. Gradually disappearing as the asci mature.

Asci: Negative in Melzer's, broadly clavate but narrowing at the tip, apex specialization not observed, 128-spored (or somewhat fewer) with spores multiseriately arranged.

Ascospores: body cell brown, smooth with a thin gelatinous outer layer, broadly ellipsoid, apex often narrowing to lemoniform and bearing a central germ pore, base rounded to slightly bulging where the pedicel is attached, body cells mostly 18 –25 µm (rarely to 26–34 µm or more, depending on the final number of ascospores/ascus) × mostly 12–16 µm (rarely to 17-20 µm or more, depending on the number of ascospores/ascus). Pedicels 10–15(–20) × 4–6 µm, hyaline with a thin gelatinous sheath, fragile and often disappearing in slide mounts.

Continued on the next page

Gelatinous caudae fugacious: cauda at the tip of the pedicel a long single whiplash that is a continuation of the thin gelatinous sheath on the pedicel; the apical cauda single with a central canal extending above the germ pore, often splitting to form 2 caudae (i.e. 'bifida', rarely 'trifid' or 'tetrafid') – sometimes only partially splitting basally. This apical gelatinous body is a continuation of the thin gelatinous sheath on the spore body (somewhat broader and less whiplash than the basal cauda). Ascospore germination (following pretreatment with 3% H_2O_2 for 25 minutes and growth on Difco CMA) was without the germination vesicle often seen in coprophilous *Podospora* species (see *Podospora bullata* in Ascomycete.org, online).

A summary of the culture work done on the nine New Zealand collections of *Podospora bifida* is provided below:

No culture work was done for collections **120**, **121**, **188** & **256** and a limited unsuccessful attempt for collection **141**. Limited successful culture work was done for collections **194** & **200** and highly successful culture work for collections **251** & **253**. Highlights of the successful culturing is summarized below.

Culture work for collections **194** & **200** was done on 29 August 1990 and 4 September 1990, respectively. In both cases perithecial contents were spread over Difco CMA containing antibiotics (100 units/ml penicillin G and 30 µg/ml streptomycin sulfate), furfural and biotin. Less than 10 ascospores germinated in **194** and none in **200**. Germinating ascospore cultures were subsequently transferred to Difco CMA + biotin universal slants and in two cases to a CMA slant to which an autoclave-sterilized (15 min at 121 C) whole wild rabbit (*Oryctolagus cuniculus*) dung dropping was added. The only perithecia in the cultures resided on the rabbit dung pellet.

Highly successful ascospore germination occurred in both collections **251** & **253** through the use of a perithecial pretreatment with 3% H_2O_2 for 25 minutes. In both collections a mature perithecium, or several perithecia, were placed in a droplet of 3% H_2O_2 on a sterilized microscope slide (in some cases on a sterilized 'cavity' slide) and after 25 minutes aseptically spread onto CMA Petri plates containing antibiotics or no antibiotics. Within 18–24 hours, over 90% (representing hundreds of ascospores) had germinated. Single ascospore transfers to various media ultimately resulted in 2 highly fertile axenic cultures in dung collection **251 (251A*** & **251C**, both in CMA universal slants – no rabbit pellet added)**. Neither CMA slants **251B** (with a rabbit pellet) nor **251D** (without a rabbit pellet) produced mature perithecia on the agar surface (perithecia were noted on the dung from the **251B** slant).

Continued on the next page

Cultures resulting from similar work with dung collection **253** support the success of germination work with **251.** In this case, however, only culture **253A*,** a CMA universal slant with one rabbit pellet, supported good fruiting and ascospore discharge. **253B** (CMA without a rabbit pellet) and **253C** (with a pellet) yielded no perithecia on the agar surface and unverified perithecia on the dung pellet). Worth noting are the recorded results (in 1995) from **253A*** growing on a CMA Petri plate to which had been added 3 sterilized rabbit dung pellets. When checked after 25 days growth there were no perithecia BUT when checked before discarding after 84 days, perithecia were numerous both on the dung and agar surface with good ascospore discharge from the pellet perithecia onto the dish lid. For whatever reason, late perithecial maturation appeared to be a feature of this culture.

The results of culture work with collections **251** & **253** indicate **homothallism**. Also, during the culture work on *P. bifida* **no anamorph** was observed on any of the culture plates.

***Podospora bifida* Lundqvist. n. sp. description and illustrations. From the publication 'Lundqvist, N. 1972. Nordic Sordariaceae s. lat. Symb. Bot. Upsal. 20: 1–374.'**

Perithecia scattered, ±immersed, non-stromatic, obpyriform, 530–720 × 335–480 μ, ostiolate, with a tapering to cylindrical neck, 95–145 × 95–115 μ, sparingly covered below with flexuous, hyaline to olivaceous brown, septate, usually simple, 2.5–3 μ

Page 182 above & page 184 below (see illustrations, p. 183, on the next page of this pdf)

thick hairs, and abundantly set on the neck with rigid, 1–2-septate, light brown, cylindrical, obtuse, non-agglutinated hairs, 25–120 × 3–3.5 μ. *Peridium* pseudo-parenchymatous, membranaceous, semi-transparent, yellowish to light brown with an olivaceous tint, except in the black, opaque neck, 3-layered; outer layer composed of angular, irregularly shaped, thin-walled cells, 5–10 μ in diam., middle layer of large, tangentially flattened, hyaline cells. *Paraphyses* short and stout, simple, soon collapsing, composed of a few large, elongated, vesicular cells. *Asci* rather few, c. 100–128-spored, 265–310 × 60–90(–110 μ, broadly clavate, with a moderately long stipe and a tapering apex, unitunicate, non-amyloid, easily bursting, costate after dehiscence; subapical chamber c. 2 μ broad; no apical ring or light-refractive membranes. *Spores* multiseriate, forming together a fusiform body, at first one-celled, hyaline, cylindrical, then somewhat dumb-bell-shaped, then swelling above, finally becoming transversely uniseptate; upper cell ranging from olivaceous, light brown to dark brown, smooth, 18–24 × 13–15 μ, broadly fusiform, equilateral, with an apical germ pore and sometimes with a small septal pore too; pedicel 11–18 × 6–7 μ, cylindrical to slightly obclavate, hyaline, devoid of plasma, at last collapsing. Whole spore, including the pedicel, surrounded by a 1.5–3 μ thick *gelatinous layer* that is apically and basally drawn out into appendages; upper cauda 30–50 × 3–5 μ, composed of two subapically attached filaments that are free from each other (bifid) below, but mostly fusing distally into a cylindrical or tapering part; basal cauda cylindrical, 30–50 × 3 μ, not divided; all cauda solid, round in cross-section, without visible microstructure, persistent, not or little swelling in water; all gelatinous equipment blackening in Indian ink. — Fimicolous.

Holotype on goat dung from Ribeira do Taborada, W of Casa das Queimadas, Madeira, 28.I.1969, Tibell 3628-c (UPS); isotypes in IMI and TRTC.

PARATYPES: **Sweden:** *Sk*, Jonstorp, Svanshall (r) 1963, J 1664-e (NY slide, UC slide, UPS). **Madeira:** same data as above, T 3629-e (UPS). — Lombo dos Pecegueiros, NW of Caldeirão Verde (gt) 1968, T 3665-j (E slide, S slide, UPS).

Most typical for this species are the rigid hairs on the perithecium, the multispored asci, and the bifid base of the upper cauda of the spore. The gelatinous equipment, especially the sheath, is rather difficult to see without treatment in Indian ink. Its configuration is very similar to that of *P. granulostriata*, which, however, is a larger species in all respects. I have been very uncertain whether *P. platensis* might be identical with *P. bifida*. Even after a study of the poor authentic remains of the former (p. 144), its morphology is still in some essential respects rather difficult to clear up. But it seems that the following differences are sufficient to distinguish the two species: *P. platensis* has agglutinated hairs, a thinner pedicel, and possibly another type of gelatinous equipment.

Podospora bifida Lundq. n. sp. (Fig. 34, a–l)

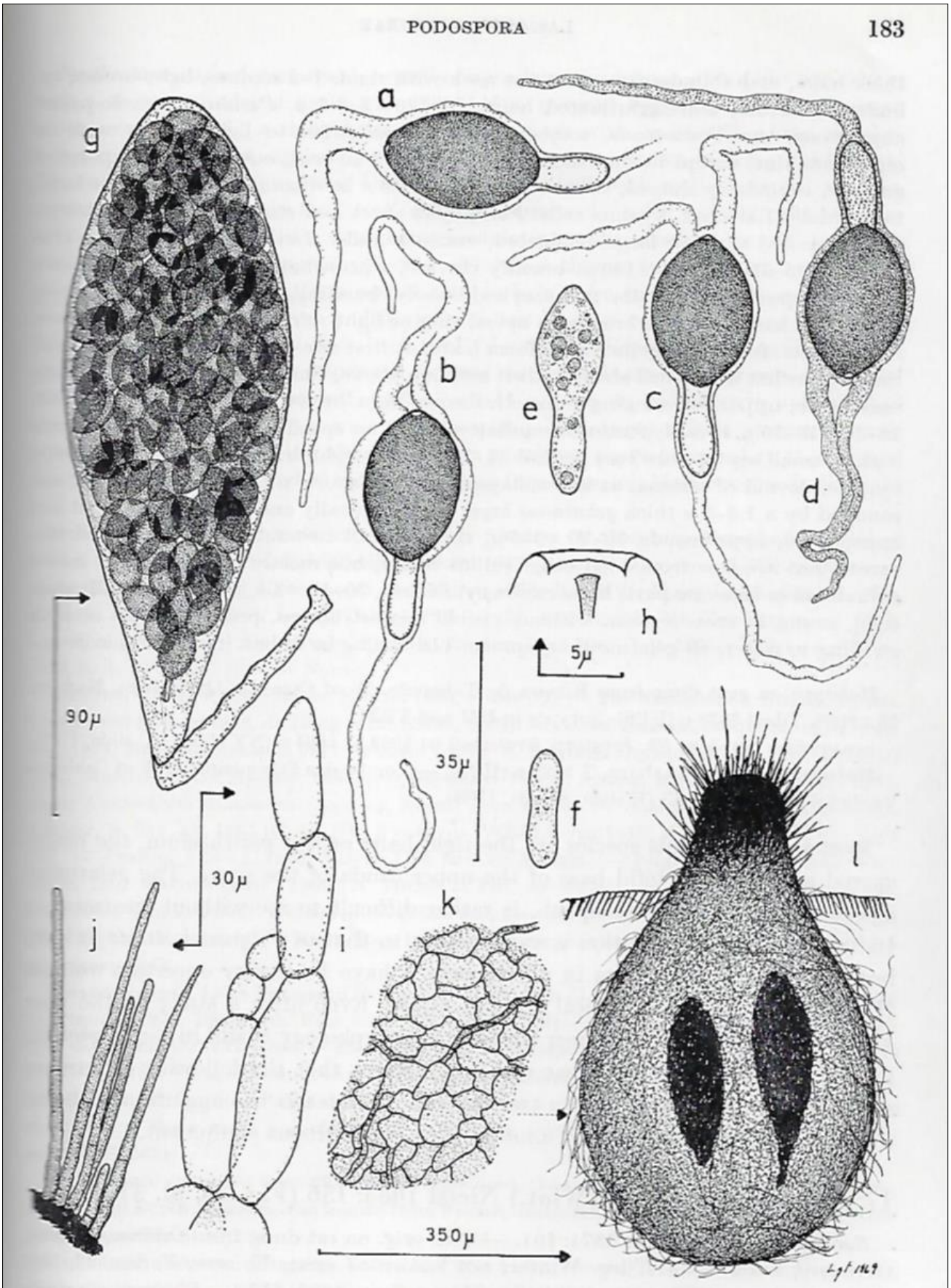
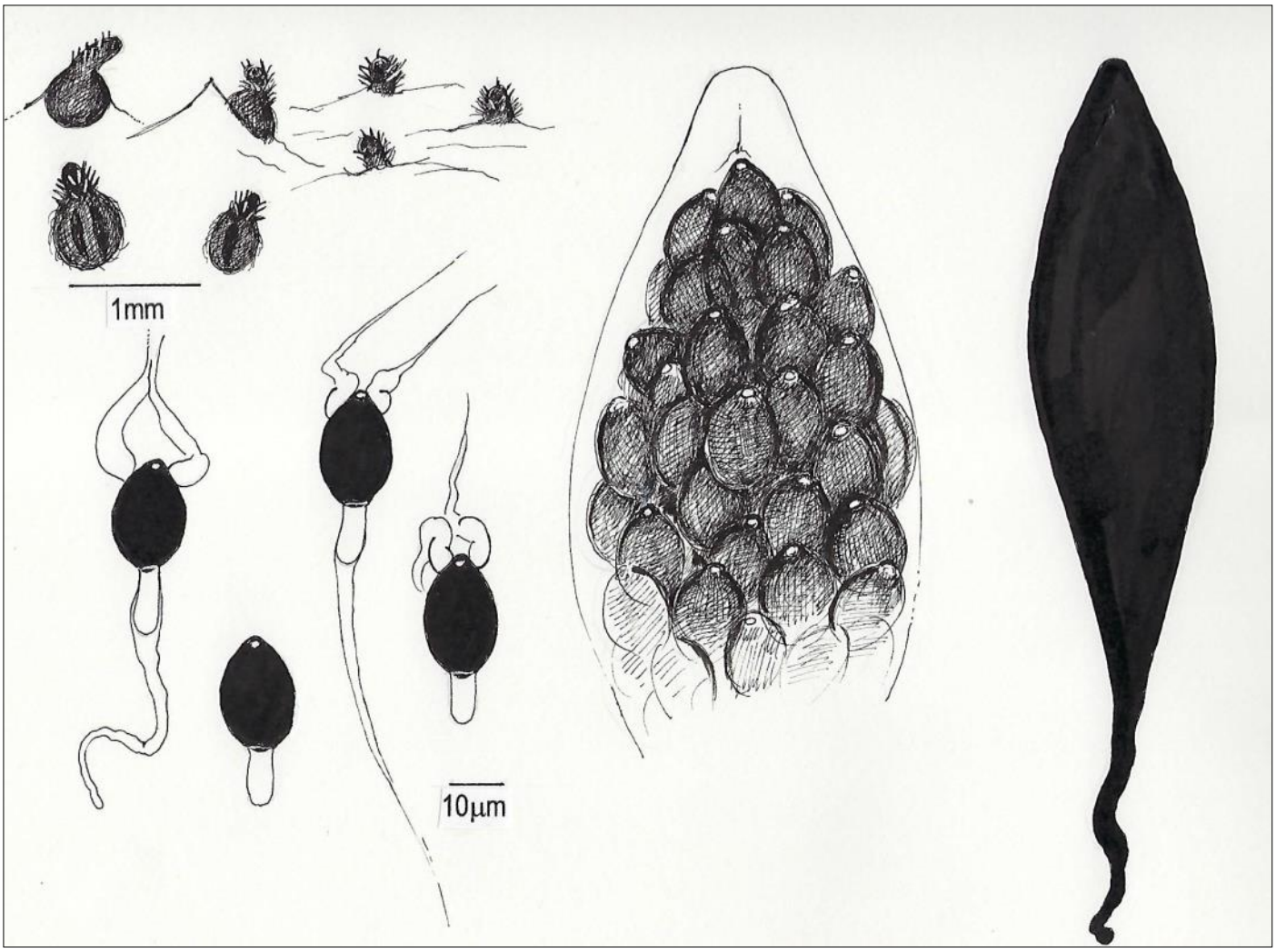
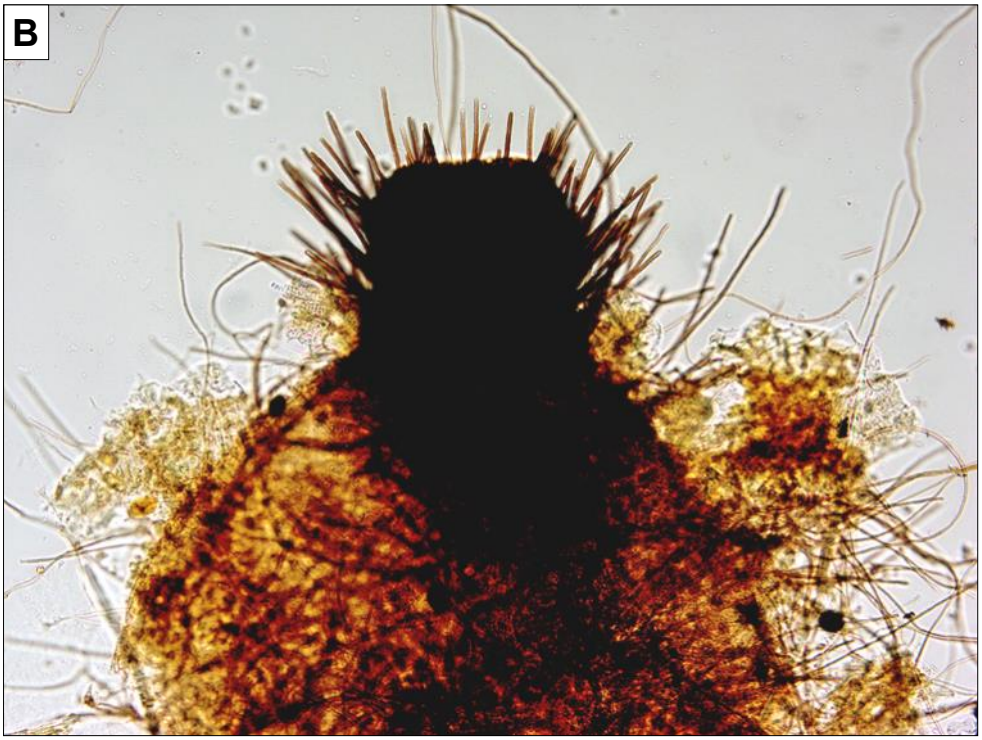


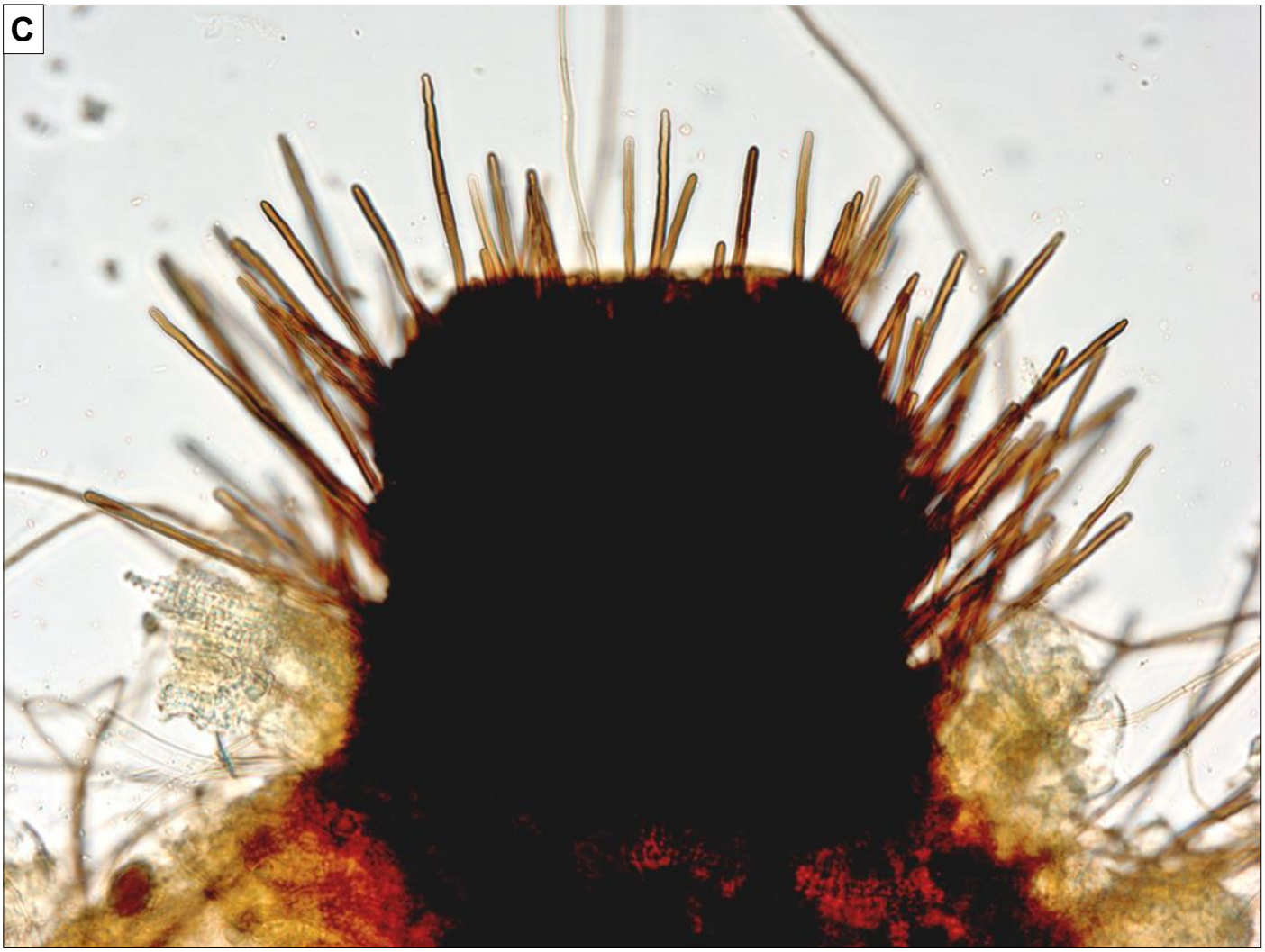
Fig. 34. *Podospora bifida*. Holotype (UPS). Drawn from living specimens. *a–d:* Mature spores; heavily stippled caudae in *d* show their appearance in Indian ink. *e, f:* Immature, hyaline spores at different stages of development. *g:* Ascus with mature spores. *h:* Invaginated ascus tip. *i:* Paraphyses. *j:* Hairs from perithecial neck. *k:* Peridium in horizontal view. *l:* Perithecium



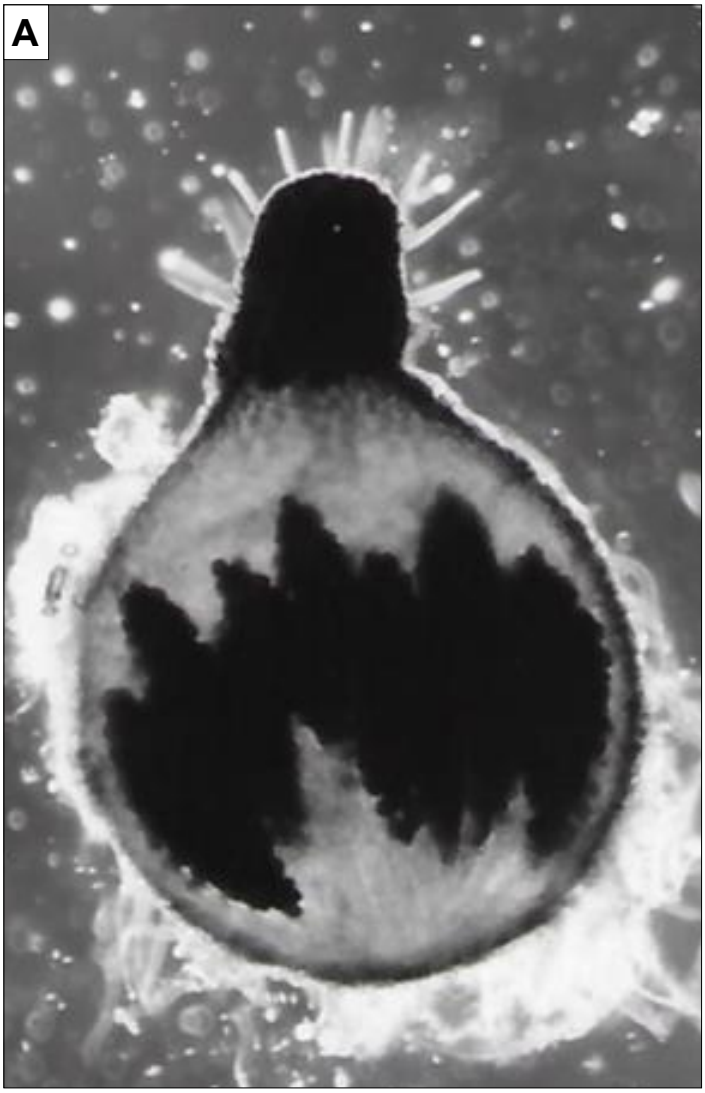
Bell, A. 2005. *An illustrated guide to the coprophilous Ascomycetes of Australia.* CBS Biodiversity Series No. 3, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, 172 p. – ISBN 90-70351, ISSN 1571-8859. Illustrations above of *Podospora bifida* are from page 121. Figures counter clockwise from the upper left are perithecia, mature ascospores, mature ascus and silhouette of mature ascus.

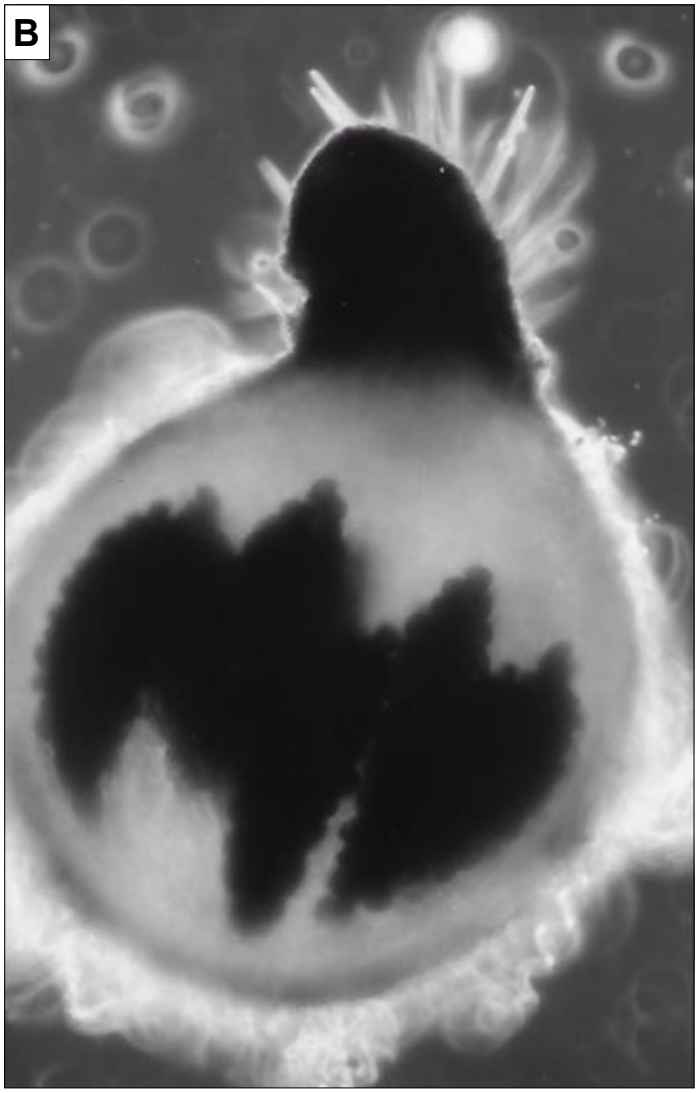
Photo above is the field from which the other photos were taken. Here young & mature multi-spored asci are visible.

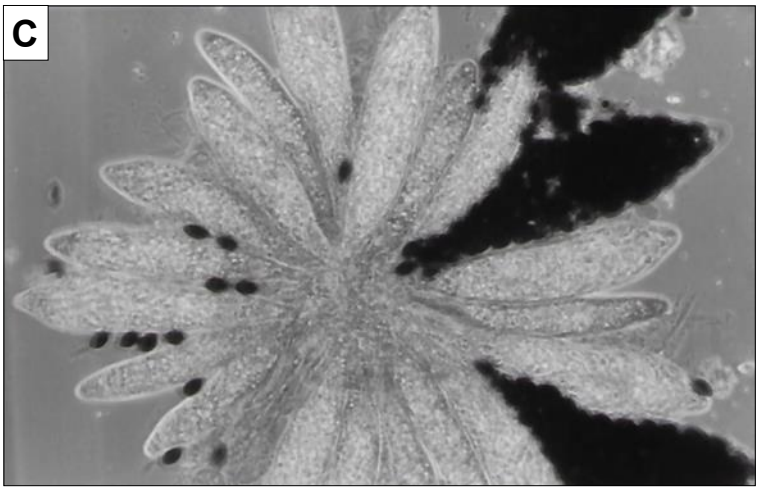




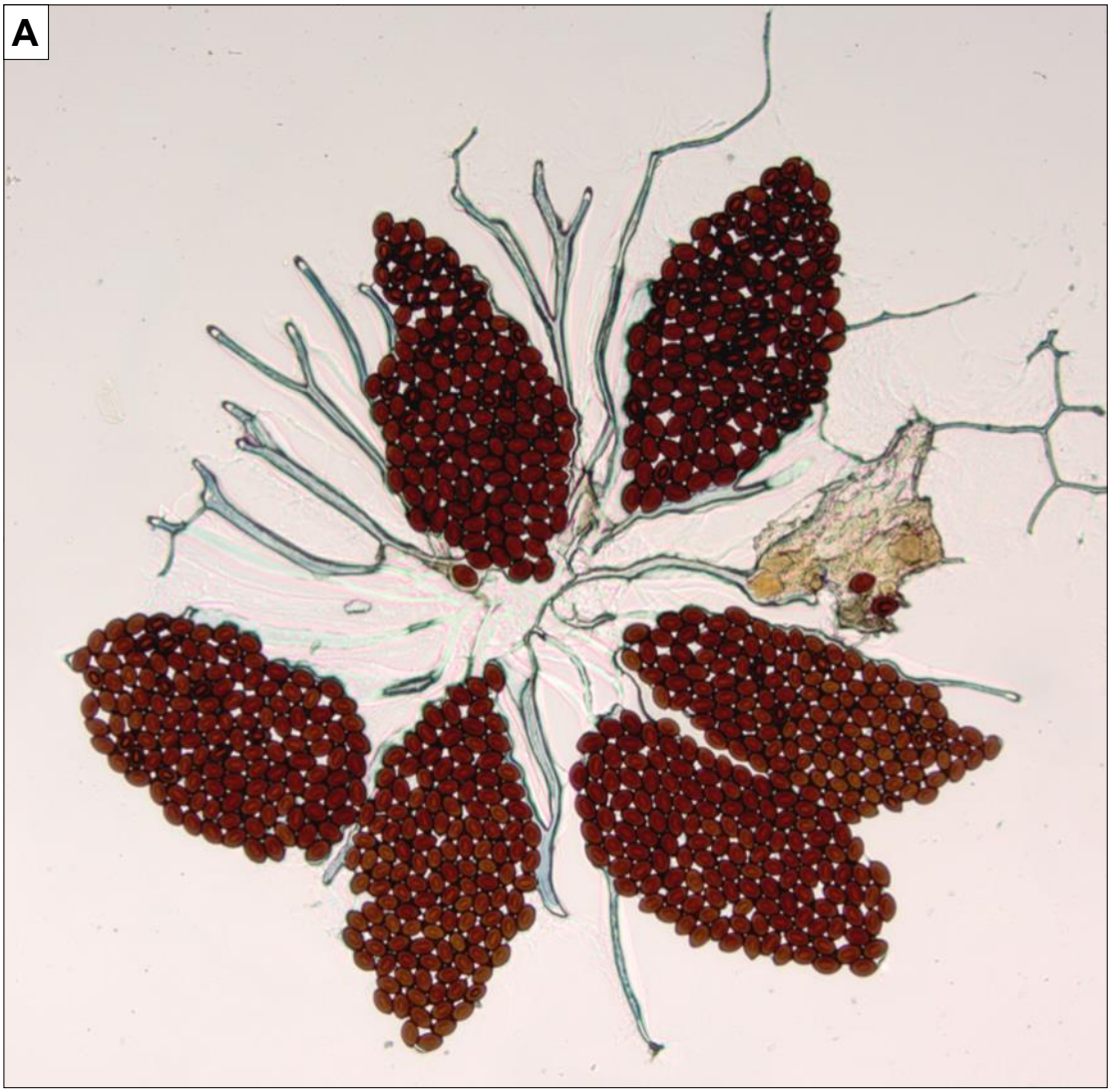
Podospora bifida **photos from dung collection A14 - one of 10 Australian dung collections from which the composite drawings in the publication 'Bell, A. 2005.** ***An illustrated guide to the coprophilous Ascomycetes of Australia.'*** **were drawn. A–C represent photos of the same perithecium under 10X, 20X & 40X objectives. A. Shows multispored asci & free ascospores squashed from the perithecium. B. Emphasizes both long flexuous hairs on the venter & shorter, stiff setae on the neck. C. Emphasizes the sparingly septate neck setae.**



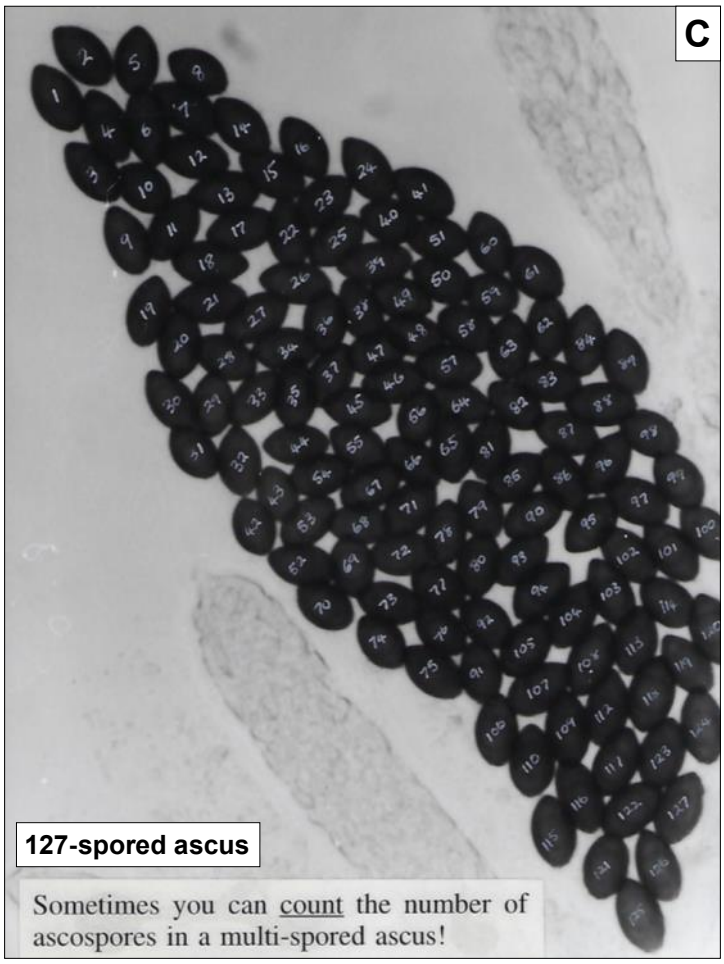




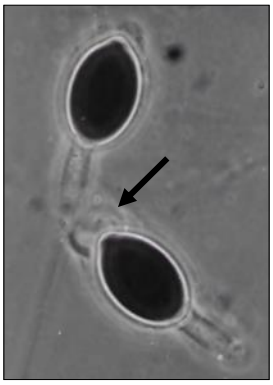
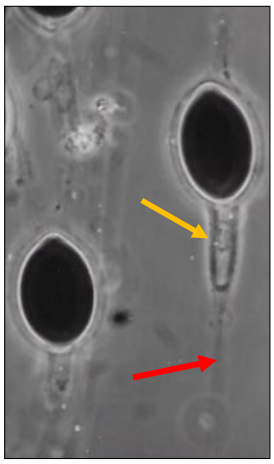
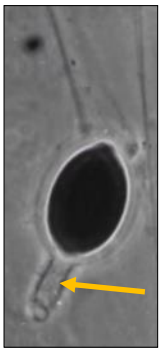
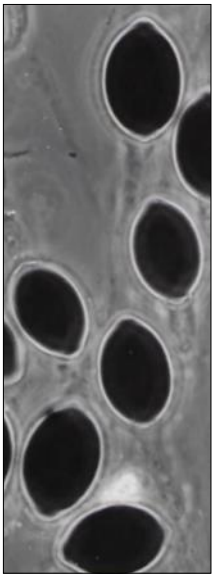
A–D. *Podospora bifida*. A–C. Photographed using Kodak T-MAX 100 (B&W film). A,B. Perithecia from NZ dung collection 121. A. X28 on the film plane (10X objective). B. X56 on the film plane (X20 obj.). Perithecium 490 × 360 µm. C. From NZ dung collection 120. Multispored asci in various stages of development. X56 on the film plane (X20 obj.). D. From NZ dung collection 251. A mature multispored ascus mounted in H_20 then irrigated with aniline blue lactic acid & heated, photos brightfield. Photographed under a 40X obj. using 35 mm slide color and that slide scanned with a Nikon 9000 film scanner.



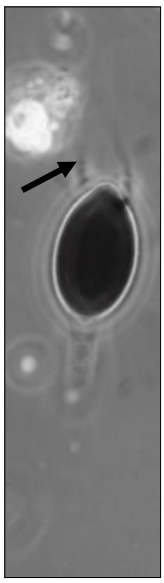
A–C. *Podospora bifida* asci in a monoplane, enabling a count of the ascospores/ascus. A. NZ Dung Collection 251, X10 objective, brightfield, water then lactophenol cotton blue & heated. Digital transfer from an Olympus BX51, DP25 camera. Natural color. B,C. Photographs from NZ Dung Collection 200 in heated lactophenol using Kodak T-MAX 100 (B&W film). Ascospore prints then labelled to allow counting, rephotographed and scanned.



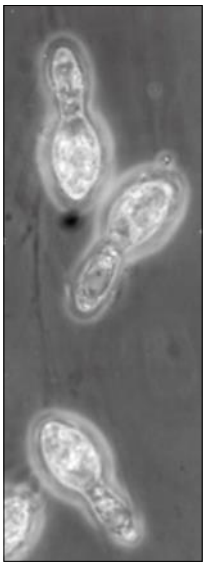
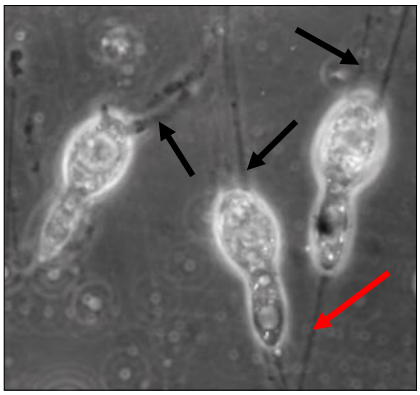
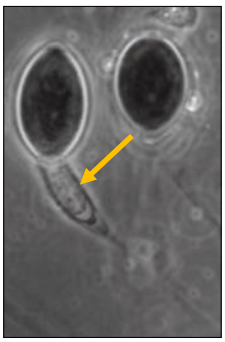
Row 1, left 4 photos - film roll 34, NZ dung collection 141

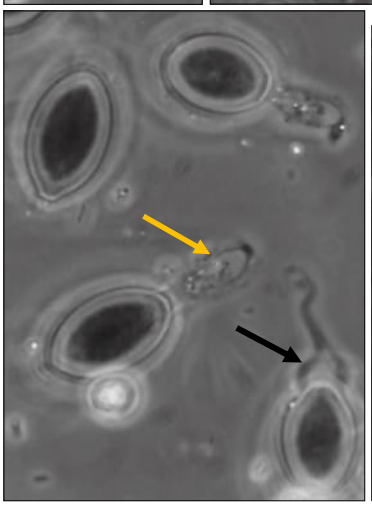
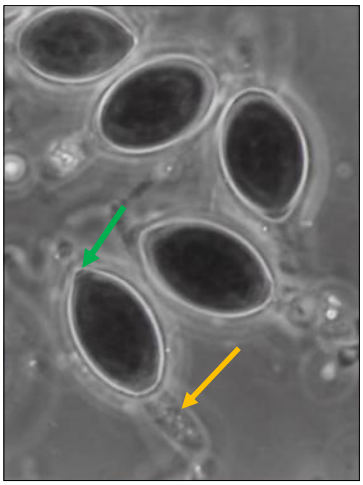
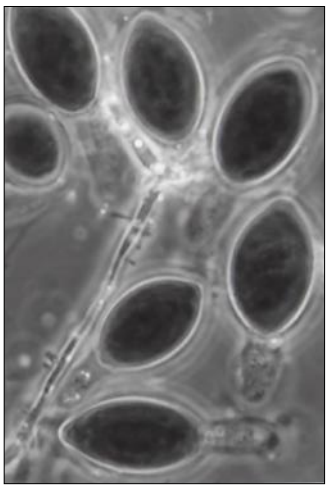





Row 1, far right photo - film roll 30, NZ dung collec-tion 120



Row 2, all 4 photos - film roll 28, NZ dung collection 120. Left 2 are young (bowling-pin shaped) with an early septum between the yet unpigmented body cell and the hyaline pedicel.

All photographed using Kodak T-MAX 100 (B&W film). All shown at the same magnification under the 100X objective. Mostly from heated lactophenol slide mounts.

Ascospore Arrows:

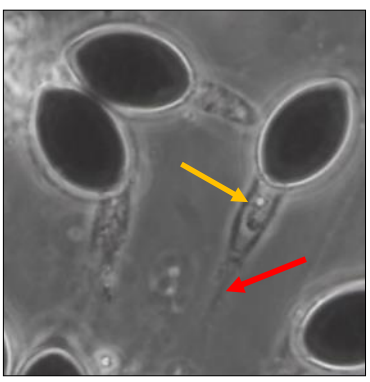
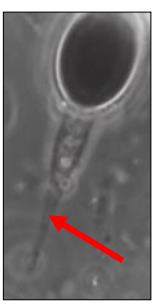
Black– pointed at one of the two 'bifid' apical gelatinous caudae

Red – pointed at the single basal gelatinous cauda

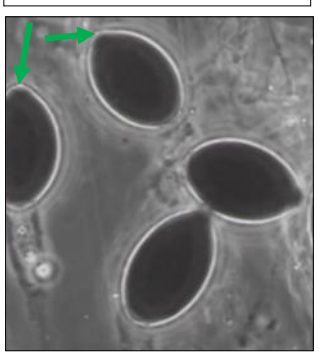
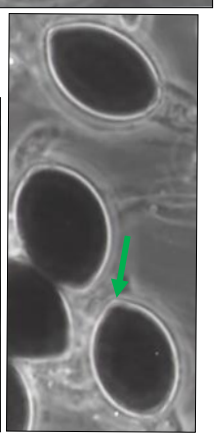
Yellow – pointed at the pedicel

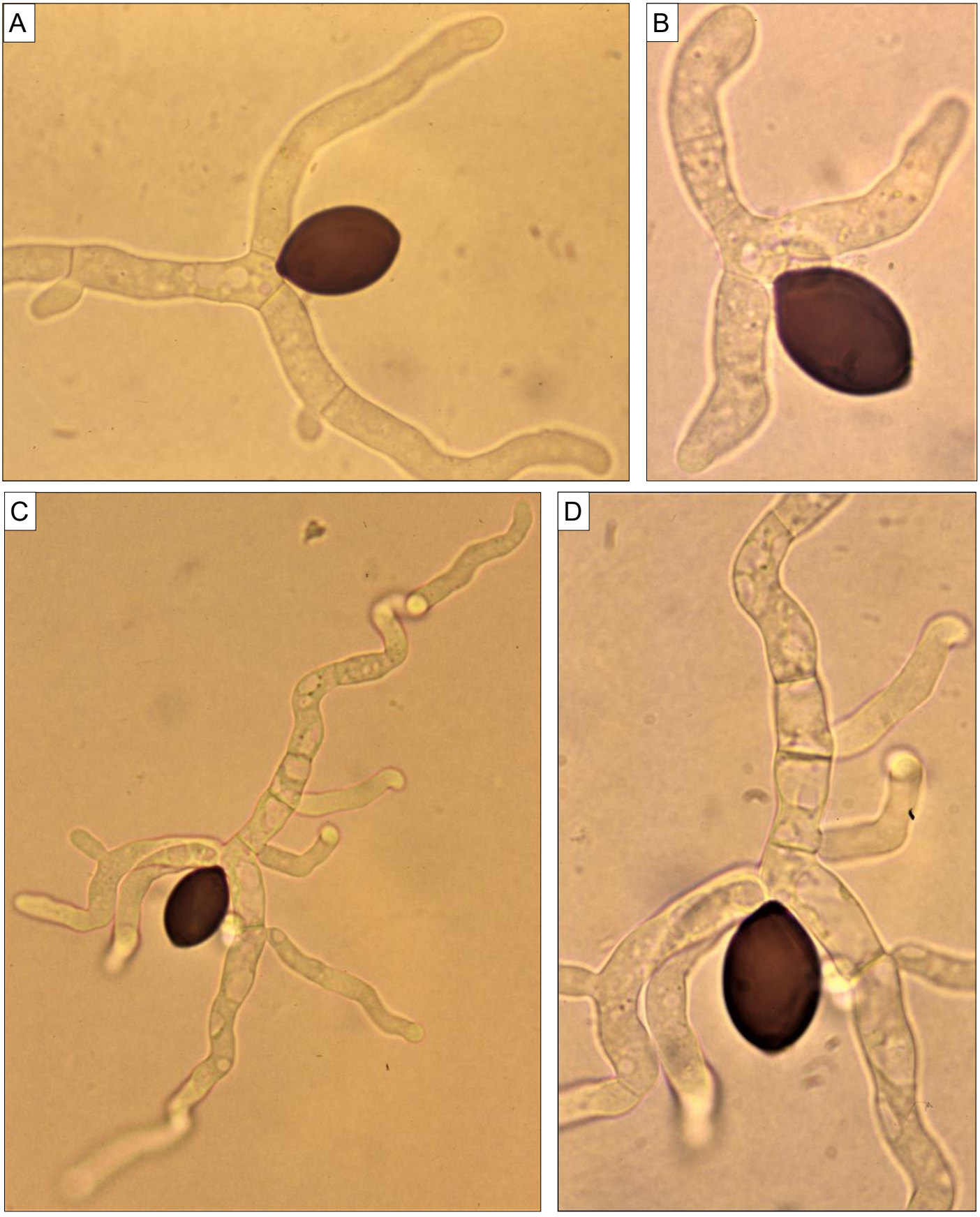
Green – pointed at the germ pore on the apex of the dark body cell. The body cell is limoniform with the germ pore situated in the center of the limoniform bulge.

Row 3, all 3 photos - film roll 27, NZ dung collection 120

Row 4, all 4 photos - film roll 26, NZ dung collection 121



A–D. *Podospora bifida* ascospore germination. Rabbit dung, NZ collection 251. Ascospore photos brightfield, directly onto Difco CMA 18 hours after a 25-minute treatment with 3% H_2O_2 (no antibiotics). A, B & D. X280 on the film plane (oil immersion 100X objective). C. X112 on the film plane (40X objective). C,D. Same field of view.

The complete absence of gelatinous sheaths, caudae and pedicel is worth noting. Photographed using 35 mm slide color and that slide scanned with a Nikon 9000 film scanner.