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Author(s): Nils Lundqvist, Daniel P. Mahoney, Ann Bell, Laura E. Lorenzo
Source: *Mycologia*, Vol. 91, No. 2 (Mar. - Apr., 1999), pp. 405-415
Published by: Mycological Society of America
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*Mycologia*, 91(2), 1999, pp. 405–415.
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# *Podospora austrohemisphaerica*, a new heterothallic ascomycete from dung

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***Abstract:*** A new species, *Podospora austrohemisphaerica,* is described from the dung of wild and domesticated herbivores. Twenty-five records are cited, including 23 from Argentina, New Zealand and Australia and 2 from England. The species is characterized by a *Phialophora* anamorph, perithecia with rigid neck hairs and large ascospores with gelatinous sheaths and multiple caudae both at the spore extremities and at the proximal end of the lower cell (= pedicel). Teleomorph descriptions from lab-incubated field dung are augmented with teleomorph and anamorph descriptions from ascospore-initiated axenic cultures. Ascospore germination, sexual compatibility, intraspecific variation, distribution, and relationships to other species of *Podospora* are discussed.

***Key Words:*** ascospore germination, coprophilous fungi, Lasiosphaeriaceae, *Phialophora,* sexual compatibility, Sordariales, systematics

## INTRODUCTION

In 1981, two of the authors (NL and AB) independently observed what each thought was a new species of *Podospora* Ces. Seventeen yr, 23 additional records and 2 more authors later, the same species is here described as new to science.

## MATERIALS AND METHODS

*Dung collection and incubation.*—Dung collections were placed in clean paper bags or other containers and incubated at room temperature, with diurnal lighting, in glass or plastic containers lined with moist filter paper (Bell 1983, Lorenzo 1992, Lundqvist 1972). Collections not incubated immediately were air-dried and stored at 5 C. The names of frequently cited collectors are abbreviated as follows: A. Bell (AB), L. Lorenzo (LL), D. Mahoney (DM) and L. Tibell (LT).

*Axenic culture.*—Nutrient agar media used were (i) Difco cornmeal agar made up to 2% agar (CMA), (ii) CMA with 5 μg/L biotin, (iii) CMA with antibiotics (100 units/mL penicillin G and 30 μg/mL streptomycin sulfate), (iv) CMA with 2–6 autoclave-sterilized (15 min at 121 C) or Γ-ray sterilized whole wild rabbit (*Oryctolagus cuniculus*) or brush-tailed opossum (*Trichosurus vulpecula*) droppings, (v) Difco potato dextrose agar (PDA), (vi) malt extract agar (MEA) (Blakeslee 1915), (vii) modified Leonian's agar (ML) and Weitzman and Silva-Hutner's agar (WSH) (Malloch 1981), and (viii) oat agar (OA: 60 g rolled oats, 20 g agar, 1 L tap water). Some of the preceding were used infrequently. Others, including CMA, CMA with antibiotics, CMA with autoclave-sterilized rabbit dung, WSH, ML and PDA were employed for all the isolates. Cultures were centrally inoculated onto 9-cm Petri dishes with agar blocks from CMA stock slants. Incubation was usually at 22–23 C under continuous fluorescent light, although some mating experiments were conducted at 17–21 C under continuous or diurnal fluorescent and incandescent lighting.

*Ascospore germination.*—Ascospores from 4 New Zealand North Island moist-chamber-incubated dung collections (2 rabbit, *AB 250* and *AB/DM 253,* and 2 horse, WELTU 651 and WELTU 652) were employed.

In one set of experiments, perithecia from *AB/DM 253* and WELTU 652 were transferred one at a time to CMA containing antibiotics and cleaned of adherent debris by pushing them through the agar. Cleaned perithecia were subsequently broken open in a drop of 3% hydrogen peroxide and left for 20–30 min. Such treatment eliminates most bacteria and thin-walled fungal propagules, and may play a role in stimulating ascospore germination. These surface-sterilized ascospores were transferred to a drop of 10% buffered (pH 6.8) pancreatin (porcine pancreas, Sigma Chemical Company) on a sterile slide in a sterile Petri dish. The dish was sealed with parafilm and placed in an incubator at 37 C for 2–2½ h (John Krug pers comm). Treated ascospores from this and other germination attempts, noted below, were spread over CMA containing antibiotics and incubated at 21–23 C under continuous fluorescent light.

In other experiments, ascospore germination attempts involved (i) no treatment, (ii) 0.5% KOH for 30 min, (iii) separate incubation of some Petri dishes from the preceding two attempts, at 37 C for 24 h, (iv) 3% hydrogen per-

Accepted for publication November 13, 1998.
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oxide only, for 20–30 min, (v) 10% buffered pancreatin at 37 C for 3–3½ h, only, and (vi) water at 60 C for 15 min.

*Sectioning of perithecia.*—Preparation of paraffin sections was as described by Bell and Mahoney (1996) except that the stain employed here was Delafield's haematoxylin. Perithecia were those removed from the agar surface of an axenic CMA-rabbit dung mating plate, inoculated with several ascospore isolates from dung collection *AB 250.*

## TAXONOMY

**Podospora austrohemisphaerica** Lundqvist, sp. nov. FIGS. 1–28

Perithecia obpyriformia, 520–980 × 300–570 μm, deorsum pilis longis, flexuosis, brunneis, sparse vel profuse, sursum pilis rigidis, obtusis, saeptatis, nigrobrunneis, 40–110 (–180) × 2–3.5 μm, instructa. Peridium membranaceum, semipellucidum, ochraceum vel laete brunneum, in collo nigrum, opacum. Cellulae externae peridii angulatae, parietibus tenuibus, 4–11 μm diam; cellulae internae tangentialiter applanatae. Paraphyses filiformi-ventricosae, crassae. Asci 8-spori, 335–450 × 52–75 μm, apice anguste rotundato; annulus apicalis carens vel indistinctus. Sporae biseriatae, maturitate transverse uniseptatae; cellula superior (43–) 50–67(–76) × (19–)25–38(–43) μm, ellipsoidea, nigrobrunnea, poro germinali apicali instructa; cellula inferior (pedicellus) (40–)48–80(–110) × 8–13(–15) μm, ±cylindracea, hyalina, collabens. Tota spora strato gelatinoso tenui cincta, ad saeptum leniter inflato, dissolventi, etiam caudis pluribus gelatinosis, persistentibus instructa; caudae superiores 3–5(–9), subapicales, basaliter applanatae, 6–7 μm latae et 5 μm crassae, sulco longitudinali instructae vel filamentis duobus compositae; caudae inferiores 2–4, basales 2–3.5 μm crassae. Caudae mediae minores parti proximali pedicelli affixae, raro distinctae, plerumque reliqua fibrillosa vel agglutinata formantes. Anamorphosis *Phialophora* sp. Fimicola.

*Etymology.* From the Latin 'auster' (= south) and 'hemisphaerium', referring to the Southern Hemisphere where the species appears to have originated.

*Habitat and distribution.* On dung of horse and pony (10), cow (2), sheep (1), rabbit (6), hare (2), kangaroo (2) and wallaby (2). Collections from Argentina (9), New Zealand (9), Australia (5) and England (2).

*Characteristics on moist-chamber dung from field collections. Perithecia* (FIGS. 1C, 2) scattered or gregarious, few (<10/collection) to moderate (<100/collection) in number, rarely numerous, dark above, lighter below, obpyriform, varying from venters semiimmersed to totally immersed with only the neck emergent, 520–980 × 300–570 μm, with rigid hairs on the neck and flexuous hairs on the venter. *Neck hairs* (FIGS. 1C, 2–5) few to many, rigid, straight or nearly so, nonagglutinated, simple, septate, infrequently constricted at the septa, smooth, moderately thick-walled, dark brown throughout or lighter brown apically, obtuse, of uniform width along most of their length, 40–110(–180) × 2–3.5 μm, with an inflated basal cell. *Venter hairs* few to many, long, flexuous, smooth, septate, thinner-walled and more lightly pigmented than the rigid neck hairs. *Transition zone hairs* (FIGS. 4, 5) usually in the lower neck-upper venter, but found elsewhere on the neck as well: some intermediate between the neck and venter hair types, some short, dark and tuberculate, some crooked, irregular and intermediate between combina-

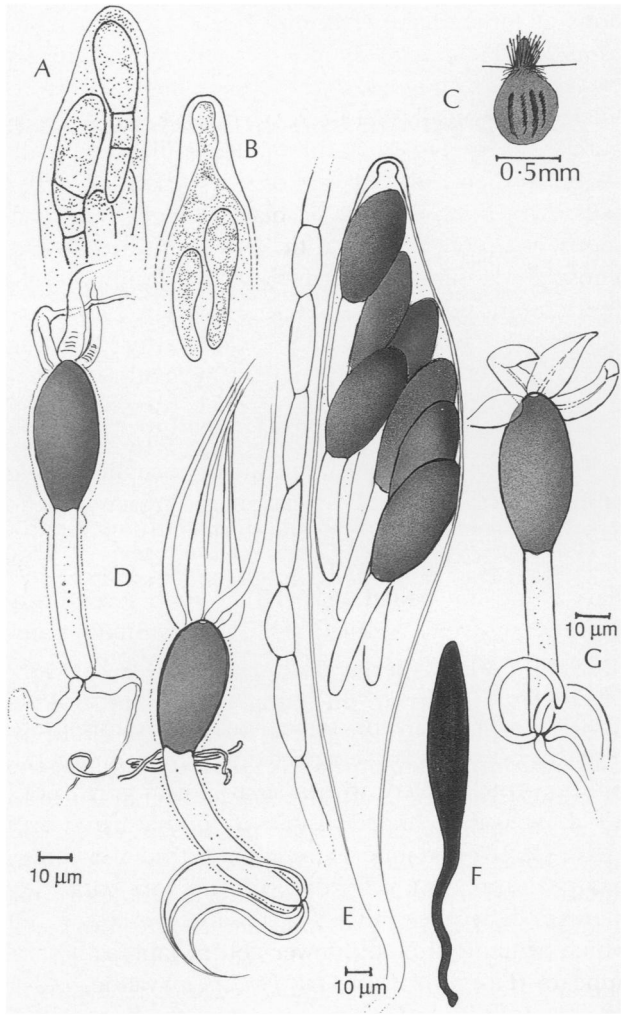



FIG. 1. *Podospora austrohemisphaerica.* A. Immature septate ascospores. B. Immature aseptate ascospores. C. Habit sketch of perithecium. D. Two mature ascospores, both with caudae at the apex of the upper cell and at the distal end of the pedicel with gelatinous sheaths covering both cells. The right spore with apical germ pore and caudae near the proximal end of the pedicel: the left spore with caudae absent, their remnants seen only as a thickening of the gelatinous sheath. E. Mature ascus and paraphysis. F. Ascus silhouette. G. Mature ascospore with apical germ pore and caudae at its extremities, but lacking a gelatinous sheath and caudae at the proximal end of the pedicel.

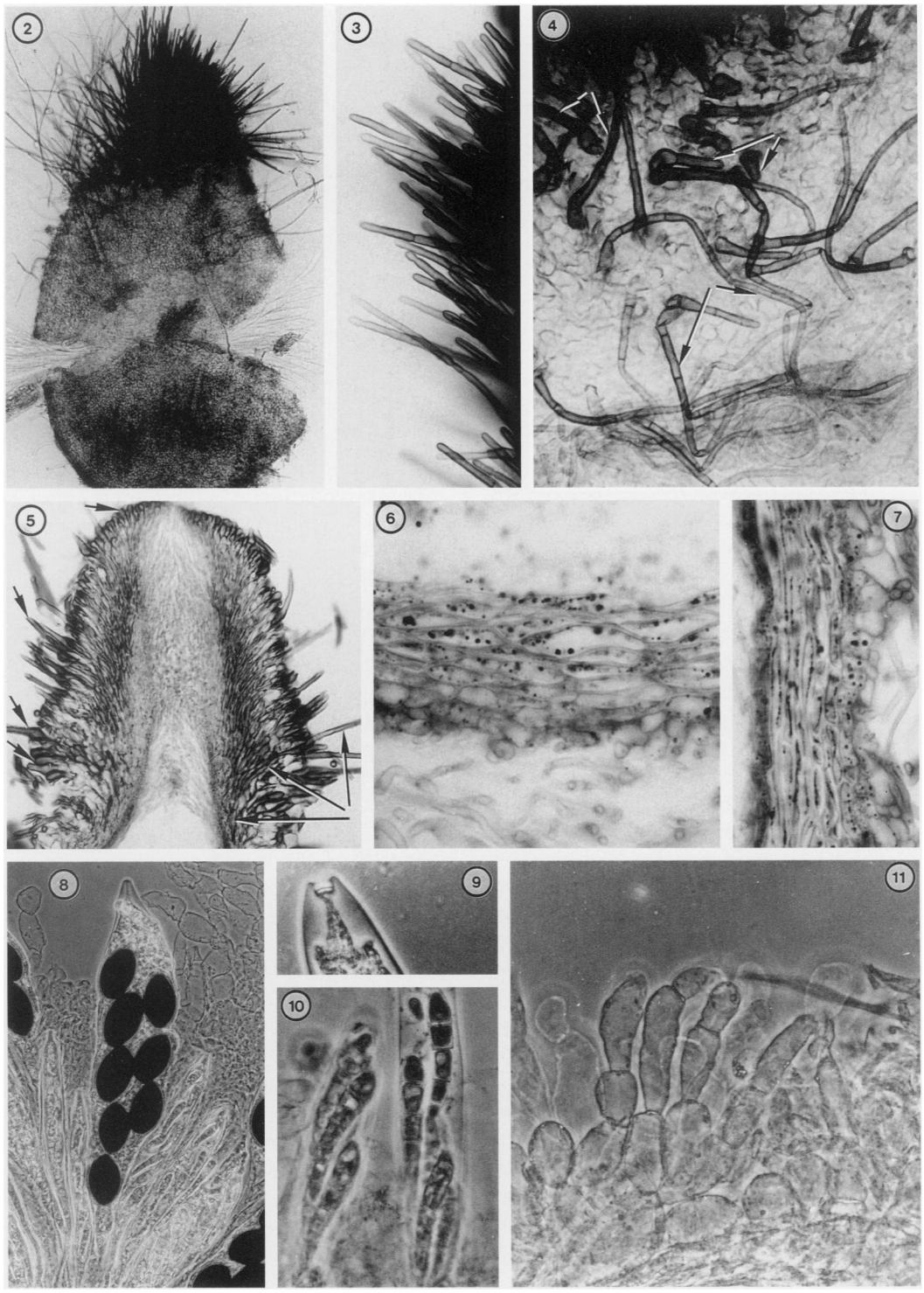
tions of tuberculate, rigid and flexuous hairs. *Venter peridium* (FIGS. 4, 6, 7) membranaceous, semitransparent, ochraceous to light brown, 6–8 cell layers thick in axenically cultured perithecia (FIGS. 6, 7), outer layer of angular, thin-walled, isodiametric cells 4–11 μm diam, inner layers of tangentially flattened cells. *Neck peridium* (FIG. 5) black, opaque, carbonaceous, of closely packed, anticlinally arranged hyphae whose dark, moderately thick-walled terminal cells (peridial surface cells) are slightly swollen. *Paraphyses* filiform-ventricose (FIGS. 1E, 8, 11), scattered among the asci, frequently collapsing as asci mature. *Asci* 8-spored (FIG. 8), occasionally 7-spored or fewer (FIG. 1E), 335–450 × 52–75 μm, with tapered apex and a fairly long stipe (FIG. 1F); apical ring usually lacking or indistinct (FIG. 1E), occasionally present at the base of a shallow apical funnel (FIG. 9). *Ascospores* biseriately arranged, at first cylindrical to narrowly clavate and one-celled (FIG. 1B) or with several evanescent transverse septa (FIGS. 1A, 10), finally transversely uniseptate (FIGS. 1D, 1E, 1G, 12–22). *Upper cell* black-brown, (43–)50–67(–76) × (19–)25–38 (–43) μm, broadly or more narrowly ellipsoid to slightly obovoid, symmetrical to slightly inequilateral, smooth, thick-walled, with a small apical germ pore which is usually more visible in immature spores (FIGS. 12, 13) and an obscure central thin area in the basal septum (FIG. 12), also with a short basal pigmented collarlike wall extension to the lower cell which remains after the lower cell collapses and disappears (FIG. 15). *Lower cell* (*pedicel*) hyaline, (40–)48–80(–110) × 8–13(–15) μm, smooth, thin-walled, cylindrical, slightly tapered or broadest in the middle, straight or slightly curved, attached symmetrically or slightly asymmetrically to the base of the upper cell, soon collapsing. *Gelatinous sheath* (FIGS. 1D, 13, 18) distinct or indistinct, evanescent, covering both the upper and lower cells, thin but often broader at the proximal end of the pedicel. *Gelatinous caudae* at each end of the spore (apical and basal caudae) and at the proximal end of the pedicel, hyaline, sticky, extensible, moderately persistent but easily disturbed and difficult to interpret, not blackening in Indian ink, defying accurate length measurements due to their sticky, extensible nature. *Apical caudae* (FIGS. 1D, G, 13, 14, 16, 19–21) 3–5(–9), often 4, arising near the apical germ pore, broadest basally and gradually tapering, appearing proximally flattened with a longitudinal furrow (see white line in FIGS. 1G, 14), or possibly composed of 2 strands, ca 6–7 μm wide and 5 μm thick; the most robust of the different types of caudae. *Basal caudae* (FIGS. 1D, G, 15–17) 2–4, sometimes seen as a single agglutinated cauda (FIGS. 18, 21), arising at the distal end of the pedicel, ca 2–3.5 μm broad at their base and gradually tapering; with a central white line (FIGS. 15, 16); narrower, longer and more evanescent than the apical caudae. *Caudae arising at the proximal end of the pedicel* (FIGS. 1D, 12, 16, 19–21) variable in number, ca 3–6, about 2–3 μm wide and much shorter than the other caudae; rarely visible in their entirety (FIGS. 1D, 21), with an interior white line (FIG. 21); usually observed as fibrillose (FIGS. 16, 20) or basally agglutinated (FIGS. 12, 19) remnants, or only as a thickening of the gelatinous sheath at the proximal end of the pedicel (FIGS. 1D, 13, 18). *Anamorph* unknown from moist-chamber cultures of field-collected dung.

*Ascospore germination.* Numerous attempts to germinate untreated ascospores failed. Likewise, heat treatments at 60 C for 15 min and at 37 C for 24 h (with or without pretreatment with 0.5% KOH for 30 min) also failed. Limited germination (one to few spores) resulted after treatment on separate occasions in 3% hydrogen peroxide (20–30 min) and in 0.5% KOH (30 min). The latter yielded its single germination (source of FGSC 8314) by growth through the central thin area in the basal septum of the upper cell. The pedicel had disappeared and no germination vesicle was formed.

Most successful was the ascospore treatment with hydrogen peroxide (28 min) followed by 10% buffered pancreatin at 37 C for 2–2½ h. Nineteen of 122 spores germinated, each first producing a conspicuous globose germination vesicle at the mouth of the apical germ pore (FIG. 22). A similar earlier treatment from the same collection (WELTU 652), without hydrogen peroxide and with incubation in pancreatin for 3–3½ h, produced no germinations. A repeat of the successful procedure for WELTU 652, this time for *AB/DM 253,* yielded 4 apically germinating ascospores. From these results it is clear that more work will be necessary before we understand what combination of treatments consistently stimulates ascospore germination.

A recent observation best exemplifies why our attempts to germinate ascospores have been so frustrating. In a mating between FGSC 8314 and 8315, we saw many recently discharged spores germinating in situ on the Petri dish lid. In fact, the germ hyphae had already produced phialides typical of the anamorph (see Bell and Mahoney 1995, Fig. 43, for a similar observation in *Podospora conica* (Fckl.) Bell & Mahoney). These untreated ascospores were germinating on a nonnutrient surface when all earlier untreated spores had failed to germinate on CMA containing antibiotics.

*Single-ascospore cultures.* The following is a collective description based on 8 single-ascospore isolates from 4 New Zealand North Island dung collections, with particular emphasis on the isolates subcultured




FIGS. 2–11. Perithecial features and centrum elements. 2. Perithecium. WELTU 649. ×84. 3. Rigid hairs on the neck of a perithecium. 4. Peridium and hairs in the transition zone between neck (above) and upper venter (below). Upper left arrows, rigid hairs; middle arrows, tuberculate hairs; lower arrows, more flexuous hairs. 3, 4. From *LT 8915-f.* ×500. 5–7. Different portions of the same perithecium paraffin section. From perithecium in CMA-rabbit dung axenic culture, WELTU 626 is part of that culture. 5. Neck, near-median longitudinal section. ×280. Arrows indicate a cell of the neck surface and various types of hairs: left side top to bottom—short apically swollen cell of the neck surface, broken rigid hair, basally swollen rigid hair, tuberculate hair; lower right—a single rigid hair originating deep within the neck peridium. 6, 7. Cell layers of the perithecium wall, from the lower venter and upper right venter (near flank of neck), respectively. Hyphae outside the perithecium wall (in the surrounding agar medium) are seen at the bottom and along the right edge, respectively. ×840. 8.

as FGSC 8314 and 8315. The anamorph *Phialophora* Medlar was present in all cultures, regardless of which nutrient medium was employed. Fertile perithecia were absent.

Colonies spreading slowly, nearly covering the agar surface in 3–4 wk; the occasional isolate growing somewhat restrictedly, especially on MEA. Colonies on the weaker nutrient media, CMA or CMA supplemented with biotin or antibiotics, translucent with low, moderately sparse, hyaline aerial hyphae; faintly brownish locally or throughout depending on the abundance of conidia. Colonies on CMA containing rabbit or opossum dung producing more aerial hyphae and conidia, somewhat darker than CMA colonies due to (i) pigments diffusing from the dung, (ii) more greyish aerial hyphae and (iii) more numerous brownish conidia particularly near the dung and on its surface. The color of conidial areas here and on richer media such as WSH, ML, PDA and OA varying from grey brown to orangish brown (plate 6 C3–C6, Kornerup and Wanscher 1978); darkest on heavily sporulating colonies of FGSC 8314. Distinctive hyphal ropes growing over the dung surface in CMA-opossum dung cultures of FGSC 8315; ropiness also a feature of colonies on richer media such as WSH and ML although varying with the isolate and the medium. Colonies on WSH, ML, OA, PDA and MEA all with luxuriant low aerial hyphae. Colony coloration among isolates on these media varied depending on the relative amounts of hyphae (white to pale greyish or light greyish brown) and conidia (brownish). Other cultural features that varied among the colonies of different isolates on these media: (i) the degree of ropiness (prominent to absent), (ii) the presence or absence of alternating light and dark concentric growth rings (more ropy, less ropy and/or heavier sporulating, lighter sporulating), (iii) the presence or absence of radial furrowing (furrows sometimes cracking the agar), (iv) the mycelial texture, varying from velvety floccose to lanose or ropy and (v) the colony-reverse coloration, normally colorless but reddish among certain colonies on PDA and varying from dull greyish red (7B4), brownish orange (7C4–7C5) to dark reddish brown (8E8) on colonies of FGSC 8314. Although each single-ascospore isolate retained its own identity, no combination of cultural features (ropiness, radial sectoring, reverse coloring, etc.) characterized the species as a whole.

Phialophora *anamorph in single-ascospore cultures.* The description below is from single-ascospore cultures on CMA and on CMA with rabbit dung. Cultures of the latter were particularly useful because they offered the opportunity to see both sparse and heavy production of conidia on the same culture, away from and nearer the dung, respectively.

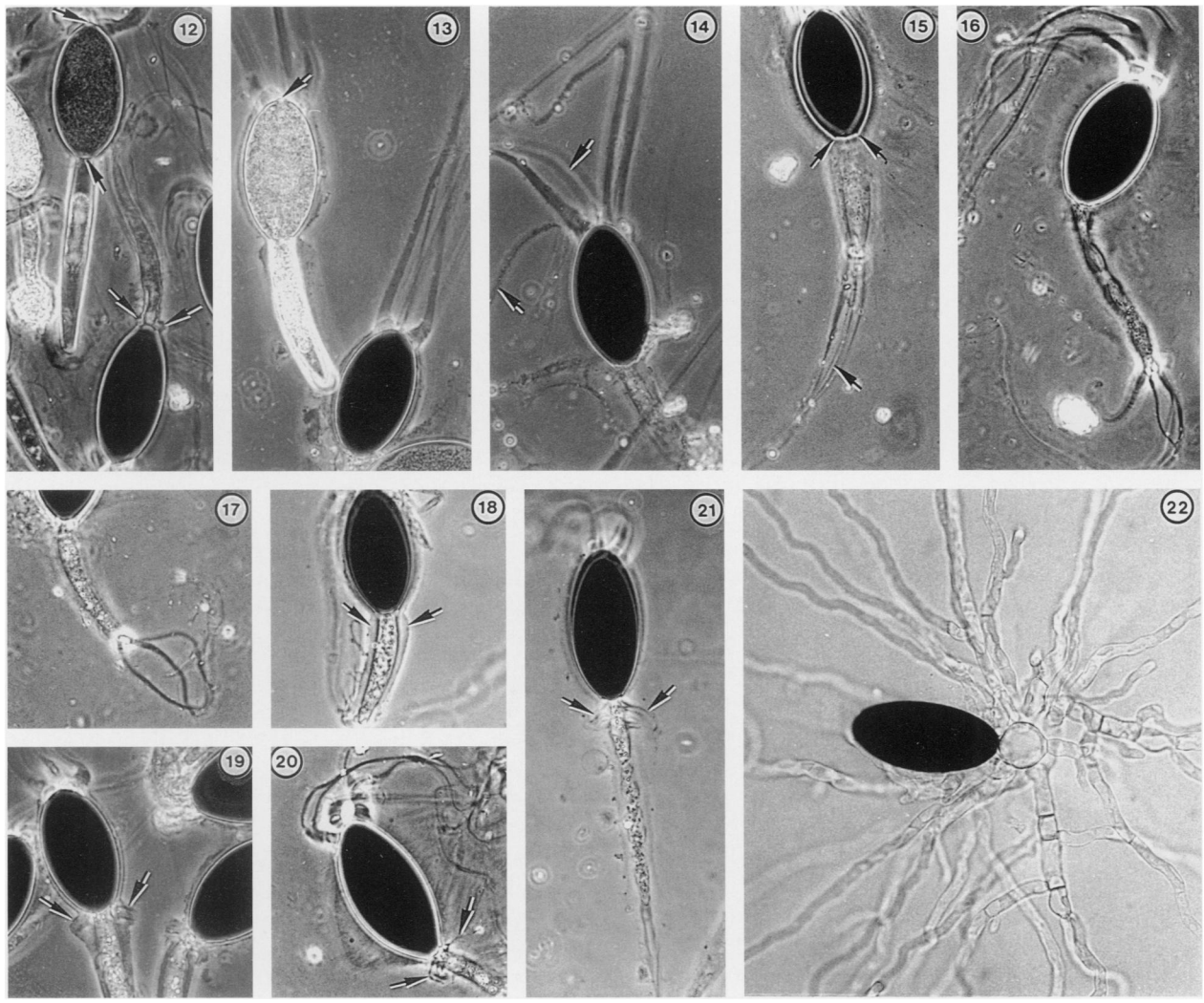
Phialides numerous, produced from hyphae on the substrate surface and from low simple or fasciculate aerial hyphae (FIGS. 23, 24), borne separately (FIG. 25) or in clusters; clustered phialides arising side by side (FIG. 26), in divergent verticillate groups of 2–5 at the tips of short simple side branches (FIG. 27) or on short irregular branch systems (FIG. 28); individual phialides and supporting branches, if present, slightly thicker-walled than vegetative hyphae, smooth and hyaline; phialide shape variable, sometimes hyphalike, straight or curved and gradually tapered from base to apex, more often widening above the base on one side only (the other side flat or slightly concave), and more abruptly tapered apically; phialide apex narrow with a widely flared, often conspicuous, collarette (FIGS. 23, 25); phialides (5–)8–15(–20) × (2–)2.5–3.5(–4) μm, basally septate. Conidia produced in glioid balls at the phialide apices (FIGS. 23, 24, 27), each conidium broadly obovoid and basally truncate (FIG. 23) but often appearing globose to subglobose in polar view, smooth, with moderately thickened walls, containing a single guttule (FIG. 25), hyaline to faintly brown individually but distinctly brownish in mass, 2.5–3.5(–4) × 2–3 μm.

No germination occurred among conidia spread onto CMA. We have not attempted to stimulate germination by any of the special treatments noted earlier under ascospore germination.

Podospora *teleomorph in single-ascospore cultures.* No fertile perithecia developed on any nutrient agar medium that was tried. However, a few sterile perithecia consistently formed in cultures of FGSC 8314 on CMA containing rabbit dung, although never on CMA alone. These perithecia were obpyriform with peridium cell detail and rigid neck hairs characteristic of the species, but asci did not form. Only perithecia whose necks developed above the agar surface

←

Mature and immature asci and paraphyses. WELTU 451. ×168. 9. Tip of mature ascus, showing a small light-refractive ring. WELTU 649. ×448. 10. Portions of two asci, showing immature septate ascospores. From *LT 8915-f.* ×500. 11. Paraphyses. WELTU 650. ×448. 2–4, 8–11. From perithecia on original moist-chamber dung. 2, 8, 9, 11. Water slide mounts. 3, 4, 10. Lactophenol cotton blue slide mounts. 2–7. Brightfield microscopy. 8–11. Phase contrast microscopy.




FIGS. 12–22. Ascospores from water slide mounts. ×336. Various views, emphasizing easily missed features of evanescent gelatinous sheaths and caudae rarely seen together in single spores. Caudae usually stretched to some degree. 12. Slightly immature spore with turgid pedicel, apical germ pore (upper arrow) and thin area in septum between upper cell and pedicel (middle arrow). Mature spore with collapsing pedicel and remnants of caudae at proximal end of pedicel (lower arrows). 13. Both spores with 4 apical caudae and gelatinous sheaths. Immature spore with apical germ pore (arrow). WELTU 650. 14. Upper cell with 4 apical caudae, one with a wide central white line (arrow). Lower arrow indicates the basal cauda from a nearby spore. 15. Pedicel with 3 caudae at its distal end. At least one cauda with a central white line (arrow). Pigmented wall extension of upper cell faintly visible at the point of pedicel attachment (arrows). 16. Nearly complete mature spore. Exhibiting 3–5 apical caudae with banding near their bases, broad diffuse gelatinous sheaths over upper cell and pedicel, a collapsing pedicel with light-refractive areas at its distal end, remnants of caudae at the proximal end of the pedicel and 4 caudae at the distal end of the pedicel (2 with central white lines). 17. Spore with 3 or 4 caudae at the distal end of the pedicel. 18–21. Different views of caudae at the proximal end of the pedicel (arrows). 18. Individual caudae not recognizable. Remnants forming a thickened gelatinous sheath. From *B. Jonsell 5246-b*. 19. Individual caudae seen only as their agglutinated basal remnants. WELTU 650. 20. Individual caudae seen as partial, somewhat fibrillose, remnants. Note also 3 artifacts: light-dark banding at the base of the apical caudae, the broad, stretched and fibrillose gelatinous sheath of the pigmented cell and the partial splitting of the left apical cauda. 21. Individual caudae clearly visible, each with a broad white central area. Showing also 4 or 5 apical caudae, collapsing pedicel with sheath and agglutinated caudae at the distal end of the pedicel. From *LT 12094-f*. 22. Ascospore germination in situ beneath a coverslip on CMA containing antibiotics, showing an apical germ vesicle from which numerous hyphae are emerging. Seen 17–18 h after treatment with $H_2O_2$ and pancreatin at 37 C. WELTU 652. All ascospores from original moist-chamber-dung perithecia, photographed under phase contrast microscopy except FIG. 22 (brightfield). 12, 14–17, 20. From WELTU 451.

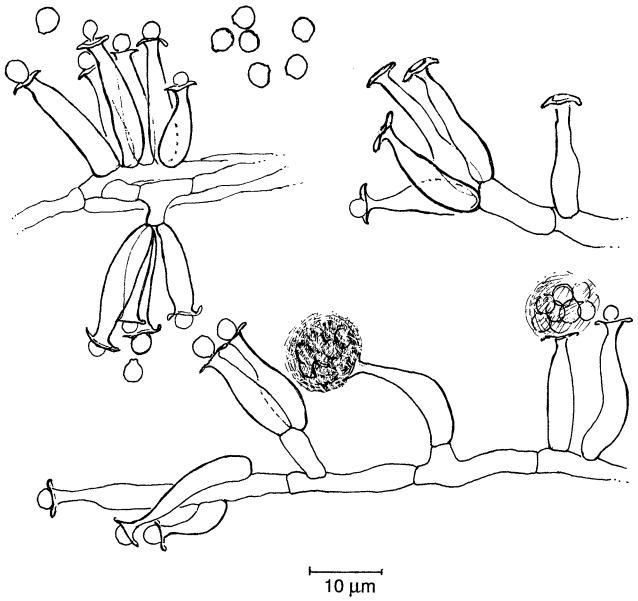
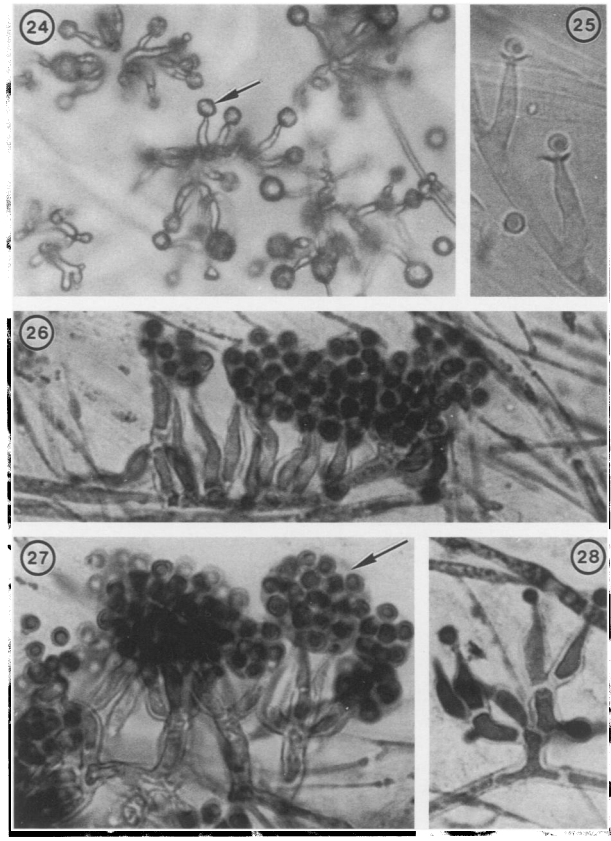



FIG. 23. *Phialophora* anamorph. Showing various arrangements of phialides with their apical flared collarettes, glioid conidial masses and basally truncate conidia.




FIGS. 24–28. *Phialophora* anamorph. 24. Phialides and glioid clusters of conidia. Arrow indicates one conidial cluster. In situ view without coverslip onto a 12 d axenic culture (CMA containing one whole Γ-irradiated opossum dropping). ×448. 25–28. Various phialide arrangements. Viewed in situ beneath coverslips on 13–14 d axenic cultures (CMA without dung). ×1120. 25. Phialides produced singly and separately, with apical flared collarettes and with young conidia each containing a single guttule. Water mount. 26–28. Phialides produced singly and clustered, in verticillate arrangements on short conidiophores and in more elaborate and irregular arrangements, respectively. Arrow indicates one glioid conidial cluster. Lactophenol cotton blue mounts. 24, 26–28. From FGSC 8315. 25. From FGSC 8314.

developed rigid neck hairs; those forming completely submerged had none.

Podospora *teleomorph in crosses between FGSC 8314 and 8315.* Fertile perithecia developed on CMA containing rabbit dung but never on CMA lacking dung. Crosses on other media were not attempted. Perithecia began to form on the agar near the dung and on the dung itself between the third and fifth wk. These were small, superficial (or occasionally submerged), hyaline to pale amber or dull brown, pseudoparenchymatous spherical masses clothed with numerous, long, flexuous, hyaline to light brownish hyphae. Transition to an obpyriform shape with a neck, rigid hairs and an ostiole followed with mature ascospores forcibly discharged after 6–7 wk. Initially fewer than 25 mature perithecia were present but during the second and third mo more perithecia formed and immature ones gradually matured. Still, after 4 mo less than 100 mature perithecia had developed. Reproductive success in this species appears to depend upon the slow staggered development of relatively few perithecia whose ascospore release occurs over an extended period of time. Perithecia, asci and ascospores from axenic cultures were identical with those observed on the original dung from moist-chamber cultures.

HOLOTYPE. AUSTRALIA. NEW SOUTH WALES: 18 km NW of Adaminaby in uppermost part of Alum Creek, in open *Eucalyptus* forest, on rabbit dung (*Oryctolagus cuniculus*), 5 Apr. 1981, *LT 12094-f* (UPS).

*Additional specimens examined.* ARGENTINA. PROV. NEUQUÉN: Dpto. Los Lagos, Paraje Rincón Chico, on horse dung, 29 Sep. 1989, *LL* (BCRU 201); Cerro Chapelco, Arroyo Pil-Pil, on cow dung, 10 Mar. 1995, *LL* (BCRU 1577). PROV. RÍO NEGRO: Bariloche, Centro Atómico, on hare dung (*Lepus europaeus*), 3 Apr. 1987, *C. Renauld* (BCRU 26); Bariloche, Llao-Llao, on horse dung, 11 May 1988, *LL* (BCRU 130, S); Dpto. Bariloche, S. C. Bariloche, Llao-Llao, on hare dung (*Lepus capensis*), 29 Aug. 1989, *LL* (BCRU 202); Dpto. Bariloche, S. C. Bariloche, Llao-Llao, on horse dung, 7 Sep. 1989, *LL* (BCRU 200); Bariloche, Bahia Serena, on horse dung, 8 Jun. 1993, *LL* (BCRU 421); El Bolsón, on sheep dung, 8 Jun. 1993, *LL* (BCRU 422); Paraje El Guadal, El Bolsón, on horse dung, 8 Mar. 1995, *LL* (BCRU 818). AUSTRALIA. NEW SOUTH WALES: 18 km NW of Adaminaby in uppermost part of Alum Creek, in open *Eucalyptus* forest, on kangaroo dung (*Macropus*

sp.), 5 Apr. 1981, *LT 12074-f* (UPS, slide only); Ku-ring-gai Chase National Park, Birrwanna Track, on swamp wallaby dung (*Wallabia bicolor*), 31 Aug. 1981, *AB* (UPS, WELTU 336). TASMANIA: Santa Maria Island National Park, just E of Darlington, on wallaby dung (*Wallabia* sp.), 12 Mar. 1981, *LT 11381-f* (UPS). WESTERN AUSTRALIA: Williams Bay, 80 km E of Albany, in coastal heath, on dung of small grey kangaroo (*Macropus* sp.), 2 Sep. 1981, *B. Jonsell 5246-b* (UPS). ENGLAND. HAMPSHIRE: Eyeworth, New Forest, on pony dung, 28 Mar. 1991, *A. Henrici* [K(M) 16993]. MIDDLESEX: Perivale Wood, on horse dung, 5 May 1991, *A. Henrici* [K(M) 17152]. NEW ZEALAND. N. ISLAND: Hokio Beach, ocean strand vegetation, on rabbit dung (*Oryctolagus cuniculus,* the only wild rabbit species in New Zealand), 5 Sep. 1987, *AB/DM* (WELTU 451); Otaki Beach, ocean strand vegetation, on horse dung, 11 Mar. 1990, *AB/DM* (WELTU 651, slides only); Waikawa Beach, ocean strand vegetation, on rabbit dung, Aug. 1993, *AB 250* (WELTU 626, a dried rabbit dropping from an axenic CMA-rabbit dung mating inoculated with several ascospore isolates of *AB 250*); same as preceding, 14 Jan. 1995, *AB/DM 253* (no herbarium record); pasture above Taupo flax swamp, near Plimmerton, on horse dung, 18 Jan. 1995, *AB/DM* (WELTU 652, slide only). S. ISLAND: Canterbury, Banks Peninsula, 7 km ESE of Taitapu along Summit Road, in grassland, on cow dung, 20 Sep. 1980, *LT 8915-f* (UPS, slide only); Canterbury, Banks Peninsula, 8 km S of Diamond Harbor in Mt. Herbert Forest, on horse dung, 6 Nov. 1980, *LT 9396-c* (UPS); Christchurch, ocean strand vegetation, on rabbit dung, 10 Sep. 1987, *J. Parkes* (WELTU 649, slides only); Westport, ocean strand vegetation, on rabbit dung, 4 Jan. 1988, *AB/DM* (WELTU 650, slides only).

*Cultures deposited.* Strain #1, a single-ascospore isolate from *AB 250* (FGSC 8315 = CBS 216.97 = ATCC 200700). Strain #2, a single-ascospore isolate from WELTU 651 (FGSC 8314 = CBS 217.97 = ATCC 200699). Freeze-dried 80 d old matings between strains #1 and #2 on CMA containing 4 whole autoclave-sterilized wild rabbit droppings were deposited as herbarium specimens (NY, WELTU 669). Fertile perithecia in these matings are typical of the species. An earlier mating on the same medium among several ascospore isolates from *AB 250* is preserved as WELTU 626.

## DISCUSSION

Considerable variation was seen within *Podospora austrohemisphaerica.* Aspects of that variation and some problems with interpretation are discussed below.

*Perithecia.*—The number of flexuous hairs on the perithecium venter varied from few to many in the original moist-chamber dung incubations. However, flexuous hairs were always numerous on perithecia of CMA-rabbit dung axenic cultures. The latter cultures maintain a higher more constant humidity and, perhaps, this condition results in more numerous flexuous hairs. Likewise, the number of rigid neck hairs was variable although the reasons for this are less apparent. These hairs do not develop on axenically cultured perithecia whose necks remain submerged in the agar medium, a phenomenon also noted among similarly cultured fertile perithecia of *Podospora curvicolla* (Wint.) Niessl (Mahoney and Bell unpubl). All neck hairs originate from the innermost portion of the neck as extensions of the anticlinally arranged peridial elements of the neck, as shown in the paraffin section of FIG. 5.

The innermost peridial layer in the upper venter wall of FIG. 7 is dark-staining while that layer is lacking from the lower venter of the same paraffin section in FIG. 6. This dark layer gradually disappears as we follow it from the upper to the lower venter area. We do not know if it was originally present in the lower venter, as it is in *Arnium olerum* (Fr.) Lundq. & Krug and *A. hirtum* (Hans.) Lundq. & Krug (Lundqvist 1972), nor do we know its function.

*Paraphyses and periphyses.*—Paraphyses (FIGS. 1E, 8, 11) and periphyses (FIG. 5) are similar developmentally to those of most *Podospora* species whose centrum development has been studied (Lundqvist 1972, Bell and Mahoney 1997), but the paraphyses differ from those of *Podospora* species with swollen agglutinated perithecial hairs (Bell and Mahoney 1995, 1996). The latter species lack interascal paraphyses and their asci grow up among cells of a pseudoparenchymatous centrum tissue. Paraphysis-like elements (jacket paraphyses) in these species form a layer, not present in *P. austrohemisphaerica,* that surrounds the centrum.

*Ascospores.*—Ascospores of *P. austrohemisphaerica* are typical of the genus *Podospora* in being bicellular, with the upper cell ellipsoidal and opaque black, and the lower cell cylindrical and hyaline. The lower cell is best seen in immature ascospores. There is variation among the collections in spore size, number of septa and the degree to which caudae and other protoplasmic features are present. The pigmented cells are atypically long in WELTU 336 [43–70(–76) μm] and atypically broad in WELTU 451 (25–40 μm) and in K(M) 17152 [31–38(–43) μm]. During maturation a transverse septum develops in the young ascospore, thus delimiting the two cells. Occasionally 2–4 additional septa are formed (FIGS. 1A, 10), but these are never visible in spores in which the upper cell has become pigmented. We cannot be certain that the opaque cell does not sometimes have more than one cell. Moreover, because asci with fewer than 8 ascospores are occasionally seen, the multiseptate ascospores may ultimately abort. Additional septa also occur in hyaline ascospores of *Podospora dasypogon* Lundq. and *P. pyriformis* (Bayer) Cain (Lundqvist 1972) and here also the extra septations are absent from mature spores.

A thin area which can serve as a germ pore is visible in the middle of the septum between the upper and lower cells (FIG. 12). *Podospora intestinacea* Lundqvist (1972) has a similar thin area in its septum, but we do not know whether this feature is widespread in the genus. A more conspicuous wall character is the short pigmented collarlike wall extension of the upper cell where it joins the lower cell. This is less apparent in spores with lower cells still intact (FIG. 15) but is conspicuous at higher magnifications in spores whose lower cell has disintegrated. Species of the *Podospora decipiens* group (sensu Lundqvist 1972) also share such a feature (Mahoney and Bell unpubl). Finally, several light refractive areas (phase contrast microscopy) are often present in peripheral protoplasmic regions of the lower cell. One to several of these were frequently observed at the tip of the lower cell just beneath the distal caudae (FIGS. 16, 17) and less frequently one to few smaller dots were seen just beneath the caudae at the proximal end of the pedicel. Their position suggests an association with caudal origin.

The gelatinous sheath and caudae were the most difficult ascospore features to observe and interpret. They were clearly visible only in water mounts viewed with phase contrast microscopy. Their presence and condition varied among the collections and within individual preparations. The large amount of sticky gelatinous material associated with each spore prevented the preparation of a flattened mount in which individual ascospores could be seen with intact caudae and sheaths. 'Normal' sheaths and caudae on a whole spore are illustrated in FIG. 1D (spore on the right) and those slightly less 'normal' in FIGS. 16 and 21. Because stretching of caudae (FIGS. 13–17) was common, length measurements were omitted.

Certain questions about caudae remain. What is the shape and internal structure of the caudae? The larger apical caudae appear slightly flattened but what is the shape of the distal and proximal caudae on the lower cell? All caudae have a central white line. Is this a longitudinal furrow as has been suggested for the upper caudae, a central canal or a point of union for two strands? Partial longitudinal splits have been observed in apical caudae, for example in the left apical cauda of FIG. 20. This and similar observations suggest that our infrequent records of more than 4 or 5 apical caudae are the result of complete longitudinal splits among existing caudae. Up to 9 apical caudae were observed in WELTU 336.

Ultrastructural detail of gelatinous sheaths and caudae for *Podospora* ascospores is yet to be determined but some views suggest a fibrillar component. Fine fibrillose strands are sometimes obvious where the gelatinous sheath appears to have moved after it stuck to the slide surface (FIG. 20). Also remnants of proximal caudae on the pedicel frequently appear fibrillose (FIGS. 16, 20). Other views of caudae may relate to underlying structure or simply to anomalies resulting from age, shrinkage or other factors. Such views include dark-light banding, pseudoseptate and accordion-like appearances of basal portions of some apical caudae (FIGS. 16, 20) and the various agglutinated views of caudae at the distal (FIG. 21) and proximal (FIGS. 12, 19) ends of the lower cell.

*Sexual compatibility.*—This is the first report of heterothallism among those species of *Podospora* with 8 or more spores per ascus. Cultures derived from single ascospores were not sexually fertile although fertile perithecia resulted from appropriate matings among these cultures. *Podospora anserina* (Ces. ex Rab.) Niessl (reviewed by Esser 1974) and *P. tetraspora* (Wint.) Cain (Raju and Perkins 1994) are 4-spored species of genetic interest whose heterothallism (pseudohomothallism or secondary homothallism) results from single ascospores each containing 2 compatible nuclei.

Two of us have attempted to culture most of the 30 species of *Podospora* in New Zealand (Bell and Mahoney unpubl). Of 22 species in single-ascospore culture, the sexual compatibilities of 13 are known. In addition to the 3 above-mentioned heterothallic species, 10 are homothallic, including 4 species with 8-spored asci [*P. decipiens* (Wint.) Niessl, *P. excentrica* Lundq., *P. fimiseda* (Ces. & DeNot.) Niessl and *P. miniglutinans* Mirza & Cain] and 6 species with more than 8-spored asci [*P. pleiospora* (Wint.) Niessl, *P. dakotensis* (Griff.) Mirza & Cain, *P. myriospora* (Cr. & Cr.) Niessl, *P. bifida* Lundq., *P. setosa* (Wint.) Niessl and *P. curvicolla*]. Studies on the sexual compatibilities of the remaining 17 species (all with 8-spored asci) are in progress.

*Relationships to other* Podospora *anamorphs.*—Twenty species of *Podospora* have a *Phialophora* anamorph. The only other *Podospora* anamorph reported has been *Cladorrhinum* Sacc. & Marchal for *P. fimiseda* (Bell and Mahoney 1997). Included among the *Podospora* species with a *Phialophora* anamorph are 12 species listed in The Whole Fungus (Kendrick and Dicosmo 1979), 3 species [*P. curvuloides* Cain, *P. glutinans* (Cain) Cain and *P. vesticola* (Berk. & Br.) Mirza & Cain] from New Zealand (Bell and Mahoney 1995), 3 species [*P. ellisiana* (Griff.) Mirza & Cain, *P. intestinacea* and *P. pyriformis*] unreported from New Zealand (Bell and Mahoney unpubl), the African species *P. serotina* Cailleux (1969) and the new species *P. austrohemisphaerica.*

The *Phialophora* state of the new species is unique

among the above-mentioned species in having its phialides often arranged in irregular or verticillate fashion on short side branches of the vegetative hyphae (FIGS. 27, 28) and in the brownish color of its conidial masses. Phialides of other species are usually produced singly, directly from the hyphae, and conidia are hyaline in mass. Only in *Podospora spinulosa* Khan & Cain (1972) are phialides described as "rarely in groups of three to five or more on short side branches," but here the conidia are hyaline. Unfortunately, no comparative studies have been conducted among the *Phialophora* states of these 20 species since emphasis has centered upon their teleomorphs.

Other *Phialophora* species and related genera such as *Acremonium* Link and *Cladorrhinum* are associated with a variety of other ascomycetous teleomorphs or have no known teleomorphic state (Domsch et al 1980, Kendrick and Dicosmo 1979). A comparison between these species and the *Phialophora* states of *Podospora* species is beyond the scope of this study, although a point about conidial function is worth making. *Phialophora* conidia from *Podospora* species are not known to germinate (Mouchacca and Gams 1993, Bell and Mahoney unpubl) while conidia of some other *Phialophora* species, for example several from decaying wood (Cole and Kendrick 1973), do. Dodge (1936), Cain (1952), Khan and Cain (1972) and Mai (1976) suggest a spermatial function for the *Podospora* 'conidia.' It may prove useful in the future to distinguish those *Phialophora* species whose conidia have a clear propagative role from those whose conidia do not.

*Relationships to other* Podospora *teleomorphs.*—A combination of ascospore characters, including the large dark cell, the long pedicel and particularly the caudal complexity, sets *P. austrohemisphaerica* apart from other *Podospora* species. Although a distinct species, it shares some similarities with other species in the genus. Among the species that have 8-spored asci, the ascospores of *P. communis* (Speg.) Niessl and *P. gwynne-vaughaniae* (Page) Cain (Mirza and Cain 1969), *P. spinulosa* (Khan and Cain 1972) and *P. dactylina* Lundq. (1970) have similar, but smaller, dark cells and pedicels, and multiple caudae at their spore extremities. Likewise, species of the *P. decipiens* group (Lundqvist 1972) have similar but smaller dark cells and quite similar pedicels and caudae at the proximal end of the pedicel. Perithecial characters, such as the nonagglutinated rigid neck hairs, are less defining of *P. austrohemisphaerica* as a unique species. Such hairs are indistinguishable from those produced by a number of species, including *P. bifida* (Lundqvist 1972) whose perithecia appeared among those of *P. austrohemisphaerica* on New Zealand rabbit dung (*AB/DM 253*). Perithecia of these two species were superficially identical under a dissecting microscope.

*Distribution.—Podospora austrohemisphaerica* is widely distributed in the temperate Southern Hemisphere. Despite a gap of 17 yr since our first observation of it and despite continuous collecting by the authors and their collaborators, only two extralimital collections have been found (in England). For this reason, and because the species has not been previously encountered in the many publications on coprophilous fungi from diverse geographic areas (Furuya and Udagawa 1972, Khan and Cain 1972, Krug and Khan 1989, Lundqvist 1972, Mirza and Cain 1969), the overwhelmingly south temperate distribution of *P. austrohemisphaerica* supports the existence of a south temperate group of coprophiles that was first suggested by Lundqvist (1972). The anomalous English collections can perhaps be explained by the transport of horses, as has been suggested for *Poronia erici* by Lohmeyer (1994).

ACKNOWLEDGMENTS

We wish to extend sincere thanks to all those individuals and institutions who helped us with this research, to Thomas Laessøe for his loan of the English dung collections from Kew, to Leif Tibell and others for their help in collecting dung, to Gerry Keating, Brett Robertson and Stan Carstensen for help with the photography, to Alan Hoverd for his assistance in establishing a staining routine for our sections, to John Krug for his suggestions on stimulating ascospore germination, to Jane Standenberge for Γ-irradiation of the dung and to Namboori Raju, David Perkins and others for information on *Podospora* genetics.

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