Pyxidiophora: life histories and arthropod associations of two species

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Based on field studies in New Brunswick and Ontario, two species of the genus *Pyxidiophora* are demonstrated to be of frequent occurrence. *Pyxidiophora* sp. and *Pyxidiophora spinuliformis* have complex life cycles involving anamorph formation and sporulation on a phoretic mite host. *Pyxidiophora* sp., the more common of the two species, appears to be parasitic on the apothecia of coprophilous Pezizales where it forms clusters of synnemata within a week of dung deposition. Later, perithecia develop among the synnemata and produce ascospores. Ascospores attach to mites that are, in turn, carried by beetles and flies to a new substrate. On the new substrate while attached to the mite, ascospores of *Pyxidiophora* sp. differentiate into linearly arranged or complex and often muriform *Thaxteriola* thalli, which produce phialoconidia. The phialoconidia appear to be the propagules that inoculate the new substrate. *Pyxidiophora spinuliformis* has a life cycle similar to that of *Pyxidiophora* sp. but differs in having a conidial anamorph with a different development and ascospores that never form muriform thalli on the phoretic mite host. The taxonomic, ecological, and evolutionary significance of these findings is discussed.

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Des études de terrain conduites en Ontario et au Nouveau-Brunswick sur deux espèces de *Pyxidiophora*, montrent qu'elles y sont fréquentes. *Pyxidiophora* sp. et *Pyxidiophora spinuliformis* ont des cycles vitaux complexes impliquant la formation d'anamorphes et la sporulation sur un hôte acarien porteur. *Pyxidiophora* sp., la plus commune des deux espèces, semble vivre en parasite sur les apothèces de Pézizales coprophiles où elle forme des groupes de synnemata, moins d'une semaine après la déposition des crottes. Plus tard, des périthèces se forment entre les synnemata et produisent des ascospores. Les ascospores s'attachent à des acariens qui sont, à leur tour, transportés sur un nouveau substrat, par des coléoptères et des diptères. Sur le nouveau substrat et toujours attaché à l'acarien, les ascospores du *Pyxidiophora* sp. se différencient en thalles de *Thaxteriola* complexes disposés linéairement et souvent muriformes, lesquels produisent des phialoconidies. Il semple que ces phialoconidies sont les propagules responsables de l'inoculation du nouveau substrat. *Pyxidiophora spinuliformis* montre un cycle vital assez semblable à celui du *Pyxidiophora* sp. mais en diffère par le développement d'un anamorphe conidial distinct et par ses ascospores qui ne forment jamais de thalles muriformes sur l'hôte acarien porteur. Les auteurs discutent la signification taxonomique, écologique et évolutive de ces observations.

[Traduit par la revue]

Introduction

Pyxidiophora Bref. & Tav. is a genus of perithecial ascomycetes poorly known in North America. About 20 species have been described from Europe, northern Africa, and South America; of these species, only *Pyxidiophora kimbroughii* Blackwell & Perry and *Pyxidiophora lundqvistii* Corlett are reported from North America.

Studies of *P. kimbroughii* (Blackwell et al. 1986*a*, 1986*b*) showed a life cycle intimately associated with mites phoretic on the southern pine beetle (*Dendroctonus frontalis* Zimmermann) and other bark beetles for dispersal. Ascospores of *P. kimbroughii* develop directly into a *Thaxteriola* anamorph on the mite integument. Conidia have seldom been seen, and germination has not been observed. Other *Pyxidiophora* ascospores (Majewski and Wiśniewski 1978*a*, 1978*b*; Lundqvist 1980; Blackwell et al. 1989) and a second *Thaxteriola* anamorph (Simpson and Stone 1987) have been reported from mites in bark beetle associations. Species of *Pyxidiophora* are known from a variety of other substrates, including dung, fungi, myxomycetes, and vascular plants. It is possible that most are fungal parasites on the substrates they occupy (Blackwell and Malloch 1989).

Because we have had difficulty in distinguishing species of *Pyxidiophora* using the existing literature and suspected that some species may have been based on immature specimens, we began this study to determine the complete life histories of these fungi. Not only were we concerned with comparing the life cycles of other species of the genus with *P. kimbroughii*, but we were also particularly interested in determining if other species are associated with arthropods for dispersal. The work reported here indicates that knowledge of the complete life cycles of these fungi and an understanding of their biological associations is necessary before a comprehensive monograph of the genus can be prepared.

Methods and materials

Moist chamber and field observations of dung

Moose (*Alces alces* (L.)) dung collected from near Meredith Settlement, N.B., Canada (UTM Grid No. 19TFA3336), and Algonquin Park, Ont., Canada (UTM Grid No. 17TPA9351), was placed in a moist chamber within 2 weeks of collection in August to October 1987. Dung from the Algonquin Park airfield and Eos Lake, Algonquin Park, was watered in the field, collected daily, and placed in a moist chamber within several hours of collection during the period of June 16–24, 1988. All moist chambers were examined initially and observed daily to determine the presence of *Pyxidiophora* species.

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The remainder of the dung heap was left in the field with small amounts collected daily and examined within several hours of collection for comparison with the original moist chambers.

Ascospores extruded to perithecium necks were collected by contact with flame-sterilized cover glass shards. These were incubated on the surface of microscope slides placed over bent glass rods in moist chambers. After conidium production, the glass shards were placed on Leonian's (Malloch 1981), dung, or potato dextrose agar.

Infested mites from dung cultures were killed with paradichlorobenzene and placed on water agar (15 g Bacto agar/L) to observe additional development of ascospores.

Mass spore cultures of anamorphs were made on 1.5% potato dextrose agar (Difco, Detroit, MI) in 9-cm plastic Petri plates. Streptomycin sulfate (Sigma Chemical Co., St. Louis, MO) and chlortetracycline hydrochloride (Sigma) were used as required to eliminate bacterial contamination.

Microscopy

Observations, photographs, and drawings were made with a compound microscope equipped with bright field, phase contrast, and differential interference contrast optics and a drawing-tube attachment. Drawings were made at an original magnification of ×3000. Fungal material was mounted in 0.05% calcafluor (Polysciences, Inc., Warrington, PA) for fluorescence microscopy. Filter excitation was in the ultraviolet range (band pass 340−380)

Fungi and mites were mounted in water or lactic fuchsin. Permanent slides were made by adding glycerine jelly. Developing ascospores and some living cultures were photographed directly on agar under a sterile cover glass to allow continuing development.

Arthropod collection

Arthropods were collected at Algonquin Park (11−23 June 1988) in pitfall traps, each consisting of a sheet-metal funnel with a bait platform near the lip and two nested 8 × 4 cm disposable drinking cups in which the open bottom of the funnel rested. Traps were buried in soil so that the lip of the funnel was flush with the soil surface. Baits included horse and cow dung collected fresh in Baton Rouge, LA (frozen until used), and fresh moose dung collected at the study site. Unbaited traps were used as controls.

Traps were emptied daily, their contents were recorded, and voucher specimens of the beetles and mites were collected and preserved in alcohol or on slides in lactic fuchsin for later identifications. Mites were screened at ×125 with a compound microscope for the presence of fungal spores.

Results

Development in moist chamber and agar cultures

Pyxidiophora sp. (see following section and Discussion for characteristics of this species) and *P. spinuliformis* (Speg.) Lundq. are apparently widespread in eastern Canada on moose dung associated with phoretic mites. They were common at the only two localities we chose for study, New Brunswick and Algonquin Park, Ont.. One specimen of *Pyxidiophora* sp. from horse dung collected at Sable Island, N.S. (unaccessioned, Malloch M109f), was known previously from Canada.

Life cycle of Pyxidiophora *sp.*

Anamorph on dung

Within 5−7 days of deposition of dung, a hyaline synnematous anamorph was apparent both in the freshly field-collected dung and in most chamber cultures. The indeterminate synnemata usually occupied the surface of young apothecia of *Ascobolus* and *Lasiobolus*. They extended 200−300 μm above the surface of the apothecia upon which they developed. Conidia were produced in a wet mass (Fig. 1). The hyphae (2−2.5 μm diam.), which bore conidiogenous cells, were regularly septate throughout their length at early stages or little septate in the region toward the conidiogenous locus in older cells. Conidium production was holoblastic, with percurrent proliferation of conidiophores marked by annellations (Fig. 2). Conidia (6−10 × 2.5−4 μm) were oblong, rounded at the apex, and truncated at the attachment to the conidiogenous cell (Figs. 2, 3). A yeast anamorph with cell division by fission developed in parts of cultures that were several days old.

Conidia from synnemata germinated readily on potato dextrose agar and Leonian's agar soon after they were formed; however, attempts to produce cultures from 1-week-old conidia were seldom successful. The period of conidium viability corresponded to the time when small fungus feeding and predaceous mites were most active on the dung surface. These animals appeared to effect the dispersal within the culture. The anamorph in culture is identical to the anamorph developed from *Thaxteriola* conidia (see following section); the similarity between the anamorphs obtained from the synnemata and from ascospores forms the basis for our belief that the synnemata are a part of the life cycle of *Pyxidiophora* sp.

Perithecia, asci, and ascospores

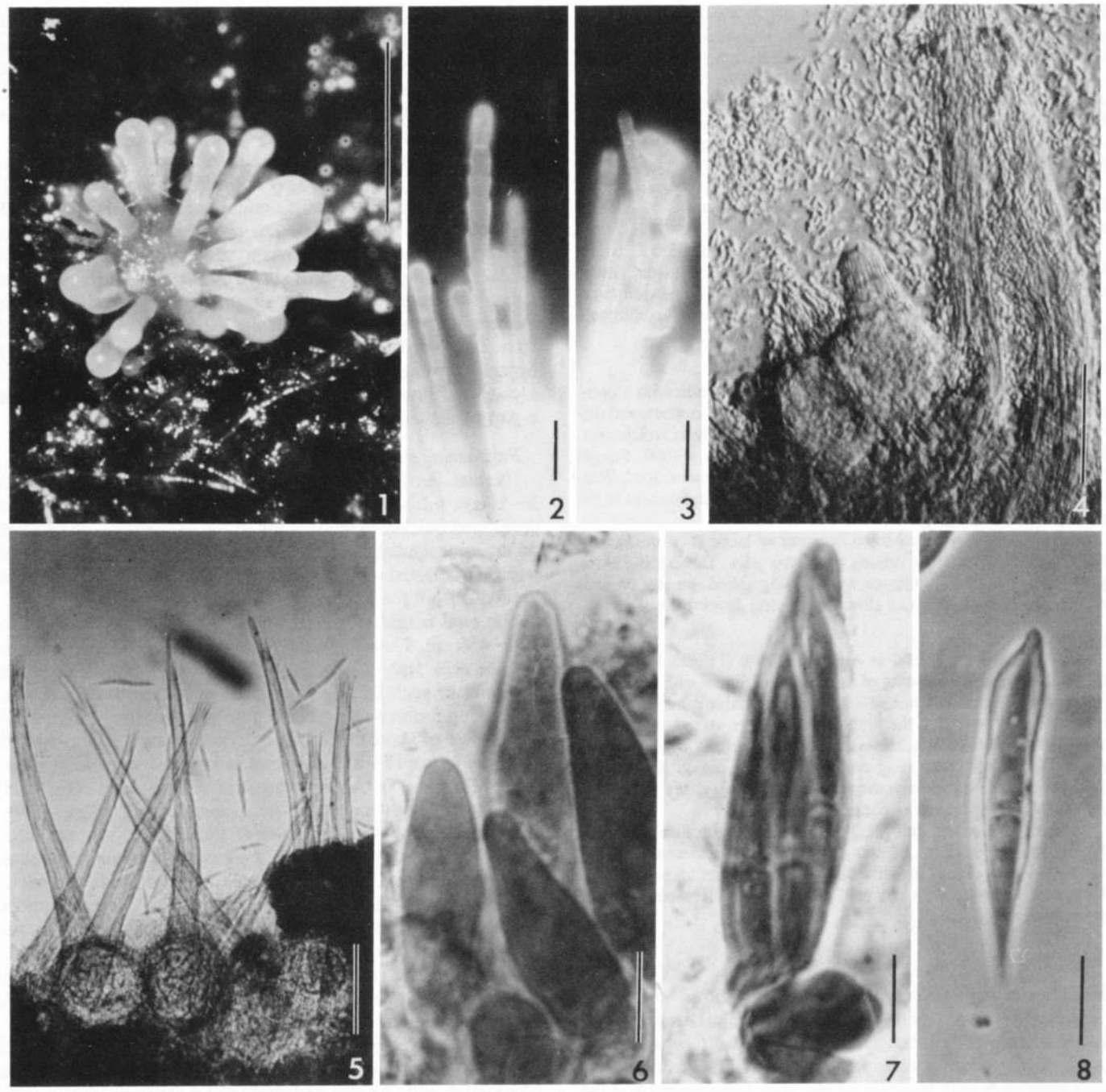
Hyaline perithecia of *Pyxidiophora* sp. always appeared 3−5 days following the appearance of synnemata. Perithecia developed quickly in the vicinity of the synnemata, sometimes on the same apothecia (Fig. 4), occasionally on bare dung, and were not detected until the necks of groups of 3−20 perithecia grew out from the apothecia (Fig. 5).

The total height of the perithecia of *Pyxidiophora* sp. was 200−450 μm. The subglobose base was 58−83 × 53−66 μm, and the neck 180−225 × 10−13 μm, with width taken at the midpoint of neck length. Neck cells were 9−40 × 3−6 μm. The young fimbriate tip tapered gradually but flared somewhat after onset of spore release (Fig. 5). The asci were 3-spored and 36−45 × 17−25 μm when ascospores were fully elongated (Figs. 6, 7). Ascospores after release were 33−48 × 2.5−4.5 μm, not including the expanded wall (Figs. 8, 9). Most ascospores were 1-septate at this time. Asci and ascospores matured sequentially. The ascospores of a single ascus were extruded as a group to the apex of the perithecium neck, usually before extrusion of the next ascus. Ascospores without a darkened attachment region at the distal end by time of extrusion developed one within 48 h. Extruded spores, which developed toward the end of ascospore production, were usually darkened when they reached the perithecium tip (Fig. 9). A single perithecium produced ascospores for 4−5 days.

Attachment-region development occurred gradually, marked by the appearance of a subterminal tan pad at one side of the blunt spore end. As this pad darkened a separate, dark horseshoe-shaped rim formed around it with the open side away from the spore apex. At its ultimate development, a large, very dark structure occupied about two thirds of the ascospore apex on the side of attachment to the arthropod (Fig. 10). Complete development was not necessary for attachment, since spores in all stages, including completely hyaline, were sometimes found on the integument of mites.

Ascospores and anamorph thalli on mites

The ascospores first seen on mites soon after perithecium development in moist chamber cultures were 0- to 1-septate with a hyaline to darkened attachment region (Fig. 10). Additional development was observed in dung cultures when mites displaying phoretic readiness behavior (those mites attaching to any moving objects introduced into the cultures) were transferred to fresh dung or held longer in the original moist chambers (Fig. 11). This corresponds to the condition of the

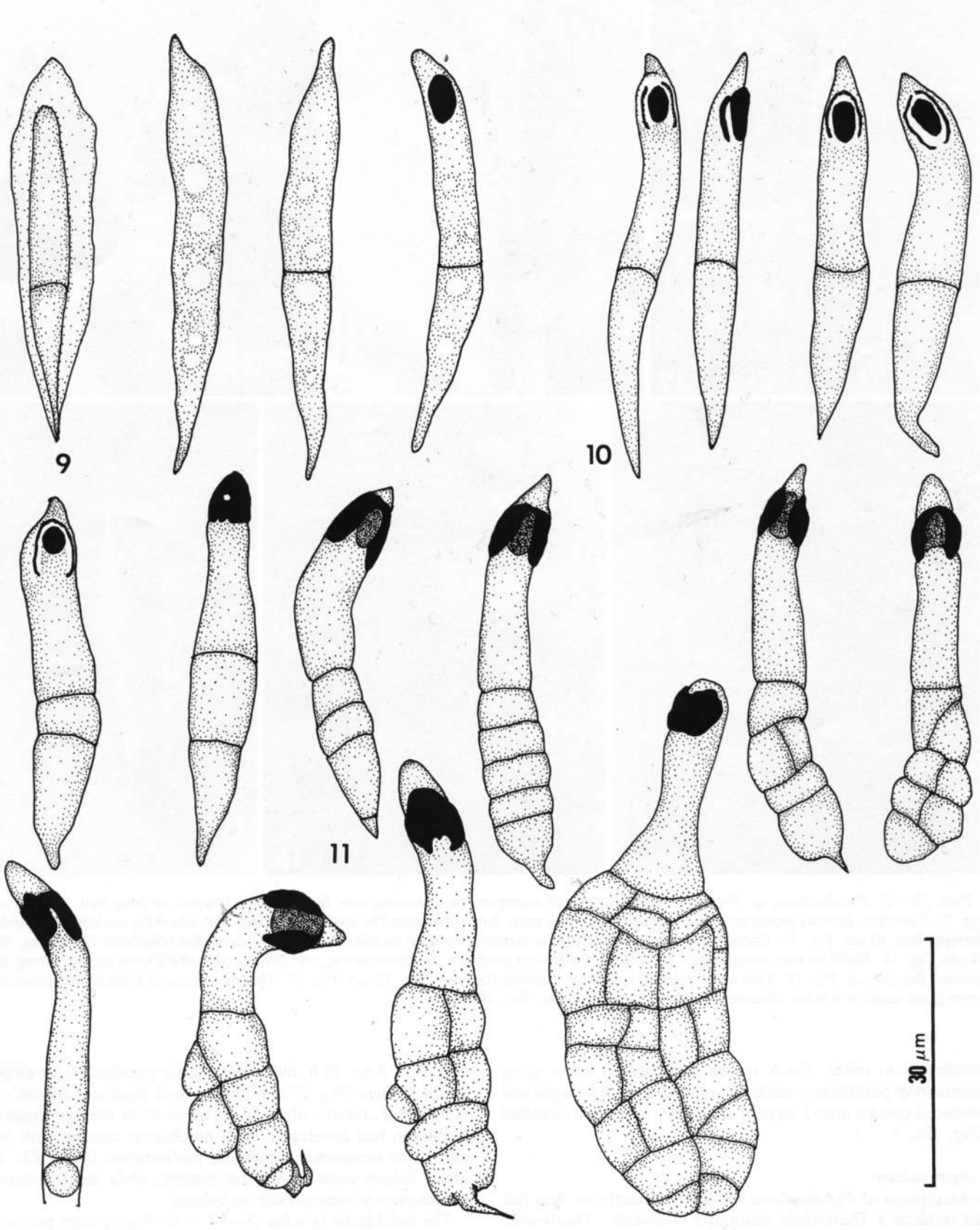


FIGS. 1–8. *Pyxidiophora* sp. Fig. 1. Synemmata developed on an apothecium in dung moist chamber. Bar, 1 mm. Figs. 2, 3. Holoblastic conidia produced on percurrently proliferating conidiophores. Calcafluor. Bar, 10 μm. Fig. 4. Perithecia before neck elongation; associated with synemmata on an apothecium. Bar, 50 μm. Fig. 5. Perithecia after onset of ascospore formation. Bar, 60 μm. Fig. 6. Asci before ascospore formation. Bar, 15 μm. Fig. 7. Ascus after ascospore formation. The three ascospores are 1-septate and have expanded walls by this time. Bar, 15 μm. Fig. 8. Immature ascospore after release from ascus. Bar, 15 μm.

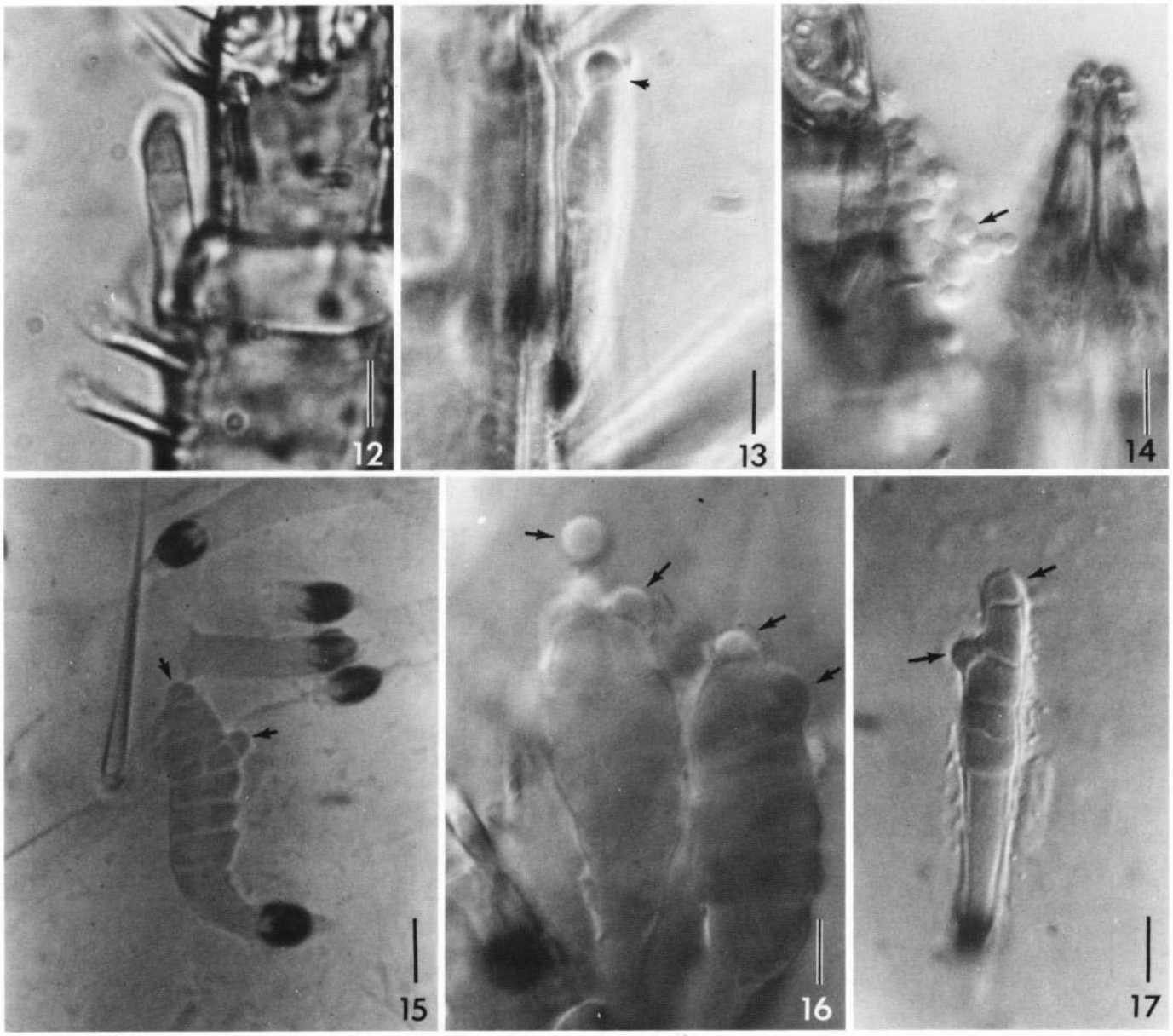
ascospores on trapped phoretic mites that were 2-septate at time of dispersal (Fig. 12).

Development of thalli from ascospores on mites in 2–3 days was marked by formation of additional septa and early swelling of the cells. Spores became linearly 3- to 7-septate by formation of transverse septa in the unattached (distal) spore cell (Figs. 13–15). Conidia formed both terminally and laterally and remained in a mass near the site of formation unless disturbed (Figs. 13–16). Conidium formation observed with a fluorescence microscope appeared to be phialidic with ring-building wall formation. The attached cell of the ascospore usually did not divide and remained as a stalk. More complex thalli of up to 40 cells often formed on unsclerotized parts of the mite integument (Figs. 11, 15, 16). The thalli became muriform by division of cells perpendicular and obliquely to the septa in the original unattached ascospore cell. Conidia were produced both terminally and laterally in these thalli (Fig. 16).

Continued development of ascospores from the 1-septate stage to a *Thaxteriola* anamorph was not dependent upon



FIGS. 9–11. *Pyxidiophora* sp. Fig. 9. Four ascospores arranged in order of maturity. Occasionally nonseptate ascospores are present. Fig. 10. Soon after attachment, most ascospores are 1- to 2-septate and have a darkened, more elaborate attachment region than earlier ascospores. Fig. 11. Ten *Thaxteriola* anamorph thalli developed from ascospores within 3 days of transferring mites to new dung substrates. Conidium formation begins at this time.



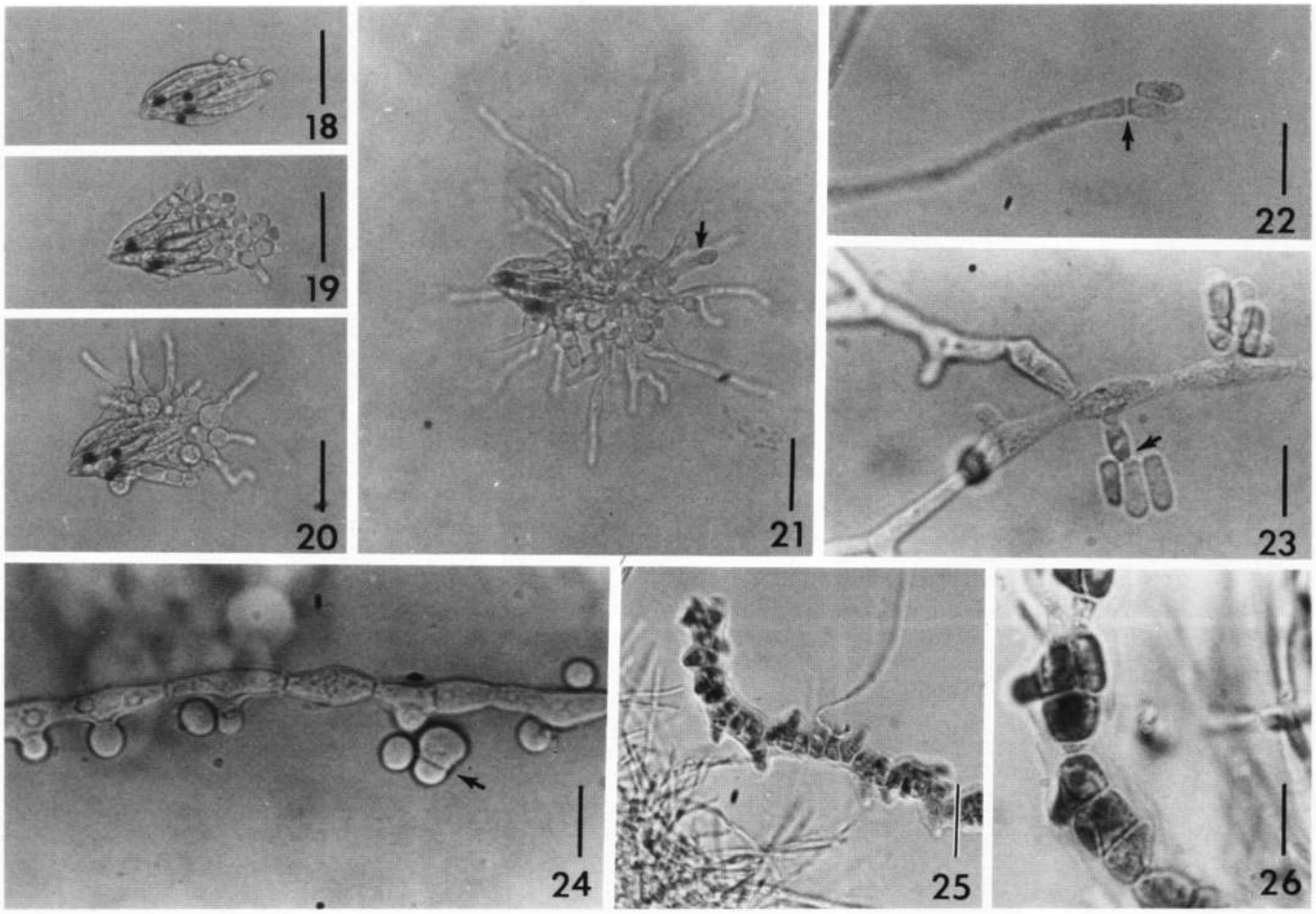
FIGS. 12–17. *Pyxidiophora* sp. Fig. 12. Dispersal stage of ascospore on a phoretic mite from a beetle trapped on dung bait. Bar, 10 μm. Fig. 13. Conidium (arrow) produced by a 2-septate thallus on a mite. Arrow indicates the broken ascospore wall where the conidium has broken through. Bar, 10 μm. Fig. 14. Conidia and yeast cell derivatives (arrow) produced on mite integument 3 days after transfer to fresh dung. Bar, 14 μm. Fig. 15. Thalli on mite integument. A muriform thallus has produced conidia (arrows), and several other thalli have broken during mite activity. Bar, 14 μm. Fig. 16. Two muriform thalli producing conidia (arrows). Bar, 10 μm. Fig. 17. Thallus developed from an ascospore on a cover glass shard in a moist chamber. Arrows indicate conidia. Bar, 10 μm.

attachment to mites. Fresh spores collected on cover glass shards from perithecium necks developed up to five septa and produced conidia after 3 days of incubation in a moist chamber (Fig. 17).

Agar culture

Ascospores of *Pyxidiophora* sp. placed directly on agar did not produce a *Thaxteriola* anamorph. However, *Thaxteriola* anamorphs did develop on cover glasses in a moist chamber. Anamorphs and the conidia they produced were placed on agar (Fig. 18). Within 15 h, conidia divided to produce a fission yeast anamorph, and some of the yeast cells had produced germ tubes (Fig. 19). Most had formed germ tubes within 30 h (Fig. 20). After 52 h, ovoid holoblastic conidia had developed on the hyphae (Fig. 21). Within several days on Leonian's or dung agar, cultures identical to those from the synnematous anamorph had developed; both holoblastic conidia with percurrent or occasional sympoidal proliferation (Figs. 22, 23) and a fission yeast stage were present. Only mononematous conidiophores were present in culture.

The holoblastic conidia (5–11 × 2–4 μm) were produced directly at the tips of certain hyphae, which in turn had developed from germ tubes of yeast cells produced by conidia of the *Thaxteriola* anamorph. Conidia developed from nonseptate lateral branches of hyphae after several days in culture. Proliferation was mostly percurrent, and conidiogenous loci were



FIGS. 18–26. *Pyxidiophora* sp. Fig. 18. Ascospores that germinated on a cover glass shard and produced conidia. Bar, 35 μm. Fig. 19. Same group of ascospores after 24 h on agar. Yeast cells derived from conidia have begun germ tube formation. Bar, 35 μm. Fig. 20. Within 30 h, most yeast cells formed germ tubes. Bar, 35 μm. Fig. 21. Within 52 h, holoblastic conidium formation (arrow) began. Bar, 35 μm. Figs. 22, 23. Holoblastic conidia (arrows) formed on mycelium in agar culture. Bar, 10 μm. Fig. 24. Yeast cells produced on mycelium in agar culture. Yeasts divide by fission (arrow). Bar, 10 μm. Fig. 25. Area of mycelium with transverse, longitudinal, and oblique cell divisions. Bar, 35 μm. Fig. 26. Cells similar to those in Fig. 25. Bar, 10 μm.

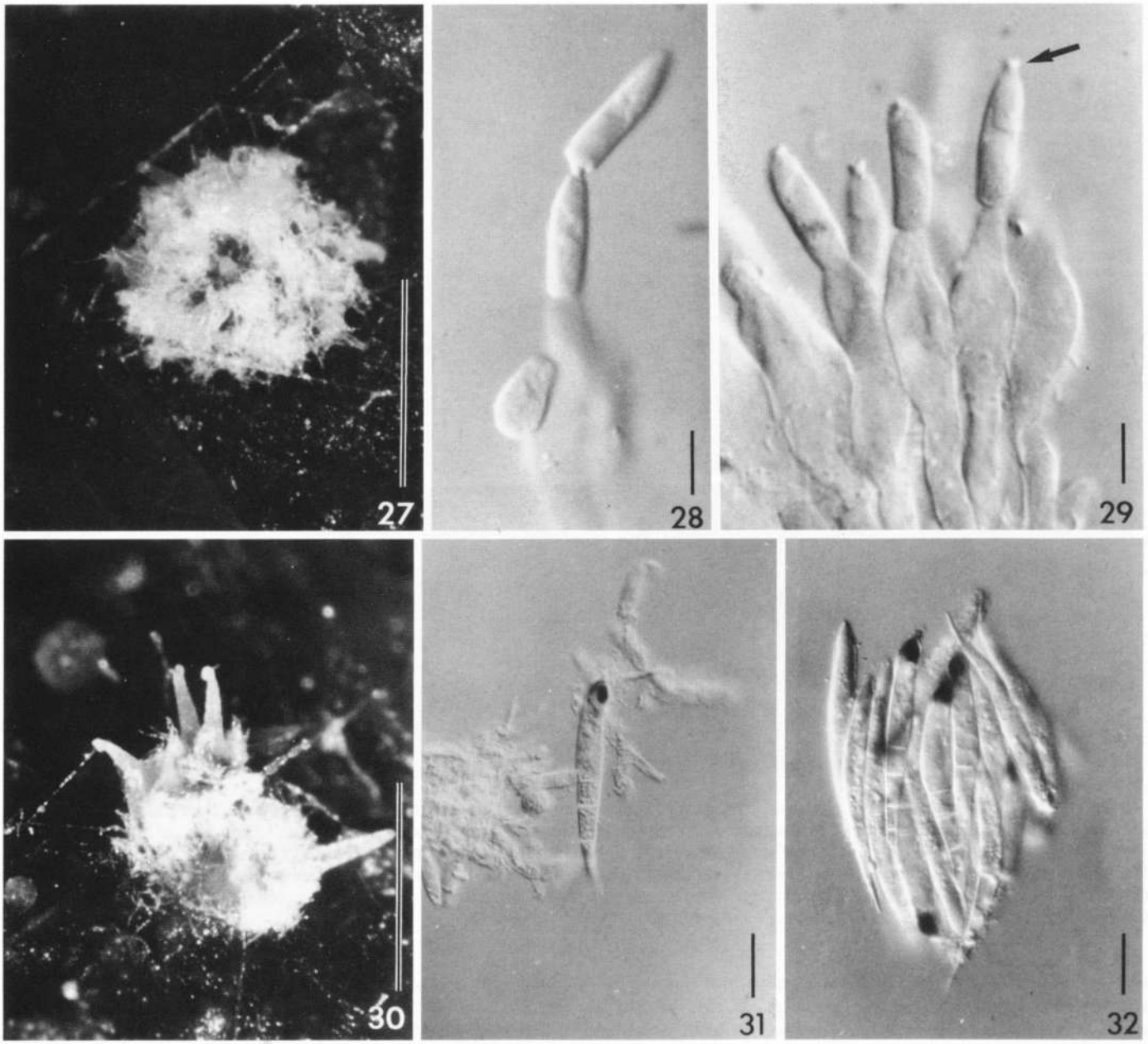
marked by annellations fluorescent with calcafluor staining, as seen in the synnematous anamorph (Figs. 2, 3). Within a week, areas of the culture were observed to have irregular bulging cells, presumably pseudohyphae, that produced yeast cells (4–8 μm diam.). Yeast cells divided by fission (Fig. 24). After several weeks, complex development of these pseudohyphae included extensive areas with transverse and oblique cell divisions similar in appearance to the development of the *Thaxteriola* anamorph from ascospores (Figs. 25, 26).

Arthropod associations

There were no differences in beetle species attracted to horse, cow, or moose dung at four sites in Algonquin Park. Beetles were rarely found in unbaited traps. After 1 week, trapping was discontinued at three sites because of dry field conditions and absence of fresh dung to provide inoculum. Trapping efforts were concentrated on the wettest site at Eos Lake (Hwy 60, km 44.15) during the 2nd week, and fresh moose dung discovered at the site was watered daily to ensure fungus development. About 200 gamasid mites belonging to several species were collected from beetles or running free in traps. Unattached mites were species known to be phoretic and are thought to have left the beetles upon arrival at the traps. One mite, a macrochelid, in a trap containing a single histerid beetle bore one ascospore of *Pyxidiophora* sp. (Fig. 12). The dispersed spore had two septa and was swollen but had not produced conidia.

During the time of earliest production of conidia by the synemmatous anamorph, fungus-feeding mites grazed on the synemmata, and they and the immature predaceous gamasids running through the cultures became covered with conidia. The conidia adhered to mite surfaces but were easily detached. Nematodes present in cultures probably serve as food for gamasids but also may disperse synnematous conidia locally.

Parasitid mites in moist chamber cultures displayed phoretic readiness behavior about 1–2 days after *Pyxidiophora* sp. began ascospore release. Infested mites transferred to new dung at this time lived about 4 more days. After 2 days the mites no longer had ascospores on their integument. Parasitid mites usually are phoretic as deutonymphs and undergo another molt on the next dung heap. The fate of ascospores on molted integuments is unknown. Macrochelid mites in the same moist chamber were ready to attach to beetles 2–3 days after onset of ascospore release. They had much larger numbers of ascospores than did the parasitid mites (121–433 versus 0–12 per mite). The macrochelid mites are phoretic as



FIGS. 27–32. *Pyxidiophora spinuliformis*. Fig. 27. Anamorph on dome-shaped mass that was associated with *P. spinuliformis*. The tip of an emerging perithecium is visible at the center. Bar, 1 mm. Figs. 28, 29. Conidia of anamorph produced in short basipetal chains from flask-shaped conidiophores. Flared appendages (arrow) remain attached to distal ends of the conidia. Bar, 5 μm. Fig. 30. Same anamorph conidioma shown in Fig. 27 after 24 h. Perithecium necks have elongated. Bar, 1 mm. Figs. 31, 32. Ascospores naturally extruded from perithecia. Most ascospores are 2- to 3-septate, and many have darkened attachment regions. Bar, 15 μm.

adults and do not undergo additional molts. They were found in new dung up to 1 week later with conidium-producing thalli developed from ascospores.

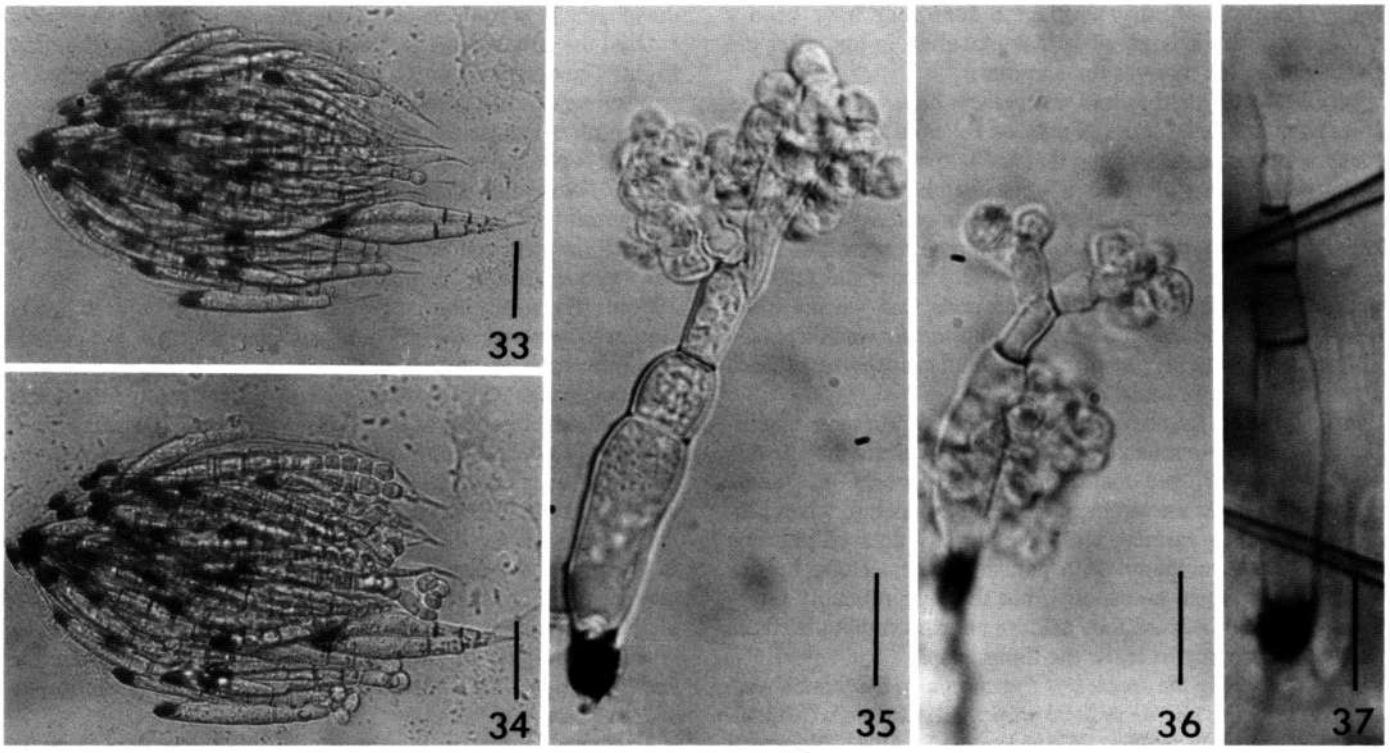
Although we have concentrated our study on phoretic mites associated with beetles because beetles are easy to trap and handle, infested mites were phoretic on flies in the dung moist chambers. The mites probably have a variety of insects with which they may be associated.

Life cycle of Pyxidiophora spinuliformis

Twelve to 15 days after the appearance of *Pyxidiophora* sp. synnemata, *P. spinuliformis* was found in the moist chamber cultures, usually producing perithecia in association with a conidial fungus, its presumptive anamorph, but sometimes directly on the dung. Perithecia of *P. spinuliformis* and *Pyxidiophora* sp. are difficult to distinguish under a dissecting microscope. Differences in substrate, anamorph, and time of perithecium development give clues to identity; however, it is necessary to rely on ascospore measurements and early septation to distinguish the two species.

Presumptive anamorph on dung

The presumptive anamorph developed on a dome-shaped mass (Fig. 27). It has not been possible to determine if the mass is a stroma or a parasitized young apothecium. Conidia (9.8–16.1 × 3.0–4.8 μm) were produced in basipetal chains at the tip of elongated or flask-shaped conidiogenus cells of variable length (Figs. 28, 29). Each conidium had a noncellu-



FIGS. 33–37. *Pyxidiophora spinuliformis*. Fig. 33. A cluster of ascospores and *Thaxteriola* anamorphs that began conidium formation after 3 days on cover glass shards in a moist chamber. Septa of some thalli have darkened. Bar, 15 μm. Fig. 34. Same ascospores and thalli as in Fig. 33 shown 24 h later on agar. More conidia have formed. Bar, 15 μm. Figs. 35, 36. After 6 weeks on agar, some thalli formed elongated protoplasts with spherical protrusions. Cells next to attachment end are noticeably swollen. Bar, 13 μm. Fig. 37. Ascospore attached to a phoretic mite from a beetle in a dung-baited trap. Septa were darkened, but conidium production had not begun. Bar, 13 μm.

lar, flared appendage at the distal end which was easily detached but was usually present on at least a few conidia in water mounts. Conidia did not germinate on Leonian's, dung, or potato dextrose agar. The anamorph was almost completely obliterated within 3–4 days of formation by development of perithecia through the conidiophores (Fig. 30) and by mite grazing.

Perithecia, asci, and ascospores

The perithecia of *P. spinuliformis* were 262–550 μm in total height at the time of ascospore production. The perithecium neck was barely delimited from the base but was about 180–355 × 16–33 μm, with width measured at the midpoint of the length. Neck cells were 15–80 × 3–6 μm, usually quite long. The basal part of the perithecium was 49–125 μm in diameter. Asci were fusoid, with three ascospores per ascus. Extrusion of ascospores was continuous, with the perithecium neck becoming filled with spores. Ascospores were 57–62 μm long before development of the darkened attachment region, 58–68 μm with darkened region present (Figs. 31, 32).

After 2 days of incubation on cover slips in moist chambers, naturally extruded ascospores had two to three often darkened septa; most had developed darkened attachment regions, and some had produced conidia (Fig. 33). Cell swelling was not observed initially. After 3 days in a moist chamber, 5–15 spherical conidia appeared to have been produced at the ascospore tip (Fig. 34). However, 6 weeks after they were placed on agar, some of these anamorphs were examined at ×1000. What appeared to have been conidia in some cases were acutally spherical protrusions of the elongated spore protoplast at the spore apex. The elongated protoplast was sometimes branched and bore up to 20 spherical protrusions 5–8 μm in diameter. The spherical bodies were not easily detached. Swelling of the attached cell and the one adjacent to it was often evident (Figs. 35, 36). No additional development occurred in 10 weeks.

Arthropod associations

Two or three ascospores of *P. spinuliformis* were found on each of a parasitid and two macrochelid mites phoretic on a large trapped scarabaeid beetle (*Geotrupes splendidus* (F.). Each spore had three septa, with the two distal septa darkened (Fig. 37).

Discussion

Arthropod associations

Dung from single depositions at the Eos Lake site left partly in the field and partly incubated in the laboratory revealed a remarkable synchrony in organismal succession during the first 10 days of our observations. The precisely timed key events were (*i*) early appearance of apothecia of several species of discomycetes and presence of nematodes, fungus-feeding mites, and young gamasid mites; (*ii*) the appearance and spread of the synnematous anamorph of *Pyxidiophora* sp., probably because of small mite activity; (*iii*) maturation and renewed activity by the gamasid mites at the time of *Pyxidiophora* sp. ascospore production; and (*iv*) phoretic readiness behavior of mites several days after onset of ascospore production and when dung beetles were ready to move to new dung. *Pyxidiophora* sp. was present more frequently in dung collected between August and October 1987 than it was in June

1988. The unusually dry weather in June may have been a factor; however, it is possible that inoculum is low after the winter and builds up over the summer.

Pyxidiophora spinuliformis was present both years at Algonquin Park, although observed only on mites in 1987. In 1988, this species was found in dung that had laid in the field for over a week before being placed in a moist chamber. It is not known if the later appearance of *P. spinuliformis* on dung was due to later dispersal to the substrate or a longer period of time required for development. We know less about the relationships of *P. spinuliformis* with arthropods that we do about those of *Pyxidiophora* sp., but *P. spinuliformis* was dispersed to fresh dung baits on phoretic mites, and ascospores adhered to gamasid mites in moist chambers.

Differences in numbers of attached ascospores on macrochelid and parasitid mites in moist chamber culture may be due to size, behavior, and maturation differences of the mites. Macrochelid mites are larger, have a higher posture when running on the dung surface, and have slightly delayed phoretic behavior when compared with parasitid mites. Dung beetles may also bear some ascospores, but they are probably rarely heavily infested because they seldom appear on dung surfaces until they are ready to leave the dung heap.

Previously, only the species of *Pyxidiophora* associated with bark beetles were known to have arthropod dispersers (Majewski and Wiśniewski 1978*a*, 1978*b*; Lundqvist 1980; Blackwell et al. 1986*a*, 1986*b*, 1989; Simpson and Stone 1987). However, Spegazzini (1918) described *Copranophilus spinuliformis* (now *P. spinuliformis* (Speg.) Lundqvist) from dung-beetle tunnels and *Thaxteriola subhyalina* Speg. (1918) (almost certainly not an anamorph of *P. spinuliformis*) from dung beetles in Argentina. Our study suggests that many species of *Pyxidiophora* in different habitats may have common associations with beetles and their phoretic mites.

Mites appear to be more efficient dispersers within the arthropod assemblage than other organisms because of their numbers and behavior. While only 1–20 beetles may invade a dung heap on a single day, some may carry up to several hundred mites. As mentioned above, mites are far more active on the dung surface than are beetles and almost certainly contact ascospores even when inoculum is low.

The large numbers of ascospores observed on mites from dung in moist chambers may be artificially high due to the larger number of perithecia produced under more moist conditions than under usually dryer field conditions. The few sparse North American collections of species of *Pyxidiophora* made in the field would seem to support this assumption. Also, containment of the mites promotes prolonged contact with ascospores. On occasion, mites from bark beetle habitats bore several hundred ascospores; this may be a natural condition in that confined habitat (Blackwell et al. 1989).

Anamorphs

Until this report, only *P. kimbroughii* from bark beetle associations was known to produce a *Thaxteriola* anamorph (Blackwell et al. 1986*a*, 1986*b*). Conidia were rarely observed in *P. kimbroughii* (Blackwell et al. 1986*a*, 1986*b*) and then only at the tip of linearly arranged thalli. The late development observed in *Pyxidiophora* sp. and *P. spinuliformis* anamorphs only after dispersal may help to explain the rarity of conidia in bark beetle associated species. *Pyxidiophora*-type ascospores have been observed in almost all cases on mites phoretic on beetles recently emerged from logs and trees. Additional development would be advantageous only when the mites entered the new habitat, not when conidia might be lost during flight. The ease with which mites on dung can be transferred to new substrates has enabled us to determine the time of direct anamorph development from ascospores; this was not possible in bark beetle habitats.

Five *Thaxteriola* anamorphs have been described previously (Spegazzini 1918; Thaxter 1920; Majewski and Wiśniewski 1978*a*; Simpson and Stone 1987); only that of *P. kimbroughii* has been linked to a telemorph (Blackwell et al. 1986*a*; 1986*b*). The anamorph of *P. spinuliformis* is morphologically similar to these species but varies somewhat in size and coloration from each of the five and, perhaps, in its potential to produce an elongated protoplast with spherical protrusions. The potential development of the *Pyxidiophora* sp. anamorph from a simple, few-celled, linearly arranged thallus to a muriform thallus of over 40 cells was unexpected. Both Thaxter (1920) and Spegazzini (1918) may have observed such forms; Spegazzini's drawings are difficult to interpret, but species described in the genera *Myriopodophila* Speg. and *Entomocosma* Speg. may be thalli similar to those we have found. Until the complete development of other *Pyxidiophora* anamorphs and the types of *Thaxteriola* species are studied, we choose to use the name *Thaxteriola* for the anamorphs of both *P. spinuliformis* and *Pyxidiophora* sp.

The production of the muriform thalli on less heavily sclerotized mite integuments may be due to earlier attachment of ascospores in these regions. However, differences in thallus development of some species of Laboulbeniales are known to be dependent upon location on the insect cuticle (see Tavares 1985). This supposes that nutrient exchange may occur from the mite to some of the fungal thalli. Anamorphs developed on cover glass shards never produced more than six cells before conidium production. We are currently investigating the possibility of haustorium formation in the more complex thalli.

When they were first observed early in this study, the muriform thalli were assumed to be immature individuals of a species of Laboulbeniales known to occur on mites. However, careful daily observations of development of the ascospores on mites and on cover glasses precludes this possibility. Blackwell and Malloch (1989) proposed a relationship between the Laboulbeniales and *Pyxidiophora*. The existence of ascospores of *Pyxidiophora* sp., with the potential to form a muriform thallus more complex than previously described species of *Thaxteriola*, strengthens the argument.

The elongated, branched apex of the *P. spinuliformis* anamorph protoplast with sphaerical protrusions bears at least a superficial similarity to trichogynes of some members of the Laboulbeniales (Tavares 1985; Blackwell 1980). However, the function of this structure is unknown in the life cycle of *P. spinuliformis*, and not all anamorphs produce this structure. The darkened septations of the anamorph also resemble those found in some Laboulbeniales.

Mycelial anamorphs have been reported previously for several species of *Pyxidiophora*. *Chalara* anamorphs (see Lundqvist 1980; Blackwell and Malloch 1989), and holoblastic conidia produced from denticles on conidiophores arising from the perithecium of *Pyxidiophora spinulo-rostrata* Webst. & Hawks. (Webster and Hawksworth 1986) are known. The presence of *Chalara* anamorphs, in fact, was considered significant enough by von Arx and van der Walt (1987) to warrant enlargement of the family Pyxidiophoraceae to include *Ceratocystis* Ell. & Halsted and *Cryptendoxyla* Malloch & Cain.

These anamorphs, as well as the yeasts and holoblastic conidia with percurrent and sympodial proliferation, produced by *Pyxidiophora* sp. broaden the range of anamorphs known in the genus but do not help to confirm the placement of the Pyxidiophoraceae in the Hypocreales (Arnold 1971). Neither yeast nor annellide-proliferating anamorphs are known in the Hypocreales (Samuels and Seifert 1987). It is interesting that von Arx and van der Walt (1987) included *Pyxidiophora* in the Ophiostomatales. Several of the anamorphs (*Chalara* and the holoblastic conidia with percurrent or sympodial proliferation) discussed herein are known to be produced by members of this order. However, Blackwell and Malloch (1989) reviewed the possibility that *Pyxidiophora* belongs in the Ophiostomatales and remain sceptical.

The yeast anamorph of *Pyxidiophora* sp. is unusual. Division by fission is known only in the few species of the ascomycetous yeast *Schizosaccharomyces* Lindner (Kreger-Van Rij 1984). Discovery of similar yeast anamorphs in other species of *Pyxidiophora* may provide an important phylogenetic character.

The presumptive mycelial anamorph of *P. spinuliformis* that we observed on dung is probably an anamorph discussed by Lundqvist (1980). The anamorph associated with *Ascolanthus trisporus* Caill. (suggested as being conspecific with *P. spinuliformis* (Lundqvist 1980)) has distinctive holoblastic conidia with deciduous connectives (Lundqvist 1980). Lundqvist pointed out that the anamorph has been found on badger dung when no perithecia were detected. If this is the anamorph of *P. spinuliformis*, it is possible that perithecium development had not yet occurred. In our experience, perithecia appeared several days after the anamorph. It is also possible that the species could be heterothallic and one mating type was absent.

The synnematous anamorph of *Pyxidiophora* sp. is difficult to identify but seems to be assignable to the genus *Graphium* Corda. *Graphium*-like anamorphs lacking dark pigmentation have been referred to *Graphilbum* Upadhyay & Kendrick, but these may not differ significantly from species of *Graphium* (K. Seiffert, personal communication).

Teleomorphs

About 15 species of *Pyxidiophora* and *Mycorhynchus* were listed by Lundqvist (1980). Five species have been described since that time (Barrasa and Moreno 1982, 1983; Corlett 1986; Webster and Hawksworth 1986; Blackwell et al. 1986*b*). Lundqvist (1980) suggested that several species he considered might have been described from immature specimens. Based upon our observations of at least nine dung-, wood-, and fungus-inhabiting species, including the two discussed here, we concur. The late complete development of ascospores, absence of information on anamorphs (both those developed directly from ascospores and mycelial anamorphs), and incomplete knowledge of arthropod associations make identification of species difficult. These problems are compounded by the difficulty involved in culturing most species of *Pyxidiophora.*

Of the known species of the genus, only *Pyxidiophora microspora* (Hawks. & Webst.) Lundq., *Pyxidiophora brunneo-capitata* (Hawks. & Webst.) Webst. & Hawks., and *Pyxidiophora caulispora* (Hawks. & Webst.) Lundq. have ascospores in the size range of the species we refer to as *Pyxidiophora* sp. We have examined the type specimens of the three species but cannot be certain if our specimens are conspecific with any of them. Until developmental studies are available for additional isolates with ascospores in the same size range, we do not feel justified in assigning a name to our specimens.

Pyxidiophora spinuliformis from Canada matches the published information on this species. Lundqvist (1980) discussed the taxonomic and nomenclatorial problems of *P. spinuliformis.* Spegazzini (1918) described *Copronophilus spinuliformis* as having completely hyaline ascospores. Lundqvist (1980) pointed out that the species is probably conspecific with *A. trisporus* and *Mycorhynchus subspinuliformis* Breton & Faurel. Of the three taxa, only *M. subspinuliformis* was described as having darkened spore apices and 2- to 3-septate ascospores. During our observations, sometimes at least 75 spores accumulated over several days before darkening of the attachment region occurred. Ascospores are 2- to 3- septate when they are released, an unusual character in *Pyxidiophora*.

We have been conservative taxonomically in the placing of the two Canadian species of *Pyxidiophora*. Many species of *Pyxidiophora* are known only from the type collections, and intraspecific variation and developmental aspects of the entire life cycle are not well understood. Some differences in perithecium development, particularly size, depending upon consistency of dung, moisture content of the moist chamber, and presence and stage of development of associated fungi have been observed and indicate that variation is to be expected.

The geographical and substrate range of *P. spinuliformis* is extended; however, this is not unexpected. Many genera and species of mammals have circumpolar distributions. The moose (*A. alces*), known as elk in Europe, with seven subspecies, is native to northern North America, Europe, and Asia (Nowak and Paradiso 1983; Franzmann 1981). Other broad distributions may be due to man's introduction of horses and other livestock throughout the world. Dung beetles and, probably, their phoretic mites and fungi have been introduced to many parts of the world, including Canada (Chagnon and Robert 1962; Ritcher 1966).

Because dung beetles have little dung substrate specificity, the occurrence of species of *Pyxidiophora* on different kinds of dung is of little use as a taxonomic character. The beetles do not distinguish between herbivore and carnivore dung. This is probably true also for fly vectors. Previously observed distribution patterns of gut-passage fungi on dung do not apply to *Pyxidiophora*, since gut-passage conditions do not influence germination and growth of these usually arthropod-dispersed species.

Some dung beetles, flies, and mites are not strictly specific for dung. The possibility exists that widely different substrates (e.g., dung and dead plant material) may not be important as a species difference. Substrate characters should be used with caution until the biology of the dispersal organisms is understood. In addition, the possibility of substrate-mediated differences in development, although presently unknown, must be considered, especially with morphologically similar forms.

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