ZOOSPORIC FUNGI FROM SOILS OF NEW SOUTH WALES

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Abstract

Despite our knowledge of the unique flora and fauna of Australia, much less is known about fungal diversity. Eighty-six species of chytrid fungi (Chytridiomycota) have been reported from soils of Australia. The primary purpose of this study was to assess the biodiversity of chytrids in four distinct vegetation types in central eastern New South Wales: subtropical rainforest, wet sclerophyll forest, dry sclerophyll forest and open heath. Attention was focused especially on newly observed species, new records of taxa in Australia, and morphological variation of known taxa. A second objective was to assess species richness and diversity of chytrids within the four habitats. Water cultures of 227 soil samples from 14 collection sites were baited with cellulose, chitin, keratin, and pollen substrates. The substrates were examined microscopically for the presence of chytrids, and 38 taxa were observed. Evaluation of species diversity among the major collection sites used a presence or absence recording technique, and indicated that the greatest number of species occurred in dry sclerophyll forest, while the least number of species occurred in the open heath habitat. Across all habitats studied, a few chytrid species were common while most were scarce to rare. Many of the 17 species recorded for the first time in Australia also are considered to be pandemic in distribution. Eight taxa were observed for the first time and were assigned provisional generic affiliation. and may be endemic to Australia. This study serves as a baseline for evaluation of chytrid biodiversity and distribution in additional natural and disturbed habitats of Australia.

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Introduction

The uniqueness of the flora and fauna of Australia is well established (Beard 1969, Specht 1970, Walker 1996). However, the diversity of fungi in Australia is understood poorly, especially the chytrid fungi (May & Pascoe 1996). The recent appearance of a chytrid responsible for an emerging infectious disease associated with global population declines and mortality of frogs (Berger *et al.* 1998, 1999, Morehouse *et al.* 2003) has brought attention to the need to learn more about the global diversity of chytrids.

The number and range of habitats that have been sampled so far in Australia have been limited and not systematically selected. For example, only one study has explored aquatic habitats (Crooks 1937). Harder & Gallwitz-Uebelmesser (1959) were the first to report soil chytrids from Australia, and further representation of chytrids from terrestrial habitats has been reported (Jeffrey & Willoughby 1964, Karling 1988, Willoughby 1965).

Crooks (1937) investigated the Blastocladiales (Chytridiomycota) from several sites in Victoria, and observed nine species, only one of which had previously been described from Australia. Her study did not include members of other orders of Chytridiomycota, such as Chytridiales, *sensu* Sparrow (1960), chytrids that had been investigated more thoroughly and were known better in America and Europe.

Harder & Gallwitz-Uebelmesser (1959) used a variety of baits (straw, cellophane, linseed and hempseed, insect parts and pollen) with 34 soil samples from across Australia and observed 35 species of Chytridiales. Several species of *Rhizophydium*, *Phlyctochytrium* and *Rhizophlyctis* that were obtained in their study subsequently occurred in Willoughby's (1965) investigation. Additionally, they charted the per cent occurrence of the various taxa observed in their soil samples.

Jeffrey & Willoughby (1964) studied the distribution of *Allomyces* (Blastocladiales) from a variety of soil groups, and concluded that the taxon could be considered a valid soil inhabitant. Willoughby (1965) investigated soils primarily from Victoria for members of the Chytridiales, for comparison with distribution of chytrids from America and Europe. Using cellulose, chitin and keratin as baits, he obtained 34 species, and found evidence of chytrid distribution patterns related to soil conditions such as type of soil, pH, and cultivation/fertilization regimes. Karling (1988) amplified Willoughby's (1965) inventory by examining 49 soil samples from 13 collection sites across Australia, and identified 15 species of Chytridiales and Blastocladiales.

The purpose of this study was to begin to characterize the chytrid biodiversity in soils of Australia within a structured and defined collecting scheme using an established protocol (Letcher & Powell 2001, 2002a) of selective baiting with a variety of substrates to obtain chytrids. We examined numerous samples of soil from four distinct plant communities—subtropical rainforest, wet sclerophyll forest, dry sclerophyll forest and open heath—and evaluated the diversity of chytrids in the four habitats studied.

Materials and Methods

Between November 2002 and February 2003, 227 soil samples were obtained from 14 collection sites among four distinct vegetation habitats: dry sclerophyll forest, wet sclerophyll forest, subtropical rainforest and open heath (Table 1). Soils were collected within National Parks under New South Wales National Parks (NP) and Wildlife Service Permit # A3392, or collected on private land with permission of the owners. Collection of soil samples and preparation of enrichment cultures were as previously described (Letcher & Powell 2001, 2002a). Global Positioning System (GPS) coordinates were obtained with a Garmin GPS 12 NAVAID. Chytrids were observed within the soils using a selective enrichment technique (Letcher & Powell 2001). Because soil chytrids exhibit a high degree of substrate specificity (Sparrow 1960), four substrates were added to cultures of each soil sample: cellulose (onion scale epidermis), chitin (purified shrimp exoskeleton), keratin (purified snake skin) and sterile gymnosperm pollen (*Pinus radiata* or *P. strobus*). Enrichment cultures were incubated at 20°C. Substrates were examined microscopically at 5–8 days and again at 14–20 days for the presence of chytrids. For each observational time, presence or absence of individual species in enrichment cultures was recorded.

Species diversity (the relative commonness and rarity of species, Morin 1999), as well as the number of species per site, was evaluated for all collection sites. Species diversity among four distinct habitats was investigated, from four collection sites where the sample size (n = 20–28 samples per collection) and repeat collections (n = 2) were sufficient to reliably indicate this ecological parameter (Letcher & Powell 2001). Each of the four collection sites represented one of the habitats studied.

Table 1. Data on collection sites.

Collection sites	Vegetation type	# Samples	GPS coordinates
1. Ourimbah, Palm Grove	subtropical rainforest	48	S 33° 18.356' E 151° 17.093'
2. Windsor, Valley Centre for Environmental Education and Research	wet sclerophyll	40	S 33° 24.825' E 150° 54.833'
3. Ku-ring-gai Chase NP, Basin Track	open heath	40	S 33° 35.671' E 151° 16.962'
4. Ku-ring-gai Chase NP, the Duckholes	dry sclerophyll	45	S 33° 39.837' E 151° 15.264'
5. Garigal NP, Deep Creek Reserve	dry sclerophyll	8	S 33° 42.400' E 151° 16.460'
6. Warringah Council, Wheeler Creek	dry sclerophyll	6	S 33° 44.193' E 151° 15.752'
7. Royal NP, Hacking River	wet sclerophyll	9	S 34° 09.185' E 151° 01.737'
8. Morton NP, Fitzroy Falls	dry sclerophyll	6	S 34° 38.798' E 150° 28.941'
9. Kangaroo Valley, Cambewarra Lookout	dry sclerophyll	2	S 34° 47.993' E 150° 34.637'
10. Morton NP, Mt Bushwalker	open heath	8	S 35° 14.459' E 150° 18.768'
11. Morton NP, Porters Creek	dry sclerophyll	2	S 35° 15.501' E 150° 22.330'
12. Morton NP, Little Forest Plateau	dry sclerophyll	4	S 35° 15.888' E 150° 21.343'
13. Morton NP, Clyde River	dry sclerophyll	6	S 35° 24.014' E 150° 16.363'
14. Murramarang NP, Cullendulla	wet sclerophyll	3	S 35° 40.109' E 150° 17.967'

Results

From 227 soil samples from 14 collection sites (Table 1), 38 species were identified. These are listed and described below. Eight of the taxa may represent undescribed chytrids (*Allomyces* sp., *Blyttiomyces* sp., *Chytriomyces* sp., *Cladochytrium* sp., *Lacustromyces* sp., *Powellomyces* sp., *Rhizophlyctis* sp. #1 and *Rhizophlyctis* sp. #2), and those taxa have been provisionally assigned generic affiliation based on available data. Seventeen additional taxa are new records in Australia of previously described chytrids. Following the name of each observed species, the reference to its description and the figure number of an illustration of the Australian isolate are given, and the notation 'New record' is included for those described taxa that have not been previously noted in Australian surveys. Notations of the substrate/s on which the chytrid was isolated (c- cellulose, ch- chitin, k- keratin, p- pollen) and the site/s where each species was collected (location numbers from Table 1) are indicated within brackets.

Taxa identified

***Allomyces* sp. Fig. 1**. [ch, 5]. This chytrid was found in a single location, in depauperate sandy soil and in association with the vascular plant *Casuarina cunninghamiana*. The sexual stage has not been observed with material from enrichment cultures or with a pure culture isolate of this fungus.

Diplophlyctis sarcoptoides Petersen (1903). New record. **Fig. 2**. [c, 4, 12]. Morphology of this chytrid agreed well with the original description; however, its occurrence on cellulose rather than chitin was unusual. Sporangia on cellulose were smaller than those of the type species, being 12–20 µm broad × 12–16 µm tall. Branched rhizoids extended from a clearly defined and visible spherical apophysis. Zoospores were discharged as a mass. Resting spores that were smaller than the sporangia were observed in older cultures.

***Blyttiomyces* sp. Fig. 3**. [p, 3, 8, 10, 11]. This organism was reported previously from Virginia, USA, soils as *Phlyctochytrium* sp. #2 (Letcher & Powell 2001). Immature sporangia were golden in colour and had a terminal apiculus. Mature, spherical (18–26 µm in diameter) to subspherical sporangia (16–30 µm broad × 12–18 µm tall) developed 3–7 prominent, lateral cone-shaped discharge tubes, and the sporangial wall was covered with orange-brown, irregularly shaped plaques. Within the substrate, a few stout rhizoids arose from a small (6–8 µm in diameter) spherical apophysis. From one or more discharge tubes, zoospores were discharged singly. They remained quiescent just external to the discharge pore for a few seconds before swimming away. Resting spores were not observed. Presence of the apical apiculus, lateral discharge pores, and an apophysis favour the provisional generic placement of this organism.

Chytridium rhizophydii Karling (1948). New record. **Fig. 4**. [2]. This chytrid was parasitic on both *Rhizophydium pollinis-pini* and *R. sphaerotheca*. Aggregations of elongate, 8–12 µm broad × 12–24 µm tall sporangia developed on the substrate. Zoospore discharge was abrupt and explosive and, following sporangial dehiscence, the zoospores remained quiescent for a few moments before dispersal. Resting spores were not observed.

Chytriomyces annulatus Dogma (1969). New record. **Figs 5–7**. [ch, p, 2, 3, 4, 5, 10, 11, 12, 13]. This organism presents a remarkable picture of distribution, morphology, and morphological plasticity. First observed in Michigan, USA (Dogma 1969), it has since been reported from several eastern USA states, Canada, and Poland (Letcher & Powell 2002b). This chytrid is readily identified on the basis of its pyriform-shaped sporangium, proximal collar-like annulations that ornament the sporangial wall, and an extramatrical rhizoidal stalk (fig. 5). The sporangial annulations also are apparent on empty sporangia. The Australian material also contained sporangia that were either clavate (fig. 6) or obovoid (fig. 7) and that were sessile upon the substrate. Additionally, the characteristic and diagnostic annulations were not observed with mature and undischarged obovoid sporangia, and only upon observation of discharged sporangia of the obovoid morphology were the diagnostic annulations revealed.

Chytriomyces hyalinus Karling (1945). **Fig. 8**. [ch, k, 1, 2, 3, 4, 8, 11, 12]. The material from Australian soils agreed well with the type material. This chytrid was encountered frequently, reinforcing its proposed cosmopolitan distribution and frequency (Letcher & Powell 2002b).

Chytriomyces poculatus Willoughby & Townley (1961). **Fig. 9**. [p, 1, 4]. Features of this chytrid corresponded well with the type description. Sporangia mostly were ovoid, 12–20 µm broad × 18–35 µm tall, and were ornamented with characteristic overlapping cupules of wall material. At zoospore discharge, the

operculum separated partially from the sporangial wall as the first few zoospores were ejected. Those zoospores remained quiescent at the exit orifice for a few minutes before slowly swimming away. The remaining zoospores began to swim in the sporangium and eventually most escaped and dispersed. Sometimes, however, one or two zoospores remained within, germinated, and proliferated within the sporangium.

It is interesting that the majority of specimens examined by Willoughby (1965) from Australian soils lacked the distinctive cupule surface ornamentation, and that many were irregular in shape as well as multioperculate. Absence of overlapping cupules has been reported by Willoughby (1965), Sparrow (1968), Booth (1971a, b), and Booth & Barrett (1971). Such morphological variation was not observed in material from New South Wales soils in this study. Longcore (1992) described *Chytriomyces angularis*, a chytrid morphologically similar to *C. poculatus* but lacking any wall ornamentation, and which in pure culture did not produce cupules of overlapping wall material. Sporangia also were irregularly gibbose and often in clusters, thus making the two species distinguishable.

***Chytriomyces* clade #1. Figs 33, 34.** [c, 2]. This chytrid was observed on cellulose bait with several soil samples from two collection sites. Sporangia were spherical (20–25 µm in diameter), subspherical, or irregularly shaped (20–32 µm broad × 30–45 µm tall). Isodiametric rhizoids, up to 6 µm in diameter, originated at 2–6 points on the sporangium, extended to 200 µm or more in length, and were sparsely branched. Upon discharge, numerous small (2–3 µm diameter) spherical zoospores emerged in a hyaline vesicle from a uniform diameter (6–8 µm) discharge tube 6–40 µm in length, and formed a quiescent cluster at the orifice prior to dispersal. Resting spores were not observed. Ultrastructural studies of the zoospore (unpublished) indicate this chytrid has a Group I-type zoospore (Barr 1980) that is found in all members of the *Chytriomyces* clade (James *et al.* 2000) thus far examined; however, its phylogenetic position is yet to be resolved via molecular analysis.

***Chytriomyces* clade #2 ('Miller's Dentate').** (Miller 1968). **Fig. 19.** New record. [ch, p, 1, 4, 6]. The spherical (15–35 µm diameter) ornamented sporangia and tubular interbiotic or endobiotic rhizoid conformed to Miller's (1968) description. Zoospores were discharged into an exogenous vesicle where they swarmed prior to dispersal into the environment. Molecular analysis (unpublished) places this chytrid in the *Chytriomyces* clade (James *et al.* 2000), but zoospore ultrastructure remains to be examined.

This organism is probably a member of a species complex (Miller 1968) that may include *Phlyctochytrium aureliae* and *P. mucronatum*. It lacks an apophysis, which is characteristic of *Phlyctochytrium*, but the dentate sporangial ornamentation is similar to that exhibited by *P. aureliae* and *P. mucronatum*.

***Chytriomyces* sp. Figs 10–12.** [p, 1]. This operculate chytrid was abundant on pollen bait, but occurred only in a single soil sample. The subspherical (12–16 µm diameter) to elongate (8–14 µm broad × 16–25 µm tall) sporangia (figs 10, 11) consisted of 2–7 lobes that served as discharge papillae. The moment of zoospore discharge was not observed, although zoospores being discharged singly following sporangial dehiscence were observed. A thin, slightly convex, almost transparent operculum remained attached to the sporangial wall after discharge. Resting sporangia (fig. 12) were elongate (7–9 µm broad × 10–14 µm tall), interbiotic, attached to the pollen grain via a long, thin rhizoidal stalk, and contained two prominent oil globules.

This fungus resembles *Chytriomyces* sp. described by Sparrow (1968), a multi-lobed, operculate chytrid that he considered to be allied to *C. poculatus*. Because so little of the development and life cycle of this organism has been observed, it is not assigned a specific epithet, but rather it is being placed provisionally in *Chytriomyces* on the basis of the operculate sporangium and epibiotic resting spore.

Chytriomyces spinosus Fay (1947). **Fig. 13.** New record. [c, 6, 10]. This distinctive chytrid was observed in soil from both dry sclerophyll forest and open heath. Consistent with the type material, it colonized only the cellulose bait. Zoospores were discharged into an exogenous vesicle, where they remained quiescent for a few moments before actively dispersing and swimming away.

Chytriomyces willoughbyi Karling (1968). **Fig. 14.** New record. [2, 4, 8]. This parasitic chytrid was observed on *Rhizophydium sphaerotheca* and *R. globosum*. The operculate sporangium partially collapsed after passive discharge of zoospores.

***Cladochytrium* sp. Fig. 15.** [c, 7]. This organism was observed on cellulose in two soil samples from a single site. The intramatrical thallus consisted of fine, highly branched rhizomycelia with one- or two-celled narrowly

elliptical turbinate organs, 7–10 µm wide × 15–18 µm long, interspersed along the rhizomycelium. Many of these cells appeared empty, while others contained light yellow globules. Some cells developed into zoosporangia, in which one half of the two-celled structure enlarged significantly in proportion to the other half, and became spherical, 15–20 µm in diameter. As they matured, sporangia assumed a granular appearance, and were light gold in color. Zoospore discharge was not observed. Resting spores were thick-walled, spherical or angular, 10–15 µm in diameter, and golden in colour.

Karlingiomyces dubius (Karling) Sparrow (1960). **Fig. 16**. [ch, 1]. Observed in two soil samples from a single site, this chytrid exhibited characteristics that conformed to the type. Sporangia were spherical or subspherical, 15–70 µm in diameter. Most sporangia had a single broad exit papilla, with a substantial hyaline region below the papilla. Rhizoids were generally isodiametric, and were extensive and profusely branched. Zoospore discharge followed a slow liberation of the operculum, which persisted on the sporangium following dehiscence. Zoospores remained quiescent for a few moments at the exit orifice prior to becoming motile.

***Lacustromyces* sp. Figs 17, 18**. [ch, 4, 11, 13]. This polycentric chytrid was observed on chitin bait in soil samples from three collection sites. It lacked the spindle organs of other certain polycentric genera (e.g. *Nowakowskiella* and *Cladochytrium*), and was similar in gross thallus morphology to *Lacustromyces hiemalis* (Longcore 1993). The robust, extensively branched isodiametric rhizomycelium bore intercalary zoosporangia that developed multiple discharge tubes. Neither zoospore discharge nor resting spores were observed. Until additional observations and analysis clarify the taxonomic position of this organism, it is placed provisionally in this genus.

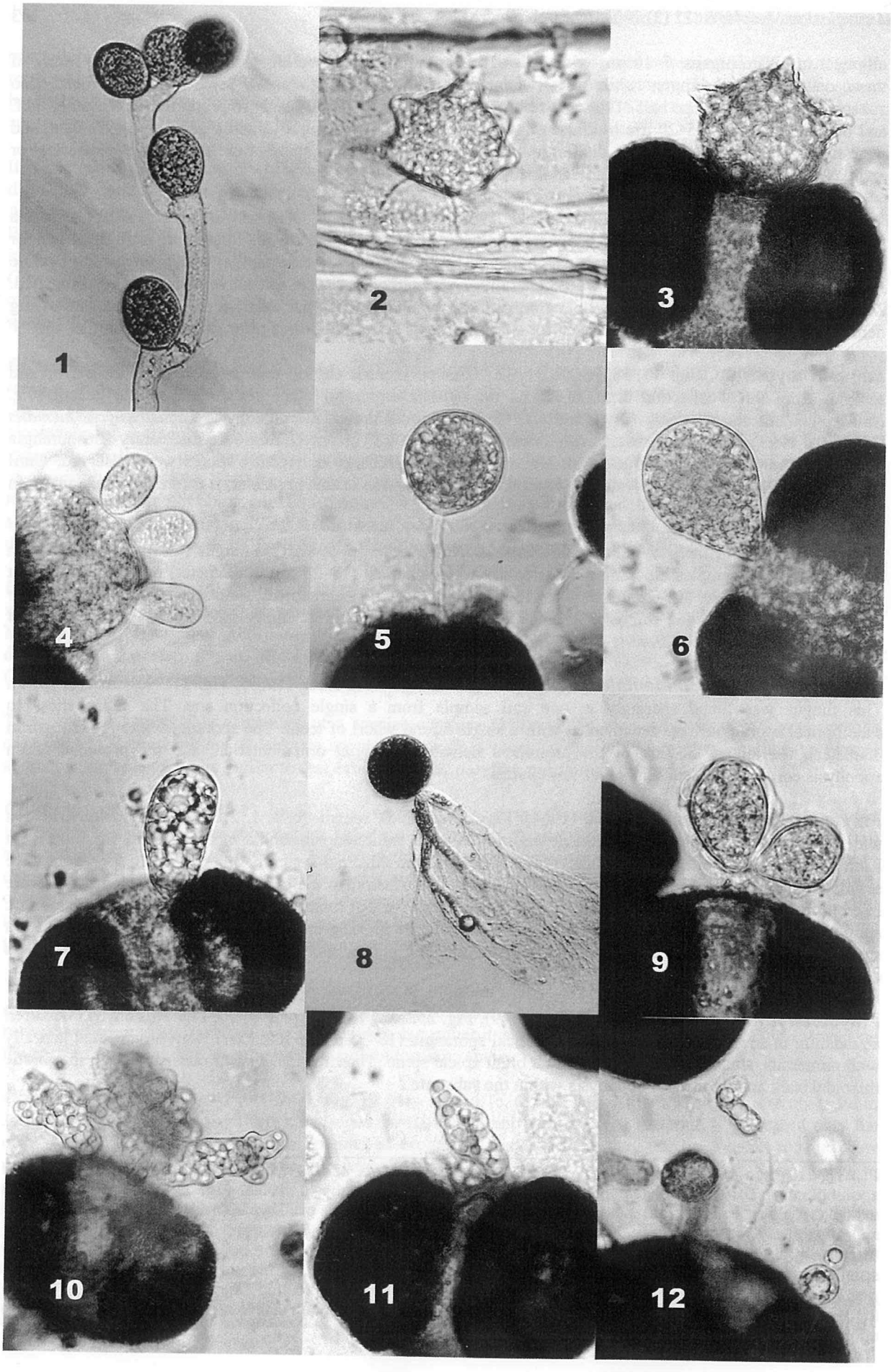
Phlyctochytrium aureliae Ajello (1945). **Fig. 20**. New record. [p, 3, 4]. This fungus was not abundant when it occurred in soil samples. Sporangia occasionally were spherical (18–25 µm in diameter) or subspherical, but generally were elongate (12–20 µm broad × 16–28 µm tall), with discernable dentate sporangial ornamentation. Zoospores were discharged singly or in small clusters. In older cultures, empty sporangia were common, but resting spores were not observed.

Phlyctochytrium circulidentatum Koch (1969) *in* Umphlett & Koch (1969). **Fig. 21**. New record. [p, 4]. This fungus was found sparingly in one soil sample from a single collection site. The subspherical to subelliptical sporangia were ornamented with a single lateral whorl of teeth. The sporangial size (18–26 µm in diameter), the lateral location of the prominent dentate sporangial ornamentation, and the presence of an apophysis confirm the identification of this chytrid.

Phlyctochytrium indicum Karling (1964). **Figs 22–24**. New record. [c, p, 1, 2, 3, 4, 10]. This chytrid was widespread in soil samples, and abundant where present. It exhibited substantial morphological variation from Karling's original description, particularly with respect to the rhizoidal system. Although many sporangia were sessile (fig. 22), as per the type, it was not unusual in the Australian material to observe sporangia that were interbiotic, with an inflated tubular (fig. 23) or apophysate (fig. 24) rhizoidal axis, in addition to the endobiotic apophysis. Zoospores were discharged in a vesicle outside the sporangium, where they remained quiescent for a short period of time before dispersing. As well as infecting pollen, this fungus occurred to a lesser extent on cellulose.

Phlyctochytrium mucronatum Canter (1949). **Fig. 25**. New record. [p, 3, 4, 10, 11]. This chytrid occurred abundantly in several soil samples. The spherical sporangia (18–36 µm in diameter) were ornamented laterally with numerous, small dentate enations and a blunt apical spine. The rhizoidal system consisted of an interbiotic rhizoidal stalk and a spherical apophysis within the substrate.

PLATE 1. (Page 104). **Figures 1–12. Fig. 1.** Asexual sporangia of *Allomyces* sp., on chitin. **Fig. 2.** Stellate sporangium of *Diplophlyctis sarcoptoides* on onion epidermis; apophysis visible. **Fig. 3.** *Blyttiomyces* sp. with multiple discharge tubes, on pine pollen. **Fig. 4.** Three sporangia of *Chytridium rhizophydii*, parasitic on sporangium of *Rhizophydium* sp. **Figs 5–7.** *Chytriomyces annulatus*. **Fig. 5.** Mature pyriform sporangium attached to pollen grain by a rhizoidal axis. **Fig. 6.** Sessile, clavate sporangium on pine pollen. **Fig. 7.** Sessile, obovoid sporangium on pine pollen. **Fig. 8.** *Chytriomyces hyalinus* on chitin. **Fig. 9.** Mature sporangia of *Chytriomyces poculatus* with distinctive cupule ornamentation; on pine pollen. **Figs 10–12.** *Chytriomyces* sp. **Fig. 10.** Two lobose sporangia on pine pollen. **Fig. 11.** Single sporangium with operculum visible at distal lobe. **Fig. 12.** Resting spore attached to pine pollen by a thin rhizoidal axis.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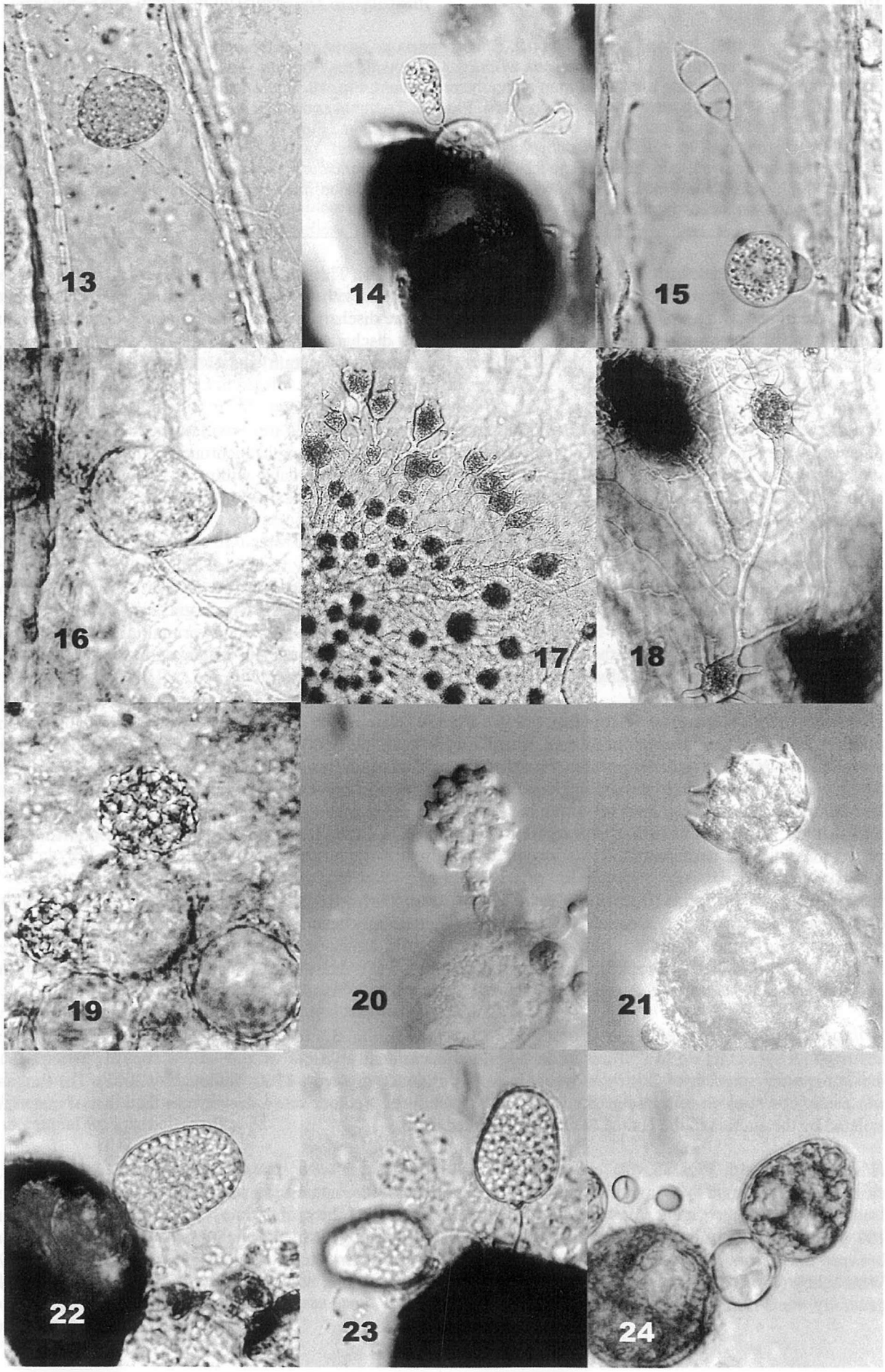
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PLATE 2. (Page 105). **Figures 13–24. Fig. 13.** *Chytriomyces spinosus* on onion epidermis. **Fig. 14.** Mature and empty, operculate sporangia of *Chytriomyces willoughbyi*, parasitic on *Rhizophydium* sp. **Fig. 15.** *Cladochytrium* sp. Sporangium and septate turbinate organ along rhizomycelium. **Fig. 16.** Sporangium of *Karlingiomyces dubius*, on chitin. **Figs 17, 18.** *Lacustromyces* sp. on chitin. **Fig. 17.** Terminal sporangia in a polycentric colony. **Fig. 18.** Two intercalary sporangia connected by isodiametric rhizomycelium. **Fig. 19.** *Chytriomyces* clade #2 ('Miller's Dentate') on chitin. **Fig. 20.** *Phlyctochytrium aureliae* on sweet gum pollen. **Fig. 21.** *Phlyctochytrium circulidentatum* on sweet gum pollen. **Figs 22–24.** *Phlyctochytrium indicum*. **Fig. 22.** Sessile sporangium on pine pollen; apophysis visible. **Fig. 23.** Two sporangia, each with an inflated rhizoidal axis. **Fig. 24.** Sporangium with two apophyses, on sweet gum pollen.

Phlyctochytrium reinboldtae Persiel (1959). **Fig. 26**. [p, 2, 4]. This isolate corresponded well with the type material. The sporangia were subglobose to broadly ellipsoidal, with 3–12 cone-shaped discharge tubes that often were tipped with a small hyaline globule prior to zoospore discharge. Occasional sporangia were interbiotic with a stout extramatrical rhizoidal stalk. Zoospores were discharged into an exogenous vesicle. A large, spherical, endobiotic apophysis that often filled the body of the pollen grain was observable in both pine and sweet gum pollen.

***Phlyctochytrium* sp. Fig. 27**. [p, 3, 10]. The elongate sporangia (14–24 µm broad × 16–30 µm tall) of this organism were ornamented with distinctive and elongate (up to 8 µm in length) bicornute teeth. Sporangia were subtended by an interbiotic rhizoidal stalk, and a spherical apophysis was visible within the pollen substrate. This chytrid is possibly an additional morphotype in the species complex with *Phlyctochytrium aureliae*, *P. mucronatum*, and 'Miller's Dentate'.

***Powellomyces* sp. Figs 28, 29**. [p, 4]. This soil-born organism closely resembled *Powellomyces* (Longcore 1995). The endobiotic chytrid occurred in pollen grains, and zoospores were released via 1 or 2 discharge tubes. Occasionally zoosporangia developed exogenously from encysted zoospores. Sporangia were spherical (20–30 µm in diameter) to irregular (15–20 µm broad × 25–30 µm tall) in shape with single or multiple rhizoidal axes arising from 1 or 2 (–5) points on the sporangium. Neither zoospore discharge nor resting spores were observed. The organism is placed provisionally in this genus pending further observations.

Rhizidium richmondense Willoughby (1956). **Fig. 30**. New record. [c, 7]. This chytrid was abundant on cellulose substrate, and corresponded well with the type material. Sporangia were spherical, subspherical, or occasionally slightly irregular in profile. A zone of clear cytoplasm occurred between the prominent apiculus and the mass of zoospores within the sporangium. The rhizoidal system consisted of thin, sparsely branched rhizoids that originated from a small swelling at the base of the sporangium and extended several hundred microns into the substrate. Zoospores were discharged passively as a mass, and they remained quiescent at the exit orifice for a few moments prior to dispersal.

Rhizidiomyces bullatus (Sparrow) Karling (1944). **Fig. 31**. New record. [c, ch, k, p, 2, 3, 4, 10, 11]. This distinctive hyphochytrid was abundant whenever found, and usually occurred on all substrates when present. The sporangia were golden to reddish brown with verrucose walls. However, sporangia assumed exceptional large size (60–100 µm in diameter) on chitin and keratin, as opposed to a much smaller average dimension (12–32 µm in diameter) on pollen and cellulose. Zoospore discharge was not observed.

Rhizophlyctis rosea (deBary & Woronin) Fischer (1892). **Fig. 32**. [c, 2, 4, 5, 6, 7]. This large, distinctive chytrid with sporangia up to 250 µm in diameter and occasionally visible without microscopy, is perhaps the most ubiquitous member of Chytridiomycota in soils worldwide (Sparrow 1960, Willoughby 2001). The fungus was easily observed on cellulose substrate, and in pure culture its colour was a deeper rose than that of material isolated by the authors in the United States (unpublished data).

***Rhizophlyctis* sp. Figs 35, 36**. [ch, 1, 2, 3, 6, 7, 13]. Sporangia were spherical (20–52 µm), subspherical, elongate (20 µm broad × 30 µm tall), pyriform or irregular, and were numerous and abundant on chitin substrate. Isodiametric rhizoids originated from 3–20 broad axes spaced about the sporangium, and extended to more than 100 µm through the substrate. Prior to zoospore discharge, a plug of hyaline material occupied the regions between the discharge tubes (1–4) and the spore mass. This organism closely resembles a chytrid described by Willoughby (1965) as *Rhizophlyctis* sp., which also occurred on chitin; however, the sporangia of material here generally were smaller than those described by Willoughby. The material here does not closely match any

previous description, but as morphological limits of species in *Rhizophlyctis* commonly are uncertain, it is placed in the genus without erecting a new species to accommodate it.

Rhizophydium coronum Hanson (1944). **Fig. 37**. [p, 1, 2]. The gelatinous corona that surrounds the sporangium is unique among the members of *Rhizophydium*. This fungus occurred in only two samples, one from wet sclerophyll soil and one from subtropical rainforest soil. Its occurrence in this study agrees with Willoughby (1965), in which this chytrid was associated with impoverished soils exhibiting high microfungal diversity. For both the type material (Hanson 1944, 1945) and Willoughby's collection, the fungus occurred on cellulose; in this study it occurred on pollen, but not the cellulose substrate.

Rhizophydium elyensis Sparrow (1957). **Fig. 38**. [k, 4]. This fungus was encountered from a single soil sample. The angular sporangium, passive discharge of zoospores as a mass, and occurrence on keratin substrate conform to the type description.

Rhizophydium globosum (Braun) Rabenhorst (1868). **Fig. 39**. [p, 1, 2, 4, 5, 8, 13]. This chytrid was common and was observed in several soil samples from the many collection sites where it was found. Sporangia were 22–42 µm in diameter, the sporangial wall was double-contoured, and 2–4 exit papillae protruded on the upper half of the sporangium. As characteristic spiny resting spores were not observed, placement here is tentative.

Rhizophydium macroporosum Karling (1967). **Fig. 40**. New record. [ch, p, 1, 2, 3, 4, 11, 12]. This fungus was identifiable by the large sporangia (30–60 µm diameter), the conspicuous broadly conical exit papillae, and the simultaneous discharge of masses of zoospores through several exit papillae.

Rhizophydium macrosporum Karling (1938). **Fig. 41**. [p, 1, 2, 10]. This large *Rhizophydium* occurred sparsely, often being interbiotic with rhizoidal branches entering several pollen grains. Sporangia were 60–90 µm in diameter, and low, inconspicuous discharge papillae were observed only occasionally. Initial zoospore discharge occurred simultaneously from one or more discharge pores. Zoospores remained quiescent in an exogenous vesicle for 1–2 minutes before dispersing; the remaining zoospores then swarmed within the sporangium and rapidly dispersed one by one.

Rhizophydium obpyriformis (Karling) Karling (1977). **Fig. 42**. New record. [1, 2, 4, 7, 14]. This parasite was common, occurring predominantly on *Rhizophydium subangulosum* and to a lesser extent on *R. pollinis-pini*, and in both hosts the sporangial contents rapidly disintegrated following infection.

Rhizophydium pollinis-pini (Braun) Zopf (1887). **Fig. 43**. New record. [p, 1, 2, 3, 4, 5, 7, 8, 10, 11, 12]. This chytrid conformed to the type description. Sporangia were spherical, 10–25 µm in diameter, with a single broad discharge papilla. The rhizoids were extensive and branched. Zoospores were observed to discharge slowly and singly, and the empty sporangium was urn-shaped with wall material slightly recurved from the broad discharge pore.

Rhizophydium sphaerotheca Zopf (1887). **Fig. 44**. [p, 1, 2, 3, 4, 5, 7, 9, 10, 11, 12]. This fungus was common on pollen bait, with spherical sporangia generally larger (17–35 µm in diameter) than those of *R. pollinis-pini*. Zoospores were discharged singularly or in groups.

Rhizophydium stipitatum Sparrow (1957). **Fig. 45**. New record. [p, 1, 4, 8, 11, 12, 13]. The fungus was distributed widely among collection sites and habitats, yet was not abundant when encountered. The majority of sporangia exhibited a single discharge pore, and following zoospore discharge into an exogenous vesicle, the sporangial wall partially collapsed.

Rhizophydium subangulosum (Braun) Rabenhorst (1868). **Fig. 46**. [c, p, 1, 2, 4, 5, 10, 13, 14]. This chytrid was perhaps the most abundant *Rhizophydium* observed, and occurred in all habitats studied. The sporangia tended to be relatively large (25–40 µm along the greatest axis), and zoospore discharge was characteristic of the taxon. A mass of zoospores initially burst through a single exit papilla, remained quiescent for 20–90 seconds, and then began to disperse. Subsequently, the remaining zoospores within the sporangium began to swarm, and then exited singly from one or several discharge pores, and the sporangium was vacated quickly. Occurrence on pollen was most common, with somewhat smaller sporangia occurring on cellulose substrate.

Septosperma rhizophydii Whiffen (1942). **Fig. 47.** [1, 3, 4, 10]. This parasite was encountered infrequently, although it was distributed widely among various habitats. Willoughby (1965) mentioned the organism only once, as occurring in peaty soil in association with Scented Paperbark (*Melaleuca squarrosa*). In our material, the resting sporangia exhibited two distinct morphologies. At the Morton NP, Mount Bushwalker site, the resting sporangia were short and less than 14 µm in length, while at the remaining sites where it was found, resting sporangia concurred with the type material, being 16–25 µm in length. The chytrid was parasitic on *Rhizophydium pollinis-pini* and *R. sphaerotheca.*

Spizellomyces kniepii Gaertner ex D. Barr (1984). **Fig. 48.** [p, 1, 4]. Sporangia were 20–25 µm in diameter, with 12–50 narrow discharge tubes, and a spherical endobiotic apophysis. This chytrid occurred sparsely in several samples from two collection sites.

Species diversity

The greatest number of species occurred at collection site # 4, the dry sclerophyll forest of Ku-ring-gai Chase NP, the Duckholes, where 24 of the 38 identified taxa were found (Table 2). In both the subtropical rainforest (site # 1, Ourimbah, Palm Grove) and wet sclerophyll forest (site # 2, Windsor, Valley Centre), 18 of the identified taxa occurred, while 14 taxa occurred in open heath (site # 3, Ku-ring-gai Chase NP, Basin Track). Those four collection sites, each representing one of the four habitats investigated, were also the sites from which a significant number of samples were obtained. Among the other 10 collection sites, where sample size varied from 2 to 9 samples per site, between 1 and 12 species were observed per site.

Five species (*Chytriomyces hyalinus*, *Phlyctochytrium indicum*, *Rhizophydium macroporosum*, *R. pollinis-pini* and *R. sphaerotheca*) were found in all four collection sites where significant sampling occurred. Seven other species (*Chytriomyces annulatus*, *Rhizidiomyces bullatus*, *Rhizophlyctis* sp., *Rhizophydium globosum*, *R. obpyriformis*, *R. subangulosum* and *Septosperma rhizophydii*) were found in three out of four of the collection sites where significant sampling occurred.

Table 2. Species diversity in all collection sites.

Collection sites[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Number of samples	(48)	(40)	(40)	(45)	(8)	(6)	(9)	(6)	(2)	(8)	(6)	(4)	(6)	(3)	
Species															**Totals**[b]
1. *Allomyces* sp.				+											1
2. *Blyttiomyces* sp.			+					+		+	+				4
3. *Chytridium rhizophydii*		+													1
4. *Chytriomyces annulatus*		+	+	+	+					+	+	+	+		8
5. *Chytriomyces hyalinus*	+	+	+	+				+			+	+			7
6. *Chytriomyces poculatus*	+			+											2
7. *Chytriomyces* sp.	+														1
8. *Chytriomyces spinosus*						+				+					2
9. *Chytriomyces willoughbyi*		+		+				+							3
10. *Chytriomyces* clade #1		+													1
11. *Chytriomyces* clade #2 ('Miller's Dentate')	+			+		+									3
12. *Cladochytrium* sp.							+								1
13. *Diplophlyctis sarcoptoides*				+								+			2
14. *Karlingiomyces dubius*	+														1
15. *Lacustromyces* sp.				+							+		+		3
16. *Phlyctochytrium aureliae*			+	+											2
17. *Phlyctochytrium circulidentatum*			+												1
18. *Phlyctochytrium indicum*	+	+	+	+						+					5

Collection sites[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Number of samples	(48)	(40)	(40)	(45)	(8)	(6)	(9)	(6)	(2)	(8)	(6)	(4)	(6)	(3)	
Species															**Totals**[b]
19. *Phlyctochytrium mucronatum*			+	+						+	+				4
20. *Phlyctochytrium reinboldtae*		+		+											2
21. *Phlyctochytrium* sp.			+							+					2
22. *Powellomyces* sp.				+											1
23. *Rhizidium richmondense*							+								1
24. *Rhizidiomyces bullatus*		+	+	+						+	+				5
25. *Rhizophlyctis rosea*		+		+	+	+	+								5
26. *Rhizophlyctis* sp.	+	+	+			+	+						+		6
27. *Rhizophydium coronum*	+	+													2
28. *Rhizophydium elyensis*				+											1
29. *Rhizophydium globosum*	+	+		+	+			+					+		6
30. *Rhizophydium macroporosum*	+	+	+	+							+	+			6
31. *Rhizophydium macrosporum*	+	+								+					3
32. *Rhizophydium obpyriformis*	+	+		+			+							+	5
33. *Rhizophydium pollinis-pini*	+	+	+	+	+		+	+		+	+	+			10
34. *Rhizophydium sphaerotheca*	+	+	+	+	+		+		+	+	+	+			10
35. *Rhizophydium stipitatum*	+			+				+			+	+	+		6
36. *Rhizophydium subangulosum*	+	+		+	+					+			+	+	7
37. *Septosperma rhizophydii*	+		+	+						+					4
38. *Spizellomyces kniepii*	+			+											2
Totals[c]	18	18	14	24	7	4	7	6	1	12	10	7	6	2	

[a] From Table I.
[b] Total number of sites where each species occurred.
[c] Total number of species that occurred per site.

Discussion

The focus of this study was to assess the biodiversity of chytrids in soils from four distinct vegetation types in central eastern New South Wales, using an established protocol. The vegetation types were dry sclerophyll forest, wet sclerophyll forest, subtropical rainforest and open heath. Soil samples were recovered from a total of 14 collection sites. A significant number of soil samples (n = 40–48) were obtained from each of four specific collection sites, and the equivalent sample size per site conveyed sampling parity to those four sites. The same four collection sites also individually represented one of the four vegetation types studied for the assessment of chytrid diversity. Conversely, from the remaining 10 collection sites, the low number of samples (n = 2–9 samples per site) precluded the inclusion of those sites in the assessment of chytrid diversity among the four vegetation types studied.

The evaluation of species diversity indicated that, among the four vegetation types investigated, the greatest number of chytrid species (25 of 38 identified taxa) was found in dry sclerophyll forests. Dry sclerophyll forests compose the largest proportion of eastern New South Wales bush, and make up more than half of Australia's native forest, occurring in every State and Territory (Parish 2001). That chytrid biodiversity would be greatest in the most expansive vegetation type of the State is a reasonable expectation, assuming that it provides opportunities for diverse or varying growth conditions. At the other extreme, the lowest number of chytrid species (13 of 38 identified taxa) was found in open heath, the vegetation type that is the scarcest of the four habitats in New South Wales. Australian heaths are among the most species-rich plant communities in the world

(Fairley & Moore 2000). However, the low nutrient, shallow sandy soil of the isolated Basin Track open heath represents the most stringent and harsh habitat of the four environments sampled, and may explain why chytrid diversity was comparatively the most limited.

In previous studies of chytrid frequency and distribution in a specific habitat, Letcher & Powell (2001, 2002a) found a few species to be ubiquitous to common, while most species were scarce to rare. That ecological principle was consistent in this study as well, in all four of the divergent habitats that were sampled. As a general indicator of chytrid frequency, 5 species were isolated from all 4 sites where significant sampling occurred; as well, 3 of those 5 species occurred in 7 or more of the 14 collection sites. Those species (*Chytriomyces hyalinus*, *Phlyctochytrium indicum*, *Rhizophydium macroporosum*, *R. pollinis-pini* and *R. sphaerotheca*) may be considered the ubiquitous to common taxa among all habitats sampled. The other 85 per cent of isolated taxa may be considered the uncommon, scarce, and rare species.

Eight of the taxa isolated in this study represent newly observed organisms (*Allomyces* sp., *Blyttiomyces* sp., *Chytriomyces* clade #1, *Chytriomyces* sp., *Cladochytrium* sp., *Lacustromyces* sp., *Powellomyces* sp. and *Rhizophlyctis* sp.), and they provisionally have been assigned generic affiliation based on currently available data. More complete observations and data on development, morphology, zoospore ultrastructure and molecular character are necessary before these entities may be delineated more accurately. In addition to the eight newly observed taxa, 17 other species represent new records for chytrids in Australia.

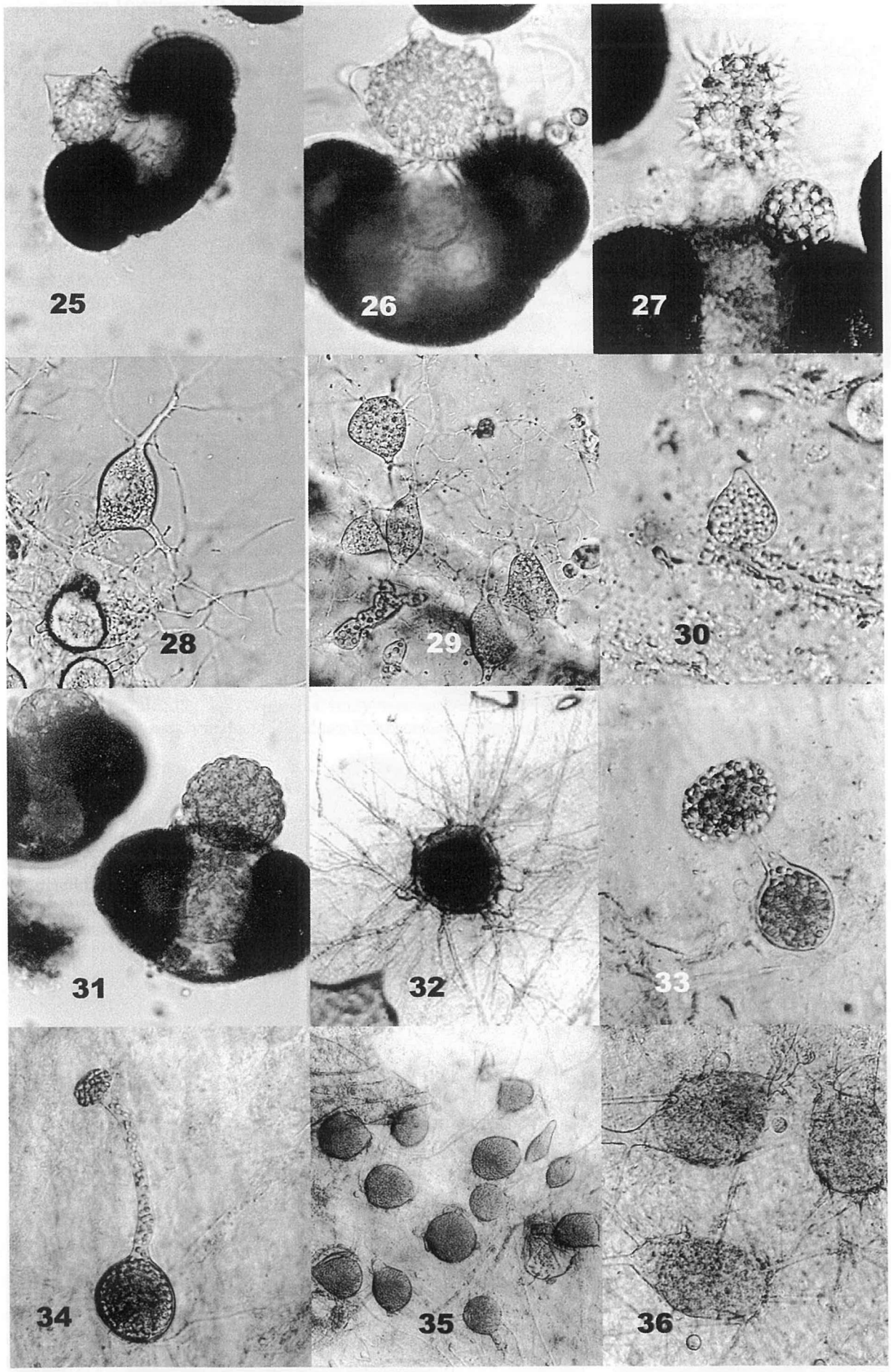
Thirteen of the species identified here also were found in one or more previous investigations (Harder & Gallwitz-Uebelmesser 1959, Karling 1988, Willoughby 1965). In total, 111 species of Chytridiomycota have been isolated from Australian soils. Only one species, *Rhizophlyctis rosea*, occurred in all four studies, attesting to the ubiquity of that organism.

Many of the taxa observed in this study, as well as other taxa recorded in the previous inventories of chytrids in Australian soils, exhibit a cosmopolitan distribution that is indicative of ancient origins of these organisms. Chytridiaceous fungi have a fossil record from approximately 400 Myr (4×10^6 years, Taylor *et al.* 1992), and these organisms may have existed as much as 1 Byr (10^9 years) ago (Simon *et al.* 1993). Thus, chytrids may have dispersed across Pangaea by the time of the separation of Pangaea into the supercontinents Laurasia and Gondwana approximately 80 Myr in the past. Subsequently, a well established chytrid flora should have existed in Meganesia (the then contiguous land masses of Australia, New Guinea and Tasmania) at the time of the Meganesian continental separation from Antarctica approximately 40 Myr in the past.

Given the long history of continental isolation, the potential for species evolution in Australia is great. With the exception of the newly described taxa in this study, none of the microfungal entities observed here appear to be endemic to Australia. However, in this study, the morphological variations expressed by *Chytriomyces annulatus*, *P. indicum* and *Phlyctochytrium* sp., as well as the general rarity of the newly described taxa, best exemplify the potential for species divergence. Additionally, morphological similarity of fungi with cosmopolitan distribution may mask greater variation at the cellular and molecular level. A concerted effort of comparison of species in pure culture, considering environmental, morphological, ultrastructural and molecular enquiries, may help to resolve this significant question.

The principal implication of both the biodiversity evaluation of this study, and the inventory analysis presented here of four surveys accomplished over the past 40 years, is of a vast and unrealized biodiversity of chytrids in Australian soils. Most of the earlier studies consisted of a limited and unstructured sampling regime. Harder & Gallwitz-Uebelmesser (1959), for example, examined 34 samples from Western Australia, South Australia, New South Wales, Queensland and Tasmania. Willoughby (1965) examined an undisclosed number of samples (perhaps no more than 50) from 12 or more sites, predominantly in Victoria. Karling (1988) examined 49 soil

PLATE 3. (Page 111). **Figures 25–36. Fig. 25.** *Phlyctochytrium mucronatum* on sweet gum pollen; apical spine and apophysis visible. **Fig. 26.** *Phlyctochytrium reinboldtae* on pine pollen; apophysis visible. **Fig. 27.** *Phlyctochytrium* sp. on pine pollen. **Figs 28, 29.** *Powellomyces* sp. in agar culture. **Fig. 28.** Single, irregularly shaped sporangium with two rhizoidal axes. **Fig. 29.** Several maturing sporangia. **Fig. 30.** *Rhizidium richmondense* on onion epidermis. **Fig. 31.** Verrucose sporangia of *Rhizidiomyces bullatus* on pine pollen. **Fig. 32.** *Rhizophlyctis rosea* on cellulose. **Figs 33, 34.** *Chytriomyces* clade #1. **Fig. 33.** Mature sporangium discharging zoospores through a short discharge tube. **Fig. 34.** Initial stage of vesicular zoospore discharge from a long discharge tube. **Figs 35, 36.** *Rhizophlyctis* sp. **Fig. 35.** Cluster of maturing sporangia on chitin. **Fig. 36.** Two sporangia with multiple rhizoidal axes originating from several points on the sporangium.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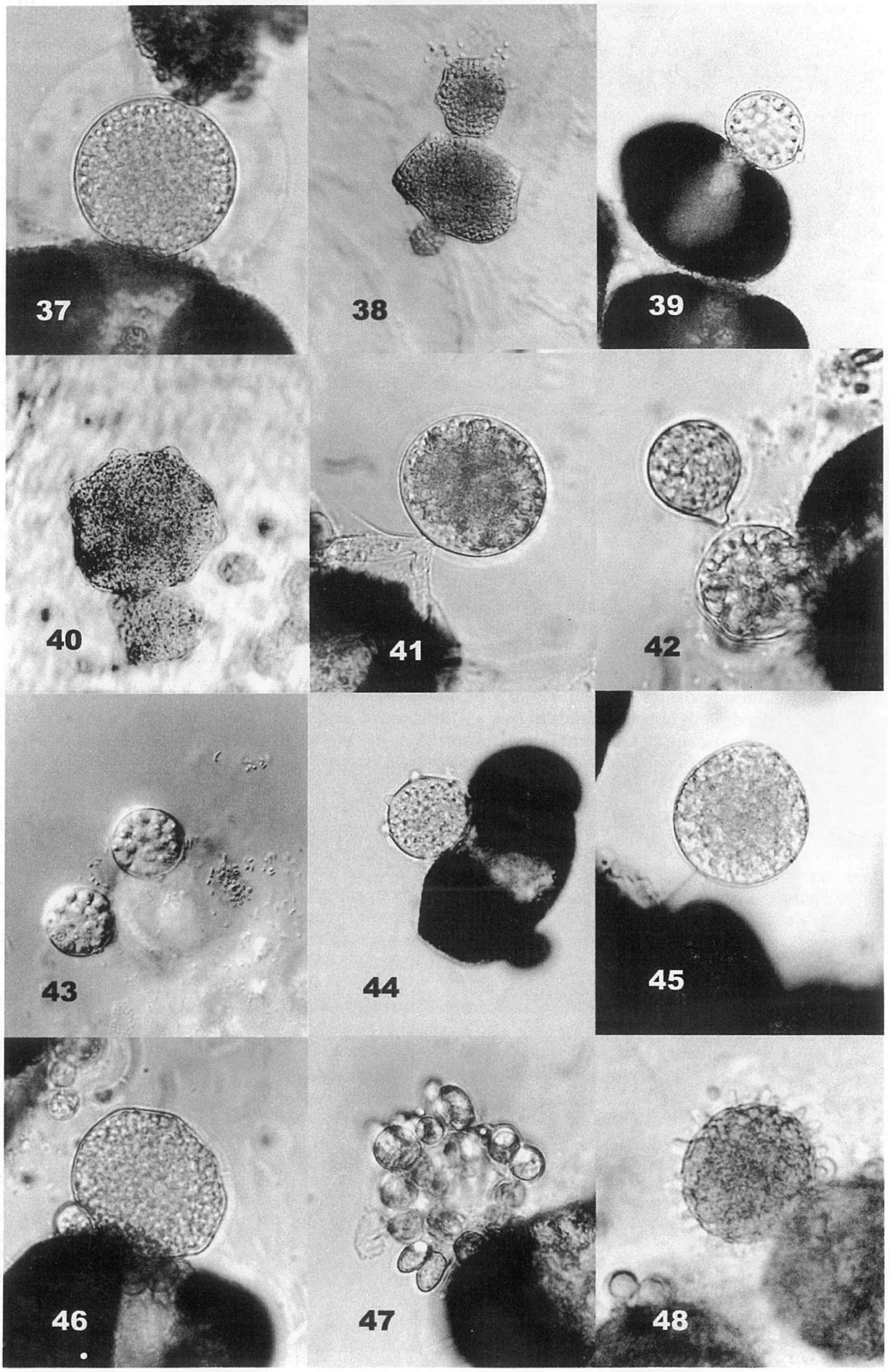
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PLATE 4. (Page 112). **Figures 37–48. Fig. 37.** *Rhizophydium coronum* on pine pollen; gelatinous corona surrounding the sporangium visible. **Fig. 38.** *Rhizophydium elyensis* on keratin; mature sporangia in initial stages of zoospore discharge. **Fig. 39.** *Rhizophydium globosum* on pine pollen, with thick-walled sporangium and a single discharge papilla. **Fig. 40.** *Rhizophydium macroporosum*, with multiple lens-shaped discharge papillae; on chitin. **Fig. 41.** *Rhizophydium macrosporum*; interbiotic sporangium on pine pollen. **Fig. 42.** *Rhizophydium obpyriformis*, parasitic on *Rhizophydium* sp. **Fig. 43.** Two mature sporangia of *Rhizophydium pollinis-pini* on sweet gum pollen. **Fig. 44.** Sporangium of *Rhizophydium sphaerotheca*, with multiple discharge papillae, on pine pollen. **Fig. 45.** *Rhizophydium stipitatum* on pine pollen. **Fig. 46.** *Rhizophydium subangulosum* on pine pollen. **Fig. 47.** Spherical sporangia and bullet-shaped resting spores of *Septosperma rhizophydii*, parasitic on *Rhizophydium* sp. **Fig. 48.** *Spizellomyces kniepii* on sweet gum pollen.

samples from 13 sites across the continent. The present study examined 227 soil samples from 14 sites in eastern New South Wales, the majority of which were within a 70 km radius of Sydney. That only one chytrid—*Rhizophlyctis rosea*—was common to the four inventory studies, while the remaining 110 identified taxa were scattered among 350 soil samples from 50 collection sites sampled at random across the continent, suggests that chytrid diversity in Australia is practically unknown and unexplored.

Chytrids occur in virtually all habitats on earth (Powell 1993). Australian natural habitats such as temperate rainforest, tropical rainforest, sedgeland swamps, grassland savannas, alpine moors, and freshwater environments have been sampled only sparsely, if at all, in any of the previously mentioned studies. No structured surveys of biodiversity in specific forest types such as those dominated by *Callitris*, *Acacia*, *Casuarina*, *Melaleuca* or *Avicennia* have been undertaken. Nor have soils been sampled from agricultural regimes, sites of volcanic activity or areas affected by fire or deforestation, and nothing is known about the effect of these disturbances on chytrid biodiversity. Additional studies are needed that can address the biodiversity of these organisms within these environments, habitats, and perturbations.

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