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Classification of anaerobic gut fungi from herbivores with emphasis on rumen fungi from Malaysia

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Abstract: Descriptive accounts and keys are given for 14 species in five genera of obligately anaerobic fungi found in the gut of herbivores. The descriptions are based entirely on thallus morphology as seen in the light microscope to permit the functional identification of genera and species. It is proposed to place *Neocallimastix patriciarum* and *N. variabilis* in synonymy with *N. frontalis*, and the status of *N. hurleyensis* as a distinct species is questioned. Two species of *Orpinomyces* are recognised: *O. joyonii* and *O. intercalaris*. *Anaeromyces mucronatus* and *A. elegans* are recognised, but the status of *A. elegans* is considered uncertain. The genus *Ruminomyces* is synonymous with *Anaeromyces*. Six species of *Piromyces* are included: *P. communis, P. mae, P. dumbonica, P. rhizinflatus, P. minutus* and *P. spiralis*. *Caecomyces* (syn. *Sphaeromonas*) comprises two species: *C. equi* and *C. communis*, but the status of *C. equi* is questioned. The present and future state of the taxonomy is discussed.

Key Words: anaerobic fungi, Chytridiomycota, gut fungi, rumen fungi, taxonomy

INTRODUCTION

Anaerobic fungi were first isolated from the rumen of a sheep and described by Orpin (1975). Until this discovery, the microbial population in the rumen was believed to be made up of bacteria and protozoa only. The significance and possible role of the rumen fungi in fiber digestion was recognised when large numbers of fungi were found to colonise fibrous plant materials in the rumen of sheep and cattle (Bauchop, 1979a, b). The fungi show a preference for the thick-walled sclerenchyma and vascular tissues (Akin et al., 1983; Ho et al., 1988a, 1991) and have been shown to contribute to the overall digestion of various forages and wheat straw (Akin et al., 1983; Gordon and Ashes, 1984; Trinci et al., 1994). All the anaerobic fungi studied so far are cellulolytic and are able to degrade structural carbohydrates of plant cell walls. They are present in large numbers when the host animal is fed a high-fiber diet but occur in low numbers when the animal is fed soft-leafy diet (Bauchop, 1979a, b). It thus seems probable that the anaerobic fungi are important in the digestion of high-fiber poor-quality forages. In tropical regions where the forages are generally fibrous and low quality, the development of methods to manipulate superior strains of fungi, naturally selected or genetically engineered, in the rumen could offer a means of improving rumen digestion of fiber in animals fed high-fiber diets.

The organisms we now recognize as anaerobic gut fungi were described by Liebetanz (1910) and Braune (1913), but they were thought to be zooflagellates. Braune (1913) named his organism *Callimastix frontalis* because of its similarity to *Callimastix cyclopsis*, a polyflagellate parasite of *Cyclops* described by Weisenberg (1912). Subsequently, Vavra and Joyon (1966) concluded that *C. cyclopsis* was likely a fungus related to the chytrids (phylum Chytridiomycota). As a result of this, *C. frontalis* was assigned to a new zooflagellate genus *Neocallimastix* (Vavra and Joyon, 1966). The discovery that *Neocallimastix* and similar "flagellates" from the rumen were in fact fungi can be accredited to Orpin's studies (1975, 1976, 1977a) on their life cycle and cell wall chemistry (1977b). Although both *Callimastix* and *Neocallimastix* are chytrids, *Callimastix* has distinctive ultrastructural characteristics similar to zoospores of the order Blastocladiales (Manier, 1978). Numerous other studies followed Orpin but the taxonomy of the rumen fungi, and their relatedness to the chytrids was not addressed until Heath et al. (1983) formally described *N. frontalis* which had not been done previously using rules of the Botanical Code of Nomeneclature. They assigned the species to a new family of chytrids, the Neocallimastigaceae[1] in the or-

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[1] The original spelling (Neocallimasticaceae) was corrected in the *Index of Fungi*. An orthographic correction of the original spelling is allowed under rules of the Botanical Code. See also *P. rhizinflatus* (as *rhizinflata*) and *P. dumbonicus* (as *dumbonica*).

der Spizellomycetales. The Neocallimastigaceae was later assigned ordinal status (Li et. al., 1993).

The taxonomy of anaerobic gut fungi (exclusive of the yeasts which are not covered in this treatment) is complicated because descriptions of the various species have been based on ultrastructure. Although ultrastructure of fungal zoospores provides valuable, and for chytrids, essential information on their classification, it is neither practical nor necessary to use ultrastructure for their identification. The purpose of this monograph is to standardise descriptions of taxa that have been described in recent years, and to provide a usable classification with keys and descriptions that are simplified but nonetheless scientifically correct.

RELATIONSHIP TO OTHER FUNGI

Anaerobic gut fungi belong to the family Neocallimastigaceae and order Neocallimastigales (Neocallimasticales). The Neocallimastigales is one of five orders in the phylum Chytridiomycota, and the only one to comprise obligately anaerobic species; however, facultative anaerobes are found in the order Blastocladiales (Emerson and Natvig, 1981). The Chytridiomycota, popularly known as chytrids, are phylogenetically related to the true fungi (Zygomycetes, Ascomycetes and Basidiomycetes) based on biochemical evidence (Bartnicki-Garcia, 1970, 1987) and on analysis of the 18S rRNA (Bowman et al., 1992; Dore and Stahl, 1991; Li and Heath, 1992).

MORPHOLOGY

All chytrids produce flagellated zoospores. Before the discovery of anaerobic gut fungi they were thought to be exclusively uniflagellate, but the gut fungi include both uniflagellate and polyflagellate taxa. In uniflagellated species, the zoospores are predominantly uniflagellated (FIG. 1) but there may be two to four flagella on some zoospores. The frequency of zoospores with two to four flagella varies between isolates of uniflagellate species from zero to about 10%. Active zoospores of polyflagellate species always have more than four flagella (FIG. 2), but upon release and prior to swimming the flagella are often clustered together and appear on superficial examination as one (FIG. 3). The flagella are shed during encystment (FIG. 4), but not always together, and often a single flagellum is left. It is important, therefore, to observe a number of zoospores when making a determination. The size of zoospores varies, not merely between isolates of the same species, but also between zoospores of a single isolate. In aerobic species of chytrids, zoospore size is affected by nutrition during sporangium development (Koch, 1968). Uniflagellate zoospores are generally smaller than polyflagellate zoospores, about 4–11 μm and 7–22 μm in diam, respectively. However, it is difficult to make accurate measurements on zoospore size because unfixed, dead zoospores tend to swell.

There are two main morphological forms, *monocentric* (one reproductive body) (FIG. 5) and *polycentric* (many centers of reproduction) (FIG. 6). These forms are determined in the earliest stages of growth and are invariable. In both, after zoospore encystment, the cyst germinates by producing a germ tube (FIG. 7). In monocentric species the nucleus does not enter the germ tube. The germ tube develops into a rhizoidal system of determinate length. The anucleate rhizoids serve a dual purpose, anchorage and absorption of nutrients. The rhizoids are of two types: a typical filamentous form in *Neocallimastix* and *Piromyces* (FIG. 5), and a bulbous form in *Caecomyces* (FIG. 8). The point between the sporangium and rhizoid is the *neck*, it may be broad (FIG. 9) or constricted and even isthmus-like (FIG. 10). The opening in the neck is the *port*; it may be broad (FIG. 9) or narrow (FIG. 11). When the sporangium matures a wall (septum) forms over the port or in the base of the sporangium (FIG. 12).

There are two further developmental events among monocentric species. In *endogenous* development the nucleus remains in the zoospore cyst which enlarges into a new sporangium (FIG. 5). In *exogenous* development there is bipolar germination; rhizoids develop from one side of the zoospore cyst and a wider outgrowth develops on the other side (FIG. 13). The nucleus moves into the broader outgrowth that develops into a *sporangiophore* (sporangial stalk), and a sporangium forms at the end (FIG. 14). The sporangiophore may be of varying lengths, sometimes short, appearing

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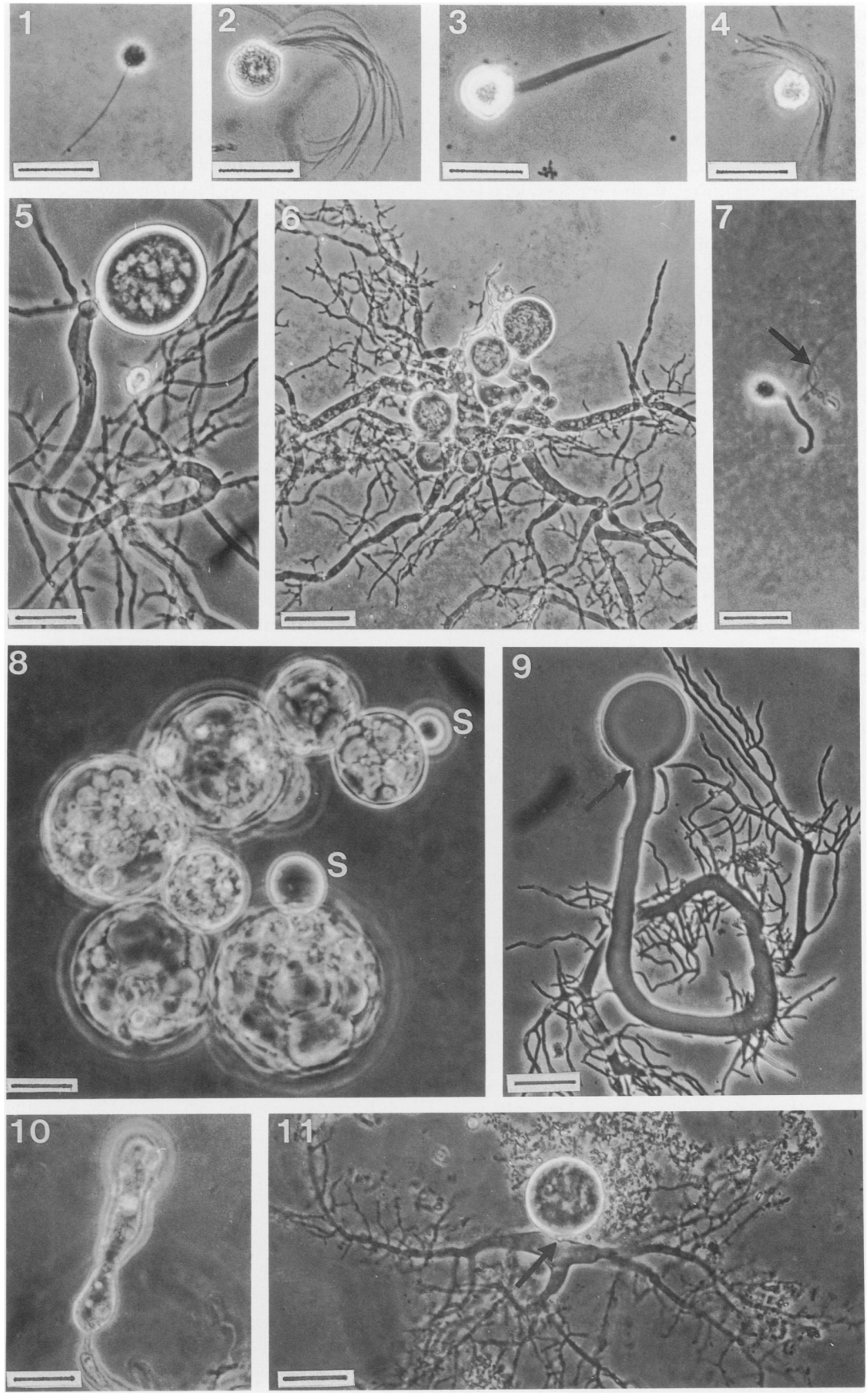
FIGS. 1–11. Morphological diversity in species of gut fungi. Bar, 20 μm. 1. A uniflagellate zoospore (*P. minutus*). 2. A polyflagellate zoospore (*O. joyonii*). 3. A polyflagellate zoospore with flagella clustered together giving the appearance of a single flagellum (*N. frontalis*). 4. A zoospore with shed flagella (*N. frontalis*). 5. A monocentric thallus with a single sporangium (*N. frontalis*). 6. A polycentric thallus with many sporangia (*O. joyonii*). 7. A germinating zoospore producing a germ tube. Note the shed flagella (arrow) (*N. frontalis*). 8. A thallus of *C. communis* showing bulbous rhizoids. S, sporangium. 9. An example of a broad neck (arrow) (*N. frontalis*). 10. An example of a constricted, isthmus-like neck (*P. rhizinflatus*). 11. An example of a narrow port (arrow) (*P. rhizinflatus*).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like an eggcup (FIG. 15), or very long, sometimes over 100 μm in length (FIG. 16). There has been confusion in the literature concerning the difference between the sporangiophore and a broad main rhizoid, and at times in a mature thallus it is not possible to be certain of the point where the zoospore germinated and commenced its bilateral growth. Nevertheless, there are two clues; the sporangiophore does not have lateral rhizoids, and in *N. frontalis* and *P. communis* the point where the cyst germinated is often swollen (FIGS. 14, 16).

In some normally monocentric species there may occasionally be a branch with two (FIG. 17) or more sporangia. These multisporangial thalli are polycentric if the term is used in its broadest sense. However, apart from *Piromyces* spp. and *C. communis* (Wubah et al., 1991a), these forms are uncommon, and most often only one or none of the sporangia develop to maturity. In *C. communis* the nucleus may move into the bulbous rhizoid (Wubuh et al., 1991a) and produce two (FIG. 18) or occasionally three sporangia, or remain in the zoospore cyst which becomes the sole sporangium (FIG. 8). The nucleus may also divide in the zoospore cyst with one nucleus entering the bulbous rhizoid. Because these species are primarily monocentric, and the number of sporangia is limited instead of unlimited as in mycelial polycentric forms discussed below, we propose they be called *monocentric-multisporangiate.*

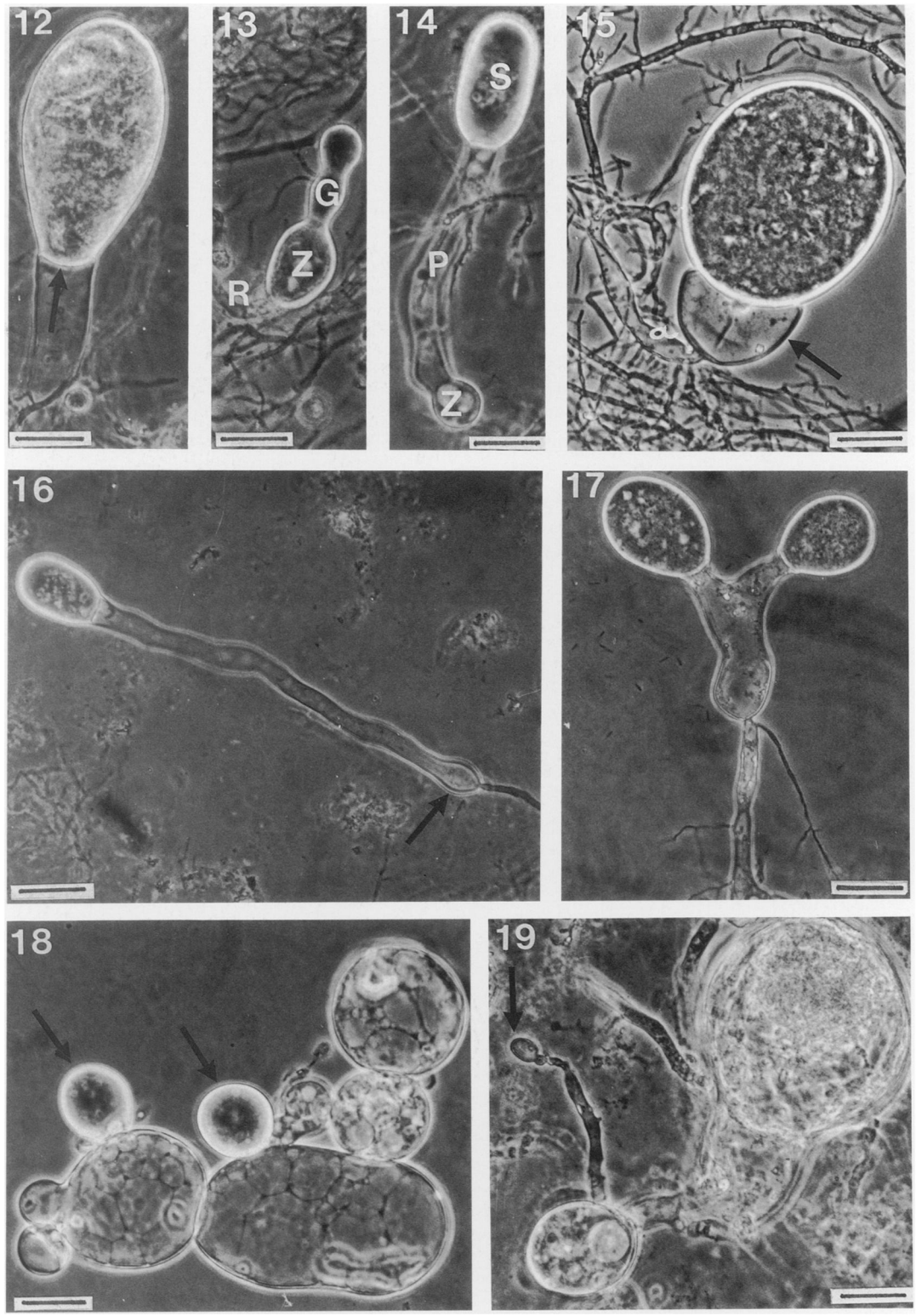
In polycentric species (*Orpinomyces* and *Anaeromyces*) the nucleus migrates out of the zoospore cyst and into the germ tube. The zoospore cyst has no further function in development, but the cyst wall may be persistent (FIG. 19). The germ tube elongates and branches into a mycelium (rhizomycelium) similar to other filamentous fungi. The nucleus divides repeatedly and migrates along individual hyphae. This form of development results in a mycelial thallus of indeterminate extent with many sporangia (FIG. 6). Unfortunately, after prolonged culture, many of the mycelial-polycentric species either produce sporangia which do not differentiate into zoospores, or no longer produce sporangia, and their identification becomes problematic.

Thallus forms may thus be classified as: i) monocentric and endogenous; ii) monocentric, exogenous and uni- or multisporangiate; and iii) mycelial-polycentric.

In the identification of chytrids, it is very important to consider their natural morphological variation. This variation makes it difficult to identify and classify these organisms. Because of temperature requirements and the anaerobic state of gut fungi, the problem is amplified and, unlike most other chytrids, the growth and development of a single thallus cannot be studied on the microscope under ordinary conditions. Differences in media probably cause more variations than any other factors. When the medium is too rich, such as in glucose or on filter paper, the sporangia often become abnormally large and abort. The identification of species is complicated as there is sufficient variation in morphology that a single thallus of one species may look like another. The extreme size difference is another problem. Small but mature thalli of monocentric species, except for *C. communis,* look alike, and it is only after observing the type of zoospore discharge and zoospore flagellation that they can be distinguished. When identifying and classifying anaerobic gut fungi it is therefore essential to ensure that the sporangia are healthy and viable, and to draw conclusions only after observations of sufficient materials.

KEYS TO THE GENERA AND SPECIES

1. Zoospores polyflagellate 2
1. Zoospores uniflagellate (occasionally with two to four flagella) 3
   2. Monocentric ..... 5. *Neocallimastix*
   2. Polycentric and mycelial ..... 6. *Orpinomyces*
3. Monocentric 4
3. Polycentric and mycelial ..... 7. *Anaeromyces*
   4. Sporangia with filamentous rhizoids ..... 8. *Piromyces*
   4. Sporangia with bulbous rhizoids ..... 12. *Caecomyces*
5. Zoospore release through an apical pore accompanied by dissolution and rupture of the sporangium wall .. *N. frontalis*
5. Zoospore release through a distinctive apical pore ..... *N. hurleyensis*
   6. Globose sporangia on simple or compound sporangiophores (branched sporangial stalks) ..... *O. joyonii*
   6. Globose sporangia intercalary (enlargement of hyphal elements) ..... *O. intercalaris*
7. Some hyphae with lobed or bead-like structures ..... *A. elegans*
7. Hyphae without lobed or bead-like structures ..... *A. mucronatus*
   8. Zoospores discharge accompanied or preceded by dissolution of sporangial wall 9
   8. Zoospores discharge through pores or papillae 10
9. Dissolution of sporangial wall accompanied with zoospore release, rhizoids not noticeably spiraled ..... *P. communis*
9. Dissolution of sporangial wall precedes zoospore release, rhizoids noticeably spiraled ..... *P. spiralis*



FIGS. 12–19. Morphological diveristy in species of gut fungi. Bar, 20 μm. 12. A sporangium with a septum (arrow) formed at the base (*N. frontalis*). 13. An example of exogenous development showing bipolar germination. R, rhizoid; Z, zoospore cyst; G, outgrowth (*N. frontalis*). 14. An example of exogenous growth development showing a sporangium formed at the end of a sporangiophore. S, sporangium; P, sporangiophore; Z, zoospore cyst (*N. frontalis*). 15. A short sporangiophore appearing like an eggcup (arrow) (*N. frontalis*). 16. An example of a very long sporangiophore; the zoospore cyst is shown by an arrow (*N. frontalis*). 17. A multisporangiate thallus with two sporangia (*P. rhizinflatus*). 18. A thallus of *C. communis* with two sporangia (arrows). 19. An example of a persistent zoospore cyst (arrow) (*O. intercalaris*).

10. Sporangia mostly less than 30 μm with a slender main rhizoid ..... *P. minutus*
10. Sporangia generally over 30 μm with a tubular main rhizoid 11
11. Mature sporangia not papilliated, neck distinctly constricted, often isthmus-like ... *P. rhizinflatus* (see also *P. dumbonicus*)
11. Mature sporangia papilliated, neck often broad ..... *P. mae*
12. Sporangium with a single bulbous rhizoid, from the caecum of the horse ..... *C. equi*
12. Sporangium with one or more bulbous rhizoids, from the rumen and hind gut of other herbivores ... *C.communis*

DESCRIPTIVE ACCOUNT OF GENERA AND SPECIES

Original diagnoses for taxa of anaerobic gut fungi comprise a diverse array of styles. Some accentuate different features such as zoospore ultrastructure or physiology. It is therefore difficult to compare one species with another. Some questionably may not comply fully with the Botanical Code of Nomenclature. The original diagnoses, therefore, have been emended where necessary or substantially rewritten. They provide only the essential information for distinguishing and separating taxa, and superfluous information has been omitted.

Order Neocallimastigales J. L. Li (Li et al., 1993).

Thallus filamentous-polycentric, monocentric-unisporangiate or monocentric-multisporangiate eucarpic; zoospores uniflagellate (occasionally bi- to quadriflagellate), or polyflagellate, flagella shed upon encystment. Obligate anaerobes in the digestive tract of vertebrates. A single family, the Neocallimastigaceae.

The anaerobic gut fungi were originally placed in the order Spizellomycetales (Heath et al., 1983), but later Heath and Bauchop (1985) and Munn et al. (1987) suggested that they were sufficiently distinctive in some mitotic and ultrastructural characteristics to warrant a separate order. Molecular analysis of 18S rRNA provided convincing evidence that the anaerobic gut fungi were only distantly related to the Spizellomycetales (Li and Heath, 1992), and the new order was later proposed (Li et al., 1993).

Family Neocallimastigaceae I. B. Heath (Heath et al., 1983).

The diagnosis is identical to that of the order. The type genus for the family is *Neocallimastix*.

Neocallimastix Vavra & Joyon ex I. B. Heath (Heath et al., 1983).

Monocentric; zoospore 8–22 μm, polyflagellate with seven to approximately 30 posteriorly inserted flagella in two rows. The type species for the genus is *N. frontalis* (Braune) Heath.

The earlier description of *Neocallimastix* by Vavra and Joyon (1966) did not include a Latin diagnosis, and therefore was invalid. Every genus requires designation of a type species which has priority when synonymies are proposed, and remains with the original genus when there is a generic split. Five species have been described, but we proposed two (*N. patriciarum* and *N. variabilis*) be reduced to synonymy with the type species *N. frontalis,* and question whether another (*N. hurleyensis*) is different. One (*N. joyonii*) was transferred to the genus *Orpinomyces* by Li et al. (1991).

Neocallimastix frontalis (Braune) Vavra & Joyon ex I. B. Heath in Heath et al. Canad. J. Bot. 61: 306, 1983. FIGS. 20-25

≡[2]*Callimastix frontalis* Braune, Arch. Protistenk. 32: 127, 1913.
=*Neocallimastix patriciarum* Orpin & E. A. Munn, Trans. Brit. Mycol. Soc. 86: 180, 1986.
=*Neocallimastix variabilis* Y. W. Ho & D. J. S. Barr in Ho et al., Mycotaxon 46: 242, 1993.

Sporangia endogenous or exogenous; exogenous sporangia spherical, 8.5–170.0 μm in diam, broadly ellipsoidal to broadly ovoid, occasionally irregular; exogenous sporangia on sporangiophores of varying length from a few microns to over 100 μm, occasionally branched with two sporangia; exogenous sporangia generally ellipsoidal, pyriform or ovoid, variable in size from 10 μm to over 100 μm in length, occasionally angular, tubular or irregular; rhizoids generally arising from one axis, occasionally two or three on the same side of the sporangium, neck not constricted or slightly constricted, main axis up to 20 μm in diam near the sporangium, extensively branched; main rhizoid often coiled and individual rhizoids may have tightly constricted points; rhizoid system over 1 mm in extent on larger sporangia; zoospore release through an apical pore accompanied by rapid dissolution and rupture of the sporangial wall; zoospores variable in size and shape, often with an equatorial constriction when first motile but becoming ovoid to globose; globose zoospores 7–22 μm in diam with seven to about 30 flagella, 28–48 μm long.

LECTOTYPE. Figs. 13–15 (Braune, 1913). Culture (PN1) located at Dr Geoff Gordon's Laboratory, CSIRO, Division

[2] A triple equal sign shows an obligate synonym (merely a name change); an ordinary equal sign indicates an opinion that species are synonymous.

of Animal Production, PO Box 239, Blacktown, NSW 2148, Australia, is here considered to be representative but currently cannot be considered as type.

Habitat. The rumen of sheep, New Zealand.

The description by Heath et al. (1983) does not give details on thallus morphology as seen in the light microscope. This makes it impossible to identify cultures with this species from the literature, and possibly the erection of subsequent new species might be attributed to this omission. By critically comparing two cultures of *N. frontalis* (PN1 and PN2) from New Zealand with a culture of *N. patriciarum* from the United Kingdom, and a culture of *Neocallimastix* from the USA, Wubah et al. (1991b) concluded that the four cultures belonged to the same species. We also conclude that *N. variabilis* from Malaysia (Ho et al., 1993a) and an unnamed culture of *Neocallimastix* from Alberta (Barr et al., 1989) fall within the morphological limits for *N. frontalis*. The diagnosis is thus emended based on the above references. Wubah et al. (1991c) reported the presence of melanized sporangia and thought these may be resting sporangia. It remains to be demonstrated whether these structures survive as resting spores, or are merely old, and possibly moribund sporangia.

The original diagnosis for *Neocallimastix* (Heath et al., 1983) included a detailed account on zoospore ultrastructure. Orpin and Munn (1986) and Munn et al. (1987) suggested that the ultrastructure of the *N. patriciarum* zoospore might be different, and, if this can be determined with certainty, it would provide convincing evidence that this species is valid. Because ultrastructural changes occur in zoospores prior to and during encystment, it is often difficult to compare fine details on zoospore ultrastructure done in different laboratories. Orpin and Munn (1986) indicated that there were differences in the physiology, such as fermentation end products, between *N. patriciarum* and *N. frontalis*; however, physiological variances without clear morphological differences do not justify species separation under the Botanical Code.

In Malaysia, *N. frontalis* has been isolated from the rumen and fecal materials of water buffalo, cattle (Ho et. al., 1993a), sheep, goat and deer (Ho, unpubl.). It has also been isolated from the rumen of cattle in New Zealand (Bauchop, 1979a), Canada (Barr et al., 1989), Australia (Phillips, 1989) and USA (Barichievich and Calza, 1990) and from the rumen, saliva and fecal materials of sheep in the United Kingdom (Orpin, 1975; Lowe et al., 1987).

Neocallimastix hurleyensis Theodorou & J. Webb in Webb and Theodorou, Canad. J. Bot. 69: 1221–1222, 1991.

This species differs from *N. frontalis* in zoospore discharge through a clearly defined apical pore in the sporangium.

TYPE. IMI 295997 (material in formaldehyde with samples in plastic prepared for transmission electron microscopy) at the herbarium of the International Mycological Institute, England.

Habitat. The ovine rumen, United Kingdom.

The abbreviated diagnosis includes only the essential differences from *N. frontalis*. The description of Webb and Theodorou (1991) was supplemented by an earlier study of the life cycle by Lowe et al. (1987) who reported and illustrated zoospore discharge. However, following the critical studies by Wubah et al. (1991b), discharge may not be unequivocally different from *N. frontalis*. Webb and Theodorou (1991) also reported that the main rhizoid was wide (avg. 15 μm in diam), but these are probably sporangiophores (see FIG. 5 in Lowe et al., 1987), therefore, establishing that the sporangia of this species are both endogenous and exogenous. Earlier, Webb and Theodorou (1988) reported on minor ultrastructural differences in *N. hurleyensis* that may support the presumption that this is a distinct species.

This species, reported from the United Kingdom, has not been reported elsewhere.

Orpinomyces D. J. S. Barr & H. Kudo in Barr et al., Canad. J. Bot. 67: 2819, 1989, emended.

Mycelium of indeterminate length arising from a zoospore cyst following monopolar germination; polycentric; zoospores polyflagellate. Type: *O. bovis* (= *O. joyonii*)

Orpinomyces joyonii (Breton) J. L. Li et al., Canad. J. Bot. 69: 587, 1991. FIGS. 26–31

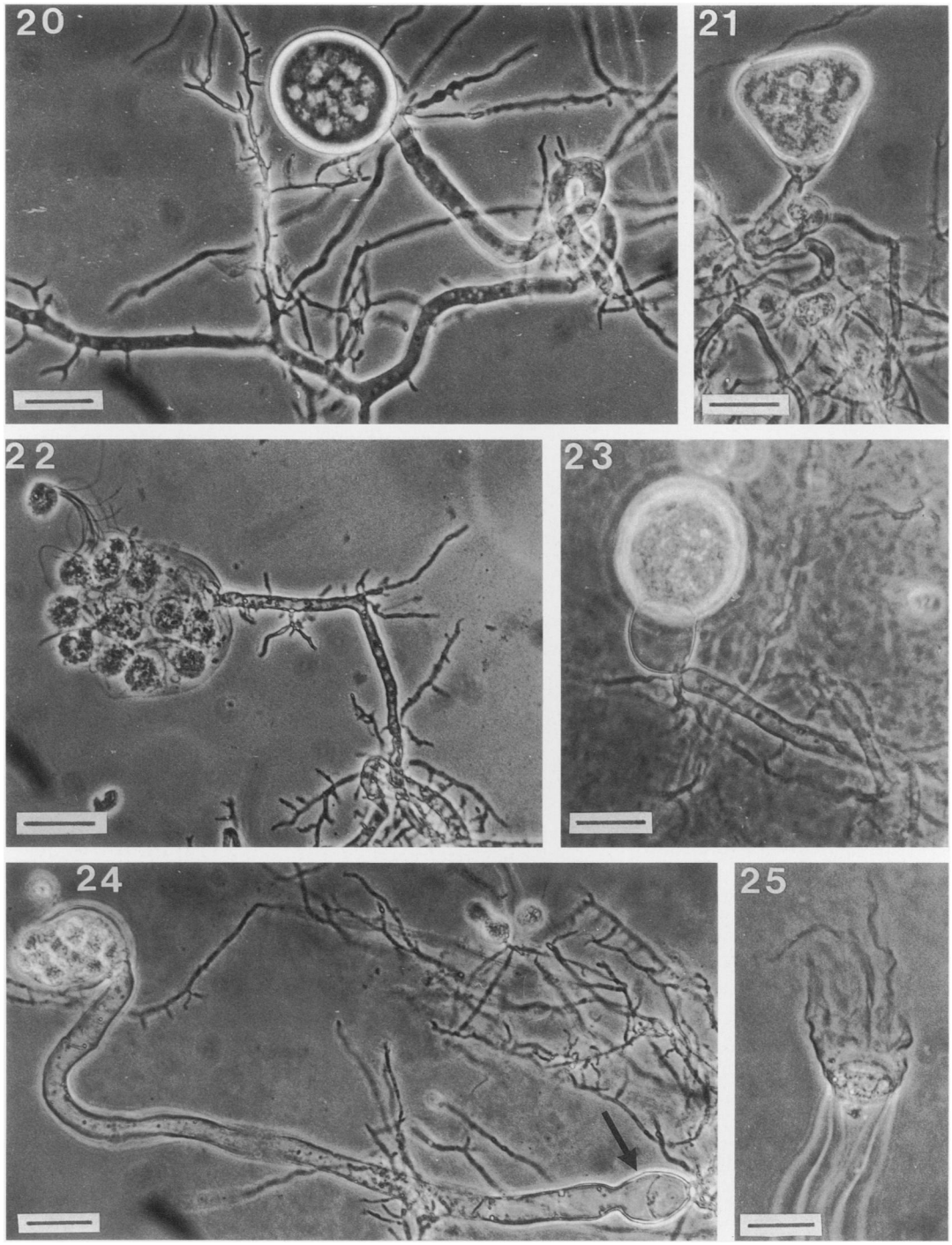
≡ *Neocallimastix joyonii* Breton et al., FEMS Microbiol. Letts. 58: 314, 1989.

= *Orpinomyces bovis* D. J. S. Barr & H. Kudo in Barr et al., Canad. J. Bot. 67: 2819, 1989.

Sporangia mostly spherical, 43–106 μm in diam; developing on simple or branched sporangiophore complexes that arise as outgrowths of hyphae or are terminal (from hyphal tips); zoospore release following rupture and dissolution of the apical part of the sporangium followed by collapse of the sporangial wall; zoospores variable in shape, about 5–15 μm across with about 14–24 flagella, 31–45 μm long; hyphae with tight constrictions.

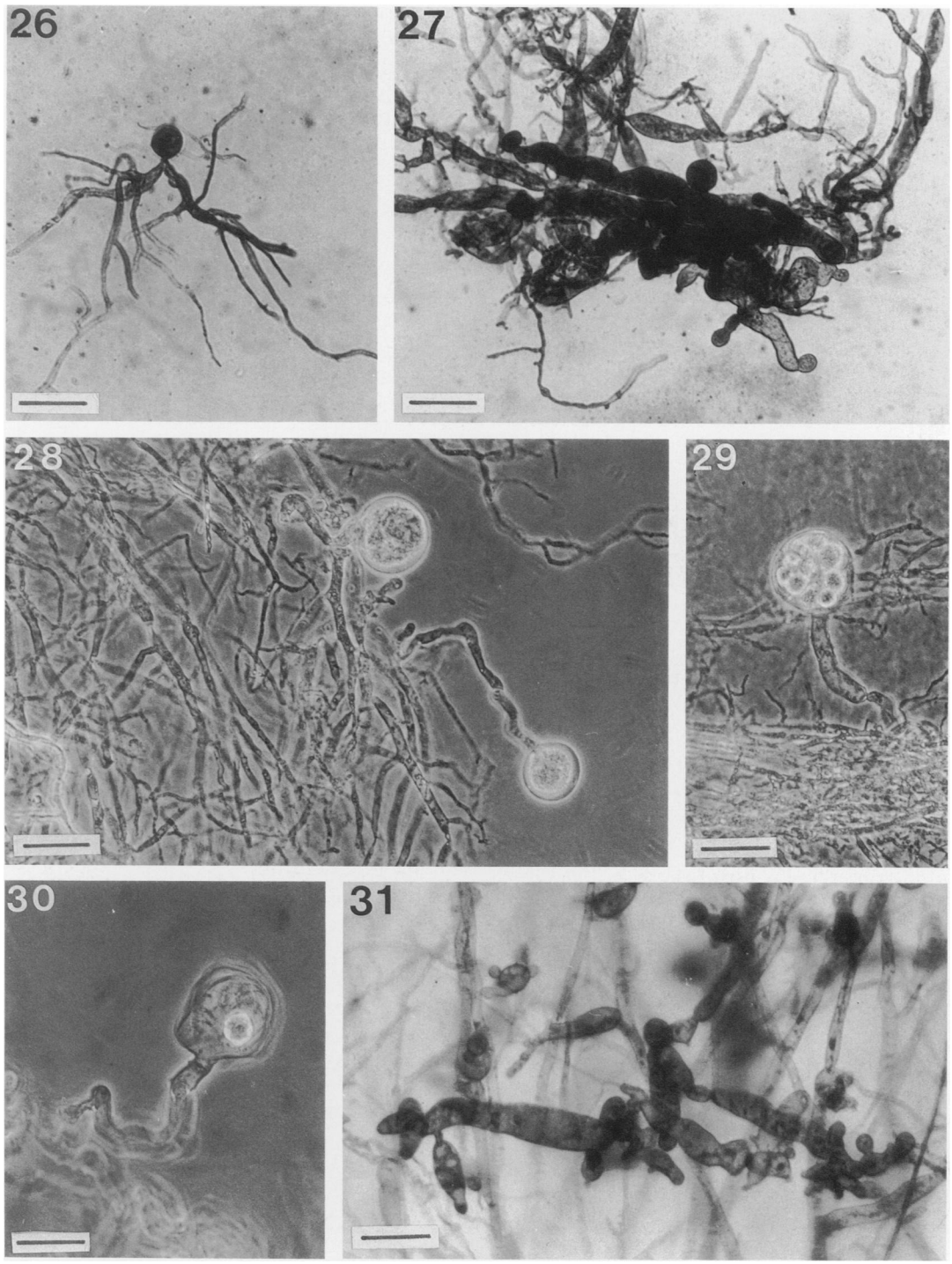
LECTOTYPE. Figs. 1, 2 (Breton et al., 1989).

Habitat. The rumen of sheep, France.



FIGS. 20–25. *Neocallimastix frontalis*. Bar, 20 μm. 20. An endogenous thallus with a spherical sporangium and coiled main rhizoid. 21. An endogenous thallus with a triangular-shaped sporangium. 22. An endogenous sporangium releasing zoospore. 23. An exogenous sporangium with a short, eggcup sporangiophore. 24. An exogenous sporangium (with zoospores) on a long sporangiophore. Note the zoospore cyst (arrow). 25. Collapse and dissolution of sporangial wall following zoospore release.

Breton et al. (1989) reported that the type was at the Laboratory of Microbiology, INRA, Theix, Ceyrat, France. We presume the type was a specimen or illustration, otherwise the name would be invalid. Cultures are not accepted as type material. As the type is no longer available, we have designated their Figs. 1, 2 (Breton et. al., 1989) as the lectotype. The original diagnoses of Breton et al. (Breton et al., 1989) and



FIGS. 26–31. *Orpinomyces joyonii*. Bar, 20 μm. 26, 27. Early stages in the development of thallus. 28. Sporangia developing on sporangiophores. 29. A mature sporangium with zoospores. 30. An empty sporangium with one remaining zoospore after zoospore release. 31. An old culture producing sporangiophore initials (swollen outgrowths from the hyphae).

Barr et al. (1989) are supplemented with additional morphological observations of Li et al. (1991) who also reported on the zoospore ultrastructure.

Old cultures often lose their ability to produce sporangia; nonetheless, many produce sporangiophore initials (swollen outgrowths from the hyphae). These structures are not known in any other species of anaerobic gut fungi.

In Malaysia, *O. joyonii* has been isolated from the rumen of cattle and buffalo (Ho, unpubl.). It has also

been isolated from the rumen of cattle in Australia (Ho and Bauchop, 1991) and in Canada (Barr et al., 1989), and from the rumen of sheep in France (Breton et al., 1989).

Orpinomyces intercalaris Y. W. Ho in Ho et al., Mycotaxon 50: 141, 1994. FIGS. 32–38

Sporangia mostly globose, 26–95 μm in diam, developed from expansion of hyphae or as a lateral outgrowth of a hypha, rarely terminal; zoospore release followed by rupture and dissolution of the sporangial wall; zoospores mostly globose, occasionally subglobose or irregular, about 8.5–14 μm in diam with 12–24 flagella, 33–48 μm long; zoospore cyst persistent; hyphae with tight constrictions.

TYPE. Figs. 1–10 (Ho et al., 1994a); a culture (C70) is maintained in the personal collection of Y. W. Ho, Universiti Pertanian Malaysia.

Habitat. In the rumen of cattle, Malaysia.

Orpinomyces intercalaris differs from *O. joyonii* in the manner that the sporangia are formed. It may also differ by having a persistent zoospore cyst, but this detail was not mentioned in the descriptions for *O. joyonii* (Barr et al., 1989; Breton et al., 1989; Li et al., 1991). This recently described species has not been reported outside of Malaysia, although Phillips (1989) mentioned a polycentric fungus (CM-1) with intercalary sporangia from the rumen of cattle in Northern Australia. Unfortunately, zoospores were not observed and hence identification was not possible.

Anaeromyces Breton et al., FEMS Microbiol. Letts. 70: 181, 1990

≡ *Ruminomyces* Y. W. Ho in Ho et al., Mycotaxon 38: 398, 1990.

Mycelium of indeterminate length arising from a zoospore cyst following monopolar germination; polycentric; zoospores uniflagellate. Type species: *Anaeromyces mucronatus* Breton.

Two similar species of polycentric anaerobic gut fungi with uniflagellate zoospores were described independently within a time-frame of a few weeks; the first name (*A. mucronatus*) has priority. The species are very similar with the exception of lobed or bead-like hyphal structures, thought to be for the penetration of plant cell wall (Ho et al., 1988b), that were reported in *R. elegans* (Ho et al., 1990). It possible that these structures were overlooked in *A. mucronatus*. Until such time that both species are critically examined together, we propose that the two species remain separate, but under the same generic name.

The sporangia of *Anaeromyces* can be readily distinguished from those of *Orpinomyces*, the other mycelial-polycentric genus. In *Anaeromyces* they are solitary, and frequently have a point-like projection at the apex (FIGS. 39, 40). Unfortunately, after several transfers these taxa often stop producing sporangia, and, although these genera cannot then be distinguished with certainty, there are distinctive characters. In *Orpinomyces*, hyphae may become lobed or lobed complexes may develop as lateral outgrowths (FIG. 31), and in *Anaeromyces* the hyphal constrictions are more numerous and often isthmus-like (FIG. 41).

Anaeromyces mucronatus Breton in Breton et al., FEMS Microbiol. Letts. 70: 181, 1990.

Sporangia solitary, ellipsoidal, 15–75 × 29–120 μm, frequently fusiform (i.e., with an apical projection), formed on sporangiophores 4–16 μm wide and 31–83 μm long; zoospore discharge not known; zoospores spherical, 7.5–8.5 μm in diam, uniflagellate, flagellum up to 30 μm long; hyphae frequently constricted.

LECTOTYPE. Fig. 1 (Breton et al., 1990).

Habitat. The rumen of a Holstein cow, France.

Breton et al. (1990) reported that the type was at the collection of the Laboratory of Microbiology, INRA, Clermont-Ferrand-Theix, Saint-Genes-Champanelle, France. We presume the type was a specimen or illustration, otherwise the name would be invalid, and as it is no longer available, we have designated their Fig. 1 (Breton et al., 1990) as the lectotype.

This species has also been found in the rumen of a Holstein steer and an elk in Alberta, Canada (Barr et al., 1995). Anaerobic rumen fungi with fusiform sporangia similar to *A. mucronatus* have been reported to occur in cattle in New Zealand (Bauchop, 1979a), in the USA (Akin and Rigsby, 1987) and in cattle and water buffalo in Australia (Phillips, 1989).

Anaeromyces elegans (Y. W. Ho) Y. W. Ho in Ho et al., Mycotaxon 47: 283, 1993b. FIGS. 39–44

≡ *Ruminomyces elegans* Y. W. Ho in Y. W. Ho et al., Mycotaxon 39: 398, 1990.

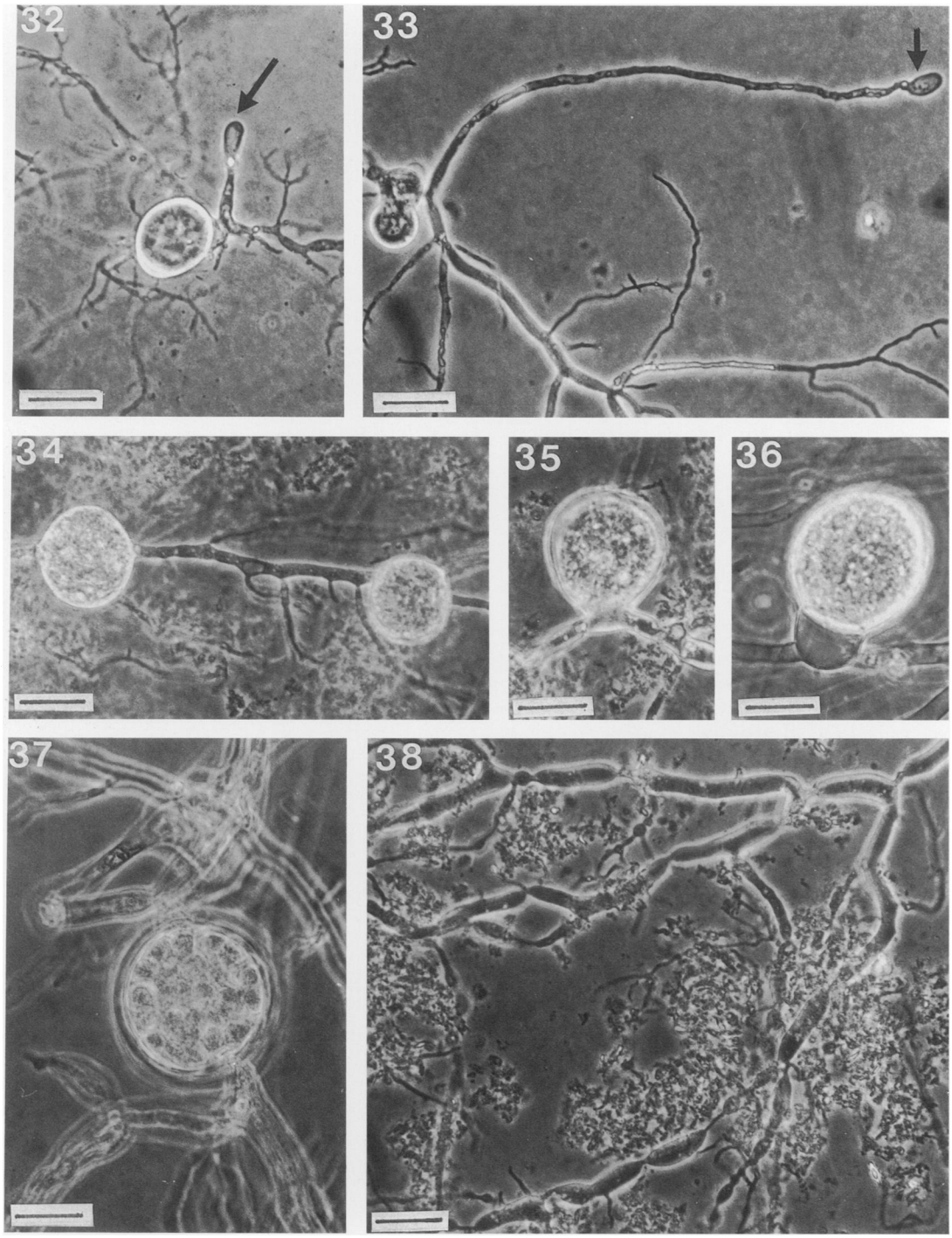
Similar to *A. mucronatus* except for the presence of lobed or bead-like structures on hyphae.

TYPE. Figs. 1–3 (Ho et al., 1990).

Habitat. The rumen of Hereford cattle, Australia.

This species is frequently found in the rumen of cattle and water buffalo in Malaysia.

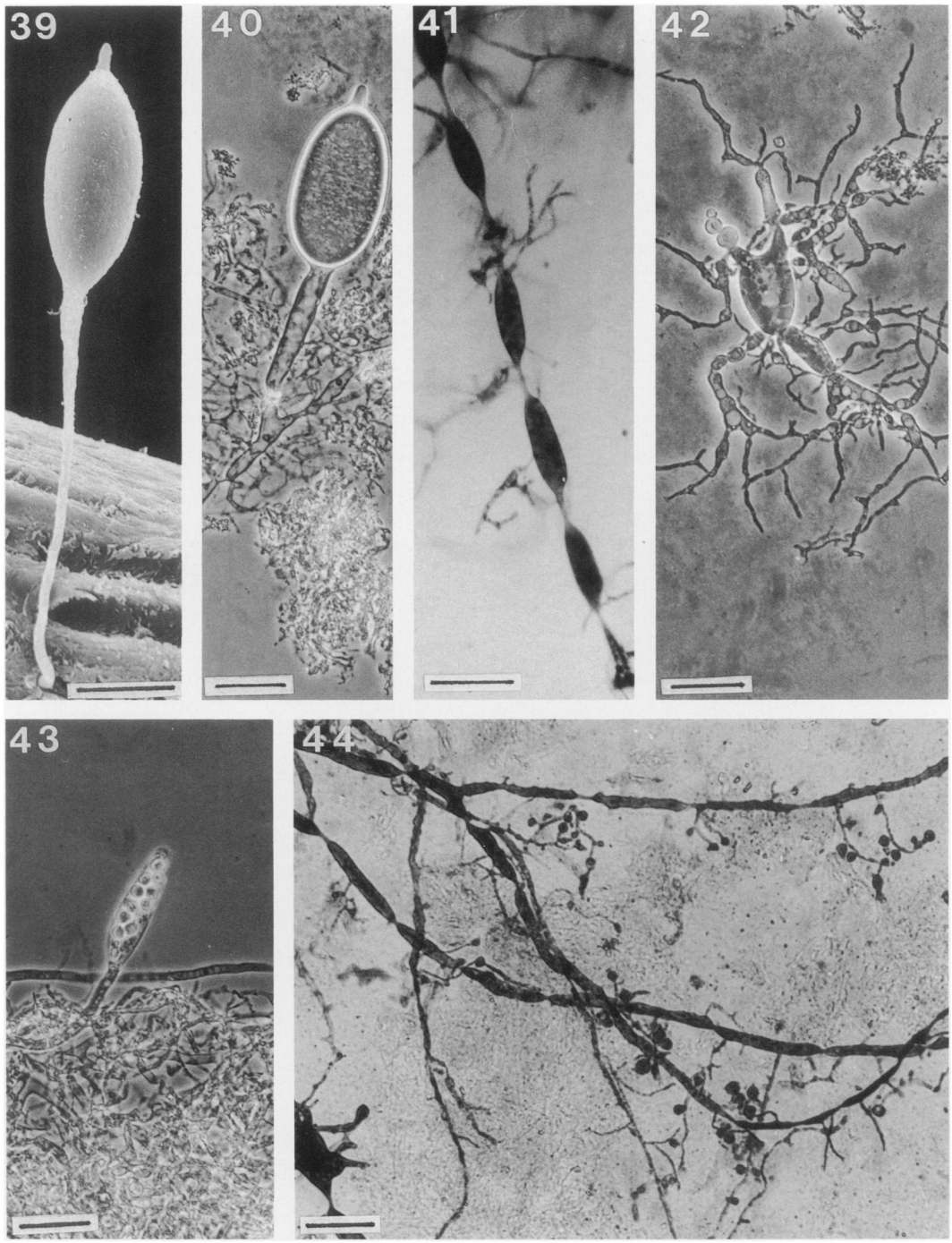
Piromyces J. J. Gold in Gold et al., BioSystems 21: 411, 1988; emend. Li et al., Canad. J. Bot. 68: 1026, 1028, 1990.



FIGS. 32–38. *Orpinomyces intercalaris*. Bar, 20 μm. 32, 33. Initial development of an intercalary sporangium as a swelling in a hyphae in a young mycelium. Note the persistent zoospore cyst (arrows). 34. Two intercalary sporangia develop in one hypha. 35. A young sporangium develops at the side of a hypha. 36. A young sporangium with a cup-shaped basal structure. 37. A sporangium with zoospores. 38. Sausage-shaped hyphae with constrictions at regular distances.

Monocentric; sporangia with a branched, filamentous rhizoidal system; zoospores spherical to ovoid, posteriorly uniflagellate, occasionally bi- to quadiflagellate. Type species: *P. communis*.

There are six species of *Piromyces* in contrast to two, or possibly one, in *Neocallimastix*. But in *Piromyces* we find that, in spite of considerable morphological variations, there are certain unique characters.

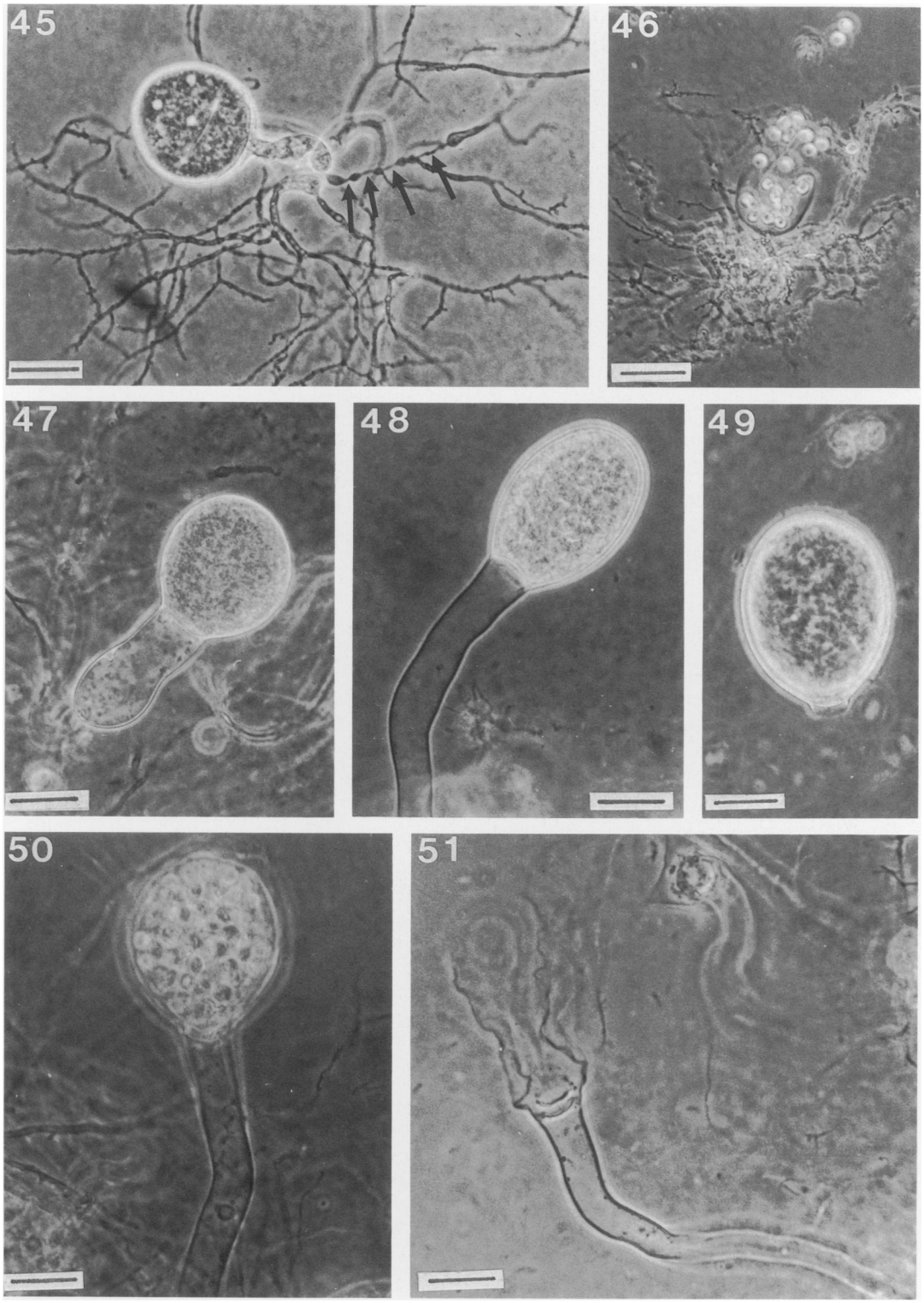


FIGS. 39–44. *Anaeromyces elegans*. Bar, 20 μm. 39, 40. Typical sporangia with pointed or acuminate apex develop on solitary sporangiophores. 41. Hyphal constrictions which are isthmus-like. 42. A young mycelial stage. 43. Mature sporangium with zoospores. 44. Constricted hyphae with lobed- or bead-like structures.

Piromyces communis J. J. Gold et al., BioSytems 21: 411, 1988 (as comb. nov.). FIGS. 45–51

≡ *Piromonas communis sensu* Orpin, J. Gen. Microbiol. 99: 107–117, 1977a, non *Piromonas communis* Liebetanz, Arch. Prostistenk. 19: 37–38, 1910.

Monocentric; sporangia endogenous or exogenous, frequently become detached when mature; endogenous sporangia spherical, 20–105 μm in diam, ellipsoidal or pyriform, generally 30–40 × 50–70 μm; exogenous sporangia generally ellipsoidal to pyriform occasionally irregular, with sporangiophores of varying length from a few microns to over 100 μm long; sporangiophores occasionally branched with two spo-



FIGS. 45–51. *Piromyces communis*. Bar, 20 μm. 45. An endogenous sporangium with coiled main rhizoid and constrictions in some of the rhizoids (arrows). 46. An endogenous sporangium liberating zoospores. 47. An exogenous sporangium with a short sporangiophore. 48. An exogenous sporangium with a long sporangiophore. 49. A detached sporangium. 50. A mature exogenous sporangium with zoospores. 51. Collapse and dissolution of sporangial wall after zoospore release.

rangia (multisporangiate); rhizoids from one axis on the sporangium base, occasionally from two axes; main rhizoid broad, 4–20 μm wide, not constricted, or slightly constricted at the neck, much branched often with constrictions along the rhizoids; zoospore discharged by dissolution of wide apical part of the wall followed by dissolution of the remaining wall; zoospores highly variable in shape from globose to irregular, 4.5–9.5 μm in diam, uniflagellate, occasionally bi- to quadiflagellate; flagella 22–29 μm long. Gold et al. cited *P. communis* as a gen. nov., comb. nov. and provided both a type and a Latin diagnosis, but failed to provide a validly published basionym. The authority is therefore corrected to Gold et al. and treated as a new generico-specific name.

TYPE. Figs. 1, 5–10 (Orpin, 1977a).
Habitat. The rumen of sheep, United Kingdom.

There is no earlier diagnosis; this one is based primarily on observations of an isolate from a Holstein steer in Canada (Barr et al., 1989) supplemented by the original observations of Orpin (1977a). The species most similar to *P. communis* is *N. frontalis*; they are easily distinguished by the number of flagella in the zoospore. The broad neck distinguishes *P. communis* from other species of *Piromyces.*

In Malaysia, *P. communis* has been isolated from the rumen of sheep, goat (Ho et al., 1994b), cattle, water buffalo and deer and from the duodenal contents of sheep (Ho, unpubl.). Coiling of the main rhizoid is frequently found in the endogenous forms. *Piromyces communis* has also been isolated from the rumen of sheep in the United Kingdom (Orpin, 1977a) and France (Gaillard and Citron, 1989) and the rumen of cattle in Canada (Barr et. al., 1989).

Piromyces mae J. L. Li in Li et al., Canad. J. Bot. 68: 1028, 1990. FIGS. 52–60

Monocentric; sporangia endogenous or exogenous, spherical, ovoid, pyriform to elongated, 26–37 × 70–125 μm, frequently with one, occasionally two distinctive papillae; exogenous sporangia on sporangiophore of variable length occasionally branched with two to three sporangia (multisporangiate); main rhizoid tubular and often swollen below the neck, neck partially to tightly constricted, port narrow; rhizoids extensively branched, extending up to 240 μm; discharge follows dissolution of one or two papillae, wall persistent; zoospores spherical to ovoid, 2.5–11.0 μm, uniflagellate, rarely two to four flagella, flagella 20–30 μm long.

TYPE. Figs. 1, 3, 5–8, 10–11, 15–16, 20–22, 25–30, 32 (Li et al. 1990); there is no culture.
Habitat. Fecal material from a horse, New Zealand.

The diagnosis of Li et al. (1990) is supplemented by a detailed account of this species by Gaillard-Martinie et al. (1992) and our own observations. The exogenous growth form is not mentioned in either. Li et al. (1990) reported elongated subsporangial swelling. Gaillard-Martinie et al. (1992) specified that the sporangium was not pedicellate, but they illustrated sporangiophores, and referred to a subsporangial swelling without constriction. Our observations of isolates with distinctive papillae similar to *P. mae* show that exogenous growth is common, and we think it is likely that the aforementioned authors misinterpreted their observations. The species most similar to *P. mae* is *P. rhizinflatus* (see discussion below).

In Malaysia, *P. mae* has been isolated from the rumen and fecal materials of cattle, buffalo, sheep and goat and from the duodenal contents of sheep (Ho, unpubl.). It has also been isolated from the rumen of an elk in Canada (Barr et al., 1995) and sheep rumen in France (Gaillard-Martinie et al., 1992).

Piromyces dumbonicus J. L. Li in Li et al., (as *dumbonica*) Canad. J. Bot. 68: 1028, 1990.

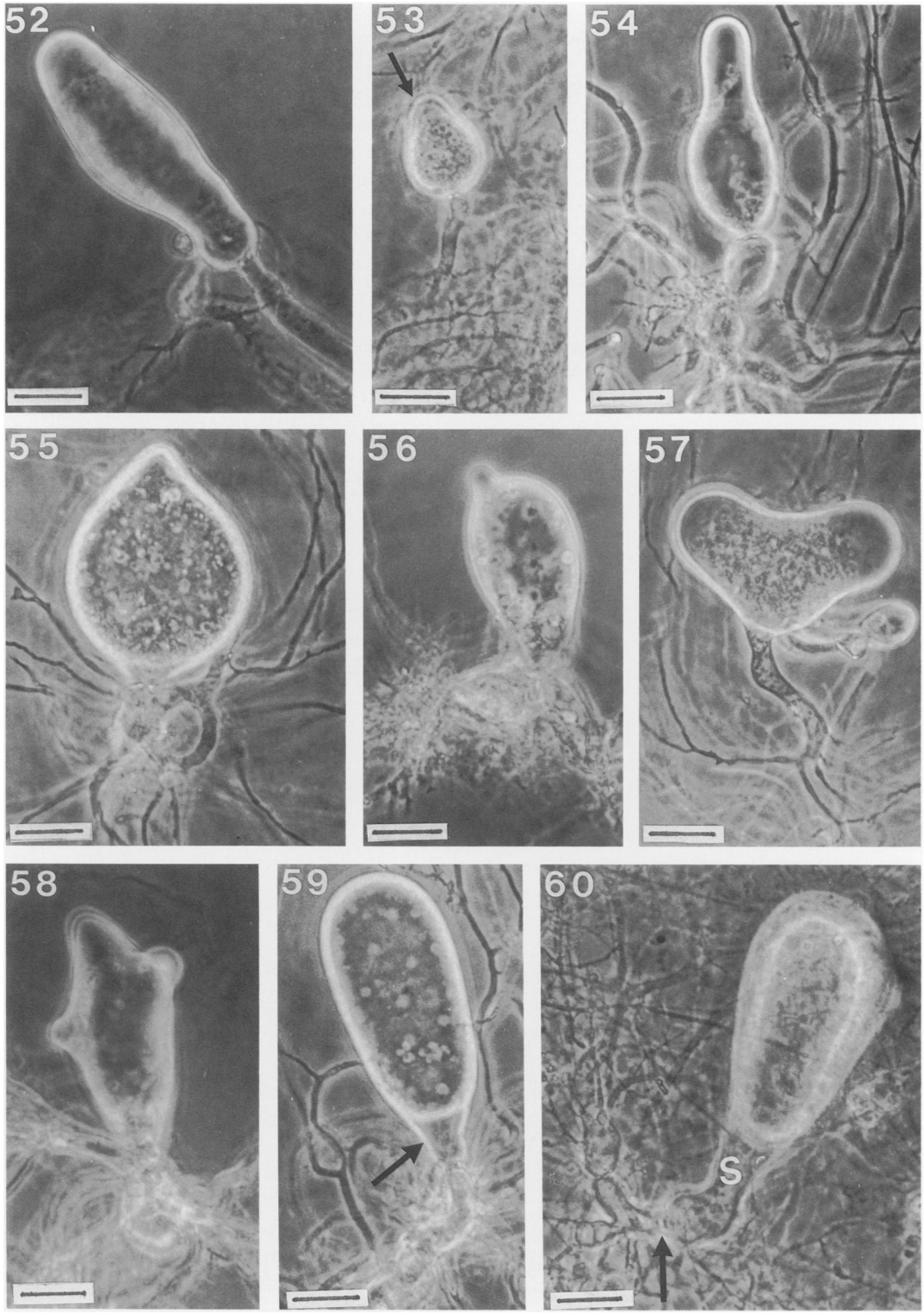
Monocentric; sporangia spherical to elongate, 30–56 × 100–112 μm, nonpapillate, with or without elongate subsporangial swelling; rhizoids extensively branched extending up to 600 μm; zoospores spherical to ovoid, 5.5–12.0 μm, uniflagellate, rarely biflagellate.

TYPE. Figs. 2, 4, 9, 12–13, 19, 23, 24, 31 (Li et al. 1990); there is no culture.
Habitat. Fecal material from an Indian elephant, New Zealand.

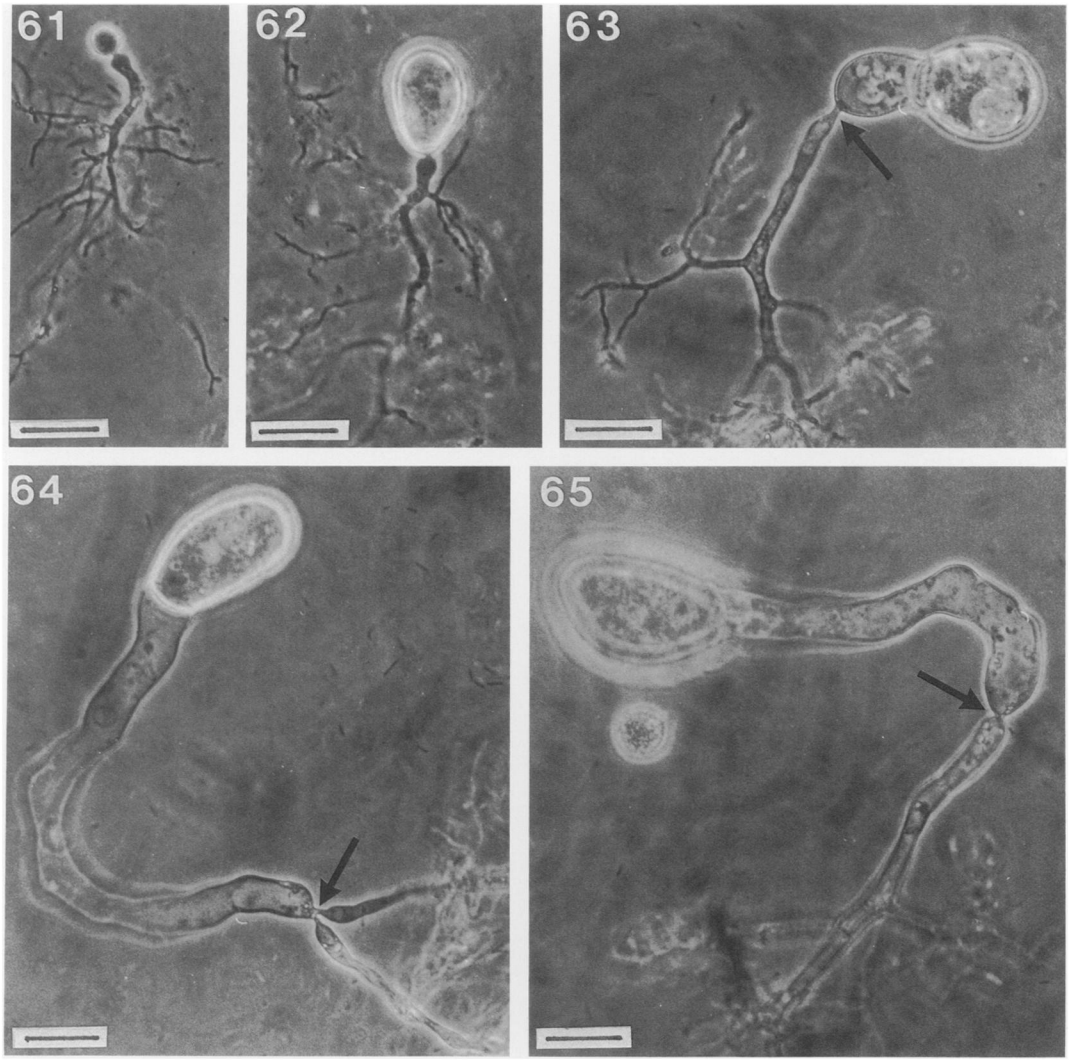
A critical analysis of the zoospore ultrastructure of this species and *P. mae* (Li et al., 1990) provides convincing evidence that these two species are different. However, the diagnosis and description provide scant information on the morphology, and it is not possible to determine whether this species is morphologically distinctive. The most closely related species is perhaps *P. rhizinflatus.* The original description is the only report of this species.

Piromyces rhizinflatus Breton et al., (as *rhizinflata*) FEMS Microbiol. Letts. 82: 8, 1991. FIGS.61–65

Monocentric; sporangia endogenous or exogenous and occasionally multisporangiate; small sporangia elongated, 8–9 × 20–25 μm, larger sporangia pyriform, ovoid, ellipsoidal, almost spherical up to 130 μm in diam; small to medium sized endogenous sporangia often with a narrow basal segment and a much larger expanded portion; neck constricted often isth-



FIGS. 52–60. *Piromyces mae.* Bar, 20 μm. 52. An endogenous ellipsoidal sporangium with a constricted neck but without a subsporangial swelling. 53. An endogenous pyriform sporangium with a papilla (arrow), constricted neck and a slight subsporangial swelling. 54. An endogenous sporangium with a papilla, constricted neck and subsporangial swelling. 55. An endogenous sporangium with papilla, constricted neck, subsporangial swelling and a coiled main rhizoid. 56. An endogenous sporangium with a very distinct papilla. 57. An endogenous sporangium with two papillae. 58. An endogenous sporangium with 3 papillae. 59. An exogenous sporangium with a short sporangiophore (arrow). 60. An exogenous sporangium with a sporangiophore (S) and constriction at the base of the sporangiophore (arrow).



FIGS. 61–65. *Piromyces rhizinflatus.* Bar, 20 μm. 61, 62. Young endogenous sporangia with constricted necks, sometime isthmus-like (FIG. 61) and subsporangial swellings. 63. An exogenous sporangium with a short sporangiophore and a constricted isthmus-like basal segment (arrow). 64, 65. Exogenous sporangia with long sporangiophores and constricted basal segments (arrows).

mus-like; main rhizoid often with a subsporangial swelling; rhizoids extensively branched, on large sporangia often over 1 mm in extent; exogenous sporangia on sporangiophores of variable length; sporangiophore often with constricted basal segment; discharge follows dissolution of a wide apical portion of the sporangium wall, wall persistent; zoospore spherical, 4.2–10.0 μm in diam, uniflagellate, flagellum 20–27 μm long with whiplash or beaded end.

LECTOTYPE. Figs. 1–2 (Breton et al., 1991); a culture is maintained at the Laboratory of Microbiology, INRA Clermont-Ferrend-Theix, France.

Habitat. Fecal material from a Saharian ass, Tunisia.

The diagnosis is based on the original description and unpublished observations of isolates from Alberta and Malaysia. As with *P. mae,* we believe that the sporangium is not exclusively endogenous. In their Fig. 1d, Breton et al. (1991) showed an exogenous sporangium with a subsporangial swelling and sporangiophore. In the Albertan material a sporangiophore is quite common, perhaps due to the substrate, but always short, whereas in the Malaysian material it is as long as 100 μm (FIGS. 64, 65). This species is similar to *P. mae* and some thalli of each species may be indistinguishable, thus requiring the observation of sufficient materials in good growing condition. Indeed, when more observations of different isolates are made, it is possible that there will be arguments to place this species in synonymy with *P. mae.* The differences appear to be in the neck constriction and discharge. In *P. rhizinflatus* the constriction is always present and

sometimes extended (isthmus-like, FIGS. 61–65), but in *P. mae,* the neck is partially to tightly constricted. The apical area of the sporangium in *P. rhizinflatus* may occasionally be slightly convex, but the wall is uniform in thickness, whereas in *P. mae* there is often a distinctive papilla, sometimes with a thickened wall and sometimes there are two papillae. Breton et al. (1991) reported that zoospores sometimes remained in a cluster on the proximal end of the rhizoid; this characteristic is similar to *P. spiralis,* but this was not seen in *P. rhizinflatus* from Canada and Malaysia.

Apart from the original description from Tunisia, this species has been isolated from elk, bison and moose in Alberta, Canada (Barr et al., 1995) and from the rumen of cattle and goat, and the duodenal contents of sheep in Malaysia (Ho, unpubl.).

Piromyces minutus Y. W. Ho in Ho et al., Mycotaxon 47: 286–287, 1993c. FIGS. 66–71

Monocentric; sporangia strictly endogenous, ellipsoidal, pyriform or spherical, predominantly 8–25 × 8.5–28 μm, occasionally 40–80 μm in diam; rhizoids from a single axis, occasionally two to four axes; main rhizoid usually unbranched terminating in a sparingly branched rhizoidal system; zoospore discharge following dissolution of a wide apical pore, occasionally two pores on large sporangia; sporangial wall persistent after discharge; zoospores globose, 5.5–7.5 μm in diam, uniflagellate, occasionally bi- to quadiflagellate, flagellum up to 31 μm long.

TYPE. Figs. 1–4 (Ho et al., 1993c); a culture (D2) is maintained in the personal collection of Y. W. Ho, Universiti Pertanian Malaysia.

Habitat. Rumen of a Sika or Japanese deer, Malaysia.

In culture this species is easily distinguished from other *Piromyces* spp. by the small size of the sporangia. It has only been reported from Malaysia where it has also been found in the rumen of Timorensis deer (Ho et al, 1993c), goat, sheep (Ho et al, 1994b) and in the duodenum of sheep (Ho, unpubl.).

Piromyces spiralis Y. W. Ho in Ho et al., Mycotaxon 48: 60, 1993d. FIGS. 72–77

Monocentric; sporangia endogenous, mostly globose to subglobose, 14–100 × 15–120 μm; rhizoid neck variable, constricted with subsporangial swelling to broad and tubular; rhizoids from a single axis, occasionally two to three, conspicuously coiled, branched and extensive; zoospore discharge by rapid dissolution of the sporangial wall; zoospores globose to subglobose, 4–7 × 4–8.5 μm, uniflagellate, occasionally bi- to quadiflagellate, flagellum 26–34 μm long.

TYPE. Figs. 1–14 (Ho et al., 1993d); a culture (G34) is being maintained in the private collection of Y. W. Ho, Universiti Pertanian Malaysia.

Habitat. The rumen of goat, Malaysia.

This species is distinctive in three characteristics. In the discharge process the sporangial wall dissolves so rapidly that the cleaved zoospores are left in a cluster at the proximal end of the main rhizoid; eventually, they swim away. The sporangia are spherical to subspherical, and elongated sporangia are exceptional. Although coiling of the main rhizoids occurs in many species, coiling of most of the rhizoids is a very consistent character of *P. spiralis.*

This species has only been reported from the rumen of goat in Malaysia.

Caecomyces J. J. Gold in Gold et al., BioSystems 21: 411, 1988.

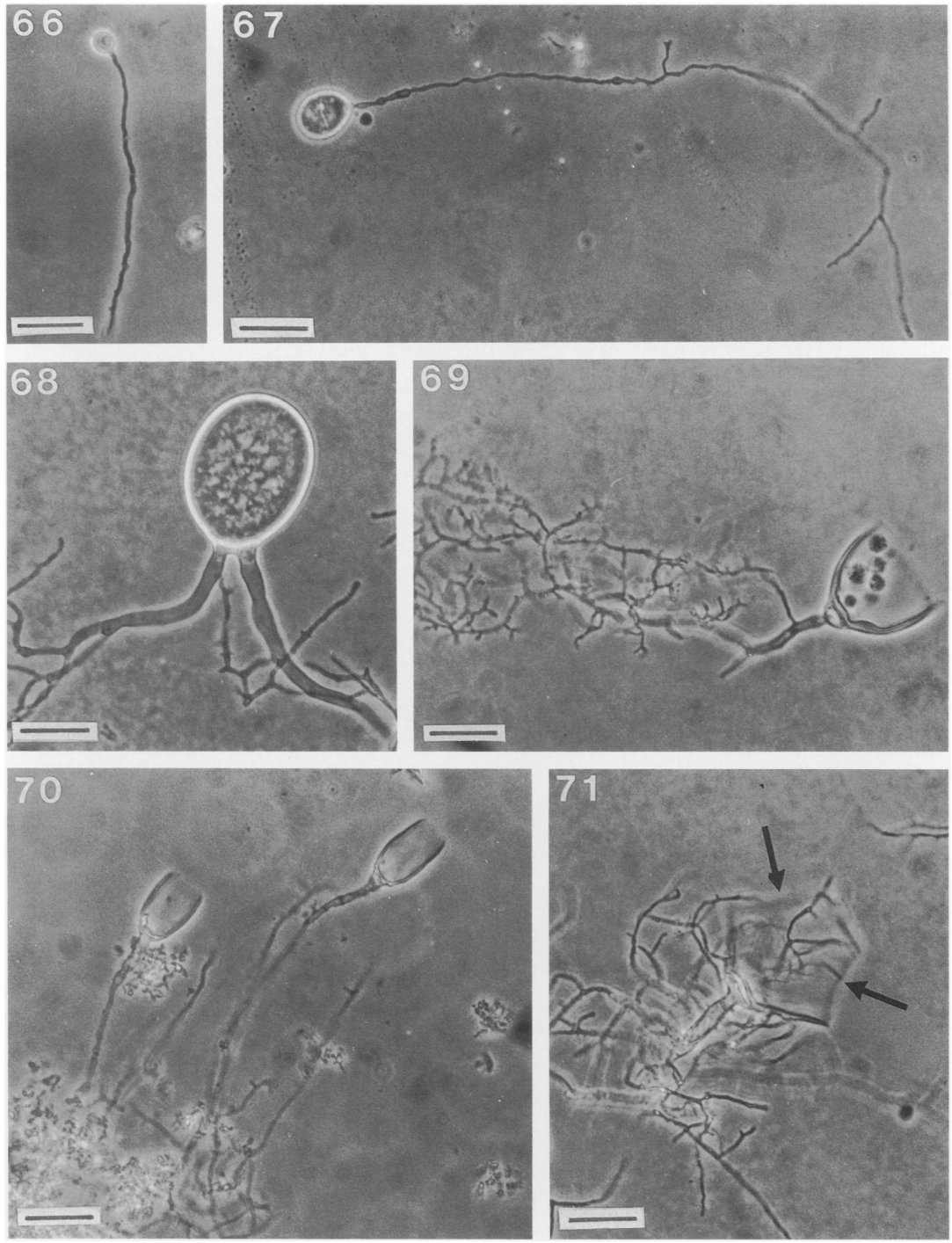
≡ *Sphaeromonas sensu* Orpin, J. Gen. Microbiol. 94: 270–280, 1976.

Monocentric and uni- or multisporangiate; unisporangiate thalli comprise a globose, ovoid or ellipsoidal sporangium attached to one, or a series of bulbous rhizoids; multisporangiate thalli like unisporangiate thalli except that two or more sporangia develop as outgrowths of the bulbous rhizoids; zoospores spherical to ovoid, uniflagellate, occasionally biflagellate. Type species: *C. equi* (? = *C. communis*).

This diagnosis is based on the work of Gold et al. (1988), Wubah et al. (1991a), and our own unpublished observations. There are two species distinguished either by the presence of a single bulbous rhizoid or having two or more rhizoids (Gold et al., 1988). However, Wubah et al. (1991a), in a critical study on the development and life cycle of *C. communis,* showed that this character was variable. Our observations show that the single form predominates in the first day of culture, and that thalli with several bulbous rhizoids, and two or more sporangia occur frequently in older cultures. The criteria for separating the two species is therefore of questionable validity. There is no longer a culture of *C. equi*; the description was based on examination of cultured material embedded in plastic. It is therefore difficult to place the two species in synonymy without further study of cultures of *C. equi* from the type habitat.

Caecomyces equi J. J. Gold in Gold et al., BioSystems 21: 411, 1988.

? = *C. communis* J. J. Gold et al., BioSystems 21:411, 1988.



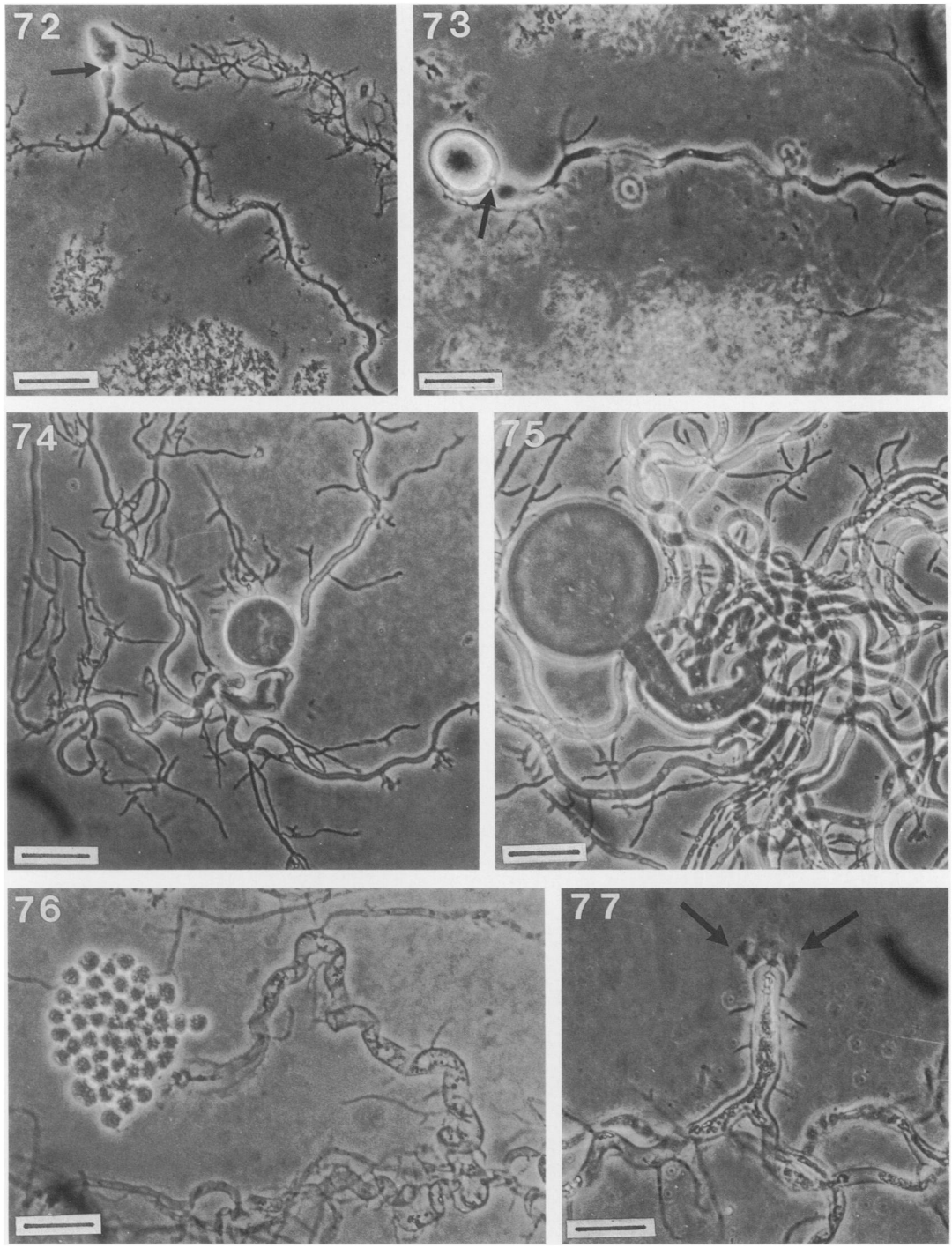
FIGS. 66–71. *Piromyces minutus*. Bar, 20 μm. 66. Early developmental stage showing a straight and unbranched main rhizoid. 67. Rhizoidal system showing straight and unbranched main rhizoid terminating in sparsely-branched rhizoids. 68. Rhizoidal system with two main rhizoids. 69. Liberation of zoospores following the dissolution of a wide apical portion of the sporangial wall. 70. Empty cup-shaped sporangia with a wide apical opening after discharge of zoospores. 71. An empty sporangium with two wide pores or openings (arrows).

Monocentric; sporangium with a single bulbous rhizoid, with attached fine fibrillar rhizoids in culture and in plant material a large lobed coralloid rhizoid.

TYPE. Figs. 1–20 (Gold et al., 1988).
Habitat. Fecal material from a horse, New Zealand.

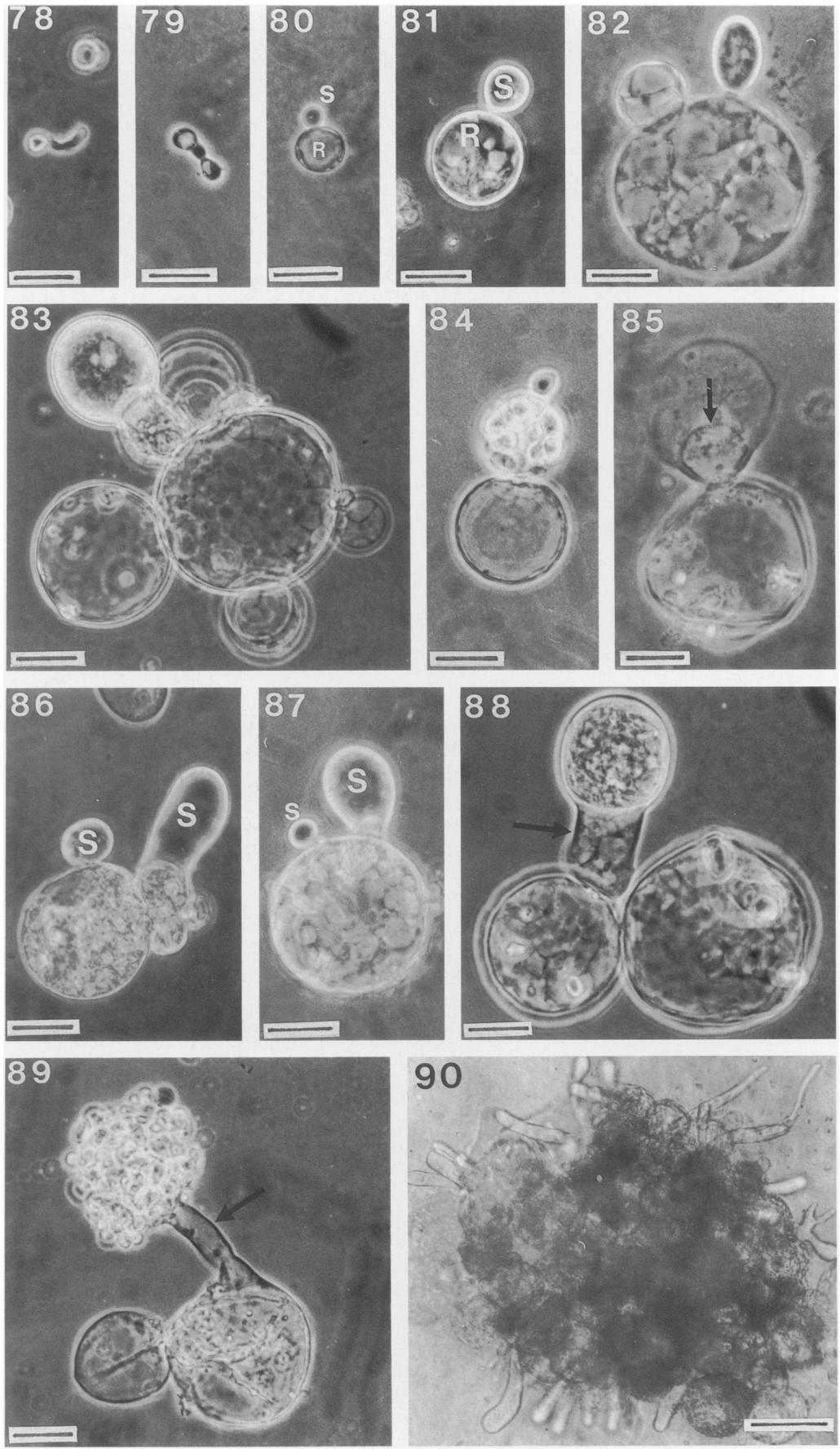
Caecomyces communis J. J. Gold et al., BioSystems 21: 411, 1988 as "comb. nov." FIGS. 78–90

≡*Sphaeromonas communis sensu* Orpin, J. Gen. Microbiol. 94: 270–280, 1976, non *S. communis* Liebetanz, Arch. Protisteuk. 19: 26–31, 1910.



FIGS. 72–77. *Piromyces spiralis*. Bar, 20 μm. 72. Young thallus showing the main rhizoid with subsporangial swelling and a constricted, elongated isthmus-like neck (arrow). 73. Main rhizoid with subsporangial swelling, short constricted neck and a moderately wide port (arrow). 74. Main rhizoid with irregularly bulbous subsporangial swelling, short constricted neck and coiled rhizoids. 75. Main rhizoid with wide neck and port. Note the coiled rhizoids. 76. A cluster or glomerule of zoospores on the main rhizoid. Dissolution of the sporangial wall is very rapid. 77. Remains of the sporangial wall (arrows) on the main rhizoid. The rest of the sporangial wall has disintegrated.

Monocentric with one, two or occasionally three to four spherical or broadly ellipsoidal sporangia, 20–60 μm in diam; rhizoids bulbous, single or multiple, 22–88 μm in diam; zoospore discharge by dissolution of a wide apical portion of the sporangial wall. Zoospore spherical to ovoid, 5–11 μm in diam, flagellum up to 40 μm long. Although cited as a comb. nov. by Gold et al., no validly published name was cited as a bas-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ionym. *Sphaeromonas communis sensu* Orpin is not a validly published name and *S. communis* Liebetanz was specifically excluded. The authority is therefore corrected to Gold et al., sp. nov. Gold et al. supplied a Latin diagnosis and designated the TYPE as Figs. 1, 2, 5–10, Orpin (1976).

Habitat. The rumen of sheep, United Kingdom.

In cultures over 20-h old, Wubah et al. (1991a) reported that multisporangiate thalli developed with coarse branches and ovoid sporangia. They also reported that occasionally a sporangium was formed on the end of a short sphorangiophore, an observation we have also detected (FIGS. 88, 89), particularly on colonies growing embedded in agar medium such as those spun on roll tubes (FIG. 90). In such a situation, sporangia are mostly formed on long tubular sporangiophores, presumably to raise them to the surface of the agar medium. Following zoospore discharge, empty sporangia show a wall-like structure in the base of the sporangium (FIG. 85). This structure is not continuous with the sporangial wall as in other chytrids with a basal septum (Ho et al. 1993c), and appears to be an extrusion from the bulbous rhizoid.

In Malaysia, *C. communis* has been isolated from the rumen and fecal materials of water buffalo (Ho et al., 1994b), cattle, sheep and goat and the duodenal contents of sheep (Ho, unpubl.). It has also been observed that *C. communis* occurs very abundantly (30 to over 90% of the total fungal population detected in the roll tube isolation) when the host animal is fed rice straw with molasses or palm press fiber with molasses (Ho, unpubl.). *Caecomyces communis* has also been isolated from the caecum of a horse and the rumen of sheep in the United Kingdom (Orpin, 1976, 1981) and France (Gaillard and Citron, 1989), and fecal material of cattle in the USA (Wubah et al., 1991a).

DISCUSSION

Systematics covers the classification and identification of organisms, and in a broader context their evolution. There is a general misconception that classification and identification require the same characters. A classification is ideally based upon characters that best reflect the evolution of organisms, thus grouping taxa of all ranks into natural clusters. In identification keys, the most convenient characters are used provided the outcome is the same. In the classification of chytrids and many other groups of lower eukaryotic organisms, such as algae and protozoa, the ultrastructure of the cell, and especially the flagellar/cilia apparatus, is generally believed to provide the best visual characteristic that reflects their natural evolution. Ultrastructure was thus introduced into the classification of the Chytridiomycetes for genera and higher ranking taxa (Barr, 1980, 1984), but these taxa can also be identified using the light microscope. Thus, once a classification is established it only becomes necessary to do an ultrastructural investigation when a substantially different organism is discovered.

Ultrastructure was never intended to be used for the classification of chytrid species. Nonetheless, any ultrastuctural difference that does exist provides compelling evidence of a significant, phylogenetic difference. Ultrastructural examination of aerobic chytrid zoospores has been done very largely on actively swimming spores. Chytrid zoospores are often nonmotile on release, and again prior to encystment when the zoospore may become ameboid. Prior to and during encystment there is depolymerization of microtubules, mitochondria may divide, and there is general reorganization of organelles. It is unfortunately difficult, although not impossible (Munn et al., 1987), to examine motile zoospores of anaerobic species. In spite of some very meticulous studies on the ultrastructure of the anaerobes, it is hard to compare small differences between species unless material is fixed at the same stage of development, and it is totally impractical to use these differences for identification purposes.

Species of anaerobic gut fungi can be identified solely on characters seen in the light microscope, but the phylogenetic relatedness of species to one another, and the classification at the subspecies level of physiological types and possibly of host specific forms, will require more exacting technology. At present, methodologies such as isozyme analysis (Ho et al., 1994b) and DNA hybridization can be used in the identification of fungal species, and are very precise. A greater level of precision in the classification of anaerobic gut fungi is required, and the future likely lies in molecular technology.

←

FIGS. 78–90. *Caecomyces communis.* FIGS. 78–80, bar, 20 μm; FIG. 90, bar, 100 μm. 78–81. Stages in the development of the sporangium (S) and bulbous rhizoid (R). 82. Thallus with two bulbous rhizoids. 83. Thallus with many bulbous rhizoids. 84. A mature sporangium liberating zoospores. 85. An empty sporangium showing a wall-like structure in the base of the sporangium (arrow). 86, 87. Examples of multisporangiate thalli. S, sporangia. 88. A sporangium forms on the end of a short sporangiophore (arrow). 89. A mature sporangium (with zoospores) develops on a sporangiophore (arrow). 90. A colony of *C. communis* growing on agar medium in a roll tube showing long tubular sporangiophores.

ACKNOWLEDGMENTS

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