Cyllamyces aberensis gen.nov. sp.nov., a new anaerobic gut fungus with branched sporangiophores isolated from cattle

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Abstract: A new genus of the anaerobic gut fungi (Neocallimastigales), ***Cyllamyces aberensis*** gen.nov. sp.nov., with bulbous holdfast, branched sporangiophores and limited polycentric thallus development is described. The fungus was isolated from fresh cattle faeces. Free-swimming zoospores were spherical, uninucleate, and uniflagellate. After encystment, zoospores germinated and gave rise to a single, bulbous holdfast. One or several branched sporangiophores were produced from different locations on the holdfast, each bearing several spherical sporangia. DAPI staining of thalli indicated that nuclei were present in the holdfast, sporangiophores, and sporangia. As many as 12 sporangia were observed per thallus on up to 5 sporangiophores, with zoosporogenesis beginning 16–18 h after encystment. Zoospore ultrastructure was examined by transmission electron microscopy and found to be similar to that reported for other anaerobic chytrid fungi. Organelles were evenly distributed throughout the cell, except for the posteriorly attached flagellum and associated attachment apparatus, the hydrogenosomes, which were mainly situated in the posterior parts of the cell and a posteriorly directed, beak-shaped nucleus. Limited polycentric thallus development (including branched sporangiophores), the possession of a single bulbous holdfast and the absence of rhizoids were stable features of this fungus that distinguished it from the other five genera of gut fungi. Therefore, we have used these characteristics to assign the fungus to a new genus, *Cyllamyces*, with the specific name *C. aberensis.*

Key words: rumen, fungal taxonomy, Neocallimastigales, chytrid, zoospore ultrastructure.

Résumé : Les auteurs décrivent un nouveau genre de champignon intestinal anaérobie (Neocallimastigales), le ***Cyllamyces aberensis*** gen.nov., sp.nov., muni d'un pied bulbeux, de sporangiophores ramifiés et développant un thalle polycentrique limité. Ils ont isolé ce champignon de fèces fraîches de bétail. Les zoospores libres sont sphériques, uninuclées et uniflagellées. Après l'enkystement, les zoospores germent et donnent naissance à un pied bulbeux unique. À partir de ce pied, un ou plusieurs sporangiophores ramifiés se développent à partir de différents points sur le pied, chacun portant plusieurs sporanges sphériques. La coloration DAPI des thalles indique la présence de noyaux dans le pied, les sporangiophores et les sporanges. On observe jusqu'à 12 sporanges par thalle sur moins de 6 sporangiophores, la zoosporogénèse commençant 16–18 h après l'enkystement. Les auteurs ont examiné l'ultrastructure des zoospores en microscopie électronique par transmission; on constate sa similitude avec celle des autres champignons chytridiens. Les organelles sont distribuées uniformément dans l'ensemble de la cellule, sauf pour le flagelle situé dans la partie arrière et les appareils d'attachement associés, les hydrogénosomes, qui sont surtout situés dans la partie postérieure de la cellule, ainsi qu'un noyau en forme de bec, dirigé vers l'arrière. Le développement polycentrique limité du thalle (incluant les sporangiophores ramifiés), la possession d'un pied unique bulbeux et l'absence de rhizoïde constituent des caractéristiques stables de ce champignon qui le distinguent des cinq autres genres de champignons intestinaux. Les auteurs ont par conséquent utilisé ces caractéristiques pour placer ce champignon dans le nouveau genre *Cyllamyces* avec le nom spécifique *C. aberensis*.

Mots clés : rumen, taxonomie fongique, Neocallimastigales, chytrides, ultrastructure de zoospores.

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Introduction

The anaerobic gut fungi were first classified as Chytridiomycetes, in the order Spizellomycetales, by Heath et al. (1983). Li and Heath (1992) and Li et al. (1993) through sequence analysis of the small (18S) nuclear rRNA region and numerical taxonomic methods, later provided convincing evidence that a new order, the Neocallimastigales (sensu Ho and Barr 1995), should be erected to accommodate these fungi. Although the taxonomy of the gut fungi has changed considerably since their discovery (Orpin 1975), 14 species are now recognised and classified into 5 genera (Ho and Barr 1995). Of the five genera of gut fungi, four contain species that are either polycentric or monocentric with a rhizoidal growth habit. Species in the fifth genus, *Caecomyces*, are monocentric with either a single or several bulbous holdfast(s), upon which a single sporangium develops. According to Barr (1983), acquisition of the polycentric growth habit (*Anaeromyces* and *Orpinomyces*) is of significance in evolution of chytrid fungi, representing a potential route for evolution of more advanced fungal forms.

The zoospores of the three genera *Anaeromyces*, *Piromyces*, and *Caecomyces* are uniflagellate, whereas those of *Orpinomyces* and *Neocallimastix* are multiflagellate. The genus *Caecomyces* contains two species, ***Caecomyces communis*** Gold et al. comb.nov. (originally called *Sphaeromonas communis*; Orpin 1976) and *Caecomyces equi* Gold et al. (Gold et al. 1988), but they have received relatively little attention. From a functional standpoint, whereas the rhizoidal fungi are reported to be potent degraders of plant fibre, producing an extensive network of branched and tapering rhizoids to aid in substrate colonization, *Caecomyces* spp. produce a more limited thallus, and it is proposed that they contribute to degradation by expanding from within and rupturing colonised tissues (Joblin 1989).

Here we report on the isolation and life-cycle characteristics of a fungus with polycentric development (notably the development of multiple sporangia borne on branched sporangiophores), the possession of a single bulbous holdfast and the absence of a distinctive rhizoidal growth habit. These were stable features of the fungus that distinguished it from the existing five genera of gut fungi. Therefore, we have used these morphological characteristics to assign the fungus to a new genus, ***Cyllamyces***, with the specific name ***Cyllamyces aberensis***.

Materials and methods

Isolation procedure

The isolation procedure involved 10-fold serial dilutions of the freshly voided faeces of a cow (animal No. 114 from the milking herd kept at the Institute of Grassland and Environmental Research) fed ad libitum on a grass silage. The dilution series was made in a complex medium under anaerobic conditions, as described by Davies et al. (1993) (medium C; 15% clarified rumen fluid, 2.5 $g \cdot L^{-1}$ yeast extract, 10 $g \cdot L^{-1}$ trypticase peptone, 6 $g \cdot L^{-1}$ $NaHCO_3$, and a basal salts solution) with cellobiose as the energy source (5.0 $g \cdot L^{-1}$) and 1% (v/v) of an antibiotic mixture containing choloramphenicol, streptomycin sulphate, and penicillin (each at 5 $mg \cdot mL^{-1}$ in the stock solution). Nine-millilitre broth cultures were inoculated with 1 mL of diluted faecal suspension and incubated at 39°C for 3 days. These were then subcultured anaerobically into fresh tubes of medium C with cellobiose. Axenic cultures of the gut fungi were picked from roll tube agar using the technique of Joblin (1981). The roll tube medium contained 1.5% (w/v) agar in addition to the other ingredients used by Orpin (1976). Colonies picked from roll tubes were transferred into broth medium, and the roll tube procedure was repeated three more times to ensure production of axenic cultures. Stock cultures were maintained in 10% glycerol stored under liquid nitrogen.

Life-cycle analysis

Determination of the life cycle of the isolate was based on the methods described by Lowe et al. (1987). Development of the isolate was followed on glass coverslips placed in anaerobic culture tubes containing Orpin's (1976) broth. Two- or 3-day-old cultures were inoculated into fresh medium through a 15-μm filter to permit passage of zoospores (the mean diameter of the zoospores was <10 μm). Inoculated tubes were incubated at 39°C without shaking and at 2-h time intervals (up to 24 h), four tubes were sampled. Fungal growth in these tubes was stopped immediately by the addition of 1 mL 25% (v/v) glutaraldehyde solution. After fixation, coverslips were removed from the culture tubes and examined by light microscopy. Representative photographs were taken using a JVC TK-C1381 video camera fitted to a Jenaval (Carl Zeiss, Jena, Germany) microscope.

Nuclear staining

Nuclei of the fungi were observed by ultraviolet (UV) epifluorescence microscopy (Olympus BH-2 UV microscope fitted with 365 nm excitation filter and 420 nm barrier filters) after staining with the fluorochrome, DAPI (4′,6 diamidino-2-phylindole; 0.3 $mg \cdot mL^{-1}$ in 50 mM Tris-HCl (pH 7.2), 100 mM NaCl, 10 mM EDTA) (Hooley et al. 1982). Samples were stained by adding the DAPI and leaving for 10 min in the dark at room temperature before microscopy, as described by Lowe et al. (1987). Photographs were taken using Elite Chrome Kodak ASA 400 film and an Olympus OM-2 camera.

Electron microscopy

Zoospores from 1-day-old culture fluids were dispensed into empty presterilized anaerobic culture tubes through a 15-μm filter under a stream of CO_2. Zoospores were pelleted by centrifugation (1000 × *g* for 5 min) and resuspended in 100 μL of ice-cold fixative (0.1 M sodium cacodylate, 0.02% (w/v) calcium chloride, 5% (v/v) glutaraldehyde, and 2% (w/v) formaldehyde). After 30 min incubation on ice, zoospores were pelleted twice more and resuspended in 50 and 25 μL of cold fixative. Finally, 1-μL aliquots were encapsulated in 20-μL droplets of 2% (w/v) molten (50°C) water agar and quickly cooled to room temperature. The agar pellets were transferred to 5 mL of cold fixative and processed for electron microscopy after 30 min.

The primary fixative was removed by washing in buffer (0.1 M sodium cacodylate, 0.02% calcium chloride, 1 h), and the agar pellets were fixed for 1 h by addition of 1% (w/v) osmium tetroxide in 0.1 M sodium cacodylate. Pellets were then left in cacodylate buffer overnight at 4°C, followed by 30 min in cold distilled water (reverse osmosis), prior to dehydration through an alcohol series (30, 50, 70, 95, 100, and 100% for 30 min each). The pellets were incubated in propylene oxide overnight and then embedded in TAAB Emix® resin (TAAB Laboratories Ltd, Aldermaston, Berkshire, U.K.) according to the manufacturer's instructions using polyethene moulds (micron®, Agar Scientific). Thin sections (ca. 70–90 nm thick with silver to silver–gold interference colours) were cut for electron microscopy with a Reichert Ultracut ultramicrotome (Leica) using a diamond knife (Diatome®) onto 5% (v/v) ethanol. Ribbons of sections were transferred to nickel slot grids and allowed to dry down on formvar films supported on steel

mesh bridges (Rowley and Moran 1975). Sections were post-stained with 2% (v/v) aqueous uranyl acetate (15 min) and Saito's lead citrate (Hanaichi et al. 1986) for 2 min. Grids were viewed on a JEM 1010 electron microscope (JEOL, Tokyo, Japan) operated at 80 kV. Electron micrographs were recorded on Kodak® 4489 film and digitized from the negatives with an Epson GT-7000 image scanner.

Results

Cyllamyces gen.nov. Ozkose et al.

Fungi semper anaerobici, thallus polycentricus, rhizoideum unicum bulbosumque, sporangia numerosa sphaericaque, sporangiophorae ramoae, zoosporae uniflagellatae. Typus *Cyllamyces aberensis.*

Strictly anaerobic fungus with determinate, multi-sporangiate thallus, single bulbous holdfast multiple spherical sporangia, branched sporangiophores, and uniflagellate zoospores. The word "cylla" is from the Welsh language, meaning "guts." Type species is *Cyllamyces aberensis.*

Cyllamyces aberensis sp.nov., Ozkose et al.

Sporangia sphaerica, numerosa (3–12), terminali, 14.7 ± 3.1 μm diametro. Zoosporae, uninucleatae, sphaericae, 7.80 ± 1.1 μm diametro, uniflagellatae, flagellum 27.33 ± 2.4 μm longum. Holotypus = figurae. 1–11), ex faece bovis, in collectu Microbiology Laboratory, Institute of Grassland and Environmental Research, Aberystwyth, Ceredigion, SY23 3EB, U.K.

Sporangia numerous (3–12; 5.8 ± 2.0 (mean ± SD), n = 27), spherical to ovoid, 14.7 ± 3.1 μm (n = 60) in diameter, terminal. Zoospore uninucleate, spherical 7.80 ± 1.1 μm (n = 80) in diameter, uniflagellate, flagellum 27.33 ± 2.4 μm (n = 30) in length. Obligate anaerobe, isolated from cattle faeces. Type = Figs. 1–11. A culture (isolate EO14) from which these photographs were made is held in the collection of the Microbiology Laboratory, Institute of Grassland and Environmental Research, Aberystwyth, Ceredigion, SY23 3EB, U.K.

Morphology and life cycle

Morphological and physiological characteristics of chytrid fungi (including members of the Neocallimastigales) may vary depending on culture conditions (Barr 1989; Wubah et al. 1991*b*). Therefore, all the observations and measurements reported here were obtained from cultures grown at 39°C on cellobiose. The morphology of this isolate (EO14) was very similar, however, when grown on a range of other substrates, including wheat straw, cellulose powder, and glucose.

The zoospores of this fungus were mainly spherical (Fig. 1*a*) but occasionally oval, having generally one but occasionally two (Fig. 1*b*) or three flagella (8% diflagellate, 2% triflagellate; n = 100), as has been reported for other uniflagellate anaerobic fungi (Ho and Barr 1995). After their release from the mature sporangium, zoospores swam for 1–2 h. The flagellum was normally shed just prior to encystment (2–4 h), but occasionally it remained attached to the cyst wall during germination. The encysted zoospore grew to form a single-bulbous holdfast without rhizoids. Within 6–8 h of germination (Figs. 2 and 3), the first of several (up to five, although usually two to four) sporangiophores (Fig. 4), each up to 85 μm in length, was formed. These sporangiophores were usually branched and invariably contained several nuclei. Although sporangiophores could develop from different regions of the bulbous holdfast (Figs. 4 and 5), two sporangiophores emanating from the same point on the holdfast and branching at the base were frequently observed (Figs. 4 and 8*a*). The single bulbous holdfast grew to a diameter of up to 54 μm (33.9 ± 6.0 μm; n = 40) after 24 h. Thallus development was considered monocentric-polysporangiate, because nuclei were present in vegetative parts of the thallus (bulbous holdfast and sporangiophore; Figs. 8*a*, 8*b*, 9*a*, and 9*b*) and numerous sporangia were consistently produced. The occurrence of branched sporangiophores giving rise to several terminal sporangia has not previously been reported in the *Neocallimastigales*. In other species forming bulbous holdfasts, sporangia are generally borne directly on the holdfast, although in *C. communis* short sporangiophores have been observed (Ho and Barr 1995; Orpin 1976; Wubah et al. 1991*b*).

The first developing sporangium was observed on the sporangiophore 8 h after release of the parental zoospore. During development of the multiple sporangia (8–14 h), nuclei migrated into these structures via the sporangiophores (Figs. 8*a*, 8*b*, 9*a*, and 9*b*). Sporangia were spherical to ovoid (Figs. 5 and 7) and 10–20 μm in diameter at maturity and delimited from the sporangiophore by a distinct septum (Fig. 5). Sporangia were always located terminally, usually on short (10–20 μm) branches arising from the main sporangiophore. Up to 12 (Fig. 6), though normally between 5 and 8, sporangia were produced from each thallus. Zoospores were seen to move within the near-mature sporangia during the hour prior to zoospore release (18–24 h). Up to 12 zoospores (5.5 ± 2.7, n = 36, based on numbers of nuclei in nearly mature sporangia) were liberated from the sporangia by localized dissolution of the apical region of the sporangium wall.

Ultrastructure of zoospores

Ultrastructure of the free-swimming zoospores of *Cyllamyces aberensis* was investigated using transmission electron microscopy. Several oval to approximately round hydrogenosomes were located posteriorly in zoospores (Figs. 10*a*, 10*b*, and 11*a*). The hydrogenosomes were less electron opaque than ribosomal aggregates. The nucleus had a clearly visible central nucleolus and regular elongation directed towards the hydrogenosomes and flagellar attachment point (Fig. 10*a*).

Serial longitudinal sections of the zoospore indicate the mode of flagellum attachment and the kinetosomal structures. The flagellum emerged from a pit behind the attachment point (Figs. 11*a* and 11*b*), a common characteristic of gut fungal zoospores (Munn 1994). The peri-kinetosomal apparatus included scoops, spurs, and circumflagellar ring (Figs. 11*b* and 11*c*), similar to those described by Munn (1994) and others.

Discussion

Cyllamyces aberensis and the members of the genus *Caecomyces* are similar in gross morphology, while being quite distinct from members of the other four gut fungal genera *Neocallimastix*, *Piromyces*, *Anaeromyces*, and *Orpinomyces*.

Figs. 1. Uniflagellate (*a*) and diflagellate (*b*) zoospores of *Cyllamyces aberensis*. **Fig. 2.** Young thallus of *Cyllamyces aberensis* (4 h after zoospore release), showing the expanding bulbous holdfast and the initiation of the first sporangiophore. **Fig. 3.** Young thallus of *Cyllamyces aberensis* (6 h after zoospore release), showing the expanding bulbous holdfast and the initiation of the first sporangiophore (arrow). **Fig. 4.** Young thallus of *Cyllamyces aberensis* (12 h after zoospore release), showing the development of five discrete sporangiophore initials from the bulbous holdfast. Arrows indicate sporangia developing from the sporangiophores. **Fig. 5.** Mature thallus (18 h after zoospore release) with six sporangia borne on a single branched sporangiophore (a developing sporangiophore is visible on the opposite side of the holdfast). Note the distinct delimitation of the sporangia from the branched sporangiophore. **Fig. 6.** Mature thallus (18 h after zoospore release) with 12 sporangia borne on a three branched sporangiophores. **Fig. 7.** Mature thallus (18 h after zoospore release) with seven sporangia.

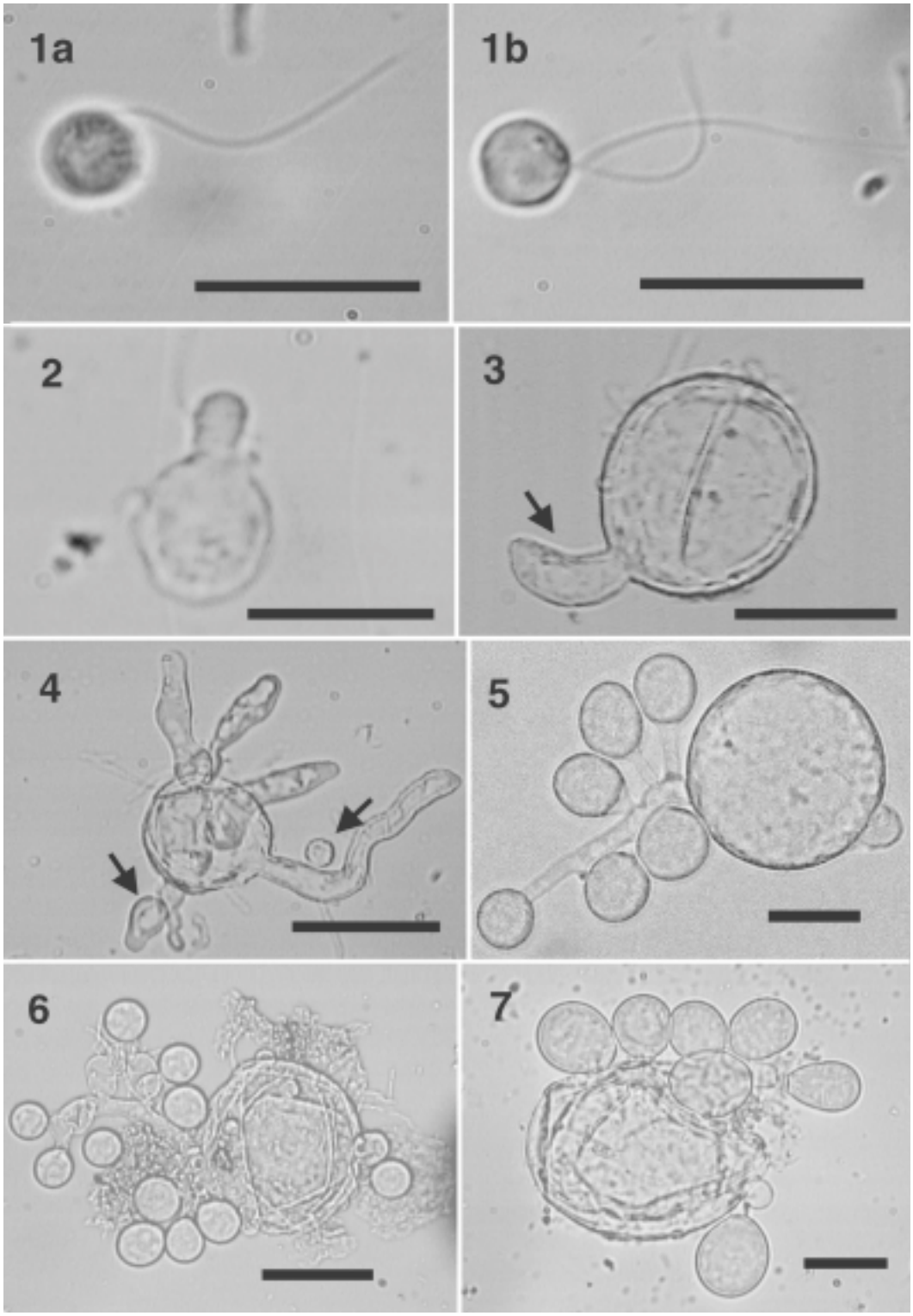
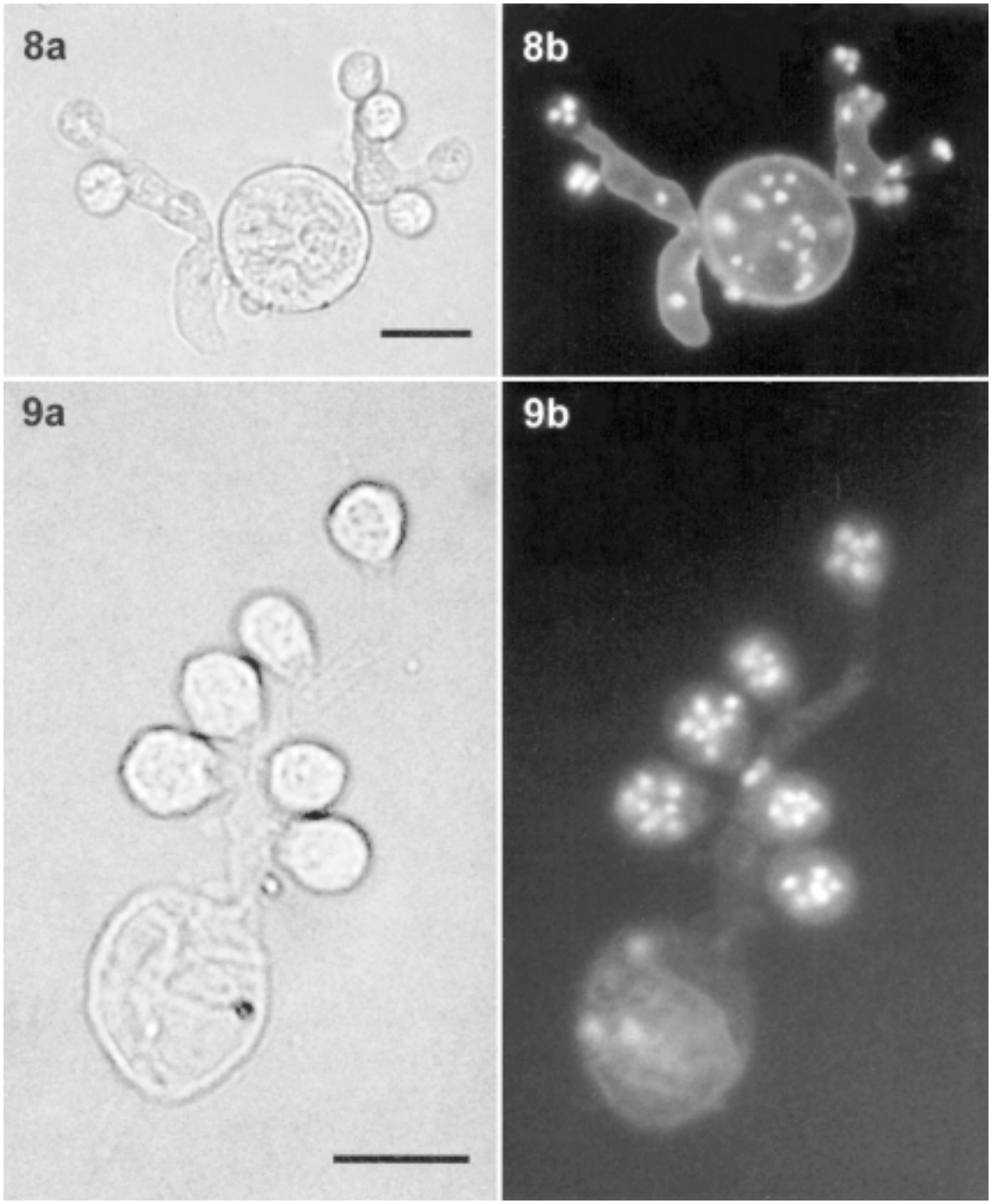
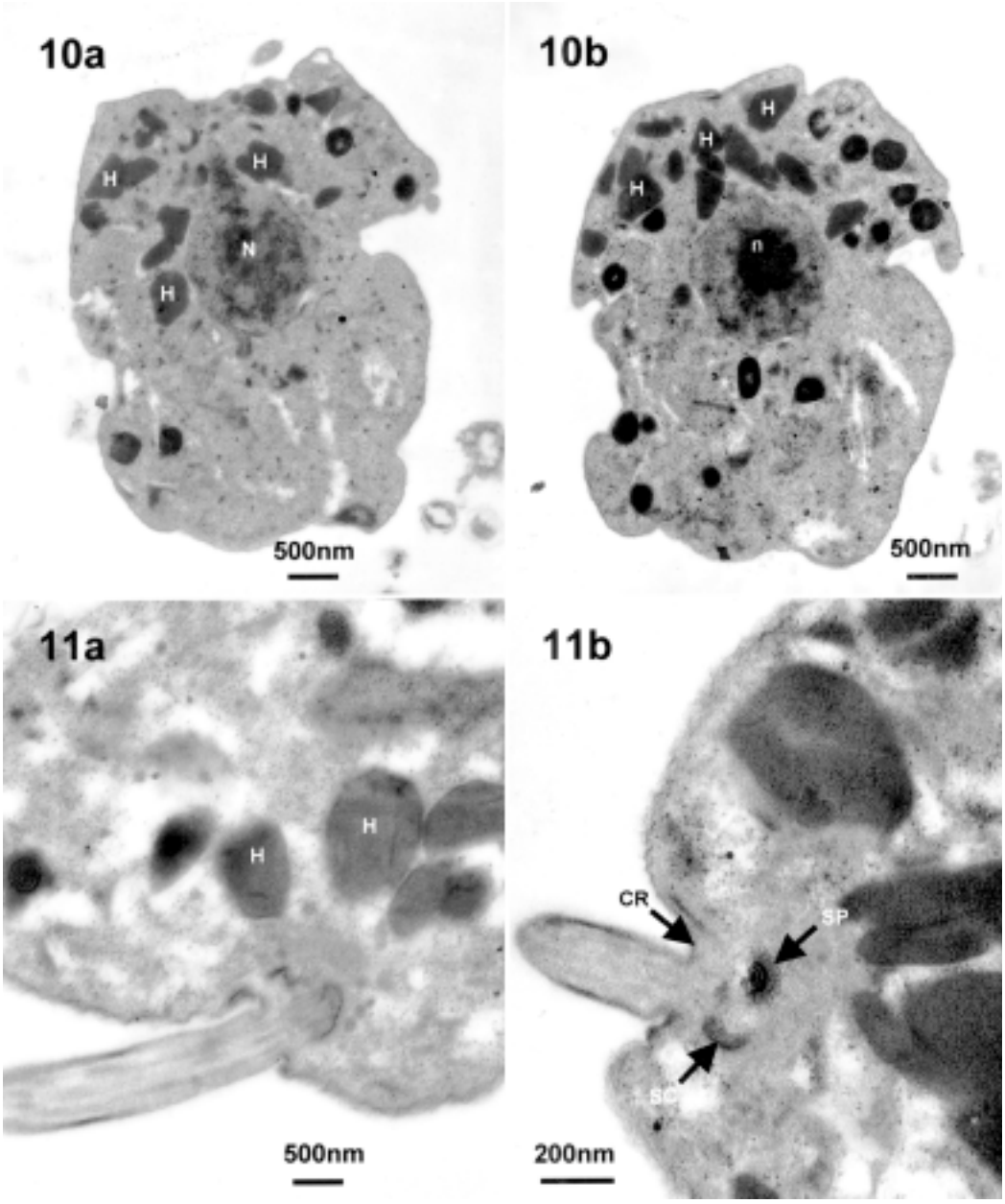


Fig. 8. Light (*a*) and fluorescence (*b*) images of an immature thallus (16 h after zoospore release) with three developing sporangiophores and several immature sporangia. Note the presence of nuclei throughout the holdfast, in the sporangiophores, and in the developing sporangia. **Fig. 9.** Light (*a*) and fluorescence (*b*) images of a mature thallus (18 h after zoospore release) with six sporangia borne on a single elongate sporangiophore.

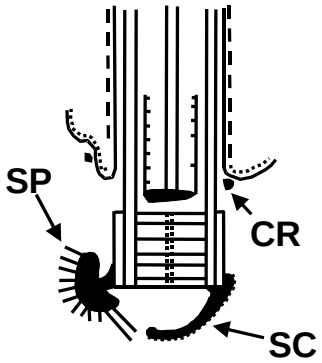


Both genera produce bulbous holdfasts rather than filamentous rhizoids. The taxonomy of the anaerobic fungi producing bulbous holdfasts is currently in a state of flux, partly because these fungi are difficult to maintain in culture (they do not preserve well under liquid nitrogen) but also because of the reported morphological variability of some isolates in pure culture (Ho and Barr 1995; Wubah et al. 1991*b*). The genus *Caecomyces* and the type species *Caecomyces equi* (producing a single sporangium borne on a single bulbous holdfast with occasional fibrillar rhizoids) was validly described by Gold et al. (1988), although the original culture is no longer available. Gold et al. (1988) also suggested that *Sphaeromonas communis* (as first described by Braune (1913), based on the description of Liebetanz (1910) and recognised as a fungus by Orpin (1976)) should be reclassified as *Caecomyces communis*. However, Orpin (1994) has subsequently disputed this reclassification because *Caecomyces equi* produces fibrillar rhizoids and only a single bulbous

Fig. 10. Electron micrographs of longitudinal sections through a zoospore. The nucleus (N) with a beak (*a*) projecting towards the flagellar attachment point is visible, as is the nucleolus (*b*; n) and the amorphous globular hydrogenosomes (H). **Fig. 11.** Electron micrographs (*a* and *b*) and a diagram (*c*) (redrawn from Munn 1994) of serial sections through a zoospore, showing the flagellar attachment point and the clustering of hydrogenosomes (H). The scoop (SC), spur (SP), and the circumflagellar ring (CR) are are shown in Figs. 11*b* and 11*c*.



11c



holdfast. More recently, Ho and Barr, (1995) have questioned the status of *Caecomyces equi* (which does not appear to have been studied since the original paper of Gold et al. (1988)), suggesting that it is synonymous with *Caecomyces* (= *Sphaeromonas*) *communis*, which is widely recognised (Wubah et al. 1991*b*) to be morphologically variable in pure culture.

The morphological variability of "*C. communis*," which has been alluded to by several authors, relates to the number of bulbous holdfasts and sporangia produced on a single thallus. Typically one of each are formed but several studies have noted the occurrence of several bulbous holdfasts and (or) two to four sporangia (Ho and Barr 1995; Orpin 1976, 1994; Trinci et al. 1994; Wubah et al. 1991*a*, 1991*b*). The term multisporangiate was suggested by Wubah et al. (1991*b*) to describe the latter forms, although it is difficult to reconcile this terminology with the definition of monocentric growth used by Ho and Barr, (1995) as having "one reproductive body" (as opposed to polycentric growth "with many centers of reproduction"), with "these forms determined at the earliest stage of growth and invariable." Further semantic confusion arises from the type of germination observed in monocentric and polycentric species, with the former exhibiting endogenous germination ("nucleus remains in the zoospore cyst which enlarges into a new sporangium;" Ho and Barr 1995). Ho and Barr (1995) recognised the ambiguous position of *C. communis* with respect to these definitions, noting that production of more than one sporangium is uncommon and that, in any case, the number of sporangia was limited with most not reaching maturity.

Our discovery of *Cyllamyces aberensis* further confuses the problems of terminology, because our isolate invariably produced numerous sporangia, as well as several branched sporangiophores. Such branched sporangiophores have not previously been reported in the Neocallimastigales. Whilst recognising that the growth of *C. aberensis* does not appear to be indeterminate like the thalli of *Orpinomyces* and *Anaeromyces* spp., we consider the thalloid nature of the branched sporangiophores and multiple sporangiophores of *C. aberensis*, to place it more closely to these polycentric species than to *Caecomyces* and other monocentric species.

We have studied a number of isolates that clearly conformed to the description of the genus *Caecomyces* (*C. communis*?) under the same conditions as *C. aberensis*. Apart from the distinctive branched sporangiophores and polycentric development of *C. aberensis*, a number of other features set this organism apart from the wide range of morphological forms observed among *Caecomyces* spp. The sporangia of *C. aberensis* are much smaller (14.7 μm as opposed to ca. 40 μm, according to Wubah et al. (1991*b*)) and the growth rate much lower than observed in *Caecomyces* spp (data not shown). Even "multisporangiate" forms of *C. communis* have not been reported to produce more than four sporangia, usually directly on the surface of the bulbous holdfast (Wubah et al. 1991*b*), most of which do not mature normally (Ho and Barr 1995). Furthermore, these additional sporangia are only produced late in the life cycle (after 24 h), whereas multiple sporangia were present on quite young thalli (8–10 h in *C. aberensis*), and most if not all of these developed to maturity. Given previous speculation about the differences in morphological development and life-cycle times in anaerobic fungi grown in vitro and in the rumen (Orpin 1976; Trinci et al. 1994; Wubah et al. 1991*b*), further work is required to examine the growth of this fungus in its natural environment.

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References

Barr, D.J.S. 1983. The zoosporic grouping of plant pathogens. *In* Zoosporic plant pathogens. *Edited by* S.T. Buczacki. Academic Press, London. pp. 161–192.

Barr, D.J.S. 1989. Phylum Chytridiomycota. *In* Handbook of Protoctista. *Edited by* L. Margulis, J.O. Corliss, M. Melkonian, and D.J. Chapman. Jones & Bartlett Publishers, Boston, Mass. pp. 454–466.

Braune, R. 1913. Untersuchungen über die im Wiederkaüermagen vorkommenden Protozoen. Arch. Protistenkd. **32**: 111–170.

Davies, D.R., Theodorou, M.K., Lawrence, M.I.G., and Trinci, A.P.J. 1993. Distribution of anaerobic fungi in the digestive tract of cattle and their survival in feces. J. Gen. Microbiol. **139**: 1395–1400.

Gold, J.J., Heath, I.B., and Bauchop, T. 1988. Ultrastructural description of a new chytrid genus of caecum anaerobe, *Caecomyces equi* gen. nov. sp. nov. assigned to the Neaocallimasticaceae. BioSystems, **21**: 403–415.

Hanaichi, T., Sato, T., Iwamoto, T., Malavasiyamashiro, J., Hoshino, M., and Mizuno, N. 1986. A stable lead by modification of Sato method. J. Electron Microsc. **35**: 304–306.

Heath, I.B., Bauchop, T., and Skipp, R. A. 1983. Assignment of the rumen anaerobe *Neocallimastix frontalis* to the *Spizellomycetales* (*Chytridiomycetes*) on the basis of its polyflagellate zoospore ultrastructure. Can. J. Bot. **61**: 295–307.

Ho, Y.W., and Barr, D.J.S. 1995. Classification of anaerobic gut fungi from herbivores with emphasis on rumen fungi from Malaysia. Mycologia, **87**: 655–677.

Hooley, P., Fyfe, A.M., Evola Maltese, C., and Shaw, D.S. 1982. Duplication cycle in nuclei of germinating zoospores of *Phytophthore drechsleri* as revealed by DAPI staining. Trans. Br. Mycol. Soc. **79**: 563–566.

Joblin, K.N. 1981. Isolation, enumeration, and maintenance of rumen anaerobic fungi in roll tubes. Appl. Environ. Microbiol. **42**: 1119–1122.

Joblin, K.N. 1989. Physical disruption of plant fibre by rumen fungi of the *Sphaeromonas* group. *In* The roles of protozoa and fungi in ruminant digestion. *Edited by* J.V. Nolan, R.A. Leng, and D.I. Demeyer. Penambul Books, Armidale, Australia. pp. 1–2.

Li, J.L., and Heath, I.B. 1992. The phylogenetic relationships of the anaerobic chytridiomycetous gut fungi (*Neocallimasticaceae*) and the Chytridiomycota. I. Cladistic analysis of ribosomal RNA sequences. Can. J. Bot. **70**: 1738–1746.

Li, J.L., Heath, I.B., and Packer, L. 1993. The phylogenetic relationships of the anaerobic chytridiomycetous gut fungi (*Neocallimasticaceae*) and the Chytridiomycota. II. Cladistic analysis of

structural data and description of *Neocallimasticales* ord. Can. J. Bot. **71**: 393–407.

Liebetanz, E. 1910. Die parasitischen Protozoen des Wiederkaüermagen. Arch. Protistenkd. **19**: 19.

Lowe, S.E., Griffith, G.W., Milne, A., Theodorou, M.K., and Trinci, A. P. J. 1987. The life cycle and growth kinetics of an anaerobic rumen fungus. J. Gen. Microbiol. **133**: 1815–1827.

Munn, E.A. 1994. The ultrastructure of anaerobic fungi. *In* Anaerobic fungi: biology, ecology and function. Vol. 12. *Edited by* C.G. Orpin and D.O. Mountfort. Marcel Dekker, New York. pp. 47–106.

Orpin, C. G. 1975. Studies on the rumen flagellate *Neocallimastix frontalis*. J. Gen. Microbiol. **91**: 249–262.

Orpin, C.G. 1976. Studies on the rumen flagellate *Sphaeromonas communis*. J. Gen. Microbiol. **94**: 270–280.

Orpin, C.G. 1994. Anaerobic fungi: taxonomy, biology and distribution in nature. *In* Anaerobic fungi: biology, ecology and function. Vol. 12. *Edited by* C.G. Orpin and D.O. Mountfort. Marcel Dekker, New York. pp. 1–47.

Rowley, C.J., and Moran, D.T. 1975. A simple procedure for mounting wrinkle-free sections on formvar-coated slot grids. Ultramicroscopy, **1**: 151–155.

Trinci, A.P.J., Davies, D.R., Gull, K., Lawrence, M.I., Nielsen, B.B., Rickers, A., and Theodorou, M.K. 1994. Anaerobic fungi in herbivorous animals. Mycol. Res. **98**: 129–152.

Wubah, D.A., Fuller, M.S., and Akin, D.E. 1991*a*. Isolation of monocentric and polycentric fungi from the rumen and feces of cows in Georgia. Can. J. Bot. **69**: 1232–1236.

Wubah, D.A., Fuller, M.S., and Akin, D.E. 1991*b*. Studies on *Caecomyces communis*: morphology and development. Mycologia, **83**: 303–310.