

System. Appl. Microbiol. 4, 114–122 (1983)

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Cystofilobasidium*: a New Genus in the *Filobasidiaceae*

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Received June 25, 1982

Summary

Description is provided of a new heterobasidiomycetous genus, *Cystofilobasidium*, based on *Rhodosporidium capitatum*. The genus is characterized by teliospores which give rise to filobasidiaceous basidia, sessile primary basidiospores which remain attached to the basidium, and a yeast phase developing from buds produced by the primary spores. Two species formerly placed in *Rhodosporidium* are transferred to *Cystofilobasidium*; these are *C. bisporidiis* (Fell, Hunter and Tallman) Oberw. and Bandoni comb. nov., and *C. capitatum* (Fell, Hunter and Tallman) Oberw. and Bandoni, comb. nov.

Key words: *Cystofilobasidium* – *Filobasidiaceae* – *Heterobasidiomycetes* – Basidiomycetous yeasts – Dimorphism – Septal pores – Ultrastructure – G + C content of DNA

Introduction

In 1968, Olive isolated *Filobasidium floriforme* Olive from dead leaves of *Erianthus giganteus* (Walt.) Muhl. that had been incubated in a moist chamber. Because of the unusual development and morphology of the basidia and spores, Olive proposed the new family *Filobasidiaceae* for the taxon. The basidium of *F. floriforme* has a slightly swollen probasidium, a narrow, hypha-like central region, and a swollen apex. Sessile spores bud from the apex, i.e. sterigmata are not produced. Haploid yeast colonies develop through budding of the basidiospores.

Basidia of *Torulopsis capsuligenum* van der Walt and Kerken (1961) were later found to be *Filobasidium*-like (Rodrigues de Miranda, 1972). This species had ori-

* Part 26 of a series, "Studies in Heterobasidiomycetes" of the Institut für Biologie I, Lehrstuhl Spezielle Botanik, Universität Tübingen and the Department of Botany, University of British Columbia.

ginally been placed in *Leucosporidium* by Fell et al. (1969). A comparable basidial type was later found in the perfect state of *Cryptococcus neoformans* by Kwon-Chung (1975), who proposed *Filobasidiella* for this species. *Filobasidiella* differs from *Filobasidium* in producing basipetal chains of basidiospores through repetitive budding of the basidium. A second species, *Filobasidiella bacillispora* Kwon-Chung, was discovered through mating strains of *Cryptococcus neoformans* B and C serotypes (Kwon-Chung, 1976b).

In 1979, we isolated an orange yeast with basidiomycetous characteristics from the mature gleba of *Mutinus caninus* (Huds. ex Pers.) Fr. (*Phallaceae*). In growing yeast colonies, some cells spontaneously produced hyphae; these bore terminal or intercalary teliospores either singly or in short chains. There was no evidence of mating prior to hyphal development, but the teliospores produce *Filobasidium*-like basidia upon germinating. The presence of teliospores and other features which clearly separate this fungus from other filobasidiaceous taxa has led us to identify it with *Rhodospodium capitatum* and to propose a new genus, *Cystofilobasidium*, to accommodate the species.

Materials and Methods

Organisms

For descriptions and illustrations of the species, the following collections were used:

Cystofilobasidium bisporidiis (Fell, Hunter and Tallman) Oberw. and Bandoni, CBS 6346 (type culture).

Cystofilobasidium capitatum (Fell, Hunter and Tallman) Oberw. and Bandoni, CBS 6358 (type culture); - FO 29337: West Germany, Kassel, isolated from gleba of *Mutinus caninus* (Huds. ex Pers.) Fr., Sept. 1979, leg. L. Kisimova-Horovitz; - FO 31572.a: West Germany, Tübingen, Schönbuch, isolated from gleba of *Phallus impudicus* L. ex Pers., Sept. 23, 1980, leg. L. Kisimova-Horovitz.

Filobasidiella neoformans Kwon-Chung; strain F. *Staib* A 117 x A 341.

Filobasidium floriforme OLIVE; on dead florets of *Erianthus giganteus* (Walt.) Muhl., Bluffton, South Carolina, Nov. 19, 1966, type (NY).

Germination of teliospores was stimulated by heating cultures up to ca. 55 °C.

Electron microscopy

For transmission electron microscopy, material was fixed in glutaraldehyde and osmium tetroxide, washed with distilled water, stained in aqueous uranyl acetate, dehydrated in an ethanol series, and embedded in epoxid resin according to Spurr (1969). Ultrathin sections were mounted on unsupported mesh copper grids, and examined in a Zeiss EM 9 S-2 transmission electron microscope.

Determination of G + C-content of DNA

For the analysis of the G + C-content of DNA strains were cultivated on a liquid yeast-malt-medium (yeast extract 4 g/l, malt extract 10 g/l, glucose 4 g/l) or on "medium I" as described by Sundström (1964), respectively, and aerated by slow shaking. Sufficient material was obtained generally within five days. Cells were harvested by centrifugation and opened by forcing them one or several times through a x-press type X 25 from AB Biox, Nacka, Sweden. Total DNA was isolated and purified according to Marmur (1961). This procedure was in some cases supplemented by precipitation of the chloroform-extracted DNA with cetyltrimethylammoniumbromide (*E. Stackebrandt*, pers. comm.) in order to remove interfering carbohydrates. Melting point (T_m) of DNA was determined in $0.1 \times$ SSC

and heated 0.5 C per 5 minutes in a Zeiss PMQ III spectrophotometer while the absorbance at 260 nm was recorded. Temperature was controlled directly in the heated cuvettes. Molar ratios of G + C were calculated from the T_m -values as described by *De Ley* (1970) and modified with respect to the lower ion strength ($0.1 \times \text{SSC}$) as that used by *Silvestri and Hill* (1965). The G + C mol% of DNA of *E. coli* K12 Hb101 (determined to 51.1% with a standard deviation of ± 0.55) was used for reference.

Results and Discussion

Cystofilobasidium Oberwinkler and Bandoni, gen. nov.

Fungi generis Heterobasidiomycetum sine carposomatibus. Basidiosporae singulis cellulis ellipsoideis germinant, deinde hyphae effibulatae formantur. Pori septorum hypharum structurae dolipororum similes efficiuntur, sed desunt parentheses. Probasidia (teliosporae, chlamydosporae) ex hyphis terminaliter vel intercalariter vel catenulatum excrescunt; mature crassitunicata et cytoplasmatibus granulosis ornantur. Post statum quietis holobasidia elongata, ad apices subglobosa vel lageniformia inflata formantur. Basidiosporae sessiles, tenuitunicatae, hyalinae, blastosporis germinant.

Typus generis: *Cystofilobasidium capitatum* (Fell, Hunter and Tallman) Oberw. and Bandoni.

Etymologia: $\kappa\acute{\upsilon}\sigma\tau\iota\varsigma$ – bladder; filum – thread; basidium.

Dimorphic basidiomycete with budding basidiospores and bright orange yeast colonies. Hyphae without clamps, septa dolipore-like, without parentheses. Teliospores terminal or intercalary, single or in short chains, cut off from supporting hyphae by clampless septa. During maturation, conspicuous guttules develop in the teliospore cytoplasm, adjacent cells of the hyphae may empty, and the teliospore wall thickens. Teliospores germinate in water, giving rise to an elongate hypha-like portion with a swollen, spore-bearing apex. The non-septate basidium bears thin-walled, sessile spores; these are not deciduous, but remain attached and germinate by budding. Teliospore germination by endospore formation may also take place.

Cystofilobasidium capitatum (Fell, Hunter and Tallman) Oberw. and Bandoni, comb. nov., basionym: *Rhodosporidium capitatum* Fell, Hunter and Tallman, Canad. J. Microbiol. 19, 650 (1973).

Hyphae thin-walled, hyaline; septa without clamps. Mature probasidia (= teliospores) thick-walled with yellowish cytoplasm, (6)–12–17–(20) μm in diam., germinating by production of a thin-walled, slender basidium, 2–3 μm in diam. basally, with a capitate apical swelling at a distance of (20)–40–80 μm from the teliospore, the capitate part 7–12 μm in diam. Basidiospores 4–10, sessile, thin-walled, hyaline, subglobose to drop-shaped, slightly attenuated basally, 5–7 \times 7–9 μm , non-deciduous, germinating by budding. Teliospores sometimes germinating by endospore formation. Yeast cells thin-walled, bright orange in mass, the individual cells 3–5 \times 5–10 μm . G + C contents of the species were determined ranging from 56.0–56.6 mol%.

The most distinctive features of *Cystofilobasidium* include the thick-walled teliospores, filobasidiaceous basidia with sessile, firmly-attached basidiospores that germinate in place, and lack of clamp connections. Basidia of *Filobasidium floriforme* Olive are only slightly inflated basally (Olive, 1968); the basidiospores are weakly attached, but may germinate in place under humid conditions. The species of *Filo-*

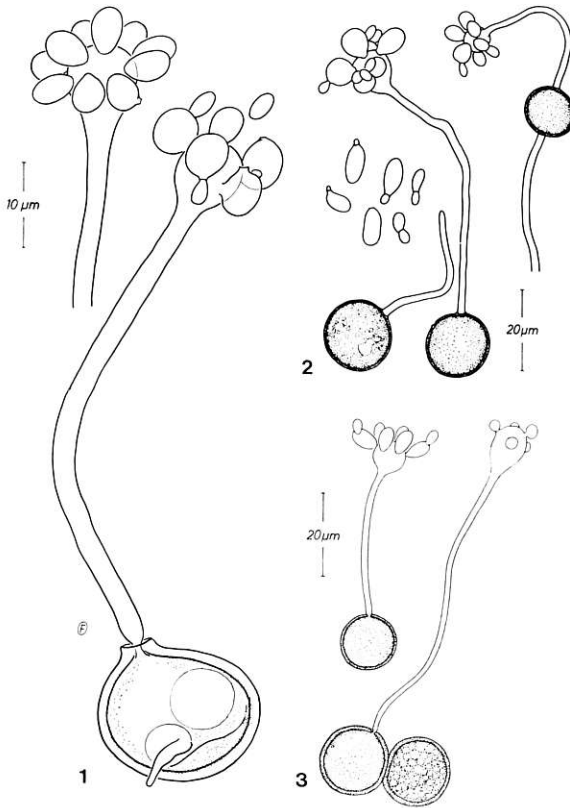


Plate I

Cystofilobasidium capitatum, germinated teliospores. Fig. 1. Details of basidia and basidiospores. Figs. 2, 3. Different stages of basidial development and yeast budding.

basidiella also lack probasidia and have deciduous basidiospores, the latter occurring in chains. Clamp connections are characteristic of *Filobasidium floriforme*, *F. capsuligenum* (Fell, Statzel, Hunter and Phaff) Rodrigues de Miranda, and in the two species of *Filobasidiella* described by Kwon-Chung (Kwon-Chung, 1975, 1976. a). *Filobasidiella arachnophila* Malloch, Kane and Lahaie lacks clamp connections as well as probasidia; it lacks also a known yeast phase. Thus, *Cystofilobasidium* is clearly separated from other taxa currently placed in the *Filobasidiaceae*. However, some species of *Rhodosporeidium* and *Leucosporeidium* have features which strongly suggest a possible affinity with filobasidiaceous fungi. These were listed by Fell (1974) and include *Rhodosporeidium bisporidiis* Fell, Hunter and Tallman, *R. capitatum* Fell, Hunter and Tallman, *R. infirmo-miniatum* Fell, Hunter and Tallman, *Leucosporeidium frigidum* Fell, Statzel, Hunter and Phaff, and *L. gelidium* Fell, Statzel, Hunter and Phaff. Fell et al. (1973) and Fell (1974) were aware of the differences separating these taxa from *Rhodosporeidium toruloides* Banno and *Leucosporeidium*

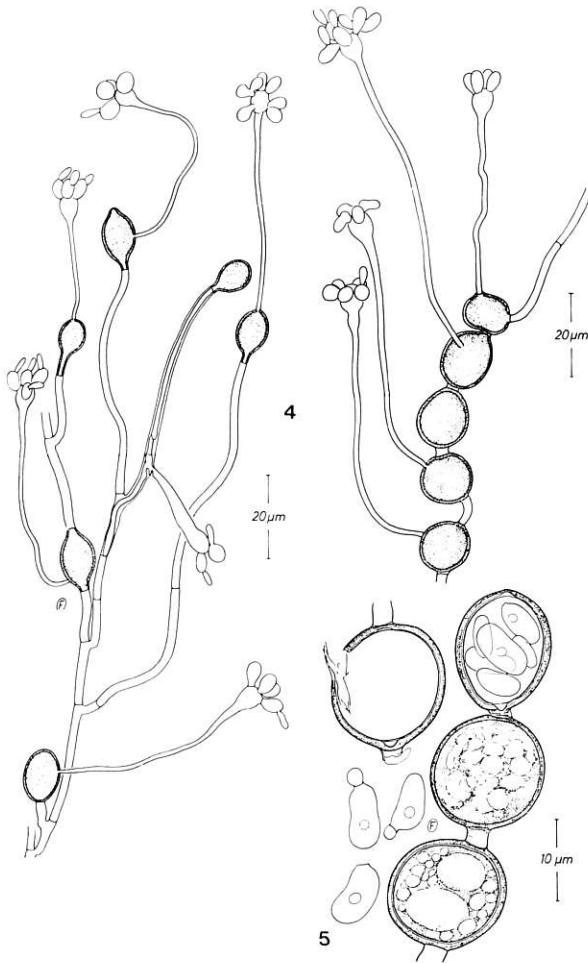


Plate II

Cystofilobasidium capitatum. Fig. 4. Generative hyphae with teliospores and synchronously developed basidia. Fig. 5. Teliospores with different stages of endospore formation, one empty teliospore, and yeast budding.

scottii Fell, Statzell, Hunter and Phaff, the type species of *Rhodosporeidium* and *Leucosporeidium*. Because of the limited knowledge of these genera, however, the taxa have not been transferred. In our opinion, two of the species can best be accommodated in *Cystofilobasidium*, *C. capitatum* (Fell, Hunter and Tallman) Oberw. and Bandoni, and

Cystofilobasidium bisporidiis (Fell, Hunter and Tallman) Oberw. and Bandoni, comb. nov., basionym: *Rhodosporeidium bisporidiis* Fell, Hunter and Tallman, *Canad. J. Microbiol.* 19, 648 (1973).

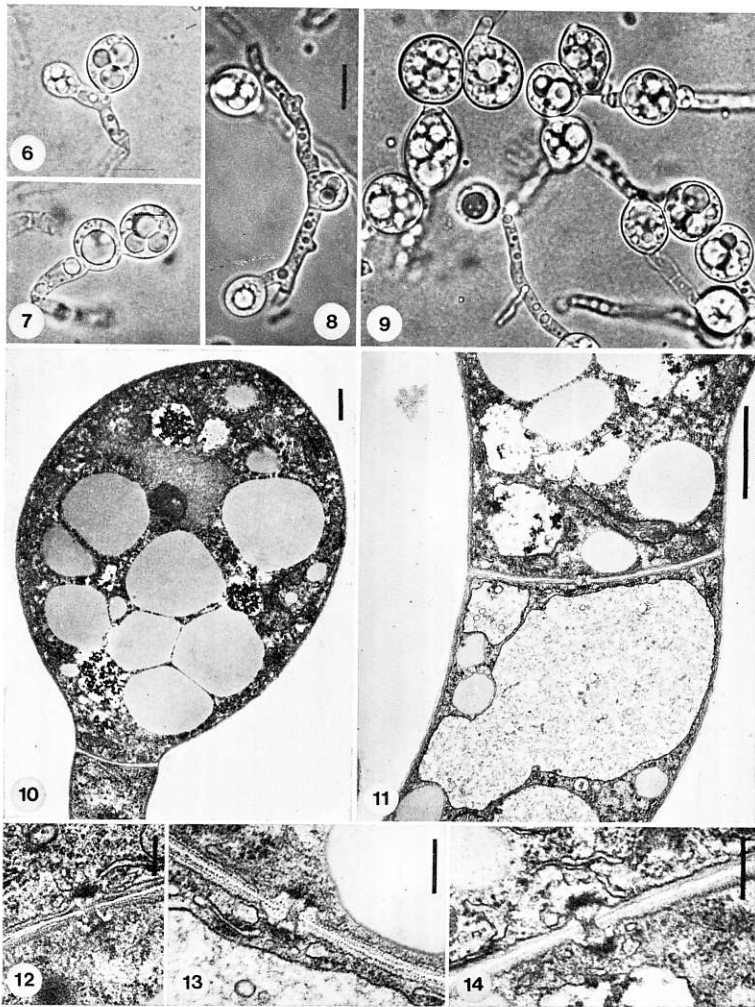


Plate III

Cystofilobasidium capitatum. Figs. 6-9. Photographs of hyphal stages with terminal, intercalary and shortly catenulate probasidia. Bar in Fig. 8 indicates $10\ \mu\text{m}$ and refers also to Figs. 6, 7, 9. Fig. 10. TEM picture of young, terminal probasidium. Bar equals $1\ \mu\text{m}$. Figs. 11-14. TEM micrographs of septal pores. Bar in Fig. 11 corresponds to $1\ \mu\text{m}$, in Figs. 12-14 to $0.2\ \mu\text{m}$.

Basidial ontogeny and morphology must be considered of high taxonomic importance here as with other groups of *Heterobasidiomycetes*. However, there is still heterogeneity within the family *Filobasidiaceae*. *Filobasidiella* species have no probasidia at all; in *Filobasidium floriforme*, the inconspicuous basal swellings may be considered as probasidia. Teliospores similar to those of *Cystofilobasidium* are also

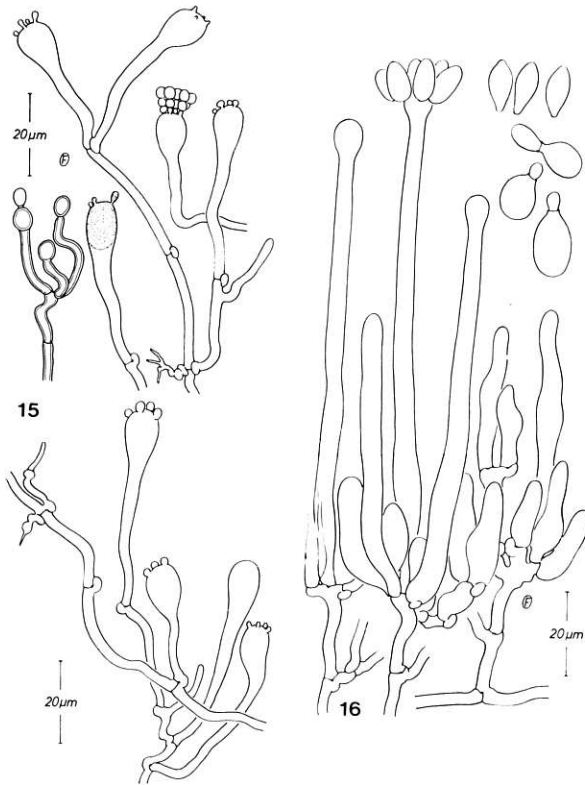


Plate IV

Fig. 15. *Filobasidiella neoformans*; hyphae, haustoria, conidia and basidia in different developmental stages. Fig. 16. *Filobasidium floriforme*; hyphae, basidia, and budding spores from type material.

characteristic of smuts, bunts, *Rhodosporidium*, *Leucosporidium*, *Tilletiaria*, and *Sporidiobolus*. However, the basidia of these groups differ from those in the *Filobasidiaceae*. Teliospores might be indicators of distant phylogenetic relationships, but it is also possible that such resistant spores have evolved independently at several times under pressure of environmental conditions. At this time, we are convinced of a natural relationship of the taxa now included in the *Filobasidiaceae*.

The lack of ballistospores within the *Filobasidiaceae* seems to us a taxonomically important feature. From this point of view, inclusion of the genera *Tilletiopsis* and *Itersonilia* in the *Filobasidiaceae* by von Arx et al. (1977) does not appear to be well-founded. The basidial types found in *Sporidiobolus* and *Tilletiaria*, and the ballistospores of these genera, and of *Bullera* and *Sporobolomyces*, would serve to separate them from the *Filobasidiaceae*. In addition, the septal pores in *Cystofilobasidium capitatum* are similar to those illustrated for *Filobasidium floriforme* (Moore and Kreger-van Rij, 1972) and *Filobasidiella neoformans* (Kwon-Chung, 1976b; Kwon-

Chung and Popkin, 1976). Dolipore-like structures are absent in *Rhodosporidium toruloides* (Johnson-Reid and Moore, 1972). We have verified the observations of Johnson-Reid and Moore, and have also detected (Oberwinkler, unpubl.) simple pores in the type species of *Leucosporidium*, *Tilletiaria*, *Sporidiobolus* and in species of the *Ustilaginales* sensu stricto. In contrast, species of the *Tilletiales* (sensu stricto) so far investigated, including *Tilletia caries* (DC.) Tul. (Deml, 1977), *Entyloma nymphaeae* (Cunn.) Setch., and *Entorrhiza casparyana* (Magn.) Lagerh. (Deml and Oberwinkler, 1981) have dolipores.

Inclusion of *Chionosphaera apobasidialis* Cox in the *Filobasidiaceae*, as was done by Cox (1976) also is unsatisfactory. This species has markedly dissimilar basidia and it lacks the dolipore septal structure (Oberwinkler and Bandoni, 1982). Similar arguments would seem to hold for exclusion of *Rogersiomyces okefenokeensis* Crane and Shoknecht (1978) from the *Filobasidiaceae*. Unfortunately, material for ultrastructural study is not available for *Rogersiomyces* at this time.

Additional features of taxonomic importance in the *Filobasidiaceae* are the presence of a yeast phase in the ontogenetic cycle, and the presence of *Tremella*-like haustoria (Olive, 1946) or „haustorial branches“ (see Olive 1968) in *Filobasidium* and *Filobasidiella*. *Filobasidiella arachnophila* Malloch, Kane and Lahaie described by Malloch et al. (1978) has no known yeast phase, but this is present in other species. *Cystofilobasidium capitatum* possesses a yeast phase, but haustoria have not been found. Since clamp connections are absent in this species, and haustorial branches typically are subtended by clamps, such structures might be represented by only slightly modified side branches.

The type strains of *C. bisporidiis* (CBS 6346) and *C. capitatum* (CBS 6358) exhibited a G + C content of 60.9 and 56.6 mol%, respectively. Our own isolates (FO 29337, FO 31572. a) of *C. capitatum* yielded values of 56.0 and 56.2 mol% emphasising the conspecificity of the *C. capitatum* strains on the one hand, and marking a significant difference to *C. bisporidiis* on the other.

Acknowledgments

We are grateful to Prof. Dr. Dr. F. Staib, Bundesgesundheitsamt Berlin, for making available the strain with sexual stages of *Filobasidiella neoformans*. Miss S. Dinkelmeier skillfully assisted the investigations with the transmission electron microscope. Portions of this study were supported by grants from the Natural Science and Engineering Council of Canada (Grant A 801), and from the Deutsche Forschungsgemeinschaft (SFB 76, Project N).

References

- Cox, D.E.: A new Basidiomycete with anomalous basidia. *Mycologia* 68, 481–510 (1976)
- Crane, J.L., Shoknecht, J.D.: *Rogersiomyces*, a new genus in the *Filobasidiaceae* (*Homobasidiomycetes*) from an aquatic habitat. *Amer. J. Bot.* 65, 902–906 (1978)
- De Ley, J.: Reexamination of the association between the melting point, boyant density and chemical base composition of deoxyribonucleic acid. *J. Bact.* 101, 738–754 (1970)
- Deml, G.: Feinstrukturelle Merkmalsanalysen an *Ustilaginales*-Arten. *Z. Pilzk.* 43, 291–303 (1977)

- Deml, G., Oberwinkler, F.: Investigations on *Entorrhiza casparyana* by light and electron microscopy. *Mycologia* 73, 392-398 (1981)
- Fell, J. W.: Heterobasidiomycetous yeasts *Leucosporidium* and *Rhodosporeidium*. Their systematics and sexual incompatibility systems. *Trans. Mycol. Soc. Jap.* 15, 311-323 (1974)
- Fell, J. W., Hunter, J. L., Tallman, A. S.: Marine basidiomycetous yeasts (*Rhodosporeidium* spp. n.) with tetrapolar and multiple allelic bipolar mating systems. *Canad. J. Microbiol.* 19, 643-657 (1973)
- Fell, J. W., Statzell, A. C., Hunter, J. L., Phaff, H. J.: *Leucosporidium* gen. nov., the heterobasidiomycetous stage of several yeasts of the genus *Candida*. *Antonie v. Leeuwenhoek* 35, 433-462 (1969)
- Johnson-Reid, J. A., Moore, R. T.: Some ultrastructural features of *Rhodosporeidium toruloides* Banno. *Antonie v. Leeuwenhoek* 38, 417-435 (1972)
- Kwon-Chung, K. J.: A new genus, *Filobasidiella*, the perfect stage of *Cryptococcus neoformans*. *Mycologia* 67, 1197-1200 (1975)
- Kwon-Chung, K. J.: Morphogenesis of *Filobasidiella neoformans*, the sexual state of *Cryptococcus neoformans*. *Mycologia* 68, 821-833 (1976. a)
- Kwon-Chung, K. J.: A new species of *Filobasidiella*, the sexual state of *Cryptococcus neoformans* B and C serotypes. *Mycologia* 68, 942-946 (1976. b)
- Kwon-Chung, K. J., Popkin, T. J.: Ultrastructure of septal complex in *Filobasidiella neoformans* (*Cryptococcus neoformans*). *Mycopath. Mycol. Appl.* 35, 329-345 (1976)
- Malloch, D., Kane, J., Lahaie, D. G.: *Filobasidiella arachnophila* sp. nov. *Canad. J. Bot.* 56, 1823-1826 (1978)
- Marmur, J.: A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. molec. Biol.* 3, 208-218 (1961)
- Moore, R. T., Kreger-Van Rij, N. J. W.: Ultrastructure of *Filobasidium* Olive. *Canad. J. Microbiol.* 1, 1949-1951 (1972)
- Oberwinkler, F., Bandoni, R.: A taxonomic survey of the gasteroid, auricularioid *Heterobasidiomycetes*. *Canad. J. Bot.* (in press)
- Olive, L. S.: New or rare Heterobasidiomycetes from North Carolina. II. - J.E. Mitchell *Sci. Soc.* 62, 65-71 (1946)
- Olive, L. S.: An unusual Heterobasidiomycete with Tilletia-like basidia. *J.E. Mitchell Sci. Soc.* 84, 261-266 (1968)
- Rodrigues de Miranda, L.: *Filobasidium capsuligenum* nov. comb. *Antonie v. Leeuwenhoek* 38, 91-99 (1972)
- Silvestri, L. G., Hill, L. R.: Agreement between deoxyribonucleic acid base composition and taxometric classification of Gram-positive cocci. *J. Bact.* 90, 136-140 (1965)
- Spurr, A. R.: A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastr. Rev.* 26, 31-43 (1969)
- Sundström, K. R.: Studies of the physiology, morphology and serology of *Exobasidium*. *Symb. Bot. Upsal.* 18, 1-89 (1964)
- Van der Walt, J. P., Van Kerken, A. E.: *Torulopsis capsuligenus* nov. spec. *Antonie v. Leeuwenhoek* 27, 206-212 (1961)
- Von Arx, J. A., Rodrigues de Miranda, L., Smith, M. Th., Yarrow, D.: The genera of the yeasts and the yeast-like fungi. *Stud. Mycol.* 14, 1-42 (1977)

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