System. Appl. Microbiol. 4, 105–113 (1983)

[1] Lehrstuhl Spezielle Botanik, Universität Tübingen, 7400 Tübingen 1, Federal Republic of Germany
[2] Department of Botany, University of British Columbia, Vancouver, Canada V6T 2B1

Trimorphomyces: a New Genus in the *Tremellaceae**

FRANZ OBERWINKLER[1] and ROBERT BANDONI[2]

Received June 25, 1982

Summary

Trimorphomyces Bandoni and Oberw. gen. nov. *(Tremellaceae)* includes a single species, *T. papilionaceus* Bandoni and Oberw. sp. nov., which parasitizes *Arthrinium sphaerospermum* Fckl. The sporocarps are mainly or entirely conidial at first; conidia and basidia are mixed in mature basidiocarps. The basidia are cruciately-septate, stalked. Basidiospores germinate by repetition or by budding to form a haploid yeast phase. Blastic conidia are formed successively; terminal pairs develop synchronously and conjugate before release. The conidia are H-shaped and dikaryotic. The dikaryotic conidia germinate by forming paired buds and a dikaryotic yeast phase is initiated. Dikaryotic yeast cells are produced in the same manner as the parental conidia and are released as H-shaped, conjugated pairs. In the presence of *Arthrinium sphaerospermum* hyphae, dikaryotic conidia or dikaryotic yeast cells germinate by hyphal and haustorial formation.

Key words: *Trimorphomyces papilionaceus* – *Tremellaceae* – Basidiomycetous yeasts – Conidial conjugation – Mycoparasitism – Haustoria

Introduction

An apparently widespread, but infrequently collected tremellaceous fungus occurs on gramineous plants. The fungus was first noted on *Dactylis glomerata* L. almost 20 years ago, but the entire collection consisted of only 3 minute sporocarps. In 1980 we were alerted to a possible association of this fungus with species of *Arthrinium* by Dr. *T.R.Nag Raj* (pers. com.). The fungus was subsequently recollected on *Dactylis*, on *Phalaris arundinacea* L., and on an unidentified bamboo cultivar (*Phyllostachys* sp.). Because of the nature of the basidia, the fungus was tentatively labelled as a *Tremella*. However, detailed studies of the fructifications and life history of this unusual fungus have led us to propose a new genus for it.

---

* Part 18 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.

Materials and Methods

Specimens examined: University of British Columbia Campus, Vancouver, Canada; on *Dactylis glomerata* L.: 16 March 1961, *R. J. Bandoni* 1574; 5 June 1980, *R. J. Bandoni* 6619; – on *Phalaris arundinacea* L.: 28 May 1980, *R. J. Bandoni* 6619; 5 June 1980, *R. J. Bandoni* 6626; – on *Phyllostachys* sp.: 11 July 1980, *R. J. Bandoni* 6626; 23 July 1980. *R. J. Bandoni* 6646 (Type in DAOM); 12 September 1980, *R. J. Bandoni* 6650; 4 June 1981, *R. J. Bandoni* 6696, 6697. All specimens other than the type are deposited in the herbarium of the University of British Columbia (UBC).

For transmission electron microscopy material was fixed with glutaraldehyde and osmiumtetroxide, washed with distilled water, dehydrated in an alcohol series and embedded in ERL according to *Spurr* (1969). Ultrathin sections were mounted on unsupported mesh copper grids, poststained in uranyl acetate and lead citrate solutions and examined in a Zeiss EM 9 S-2 transmission electron microscope.

Results

Trimorphomyces Bandoni and Oberwinkler, gen. nov.

Genus Heterobasidiomycetum. Carposomata minute pustulata, gelatinosa, primo cellulis conidia gerunt deinde basidiis composita. Hyphae hyalinae, tenui-tunicatae, fibulatae, cum haustoriis propriis tremelloideis. Conidia ex locis oppositis bina simul oriuntur, quorum fusione cellulae papilionaceae et binucleatae efficiuntur. Probasidia stipitata, subglobosa, mature septis cruciatis divisa. Basidiosporae hyalinae, tenui-tunicatae, tunicis levibus, cellulis singulis vel per repetitionem germinant.
Typus generis: *Trimorphomyces papilionaceus* Bandoni and Oberwinkler.
Etym.: τρι – three; μορφή – form; μύκης – fungus; because of the three assimilative states (haploid yeast, dikaryotic yeast, hyphae) in the life history.

Fructifications minute, gelatinous, tuberculate, at first entirely conidial, later of mixed conidiophores and basidia. Hyphae hyaline, with clamps and haustoria, radiating, terminated by conidiogenous cells, basidia, or both of these; conidiogenous cells short, phialide-like, or elongate, cylindric. Conidia developing blastogenously and synchronously in closely situated apical pairs, conjugation occurring between members of the pair as they develop, the conidia released as H-shaped dikaryotic cells; conidia germinating by the synchronous formation of paired conjugating cells, a dikaryotic yeast phase resulting; alternatively, haustoria are produced in the presence of host hyphae. Basidia stalked, the upper portion pyriform to ellipsoid or subglobose, becoming cruciate septate. Basidiospores subglobose, germinating by repetition or by budding to form a monokaryotic yeast phase. Septa of the dolipore type, the parenthesome consisting of poorly developed (vestigial) structures.

Trimorphomyces papilionaceus Bandoni and Oberwinkler, sp. nov.

Carposomata stillata, gelatinosa, hyalina vel plus minusve lactea, interdum subviridia, usque ad 1 mm, raro 2 mm in diam.; siccata inconspicue maculata, membranacea corneaque, brunnea. Hyphae breves et dense aggregatae, hyalinae, fibulatae, 2.5–3 μm raro ad 4 μm in diam., cum haustoriis; hyphae fertiles conidia vel basidia gerunt. Conidiophora 6–15–(20) × 2.5–4 μm, cylindrica vel inflata, apicibus bifurcatis conidiis separatis oreundis deinde per fusionem unum solum conidium papilionaceum, binucleatum, producunt. Conidia inflata similiter geminantur vel

hyphis fibulatis frequenter haustoriis praeditis. Probasidia maturitate plerumque cruciatim septata, (9)–12–18–(22) × 7–9.5–(11) μm. Epibasidia angusta, cylindracea, 120 × 1.5–2.5 μm, parce inflata apicaliter. Basidiosporae subglobosae vel late ovales, 6–8 × 5–7 μm, oblique apiculatae, gemmis vel per repetitionem germinandae. Cellulae fermenti ovales ad fusiformes an cylindraceae, 5–8–15 × 3–4.5 μm.

Habitatio: Species graminicola Arthrinio ssp. consociata est.

Typus: *R. J. Bandoni* No. 6646, in culmis emortuis Phyllostachidis, 23.7.1980, in campo universitatis Columbiae Britannicae, Canada.

Fructifications pulvinate (Fig. 8), hyaline to slightly milky or sometimes faintly greenish, almost transparent, gelatinous, mostly less than 1 mm wide, sometimes anastomosing and exceeding 2 mm, drying to thin, faintly brownish, horny spots. Hyphae of sporocarp short, compactly arranged, radiating (Fig. 9), hyaline, with clamps, mostly 2.5–3 μm in diam., sometimes swollen to 4 μm, with haustoria, giving rise to conidiogenous cells and/or basidia terminally, proliferating laterally. Conidiogenous cells (Figs. 6, 7) 6–15–(20) × 2.5–4 μm, the shortest ones often bottle-shaped and phialide-like, longer cells more or less cylindric; conidia developing blastogenously, from two closely-situated, apical loci (Figs. 6, 7, 17–19, 23, 24), the loci at first inconspicuous, elongating with successive conidial formation, the conidiogenous cell apex eventually becoming bifurcate; conidia at first narrowly elliptic to cylindric, conjugating before maturity (Figs. 6, 7, 16–18, 23), papilionoid in face view when released (Fig. 20), 3.5–7 × 3.5–5.5 μm. Conidia germinating (Fig. 10) by formation of synchronous, paired buds, the paired budding loci commonly subpolarly on one of the two conjugated parental cells, a dikaryotic yeast phase developing in culture; budding dikaryotic cells at first H-shaped, enlarging, the isthmus finally visible only as a slight constriction or becoming obliterated (Figs. 22, 24, 25); budding loci becoming prominent with repeated budding, a short tubular process slowly forming between the parent cell and the loci; secondary budding sites often initiated on parental cells, these developing at the wall itself or apically on a clamped, short tubular process, the latter with the potential to develop into a short dikaryotic hypha, a haustorium (Figs. 21, 22, 24), or a conidiogenous cell (Figs. 21, 23–25); infrequently, 2 pairs of budding loci occur side-by-side. Probasidia (Figs. 1, 2, 11) at first narrow to broadly clavate, becoming capitate or pyriform, usually tapering to a long basal stalk; mature basidia (Figs. 2–4) diagonally to irregularly cruciate-septate at maturity, (9)–12–18–(22) × 7–9.5–(11) μm; epibasidia narrow, cylindric, to 120 × 1.5–2.5 μm, slightly swollen apically. Basidiospores subglobose to broadly oval, 6–8 × 5–7 μm, the apiculus oblique or sometimes situated at right angles to the long axis; germination by repetition or by budding, in the latter case, budding directly or from apices of one to several elongate cells which remain attached to the basidiospore, yeast cells oval to fusiform or cylindric, 5–8–12 × 3–4.5 μm.

Habitat: Associated with *Arthrinium* spp. on gramineous plants.

Discussion

The constant association with *Arthrinium* state (= *Papularia arundis* (Corda) Fr.) of *Apiopora montagnei* Sacc. suggested that *Trimorphomyces papilionaceus* might be a parasite of these dematiaceous fungi. When grown in culture with the *Arthrinium,* dikaryotic yeast cells and conidia of *T. papilionaceus* germinated by the formation of a short hypha and a haustorium of the *Tremella* type (*Olive,* 1946;

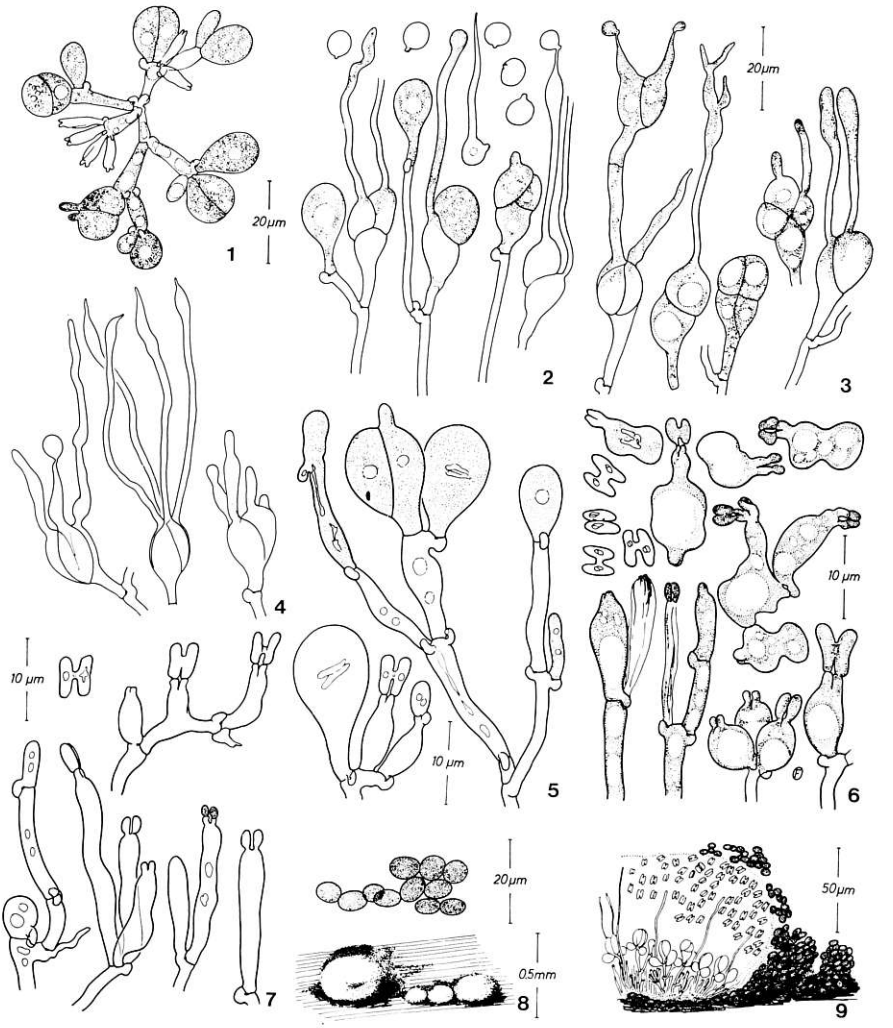

Plate I

Trimorphomyces papilionaceus. Figs. 1–5. Fertile hyphae with basidia; developing basidiospores, and mature basidiospores (Fig. 2, top); note empty conidiogenous cells in Figs. 1 and 5, and aberrant basidia in Fig. 3. – Figs. 6, 7. Conidiogenous cells, conidia, and production of synchronous, conjugating buds (Fig. 6, top). Fig. 8. Habit sketch of basidiocarp and associated Arthrinium conidia.

Bandoni, 1961). Numerous *Trimorphomyces* cells adjacent to the host hyphae formed a radiating pattern, similar to that shown in Fig. 9, around the host hyphae. In addition to, or in the place of the haustorium, the germ tube often produced a conidiogenous cell (Fig. 7). Haustoria also are present on fertile hyphae in the

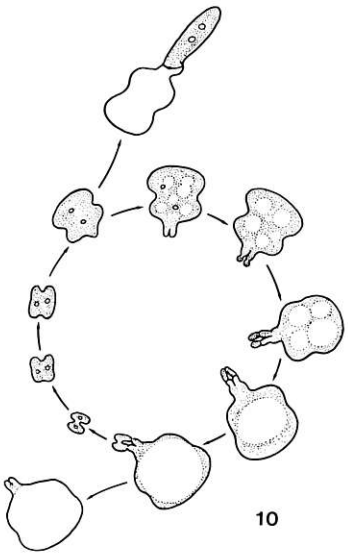

Plate II

Fig. 10. Dikaryotic yeast phase of *Trimorphomyces papilionaceus*. Diagrammatic representation of budding of dikaryotic conidium. Young cell (arrow) enlarges, budding repeatedly. Old cells may die (below); some cells produce short, clamped tubular extensions (top) which may either develop new apical budding loci or, presumably, can continue hyphal growth.

hymenia of mature fructifications, as in some Tremellas, but they are lacking or infrequent in immature fructifications.

The most distinctive features of *T. papilionaceus* are the formation of paired conidia which conjugate during development, and the germination of these conidia by a kind of repetitive budding process to produce a dikaryotic yeast phase. The species thus is characterized by the production of two unicellular phases, one dikaryotic and the other monokaryotic, and a dikaryotic hyphal phase. Synchronous development and conjugation of paired conidia of this type is known only in the holobasidiomycetous genera, *Syzygospora* Martin (1937) and in the related *Christiansenia* Hauerslev (1969). Development of paired, blastic conidia also takes place in *Platygloea peniophorae* Bourd. and Galz., but conjugation is not known to occur. The species of *Christiansenia* and *Syzygospora,* as well as *P. peniophorae* are all parasites of basidiomycetous fungi. Blastic conidial production in *T. papilionaceus* appears to be identical to budding in other basidiomycetous yeasts (*Marchant* and *Smith,* 1967; *Prusso* and *Wells,* 1967; *Bandoni* and *Bisalputra,* 1971; *Kreger–van Rij* and *Veenhuis,* 1971). Walls of developing buds or conidia are continuous with the inner wall layer of the mother cell, the outermost wall layer rupturing. The ruptured outer layer is often visible by light microscopy, but is much more conspicuous in TEM preparations (Figs. 17, 19). The fertile locus typically extends for a short distance between production of successive buds or conidia and the outer wall vestiges are present at each productive site. In many budding *Basidiomycetes,* these vestiges are visible as annellations on the tubular extensions that develop with repeated budding at a locus.

Although synchronous nuclear division occurs during formation of the dikaryotic conidia, the overall developmental pattern does not suggest that *T. papilionaceus* conidia are of the type designated as modified clamps or disarticulated clamps (*Kendrick* and *Watling,* 1979).

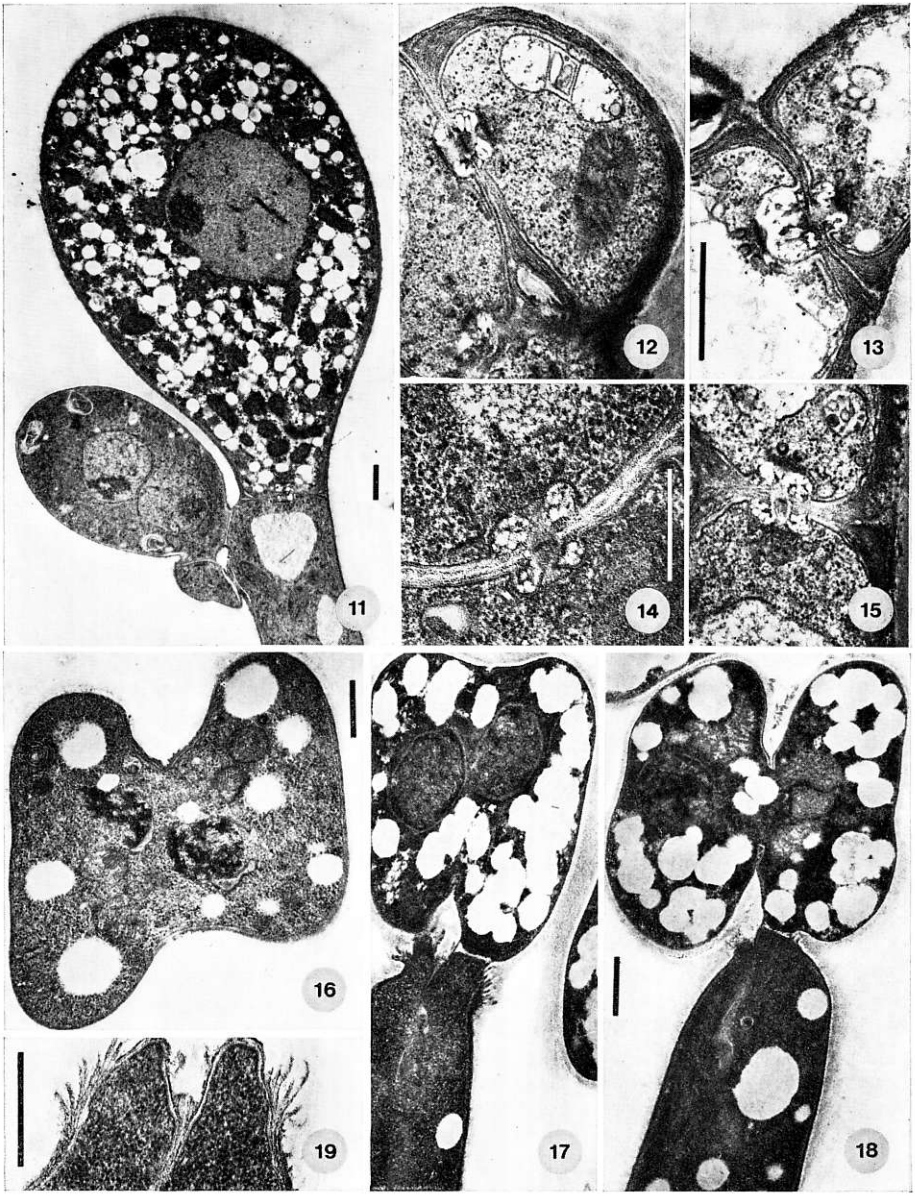

Plate III

TEM photomicrographs of *Trimorphomyces papilionaceus*. Fig. 11. Section through two young probasidia, one dikaryotic, the second with a fusion nucleus. Figs. 12–15. Sections through septal pores showing dolipore and poorly developed parenthesomes. Fig. 16. Section through dikaryotic conidium showing two nuclei. Fig. 17. Median section through mature conidium and conidiogenous cell apex, the conidium with paired nuclei and broad attachment of parental cells. Fig. 28. Non-median section of conidiogenous cell apex and conidium.

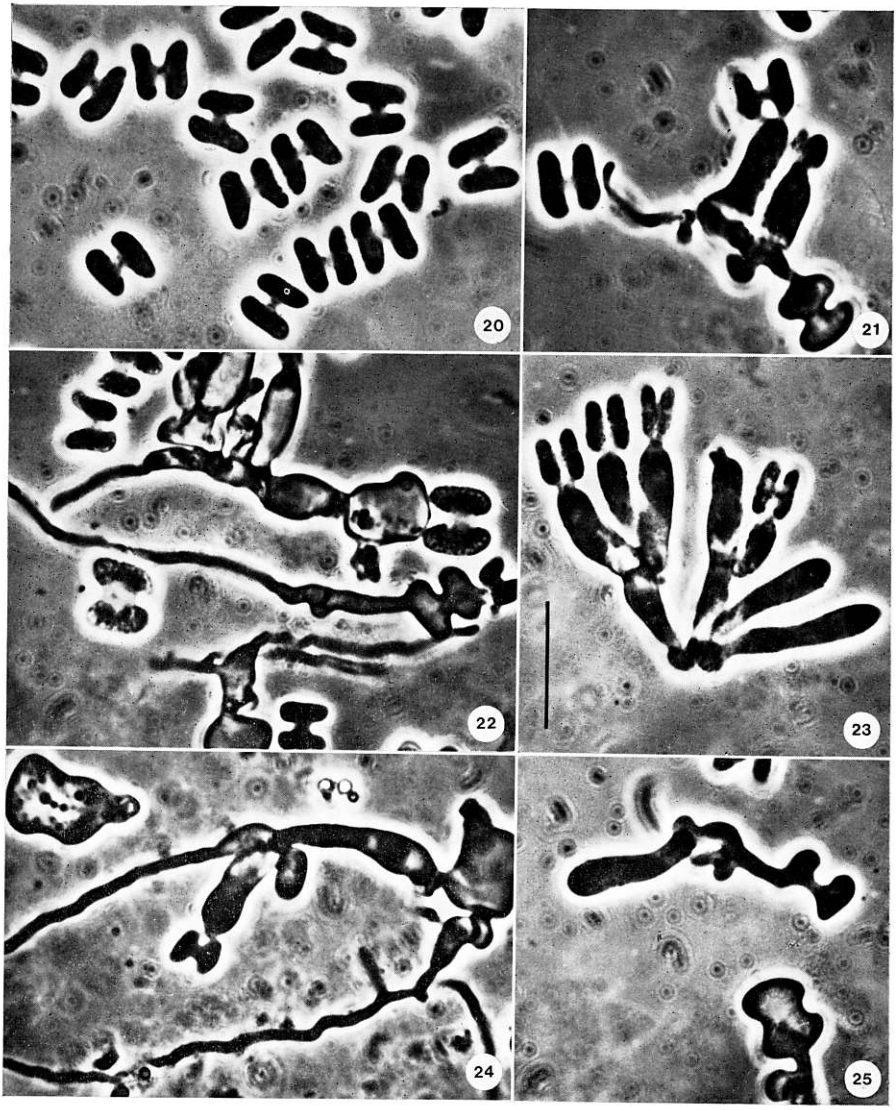

Plate IV

Phase contrast photographs of *Trimorphomyces papilionaceus* (all figures at the same magnification; bar in Fig. 23 = 10 μm). Fig. 20. Young conidia as released from conidiogenous cells. Figs. 21–25. Germination of conidia as seen in dual culture with *Arthrinium sphaerospermum;* Fig. 21. Conidium (lower right) has produced short germ tube, 2 conidiogenous cells, one of which bears a mature conidium; a haustorium terminates the hypha (left); Fig. 22. Two conidia, the older one (above) of which has lost the H-shape and has a nearly mature conidium attached opposite the germination hypha; the younger conidium (center) has produced only a short hypha terminated by a clamp and haustorium. Fig. 23. Cluster of conidiogenous cells after germination of dikaryotic conidium, the latter not visible. Fig. 24. Old dikaryotic conidium (far right) with haustorium arising directly from its base, an elongate budding locus above, and a germ tube bearing a conidiogenous cell and a haustorium, the latter only partially visible.

The septal pore apparatus of *T. papilionaceus* is similar to the type found in a *Tremella* sp. by *Khan* (1976), *Filobasidium capsuligenum* Olive (*Moore* and *Kreger-van Rij*, 1972) and *Tetragoniomyces uliginosus* (Karst.) Oberw. and Bandoni (*Oberwinkler* and *Bandoni*, 1981). In contrast to *Tremella mesenterica* Fr. (*Moore*, 1978) and *Sirobasidium magnum* Boedijn (*Moore*, 1979) the parenthesome consists only of a few shallow, cupulate ampullae (Figs. 12–15).

T. papilionaceus resembles some of the small, mycoparasitic Tremellas in having a haploid yeast phase, in its haustorial structure, and in the general appearance of its basidiocarps and basidia. However, it is clearly distinct from the *T. mesenterica* group in having a dikaryotic yeast phase, in conidial structure and development, and in details of its septal pore apparatus. Our cultural studies gave possible indications of a structural difference in development of basidiocarps of *Trimorphomyces* and *Tremella*. In the latter genus, basidiocarps presumably originate from a single, extensively-branched dikaryotic hypha. Individual *Trimorphomyces* fructifications produced in culture, developed from numerous dikaryotic conidia and yeast cells adjacent to one another and to a host hypha. It is not known whether this also occurs in natural collections, but basidiocarps of such collections also are made up of rather short, radiating, infrequently-branched hyphae.

Acknowledgments

We thank Dr. *T.R. Nag Raj* for the information that led to our finding abundant *Trimorphomyces papilionaceus* material. The assistance of Miss *S. Dinkelmeyer* with electron microscopy is gratefully acknowledged. Portions of this study were supported by grants from the Natural Sciences and Engineering Council of Canada (Grant A 801) and from the Deutsche Forschungsgemeinschaft (SFB 76, Project N).

References

Bandoni, R.J.: The genus *Naematelia*. Am. Midl. Nat. *66*, 319–328 (1961)

Bandoni, R.J., Bisalputra, A.A.: Budding and fine structure of *Tremella mesenterica* haplonts. Canad. J. Bot. *49*, 27–30 (1971)

Hauerslev, K.: Christiansenia pallida gen. nov., sp. nov. A new parasitic Homobasidiomycete from Denmark. Friesia 9, 43–45 (1969)

Kendrick, B., Watling, R.: Mitospores in *Basidiomycetes*, pp. 473–545. in: *Kendrick, B.* (ed.), The whole fungus. 2. Nat. Mus. Nat. Sci. Canada (1979)

Khan, S.R.: Ultrastructure of the septal pore apparatus of *Tremella*. J. gen. Microbiol. *97*, 339–342 (1976)

Kreger-Van Rij, N.J.W., Veenhuis, M.: A comparative study of the cell wall structure of basidiomycetous and related yeasts. J. gen. Microbiol. *68*, 87–95 (1971)

Marchant, R., Smith, D.G.: Wall structure and bud formation in *Rhodotorula glutinis*. Arch. Mikrobiol. *58*, 248–256 (1967)

Martin, G.W.: A new type of heterobasidiomycete. J. Washington Acad. Sci. *27*, 112–114 (1937)

Moore, R.T.: Taxonomic significance of septal ultrastructure with particular reference to the jelly fungi. Mycologia *70*, 1007–1024 (1978)

Moore, R.T.: Septal ultrastructure in *Sirobasidium magnum* and its taxonomic implications. Antonie v. Leeuwenhoek *45*, 113–118 (1979)

Moore, R.T., Kreger-Van Rij, N.J.W.: Ultrastructure of *Filobasidium* Olive. Canad. J. Microbiol. *18*, 1949–1951 (1972)

Oberwinkler, F., Bandoni, R.J.: *Tetragoniomycetaceae*: a new family in the *Tremellales*. Canad. J. Bot. 59, 1034–1040 (1981)

Olive, L.S.: New or rare *Heterobasidiomycetes* from North Carolina. J.E. Mitchell Sci. Soc. 62, 65–71 (1946)

Prusso, D.C., Wells, K.: *Sporobolomyces roseus*. I. Ultrastructure. Mycologia 59, 337–348 (1967)

Spurr, A.R.: A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastr. Res. 26, 31–43 (1969)

Prof. Dr. *F. Oberwinkler*, Lehrstuhl Spezielle Botanik der Universität Tübingen, Auf der Morgenstelle 1, D-7400 Tübingen 1, Federal Republic of Germany