

## BASIDIAL MORPHOLOGY AND HYMENOPHORAL DEVELOPMENT IN RHIZOPOGON

P. H. B. TALBOT

*Waite Agricultural Research Institute, Glen Osmond, S. A., Australia*

(With 14 Text-figures)

Species currently assigned to *Rhizopogon* Fr. & Nordh. can be divided into at least two groups on the basis of basidial morphology and hymenophoral development. It is desirable that two genera should be recognized but problems of typification preclude a formal proposal to this effect. One group contains species with clearly dimerous basidia which are commonly associated with swollen, thickened or gelatinized basidioles and often arise laterally from these. The other is composed of species with clavate or cylindrical non-dimerous basidia which are not associated with modified basidioles. These observations show the potential value of using the developmental anatomy of the hymenophore in the taxonomy of *Gasteromycetes*. Fresh specimens in various stages of development are desirable; mature or dry specimens usually prove unsatisfactory.

### Introduction

In reference to early work by Fischer on the classification of *Gasteromycetes*, Lloyd (1902: 5) stated: "It seems to be the tendency of some writers to select the most obscure and difficult points on which to base classification. This has one advantage, it gives an air of greater learning. For our part we feel that a system based on points of difference of the mature plant obvious to the student, is more satisfactory and rational." Lloyd (1902: 4) also said: "... it is not a matter of policy to classify plants by minute anatomical differences which only an expert microscopist can trace. . ." Few mycologists would still agree with Lloyd on this issue, yet the fact is that most work on *Gasteromycetes* still relies heavily on mature fructifications and makes little use of detailed developmental anatomy. One wonders how much this may be due to the lingering influence of Lloyd's sharp pen, and how much to technical difficulties presented by *Gasteromycetes*. The basidia and other hymenial structures are best seen in young, fresh collections or those preserved in liquid, and usually collapse well before the fruitbodies reach their maximum size. Thus there are difficulties in using dry herbarium material, especially if the collector has carefully selected only older or larger specimens.

What follows is intended primarily to demonstrate the potential value of using hymenophoral anatomy and basidial morphology in the taxonomy of *Gasteromycetes*, and to show that it is also essential to take development into account. For this purpose a few species of the genus *Rhizopogon* have been studied.

## RHIZOPOGON Fr. &amp; Nordh.

Only three species of *Rhizopogon* have so far been recorded in South Australia: *R. clelandii* G. H. Cunn., *R. luteolus* Fr. & Nordh. and *R. rubescens* Tul. (Cleland, 1935; Cunningham, 1944). Thus it would seem easy to identify the species collected locally by my colleagues in mycorrhizal associations with *Pinus*. However, Smith & Zeller (1966) detected at least fifteen species in the North American flora masquerading under the name of *R. rubescens* and indicated that *R. luteolus*, the type species of the genus, has been similarly confused by various authors. A further complication is that the litter even under a single tree may sometimes harbour more than one species of *Rhizopogon*, each with basidiocarps at various stages of development and thus very difficult to sort out. Or, again, isolated basidiocarps may be found. The problem is this: although the pattern of macroscopic change with aging has been recorded for some species (particularly by Smith & Zeller, 1966), isolated basidiocarps cannot reveal the full pattern and so can only tentatively be determined to species, while a range of material of all ages cannot in any simple way be recognized as belonging to one species unless there is a striking macroscopic character (such as a particular colour reaction to bruising or chemicals) which is present in all stages. It is probably fair to say that microscopic changes with aging, especially in the hymenium, have never been adequately investigated. Yet the importance of basidial morphology as a stable microscopic character has been demonstrated repeatedly with other basidiomycetes and should apply in *Rhizopogon*.

The purpose of this study was to investigate the development of the hymenium, basidia and associated structures in local specimens because the literature shows that these features have not been clearly understood in the past. The hymenium and basidia are difficult to examine in the more gelatinized species. Thin freezing-microtome sections of fresh specimens, mounted in ammoniacal Congo red, teased out and gently squashed under the coverslip, were found to give the best results; but even then it was not easy to determine the exact relationships and morphology of the various elements present. Pickled material gave reasonably good results, but dried specimens were virtually useless for this purpose (see below).

Most accounts of *Rhizopogon* state that the basidia are clavate or cylindrical and usually soon collapse; they usually have 6–8 sessile or subsessile spores, though there have been reports of basidia with 2–4 spores. It is indeed odd that the basidia should be described thus in generic diagnoses, and often in descriptions of species, when illustrations by various authors show that within the genus there are some species with clavate to subcylindrical basidia, e.g. *R. reticulatus* Hawker (1955: Fig. 1), *R. rubescens* sensu Hawker (1954: Fig. 29,  $d_1$ – $d_5$ ), and others with clearly dimerous basidia having a ventricose base and a cylindrical prolongation with an expanded apex bearing the spores, e.g. *R. rubescens*, *R. luteolus*, *R. roseolus*, and *R. nigrescens* sensu Coker & Couch (1928: Pls. 106–107). In some cases the descriptions do not match the illustrations for particular species. Smith & Zeller (1966) report the true situation: "... in most species the basidiole is clavate to subcylindric and as the spores

form it may remain clavate, it may elongate to cylindric, or the apex may elongate and the spores form on the narrow apex of the neck."

For the sorts of reasons given above I am still unable to determine local species of *Rhizopogon* and indeed do not yet know how many species are represented by our material. Thus I have to report this work on the basis of collections.

### Details of fresh collections

- No. 1: ADW 16239, E. Davison, under *Pinus radiata*, Penola forest, Nov. 1971.  
No. 2: ADW 16241, J. H. Warcup, under *Pinus radiata*, Kuitpo forest, Nov. 1971.  
Nos. 3, 4, 5, 6: ADW 16242, 16243, 16244, 16245 (respectively), J. H. Warcup, under mixed *Pinus* and *Eucalyptus*, Kuitpo forest, Nov. 1971 (collected separately in areas not close to one another).  
No. 7: ADW 16240, J. H. Warcup (E. M. 275), under mixed *Pinus* and *Eucalyptus*, Kuitpo forest, Oct. 1971. (Shown experimentally by Dr Warcup to be mycorrhizal with *Pinus radiata*.)

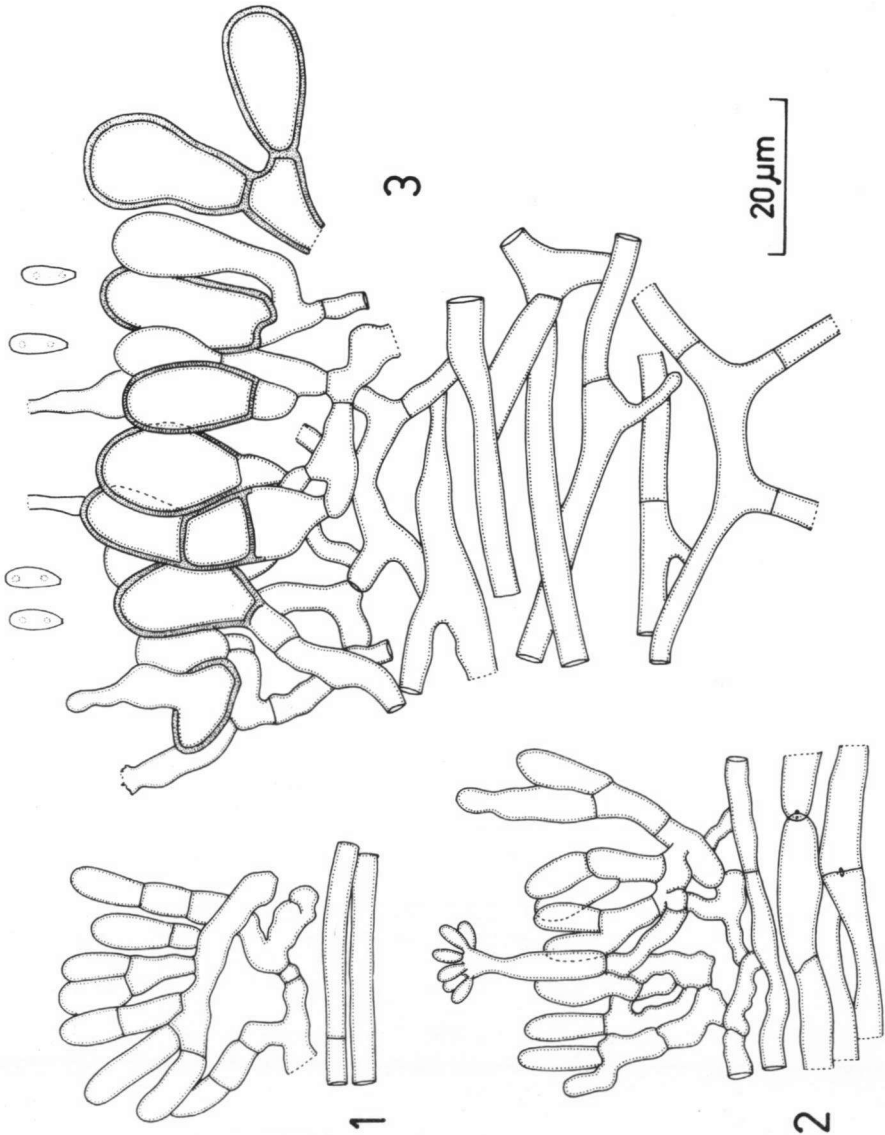
### Development in collection No. 1

This was a large collection of forty-five basidiocarps all found in a restricted area. Although various stages are represented it is certain that all belong to one species, there being a very characteristic bruising reaction common to all stages.

*Basidiocarps* 0.5–3.5 cm diam. *Peridium* externally white when young, later developing bright yellow areas, finally yellow all over, staining vinaceous in all stages when bruised, the bruised parts drying vinaceous-brown, not affected by  $\text{FeSO}_4$  or ethanol, darkening to medium reddish-brown in KOH; peridium in section composed of a single layer of prosenchymatous hyphae with reddish granules, browning in KOH; hyphae of peridium thin-walled or slightly thickened, without clamps or inflated cells, septate, 3–10  $\mu\text{m}$  wide; epicutis and latex vessels absent. *Rhizomorphs* pale at first, brown when old or dry, composed of hyaline to light brown septate hyphae without clamps, thin-walled, not inflated, mostly 2–5  $\mu\text{m}$  wide, often branching at a wide angle; some central hyphae less branched, 6–11  $\mu\text{m}$  wide, with the wall thickened to 1  $\mu\text{m}$ . *Gleba* soft and gelatinous when fresh, becoming firm but not excessively hard on drying, whitish then pale yellowish and finally medium brown, with small meandering labyrinthiform locules. *Trametal hyphae* thin-walled, 2.5–6  $\mu\text{m}$  diam., much branched, septate, without clamps, with occasional visible dolipores, without latex vessels. *Spores* present in basidiocarps from about 1.5 cm diam., fusoid-ellipsoid, thin-walled, light olivaceous, smooth, non-amyloid, with a truncate base and usually a pair of refractile polar inclusions, borne 6 per basidium, 6–8  $\times$  2–3  $\mu\text{m}$ .

*Hymenial development*.—The hymenium arises vertically from a more or less horizontal layer of hymenophoral hyphae, the vertical branches forming two or three subcymose or irregular ranks. The distal cells of these branches (probasidia) are cylindrical with rounded apices (Fig. 1). Some of these develop into metabasidia by putting out a short prolongation separated by a constriction from the lower part of the metabasidium and terminating in an expansion bearing six sessile basidiospores formed as buds from the apex (Fig. 2). These basidia measure about 6–18  $\times$  4  $\mu\text{m}$ . In a later stage such basidia are still found, but in addition some of the probasidial cells, and some of the proximal cells which bear them, have enlarged

considerably into ellipsoid or more often obovate cells  $(13-16-22 \times 7-10-13 \mu\text{m})$ , with walls thickened to  $1 \mu\text{m}$  or slightly more (Fig. 3). At this stage further basidia arise laterally from some of the enlarged cells and develop a much longer prolongation (more than  $10 \mu\text{m}$ ) than the original basidia. The basidia in this species are thin-



Figs. 1-3. — Development of dimerous basidia and hypertrophied basidioles in Collection No. 1.

walled at all times. An old hymenium consists mostly of enlarged thickened cells supported by a narrow hymenophoral layer. The median part of the hymenophoral trama consists of irregularly arranged hyphae which vary greatly in width over a short distance and are intricately branched.

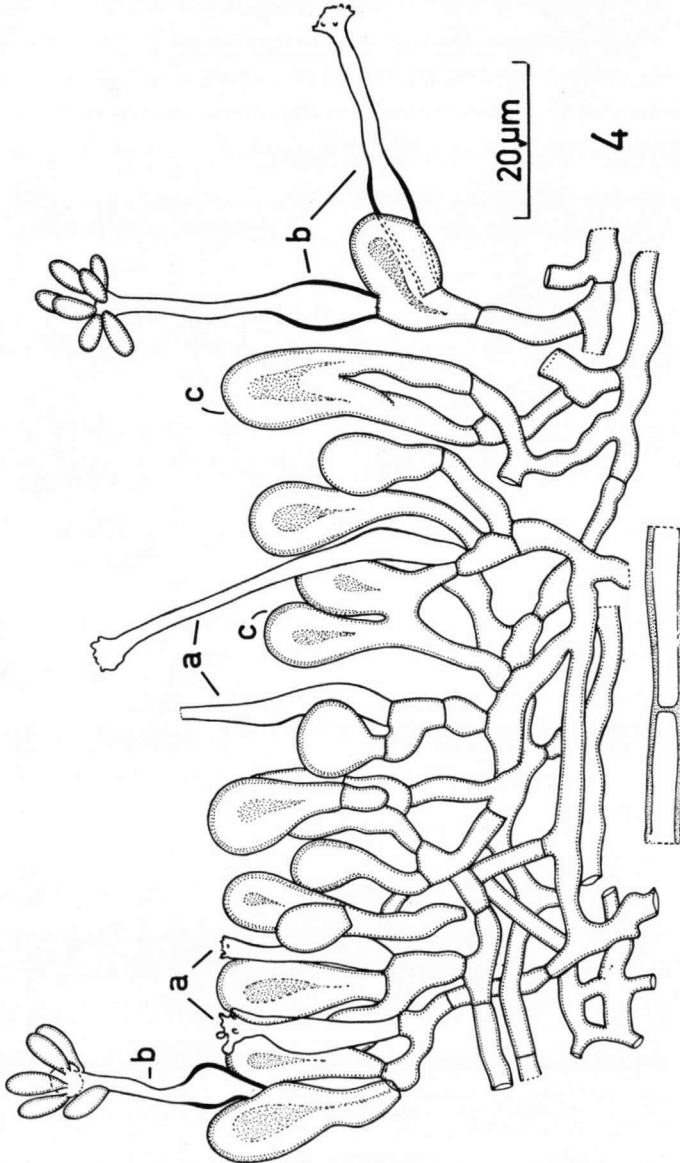
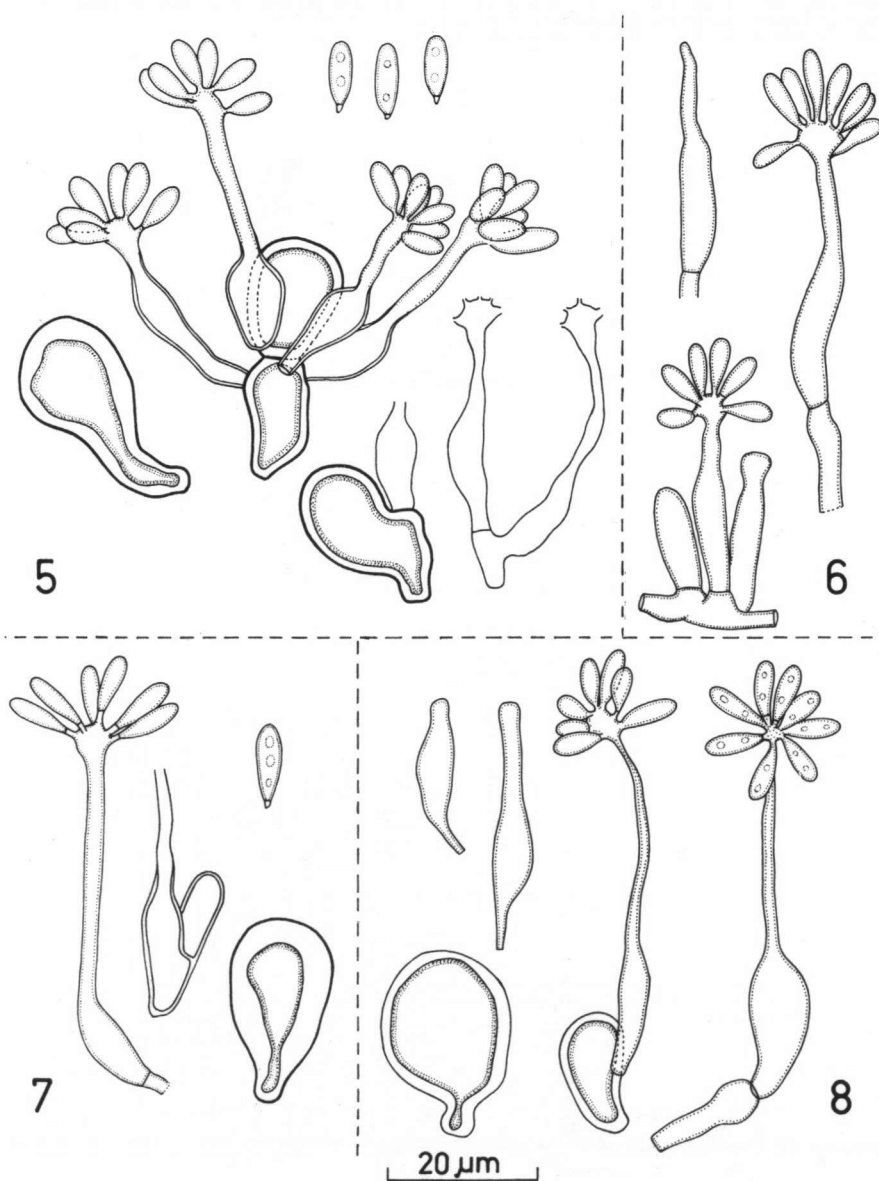


Fig. 4. — Mature hymenium in Collection No. 2. — a. Dimerous basidia arising from hyphae. — b. Dimerous basidia with thick-walled bases arising laterally from swollen gelatinized basidioles. — c. Anastomosed basidioles.



Figs. 5-8. — Collections Nos. 3-6 respectively. See text for details.

The basidia in this species are quite clearly dimerous, whether they arise directly from hymenial hyphae or laterally from the enlarged hymenial cells. The latter type of cell has usually been referred to in the literature as a 'paraphysis' but in origin it is an hypertrophied probasidium or basidiole. The hymenium changes so much in microscopic appearance with aging that young and old basidiocarps would probably be assigned to different species if the pattern of development were not known.

### Other collections with similar hymenia

Fig. 4 (Collection No. 2) shows a mature hymenium in which the basidia arise either directly from hymenial hyphae (4a) or laterally from hypertrophied basidioles (4b). The latter are strongly gelatinized, very thick-walled, and occasionally anastomosed (4c). The basidia are strongly dimerous, sometimes with the ventricose base becoming firm-walled (4b).

Fig. 5 (Collection No. 3) shows dimerous basidia arising either from hyphae or from hypertrophied basidioles. The main points of interest are that several basidia may be formed in a cluster from a single basidiole, and that the basidial walls become appreciably thickened towards the base.

The development of dimerous basidia is shown in Fig. 6 (Collection No. 4). No hypertrophied basidioles were found in this material which consisted of a single basidiocarp. The basal part of the metabasidium was subcylindrical rather than ventricose.

The basidial prolongations shown in Fig. 8 (Collection No. 6) are notable for being unusually tapered towards the distal end, and narrow in comparison with those in other collections.

In Fig. 7 (Collection No. 5) it is seen that the spores are clearly not sessile in this species but are borne on short sterigmata. The spores themselves, as in several other collections examined, are shortly pedicellate at the base, instead of truncate as in Fig. 3.

### Development in collection No. 7—Fig. 9

*Basidiocarps* 1–1.5 cm diam. *Peridium* externally pink, lacking an epicutis, in section composed of a single prosenchymatous layer of thin-walled to slightly thickened hyaline hyphae, 3–5  $\mu$ m wide but inflated in short-celled portions to 15  $\mu$ m wide, lacking clamps. *Rhizomorphs* whitish to reddish-brown, composed of parallel thin-walled septate hyphae, 2.5–5  $\mu$ m wide, with frequent septa, lacking clamps, a few central hyphae becoming 10  $\mu$ m wide. *Gleba* soft, gelatinous, white when fresh, with small meandering locules. *Trametal hyphae* 2.5–6.5  $\mu$ m wide, thin-walled, hyaline with frequent septa and exceptionally large dolipores, lacking clamps. *Latex vessels* present throughout the trama though rather scanty, 3–7  $\mu$ m wide, rarely septate, with homogeneous contents and walls that cannot be distinguished from contents. *Spores* fusoid-ellipsoid, 7.5–8.5  $\times$  3–3.5  $\mu$ m, hyaline to faintly tinted, not amyloid, thin-walled, smooth, with a truncate base, borne (2–4)–6–8 per basidium on sterigmata up to 2  $\mu$ m long.

*Hymenial development.*—The hymenial branches arise vertically in 2–3 ranks of

subcymose or irregular branching from the subhymenial hyphae. The distal cells (probasidia) are subcylindrical with rounded apices. The spores develop directly from the apex from bud-like processes whose proximal part remains as a narrow sterigma which is eventually separated from the spore by a septum. The metabasidia

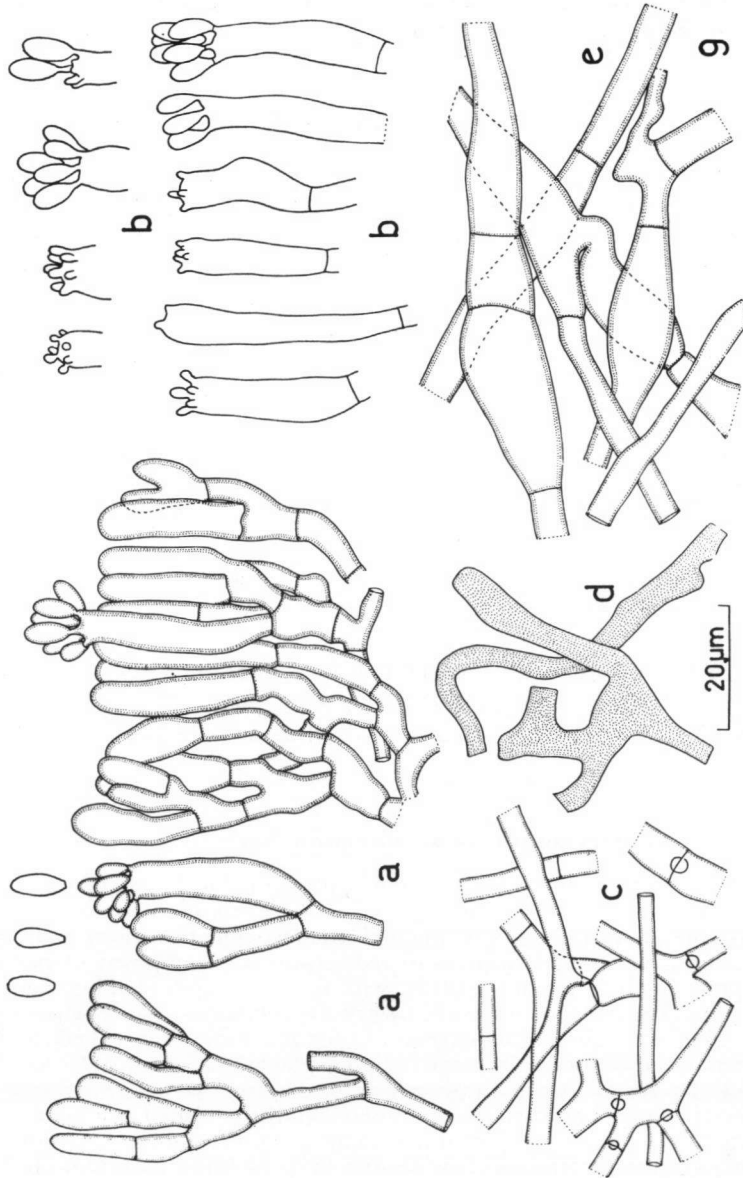


Fig. 9. — Development of the hymenium (a) and cylindrical basidia (a and b) in Collection No. 7. — c. Tramal hyphae with exceptionally large dolipores. — d. Laticiferous hyphae. — e. Peridial hyphae.



in this species are subcylindrical, not dimerous nor constricted,  $23-27 \times 5.5-7 \mu\text{m}$ , and they always develop terminally from hymenial hyphae. Hypertrophied basidioles are absent. The hymenium is finally composed of subcylindrical probasidia and scattered fertile subcylindrical metabasidia.

### Information from dried herbarium specimens

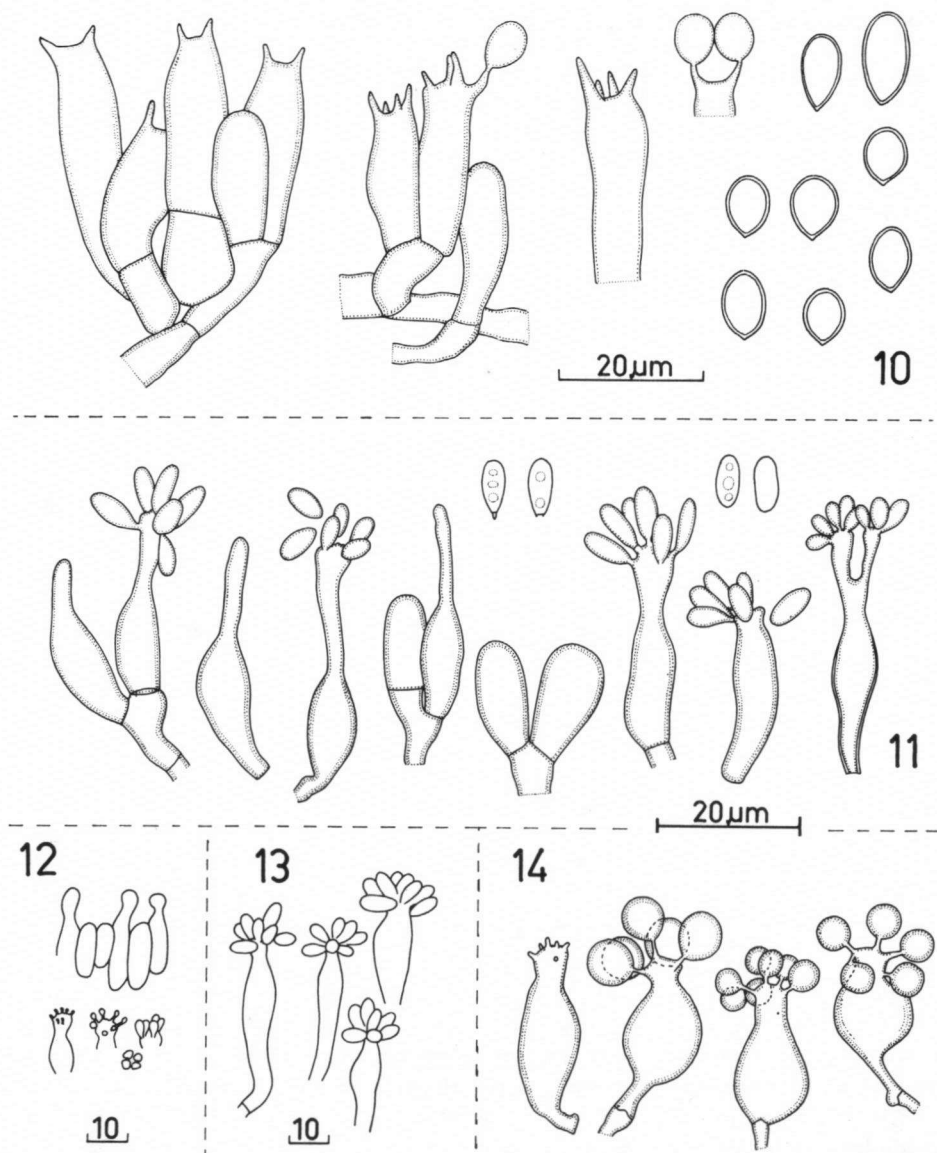
Twenty-one herbarium specimens of *Rhizopogon* species were examined. In only one collection was the state of preservation almost as good as in a fresh specimen. In general, the details of hyphae and spores were quite well preserved, but the hymenial structures had collapsed and were badly distorted. The presence of thickened basidioles, but not their details, could be determined in eighteen collections. In only nine of these was it possible to infer the presence of dimerous basidia from collapsed metabasidial prolongations emerging above the level of the hymenium. In the other collections basidia could not be seen. It was noted that the spores in some collections, even in a single preparation, varied from shortly pedicellate at the base to notched or truncate. Thus dried specimens were found to be of little use for determining details of hymenial structure.

Among the dried material examined was the type specimen of *Rhizopogon clelandii* G. H. Cunn. (Herb. ADW 6009). This, as shown in Fig. 10, has subcylindrical basidia,  $19-36 \times 7-8 \mu\text{m}$ , with two or four substantial sterigmata. Its spores are smooth, non-amyloid, thick-walled, non-guttulate, broadly ellipsoidal or pip-shaped, somewhat narrowed towards the small pointed hilum,  $8-13 \times 5.5-6.5 \mu\text{m}$ . I consider that it should not be regarded as a species of *Rhizopogon* sensu lato.

### Discussion

Among representatives of the genus *Rhizopogon* sensu lato there are at least two different types of hymenial development with associated differences in basidial morphology. Most species will probably be found to have dimerous basidia and a hymenium in which some or most of the basidioles finally become enlarged, thick-walled or gelatinized, and able to produce further basidia laterally. In this way the hymenium thickens both by swelling and gelatinization of the basidioles and by addition of new basidia, until the glebal locules may become almost filled. I am not certain that this is so in the type species, *R. luteolus*, because there is confusion as to what this really represents. However, the illustrations by Coker & Couch (1928, Pl. 106, Figs. 15, 17) show dimerous basidia, some arising laterally from swollen basidioles, while Morten Lange's (1950) description mentions long basidia projecting above the hymenium (presumably the prolongations from dimerous basidia). Dr. D. M. Dring of Kew Herbarium has favoured me with liquid-preserved material of what he regards as *R. luteolus*. This (Fig. 11) has dimerous basidia, but in the single basidiocarp examined there was no evidence of hypertrophied basidioles, though possibly these might develop in older material.

The other type of development is that where the basidia are subcylindrical to



Figs. 10-14. — 10. *Rhizopogon clelandii* (holotype), showing basidia and spores. — 11. *Rhizopogon luteolus* fide Dring, Scotland, showing dimerous basidia and spores with pedicellate, notched or rounded bases; extreme right, a basidium with two apical prolongations, not uncommon in this material — 12. *Rhizopogon flavum*, Dehra Dun, Bakshi 113/65, showing "waisted" basidia (courtesy of D. M. Dring). — 13. *Rhizopogon* sp., Hampshire, showing "waisted" basidia (courtesy of D. M. Dring). — 14. Basidia and spores of *Geastrum* sp.

clavate, not dimerous, and are not associated with modified basidioles. If hymenial thickening occurs it must be by growth of hyphae from below the layer of current basidia; this was not apparent, however, in the example studied, nor were the glebal locules filled. This type of development appears to be illustrated by Coker & Couch (1928, Pl. 20) for *Rhizopogon parasiticus* Coker & Totten, and also by Hawker (1954, Fig. 29) for *R. rubescens*. However, Coker & Couch's illustration for *R. rubescens* (1928, Pl. 106, Fig. 7) is at variance with Hawker's.

In both types of development the initial basidia are formed at the surface of the hymenium; they are not embedded in either a hyphidial or a gelatinous matrix which, in many genera of Aphyllophorales, appear to be responsible for the development of dimerous basidia (Donk, 1964: 215). Also, in both types the gleba is entirely enclosed by the peridium during development. The basidia in both instances would thus appear to occupy the same sort of ecological situation and be subject to the same types of spatial pressures. This suggests that the differences between the basidia are fundamental and not simply adaptive to the situations in which they occur.

Dr. D. M. Dring has drawn my attention to what he calls "waisted" basidia in *Rhizopogon flavum* Petch and in an undetermined British collection (Figs. 12, 13). These are probably dimerous in their development but notable for the shortness of the apical prolongation. Similar basidia occur as rarities among the elongated dimerous basidia in some South Australian collections but are evidently the dominant form in some species investigated by Dr. Dring.

Among other Gasteromycetes, dimerous basidia are known in *Nigropogon asterosporus* Coker & Couch (1928, Pl. 108, Figs. 4, 5) and in *Galeropsis paradoxa* (a gasteroid *Bolbitius*; Dring & Rayner, 1967). What appear to be dimerous basidia are also well known in *Geastrum* (Fig. 13). The basidia in *Lycogalopsis* E. Fisch. (Martin, 1939) are compared by Martin with those of *Rhizopogon* and *Geastrum* as well as certain Aphyllophorales. The differences between urniform and utriform dimerous basidia (Donk, 1964) are primarily cytological though Donk gives morphological pointers to their differentiation. In the absence of cytological data I would hazard the opinion that the dimerous basidia of *Rhizopogon* are utriform. *Lycogalopsis* basidia are also probably utriform: if so I suggest that they (and the basidia of *Coniophora*) may represent a subtype in which the basidium instead of being ventricose and rounded at the base, is tapered below the ventricose part.

There appears to be a good case for dividing *Rhizopogon* sensu lato into two genera primarily on the basis of basidial and hymenophoral morphology once the problems of typification have been solved. There are several instances in the Aphyllophorales where genera have been so divided and a wealth of confirmatory features found only after the initial division gave the impetus to further investigation.

## ACKNOWLEDGMENTS

I am grateful to my colleagues Dr. J. H. Warcup, Dr. Elaine Davison and Mr M. Bumbieris for collecting fresh specimens for examination; also to Dr. D. M. Dring for helpful discussions and for providing materials of use in this study.

In this publication dedicated to Dr. M. A. Donk it is fitting that I should acknowledge my profound indebtedness for the friendly help he has given me for many years, and especially for first interesting me in problems of basidial morphology and terminology, and their application in taxonomy.

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