

CONTRIBUTIONS TO THE CYTOLOGY OF
HYMENOMYCETES :

II. KARYOLOGICAL OBSERVATIONS IN *STEREUM*
FUSCUM (SCHRAD.) QUÉL.

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(With 2 Text-figures)

This investigation was undertaken in 1954 with a view to study the nuclear behaviour in the life-cycle of *Stereum fuscum*, a very common species of Thelephoraceae occurring as a saprophyte on logs and posts of *Shorea robusta* in West Bengal, during the wet months of July to September.

It has been found that its karyological features are consistent with its sexually 'heterothallic' and 'bipolar' nature.

The typical dikaryon in the young basidium fuses to form a large fusion-nucleus in which reticulate structures of chromatin materials appear in the early stages of meiosis. The reticulum condenses greatly during the subsequent stages in which there appear chromosome-like structures and ultimately, three chromosomes constituting the haploid complement segregate into each of the two daughter nuclei formed at the poles of a transverse spindle. Two subsequent divisions in each of them eventually produce eight daughter nuclei within the tetra-sterigmatic basidium on which are developed four basidiospores. Each of the spores receives a single basidial nucleus and the remaining four nuclei degenerate within the basidium which subsequently becomes empty after the spores have been shed.

Mature basidiospores are always uninucleate. On germination, the nucleus in each divides and one daughter nucleus passes into the germ-tube. This nucleus divides further and its descendants eventually produce a four to five-nucleate hypha which is at first aseptate and sparsely branched. In later stages, only the tip-portion of this hypha becomes septate and is resolved into typical monokaryotic cells while its distal part remains aseptate and multinucleate.

The primary mycelium developing from a single spore always lacks clamp-connections and is in all cases composed of uninucleate cells. The secondary mycelium, on the other hand, shows abundant clamp-connections due to conjugate division of the heterodikaryon in each cell. Cytoplasmic chromatic granules, well distinguishable from the nuclei, are present in all the afore-said stages in the life-cycle of the fungus.

INTRODUCTION

From earlier times efforts were being made by workers like Strasburger (1884), Rosenvinge (1886), Wager (1891-1899) and Dangeard (1895) to reveal the karyological phenomena in the life-cycle of Hymenomycetes and the latter worker reported nuclear fusion in the basidia of *Polyporus versicolor* (L.) Fr. Our knowledge in the cytology of Hymenomycetes

made considerable progress due to subsequent researches of Maire (1902), Lévine (1913), Kniep (1913-1916), Juel (1916), Sass (1928-1935), Wakayama (1930, 1932) and others. Chromosome numbers of a number of Hymenomyces were also recorded. Since then, investigations on fungal cytology as applied to Hymenomyces have been steadily increasing in number as will be evident from Olive's (1953) critical review on the subject. In so far as Indian works on Hymenomyces are concerned, it was Bose (1937) who first described the basidial cytology of eleven species of polypores and four species of agarics with special reference to some extranuclear protoplasmic elements. Cytological studies on several Hymenomyces occurring in India have recently been undertaken in this laboratory and the first contribution of the series was made by Banerjee and Mukherjee (1955).

In recent years there has been a growing tendency to look for a cytological basis which underlies the sexual reproduction in fungi. Considering the broad use of the term heterothallism as applied to fungi, Whitehouse (1949) has expressed his judgement that the essential criteria of sexual reproduction are nuclear fusion and meiosis in the life-cycle irrespective of the presence or not of differentiated sex organs or gametangia. This is particularly suggestive in the case of Hymenomyces where sex organs are not differentiated and the great majority of which are heterothallic with various means by which the monokaryophasic mycelia are diploidized. The compatible monokaryophasic mycelia are superficially indistinguishable from each other and it will be evident from Olive's (1953) review that instances are not uncommon in Hymenomyces where clamp-connexions are absent in the hyphae and yet the cells composing them are not monokaryotic. Incidentally, it has been observed by the writers in course of their studies on the biology of *Stereum fuscum* (Schrad.) Quél, a common species of Thelephoraceae occurring in India, that the fungus in question is 'heterothallic' like the majority of Hymenomyces and is sexually 'bipolar'. But as far as informations are available, karyological conditions in *Stereum fuscum* have not been studied. The object of the present investigation was, on the one hand to study in general the cytology of *Stereum fuscum*, and on the other to examine its sexuality in the light of the nuclear phenomena in the life-cycle of the species. In the following pages will be described the karyological conditions of the spores, germinating spores, primary and secondary mycelia as well as the cytology of the basidia of *Stereum fuscum*.

MATERIALS AND METHODS

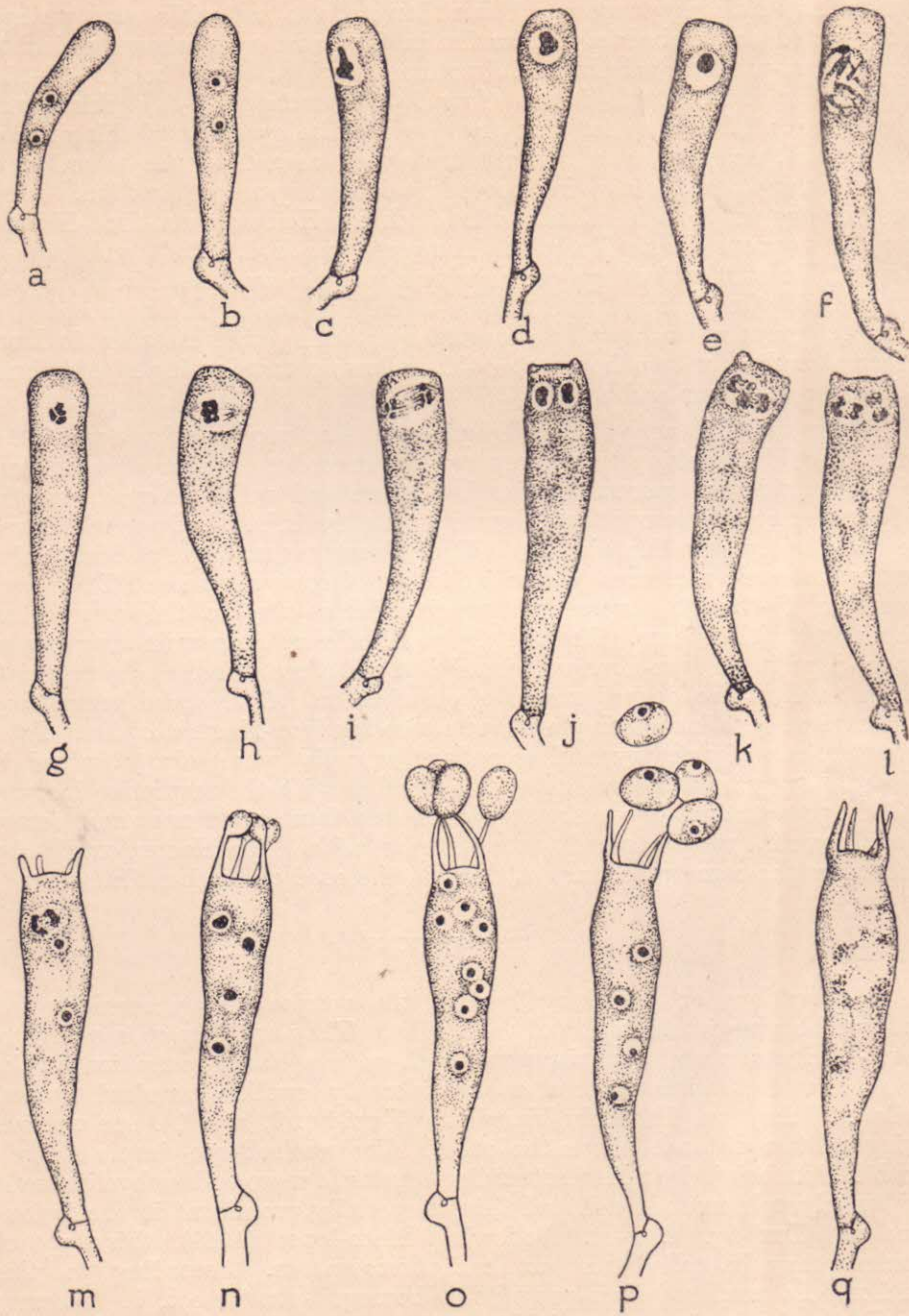
For studying nuclear conditions within the basidium, fresh fruit-bodies of *Stereum fuscum* were collected from Calcutta, after a brief shower, and immediately fixed at different intervals of time, viz., at 11 a.m., 12.30 p.m. and 2 p.m. Of the fixatives used were 'Bouin-Allen', 'Formal-Acetic-Alcohol', 'Navashin (A & B)', 'Sass' and 'La Cour (2BD)'. The results of staining varied with the nature of the fixative and also with the stain used. 'Bouin-Allen' and 'Sass' proved to be the most satisfactory fixatives

for general staining purposes. After fixation, the materials were washed, dehydrated and embedded in paraffin in the usual way following alcohol-chloroform-paraffin schedule. Microtome sections were cut at 6-12 μ thicknesses and by trial, it was found that sections 6-8 μ thick were the most suitable ones for studying the basidial cytology of this species. Difficulties, however, were encountered with regard to the selection of proper stains and the staining procedure following proper fixation. After preliminary trials with several stains such as Heidenhein's Iron-Haematoxylin, aqueous Basic Fuchsine (Feulgen's method, 1924) and aqueous Crystal Violet, the first one could bring about the most satisfactory result when used after fixation with 'Bouin-Allen' or 'Sass'. It was noted that the best preparations may be obtained by staining the materials in Iron-Haematoxylin for two hours at 40°C. following mordanting in 4% Iron-Alum for one hour and a half.

Kniep's (1913) agar-film technique and that modified by Sass (1929) were tried for making total preparations in order to study the nuclear phenomena in the spores, germinating spores and well-developed mycelia, both primary and secondary. Spores from a fresh fruit-body were allowed to fall directly on a thin film of sterilized clear agar made on a slide, taking extreme aseptic measures to avoid chances of contamination. They were immediately fixed in several fixatives in order to observe the karyological conditions within them. For the study of nuclei in the germinating spores, spore-deposits on agar-films were incubated and these served as the materials when fixed at the desired stages of germination. Spores and germinated spores were best fixed with 'Sass' and stained in Iron-Haematoxylin. Total preparations of primary and secondary mycelia were also obtained in the same way by inoculating sterile agar-films with the respective inocula. All the preparations of germinating spores and those of primary and secondary mycelia were stained satisfactorily with Iron-Haematoxylin by following the same procedure as done in the case of the basidia.

OBSERVATIONS

Observations on the karyology of the species under consideration may well begin with the study of the basidia. They are more or less clavate, tetra-sterigmatic and quadrisporous, each with a clamp-connexion at the base. Young basidia appear as considerably swollen terminal hyphal cells and they are distinctly binucleate (Text-fig. 1, a & b), one nucleus lying a little distance above the other. The nucleoli are deeply stained with Heidenhein's Iron-Haematoxylin, leaving a more or less hyaline zone of karyolymph around each and this is specially prominent in the fusion-nucleus (Text-fig. 1, c-e). Following Pinto-lopes (1949), this type of nucleus may be called *compact and homogeneous*. According to this author, the nucleus when presents a homogeneous aspect, it becomes small, its chromatin and nucleolar-matter are contracted in an intensely stainable homogeneous body which is feulgen-positive and the major part of the nucleoplasm is free from the chromatin. In the *disperse-expanded* type according to him, on the other hand, diffused chromatin and chromocentres are to be found. The binucleate condition of the basidium is

Text-fig. 1. Karyological phenomena in the basidia of *Stereum fuscum*. $\times 3000$.

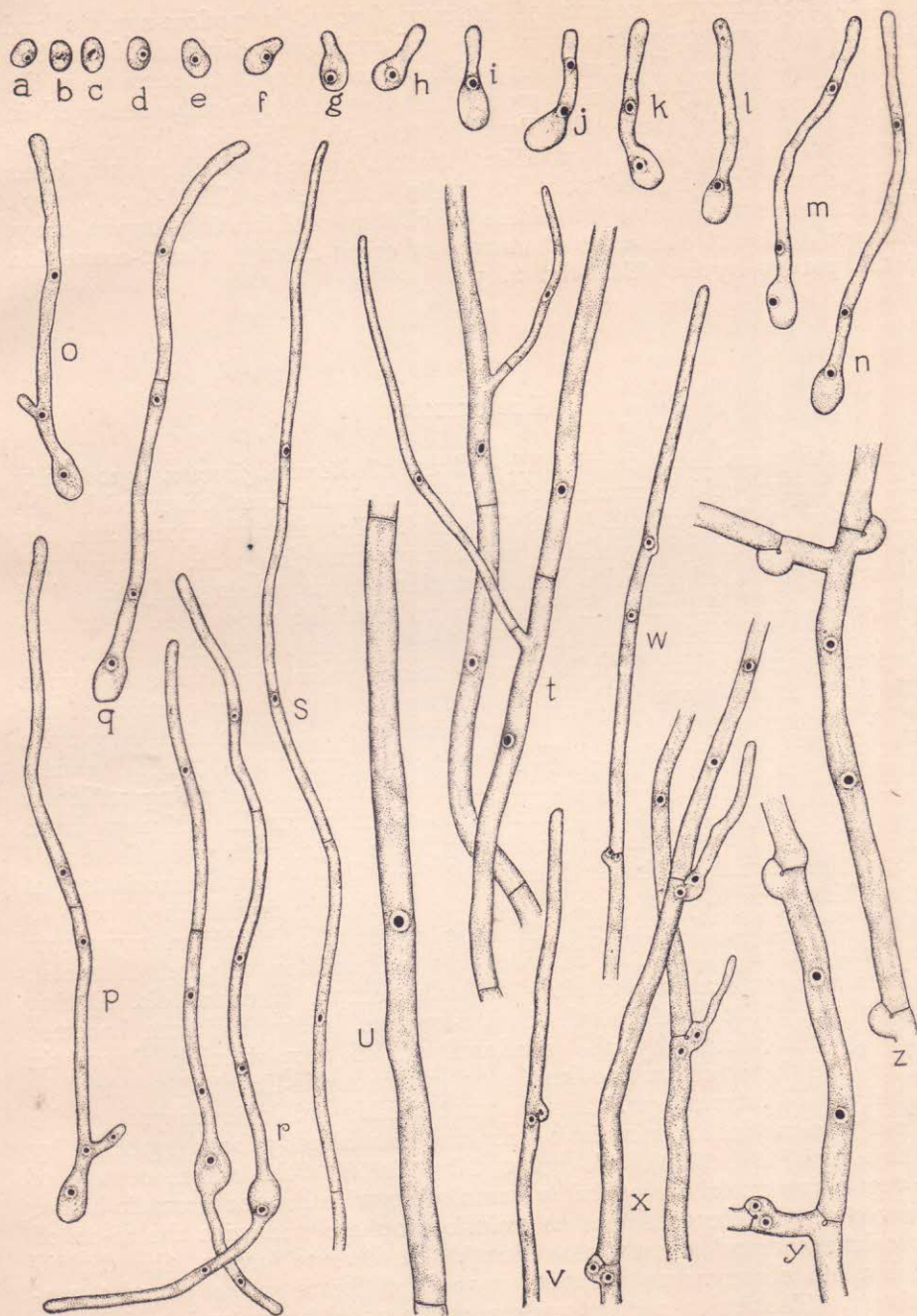
(For explanation, vide text).

maintained until it attains a fairly clavate form after which karyogamy occurs. During karyogamy, the two nucleoli with their respective chromatin-matter fuse to form a large deep-staining body within the more or less hyaline nucleoplasm (Text-fig. 1, c-e). The synkaryon then moves towards the upper part of the basidium. Subsequently, the fusion-nucleus presents a distinct reticulate structure, somewhat similar to that reported by Wakayama (1930, 1932) in a number of *Hymenomyces*. The fusion-nucleus eventually takes up a very characteristic appearance (Text-fig. 1, f). The reticulum condenses greatly and the strands begin to thicken, as if they are going to be resolved into individual chromosomes. The thickening and condensation, however, are not uniform; but ultimately distinct heterochromatic bodies are produced by the condensing reticulum (Text-fig. 1, f). These heterochromatic bodies are provided with 'chromatic tails' which gradually fade away after a short distance. Such heteropycnotic chromatic bodies are observed only in the first meiotic prophase and much simulate chromosomes on which the allocyclic heterochromatin appears more condensed. According to Olive (1953), these heterochromatic bodies observed on the chromosomes of meiotic prophase and the so-called 'protochromosomes' of certain authors are probably homologous with the chromocenters which have been demonstrated by Heim (1951a, 1951b, 1952) in the vegetative nuclei of a large number of *Ascomycetes*. Various stages of heteropycnosis may be observed during the early meiotic prophase and it may be concluded that this is a long process relative to other stages of nuclear division. Exact count of the so-called *protochromosomes*, can not be taken. With chance of inaccuracy, due to close mingling, their number comes out to be 6-8 (Text-fig. 1, f). The haploid chromosome number has been verified in all cases to be 3 by counts from the first meiotic metaphase at which 3 closely associated bivalents may be seen in the polar and side views (Text-fig. 1, g & h). Later on, the 3 bivalents separate at anaphase-I, three chromosomes constituting a genome going to each of the two poles of a transverse spindle (Text-fig. 1, i). After completion of the first meiotic division, the resulting two nuclei remain side by side in the terminal portion of the basidium (Text-fig. 1, j). At this stage, the basidia have nearly reached their full dimension and show rudiments of sterigmata. A further division of both the nuclei then follows. These divisions are synchronous in most of the cases (Text-fig. 1, k & l), but rarely the division of one of the two nuclei is little belated (Text-fig. 1, m). Orientation of spindles in these divisions is variable but at least one of the spindles appears to be transverse. The other has been found to be transverse, obliquely transverse or nearly longitudinal and one usually remains a little above the other. These findings may be a strong support to the current interpretation that the types of spindle-orientation in *Basidiomycetes* are due to spacial reasons and necessarily do not always bear absolute phylogenetic and taxonomic value. By the time the second meiotic division is complete, four sterigmata appear and the four nuclei may be seen within the basidium without any definite arrangement. A third division in each of these four nuclei gives rise to the 8-nucleate stage of the basidium (Text-fig. 1, o). Meanwhile, four basidiospores are developed on four sterigmata and each of them receives

a single nucleus from the basidium, which still retains the remaining four nuclei within it (Text-fig. 1, p). The fate of these four supernumerary nuclei has not been studied in detail but, undoubtedly they do not enter into spores. In all probability, the four extra nuclei degenerate within the basidium which eventually becomes empty after the spores have been shed (Text-fig. 1, q). In several cases, basidia have been found to be collapsing even before the retained nuclei within them have fully degenerated.

The mature spore has always been found to contain a single nucleus within it (Text-fig. 2, a-d). The nucleolus and the chromatin are condensed into a central deep-staining body with a hyaline zone around it. Adjacent to the nucleus there are one or two vacuoles in the surrounding cytoplasm and these appear hyaline in the preparations (Text-fig. 2, a-d). When germination starts, the nucleus slightly enlarges, its chromatic material becomes more deep-staining but the reticulum is absent. At first, a short germ-tube is produced from one end of the spore while the nucleus remains within the spore-case. A few spores, however, have also been found to produce two germ-tubes at opposite ends of the spore-wall (Text-fig. 2, r). As the germ-tube elongates a little, the nucleus moves up to the base of the germ-tube and the cytoplasm within the spore becomes distinctly vacuolate (Text-fig. 2, f-i). After the germ-tube has attained a considerable length, the nucleus divides into two and a daughter nucleus passes into the germ-tube (Text-fig. 2, j & k). Septum-formation is deferred for the time being and the nucleus within the germ-tube divides once again (Text-fig. 2, l-n). Division of the basal nucleus within the spore-case can also take place when two germ-tubes are produced at opposite ends. The first two nuclear divisions take place in rapid succession after which the germ-tube elongates considerably (Text-fig. 2, m-o) and further nuclear divisions are somewhat delayed. The germ-tube does not usually branch even after it has attained a considerable length. Occasionally, however, a branch may be found to develop from the lower part of the germ-tube (Text-fig. 2, o-p) and it eventually receives the nuclear supply from the nearest nucleus of the germ-tube. The resulting hypha ultimately becomes 4 to 5-nucleate after which septation begins only in its upper part, resolving the tip-portion of it into uninucleate cells while the lower part of the hypha still remains multinucleate (Text-fig. 2, p-r). No clamp-connexion has been observed in this primary mycelium arising from a single germinated basidiospore. Such a finding is in harmony with the observations that *Stereum fuscum* is heterothallic in its sexual behaviour and has clamp-connexions only in the secondary mycelia obtained through pairing of two compatible monosporous mycelia.

Primary mycelia developed from several single-spore-cultures and also belonging to opposite sexual strains, when examined, appear to have the same essential morphological and karyological characteristics and the cells composing them are always uninucleate (Text-fig. 2, s-u). Chromatic materials of variable shape and size are also abundantly present in the cytoplasm. These chromatic granules have also been observed in spores, secondary mycelia as well as in the basidia. They are readily distinguished from the chromatic body of the nucleus on account of their much smaller size and lack of any hyaline zone around them.



Text-fig. 2. Nuclear phenomena in basidiospores, germinating basidiospores and in mycelia (primary and secondary) of *Stereum fuscum*. $\times 750$.

(For explanation, vide text).

The secondary mycelium of *Stereum fuscum* is nodose-septate and possesses clamp-connexions as a result of conjugate division of the two nuclei in each cell. The nuclei are of the same general structure as in the case of primary mycelia and spores but they are typically arranged in conjugate pairs in each cell (Text-fig. 2, v-z). Occasionally, a clamp-connexion has been found to proliferate into a branch, which in turn forms clamp-connexions immediately after its emergence (Text-fig. 2, x-y). The subsequent increase in diameter of the proliferating hypha may, ultimately, almost obliterate its nature of origin from a clamp-connexion of the parent hypha (Text-fig. 2, y).

DISCUSSION

Nuclear phenomena in the life-history of *Stereum fuscum* agree fully with the nature of its sexuality in as much as it has been observed by the writers to be 'heterothallic' and 'bipolar'. The mature spores and the cells of the primary mycelium are always monokaryotic. Spores and primary mycelia of opposite sexual strains are morphologically identical and have nuclei of similar morphology and staining properties. Furthermore, clamp-connexions are entirely absent in such mycelia. The basidiospore germinates to form a short 'homokaryotic' multinucleate hypha with little or no branching. In later stages, only the tip-portion of the hypha becomes segmented into uninucleate cells from which develops the monokaryophasic mycelium. Haploid nuclei present a more or less generalized aspect in their structure with a central deep-staining nucleolar body around which lies a hyaline zone of karyolymph. Dikaryophasic hyphae always have clamp-connexions and the affinity between the nuclei of opposite sexual strains is illustrated by the conjugate association of 'heterodikaryons' within each cell of the secondary mycelium as well as within the initial of basidium. Karyogamy in the basidium represents the true sexual act and is followed by segregation of parental nuclei, perhaps with some re-combinations, after the completion of meiosis. During the early stages of meiosis, the fusion-nucleus in the basidium becomes distinctly reticulated by the condensation of deep-staining chromatin matter and heterochromatic knots on chromosome-like bodies may be located in the later stages. A somewhat similar cytological aspect of the diploid nucleus in early meiotic prophase has already been reported by Wakayama (1930, 1932). To quote Olive (1953), "the chromocentres of the vegetative nuclei are probably homologous with heterochromatic bodies observed on the chromosomes at meiotic prophase and with the so-called 'protochromosomes' of certain authors". In *Stereum fuscum*, the diploid and haploid chromosome numbers have been found to be 6 and 3 respectively. The fusion-nucleus undergoes the usual heterotypic and homotypic divisions of meiosis resulting in the production of four nuclei. A third division in all these four nuclei, ultimately gives rise to an eight-nucleate tetra-sterigmatic basidium, on which are developed four basidiospores, each receiving a single nucleus from the basidium. The remaining

four nuclei within the basidium degenerate. The existence of three successive nuclear divisions within the basidium is nothing unfamiliar and has been reported previously in *Craterellus cornucopioides* (Juel, 1916; Wakayama, 1932), *Corticium rolfsii* (Goto, 1936), *Peniophora ludoviciana* (Biggs, 1938), *Sistotrema confluens* (Kühner, 1947) and in a number of other Hymenomyces. Rogers' (1934) challenge on the validity of the type of orientation of spindles during meiosis as the primary criterion in maintaining chiasmobasidial and stichobasidial series within the Basidiomycetes seems justified, in Homobasidiae at least, when the inconstancy in the orientation of spindles during the three successive nuclear divisions in the basidium of *Stereum fuscum* is taken into consideration. In this species, it has been observed that the spindle, during the first meiotic division is transverse; but in the second homotypic division, one of the spindles is transversely oriented but the other has been found to range in orientation from vertical to transverse or oblique. This may suggest that the pattern of spindle-orientation may be determined by space within the basidium and it tends to be transverse when the basidium is broad or becomes vertical when it is long and narrow.

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