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STUDIES ON *SORDARIA HUMANA* (FUCKEL) WINTER: THE CYTOLOGY OF ASCUS DEVELOPMENT AND DEVELOPMENTAL MORPHOLOGY OF PERITHECIUM

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Abstract

Studies on the cytology and developmental morphology of *Sordaria humana* revealed that the ascognia arise as coiled multinucleate side branches without trichogyne. No antheridium was observed though anastomoses were noted between somatic hyphae. The archicarp consists of 4—12 binucleated cells. The peridium and sterile elements of the centrum develop from the hyphae arising from the subtending hypha of the ascogonium. The peridium consists of an outer exoperidium of dark, thick-walled cells and an inner endoperidum of hyaline, thin-walled cells. A third layer, the perilocular layer, is instrumental in the formation of the neck and ostiole, which develops schizogenously. Ascogenous hyphae develop from the cells of the ascogonium. The asci develop from the ascogenous hyphae in the usual manner. The centrum is "*Diaporthe*" type in the sense of Parguey-Leduc, the asci forming a fascicle on a ridge at the base of the centrum.

Introduction

The classification of Ascomycetes in general and Pyrenomycetes in particular, constitutes one of the most difficult problems in mycology. The morphological complexities and variations in Pyrenomycetes have led to an understandable hesitancy on the part of mycologists to accept revisions of the classification of this group which attempt to incorporate the new data that have been obtained from time to time. Recent work has tended to shift the emphasis in the classification of this group from such purely morphological criteria as habit, insersion of ascocarp, presence or absence of stroma and colour and consistency of perithecial wall and stroma to what seem more fundamental criteria such as developmental morphology, ascus structure and the organization of centrum. The importance of centrum structure not only as a taxonomic criterion but also in pointing out phylogenetic relationships between various taxa became increasingly evident with the publication of Miller's paper (1949) on the classification of Ascomycetes with special emphasis on Pyrenomycetes and with the appearance of 'Taxonomy of the Pyrenomycetes' by Luttrell (1951).

Both Luttrell (1951) and madame Parguey-Leduc (1967) are of the opinion that '*Diaporthe*' type of centrum is found in species of *Sordaria*. Luttrell, thinks that paraphyses are absent in '*Diaporthe*' type of centrum but present in '*Xylaria*' type. Parguey-Leduc (1967), however, differs from him when she says that at first paraphyses are not present, but may develop later in '*Diaporthe*' type of centrum. 'Nutritive cells', according to her, are found in '*Diaporthe*' type of centrum but they are absent in '*Xylaria*' type. In this paper, observations on cytology, developmental morphology and centrum type in *Sordaria humana* are described.

Materials and Methods

Cultures obtained from buffalo dung were maintained on Malt extract agar (McLean & Cook, 1952) and Dung agar (Page, 1939), since perithecial production was best on these media. The perithecia were harvested at intervals of twenty-four hours from

third to sixth day. Perithecial development was complete after six days when Dung agar was used as a substrate. Perithecia at this stage usually contain all the stages of ascus development from crozier initiation to mature ascospores.

Pieces of agar medium containing perithecia were fixed in Cornoy's fluid, washed in several changes of 70% alcohol and finally embedded in wax, using tertiary butyl alcohol method (Johansen, 1940). Sections were cut to a thickness of 8μ on a Spencer rotatory microtme. Slides thinly coated with Mayer's albumin adhesive were allowed to air dry for three minutes, and then flooded with distilled water. Ribbons of serial sections placed thereon were judiciously heated. These sections were then sprayed with a fine mist of Xylene from an automiser to stretch them (Lin & Corlett, 1969) and were finally stained in 2% solution of Heidenhain's iron haematoxylin and differentiated with 3% iron alum.

For studies of ascus development acetocarmine—aceto-orcein squash preparations showed better results than the two stains separately. The procedure adopted for staining was as follows: —

Perithecia, scraped from a 3 to 6 days old culture were placed in a drop of aceto-carmine and one drop of aceto-orcein and gently pressed to release clusters of asci and the perithecial walls was removed. A glass cover was placed and a judicious pressure was applied to spread out the asci uniformly without breaking the glass cover. The slide was warmed over burner and was kept at room temperature for 24 hours during which period the nuclei took a deep red stain.

Results

ORIGIN OF ASCOGONIUM AND EARLY DEVELOPMENT OF PERITHECIUM

(i) *Formation of ascogonium and ascogonial coil.*

Ascogonium formation begins when an apparently ordinary hyphal cell sends out a short coiled side-branch, the ascogonial coil, which may be distinguished in its early stages of development from the vegetative mycelium only by its greater ability to absorb stain. Normally only one ascogonial coil is found in a perithecium.

It could not be ascertained whether only a single pair of nuclei enters the ascogonial coil and then undergoes several mitotic division or whether several nuclei enter the ascogonial coil. However, it was found to contain several nuclei (Fig. 1). The coil by further elongation and septation results into an archicarp (Woronin hypha) consisting of four to twelve cells, but more commonly seven to ten cells. Archicarp at this stage is composed of binuleate cells (Fig. 2-5).

(ii) *Antheridium and fertilization*

Inspite of careful examination, no structure which could be interpreted as an antheridium was ever observed However, two vegetative hyphae were rarely observed to lie side by side and fused at their tips. No trichogyne was observed. Anastomoses between adjacent hyphae were quite common (Fig. 6).

(iii) *Early stages for perithecial development*

While the archicarp is being formed, the subtending hypha begins to proliferate, its branches growing upward around the archicarp to form a cover and produce a spherical mass of pseudoparenchymatous cells with the archicarp at its centre. Formation of sterile elements is accompanied by a considerable enlargement of the ascocarp, so that by the time the young perithecium is morphologically distinct, it has at its centre an easily distinguishable coiled, multiseptate, densely staining archicarp whose cells are binucleate (Fig. 4-5).

Examination of numerous young perithecia has shown that the outer most layers of the sheath surrounding the archicarp even at such an early stage have already begun to differentiate into future peridium, the cell-walls in the outer peridial layers being thicker and darker (Fig. 7). The cells of the young peridium begin to undergo repeated divisions and elongate tangentially, causing a gradual enlargement in the size of perithecium. As the perithecium enlarges the area around the centrally located archicarp is filled by numerous pseudoparenchymatous thin-walled cells.

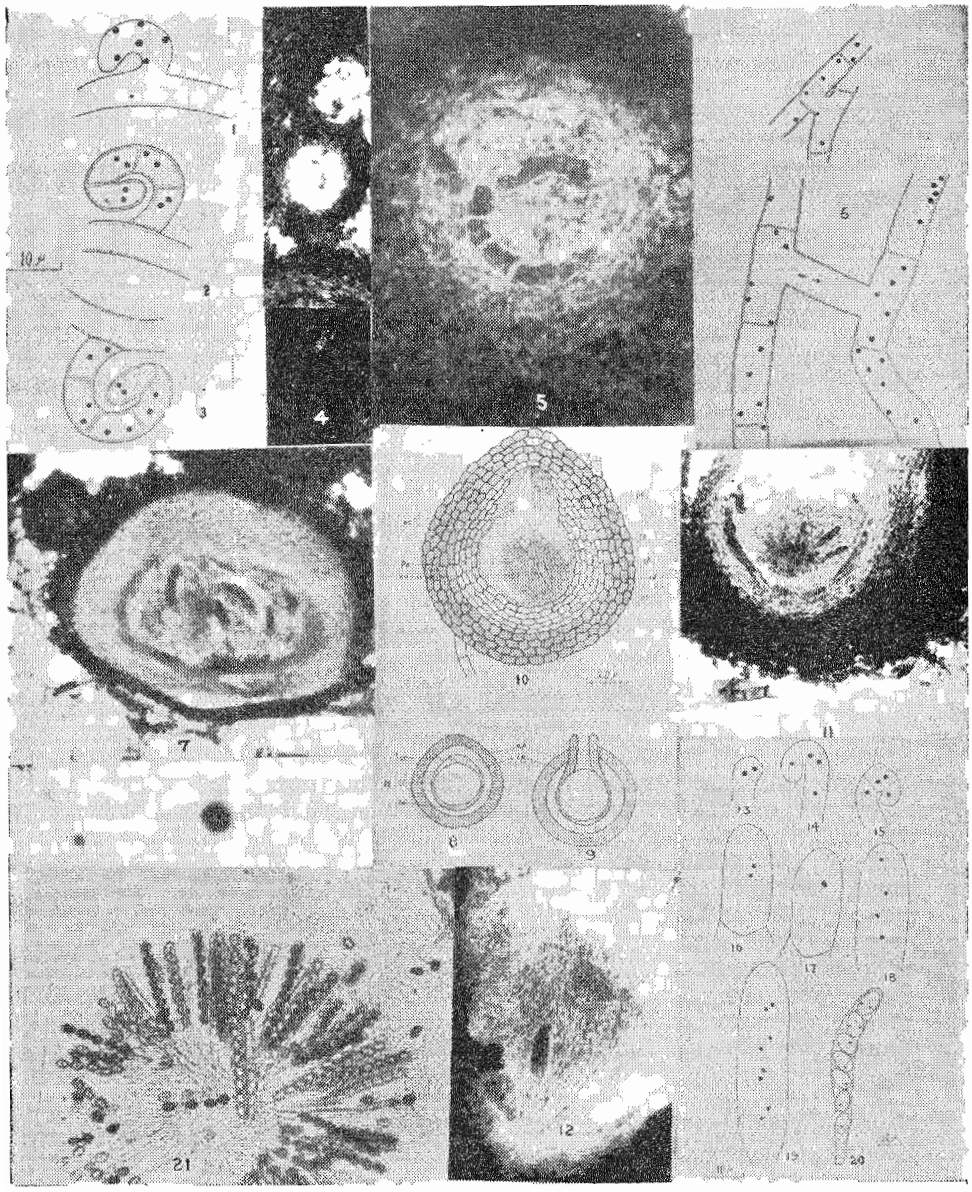
ORGANIZATION OF THE CENTRUM

When the archicarp has matured further the region outside the centrum becomes differentiated into two zones (Fig. 8-10). The outer dark coloured layer, the exoperidium (ep), is mostly two to four cells in thickness. It consists of more or less elongated cells with thickened and pigmented walls. The next inner layer, the endoperidium (ip), is about five to six cells across comprising of tangentially compressed cells with thin, hyaline walls.

ORGANIZATION OF THE PERIDIUM

Centrum includes ascogenous hyphae, asci and sterile tissue occupying the perithecial cavity inside the peridium within which the asci develop (Fig. 8-10). Inside the two layers of peridium is a layer, the perilocular layer (pl) consisting of thin-walled tangentially elongated cells which are smaller than those of the peridial layers and have greater ability to absorb stain, hence take a darker stain. It is often single cell in thickness in the lateral region and forms an epihymenial cone (ce) near the apical region of the ascocarp. At the base, it is usually three to four cells in thickness resulting in a subhymenial disc(sd) which gives rise to a pseudoparenchymatous ridge (Fig. 11) on which the asci develop from the cells of archicarp in a fascicle. Meanwhile, the other cells from the sides and base of the perilocular layer seem to give rise to cells which invade the central cavity. As they grow inward and upward to form the sterile tissue of the centrum they push the ascogenous system even further towards the centre of the perithecium.

Next to the perilocular layer is a core of sterile more or less rectangular or polygonal pseudoparenchymatous cells(nc) with hyaline evanescent walls surrounding the developing ascogenous system and with intercellular spaces. These cells are often referred to as "nutritive cells". It can be observed by focussing at different levels that the slender young asci and paraphyses grow upward in between the sterile cells of this pseudoparenchymatous core (Fig. 12). As more asci and paraphyses develop and their subsequent expansion takes place many of the so called 'nutritive cells' are apparently crushed, but others continue to enlarge and in sectioned material they can be observed scattered among and around the asci (Fig. 8).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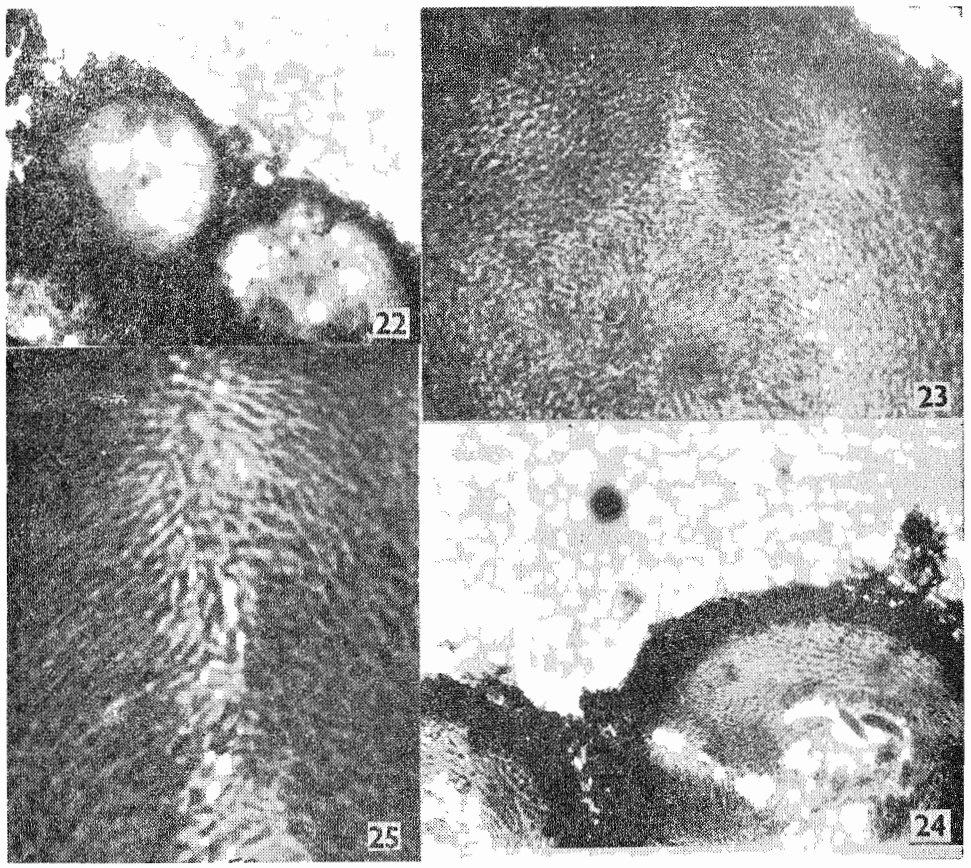

EXPLANATION OF FIGURES 1-25.

Fig. 1. Young ascogonial coil with 6 nuclei.
Fig. 2-3. Ascogonial coil showing later stages of development.
Fig. 4-5. Section through a young perithecium showing coiled and septate archicarp.
Fig. 6. Anastomoses between adjacent hyphae showing spherical (static) and elongated (migratory) nuclei.
Fig. 7. Vertical section of a young perithecium showing different layers shown diagrammatically in Fig. 10.
Fig. 8-9. Camera lucida drawings (diagramatic) of vertical radial sections of young and mature perithecia, respectively, showing different layers.
Fig. 10. Camera lucida drawing (semi-diagramatic) of a vertical section of a young perithecium. ep. exoperidium, ij. endoperidium, pl. perilocular layer, ce. epihymenial cone, ll. lateral part of perilocular layer, sd. subhymenial disc, as. ascus, pa. paraphyses, ne. nutritive cells. pi. periphyses.
Fig. 11. Vertical section of a perithecium showing a ridge with fascicle of asci.
Fig. 12. One ascus and paraphyses.
Fig. 13-20. Different stages in the development of asci from crozier formation to the formation of ascospores.
Fig. 21. Eight uniseriately arranged ascospores in asci.
Fig. 22-24. Formation of perithecial neck.
Fig. 25. Vertical section through the neck of an immature perithecium showing the formation of periphyses.

(i) *Origin and development of ascogenous hyphae*

Ascogneous hyphae radiate out from the cells of the archicarp and typically grow out in a basal fascicle (Fig. 11). Immature ascocarps contain a large number of branched ascogenous hyphae in the basal portion of the centrum. In sectioned material fragments of the ascogenous hyphae are easily recognizable because of their prominent nuclei and dense and deeply staining cytoplasm.

(ii) *Ascus formation*

The short ascogenous hyphae terminate in a hook-like structure, known as crozier. A binucleate cell of ascogneous system grows up and becomes bent over. The two nuclei divide conjugately to form a four nucleated hook cell (Fig. 13-14). Two septa are then laid down delimiting three cells, an uninucleate ultimate cell, a binucleate penultimate cell and an uninucleate basal cell (Fig. 15). The ultimate cell grows down and fuses with the basal cell to form a single binucleate cell which can grow out and form another crozier. Thus it is possible for a number of asci to form at one point. The penultimate cell known as the ascus mother cell is destined to become the ascus (Fig. 16). Karyogamy of the two nuclei takes place shortly after the ascus mother cell has begun to enlarge (Fig. 17). Shortly after the fusion of the two nuclei, the ascus mother cell grows into a broadly clavate structure, the ascus. There follows now a period during which the ascus undergoes an enormous extension and finally becomes cylindrical. The fusion nucleus divides into eight nuclei by three successive divisions, the first two are meiotic and the third one is typically mitotic. The four haploid nuclei (Fig. 18) produced as a result of the meiotic divisions, undergo a third (mitotic) division to give rise to eight nuclei (Fig. 19). The apical apparatus of asci is fairly well developed by this time and can be seen as a dark-red ring in aceto-carmine—aceto-orcien preparations. Spore formation begins with cleavage of cytoplasm around each of the eight nuclei. A thin hyaline wall is then formed around each uninculeated protoplast delimiting the ascospores. The wall finally thickens and becomes pigmented. The mature ascospores usually become binucleae due to a fourth division which is mitotic and occurs in young spores. The eight ascospores thus produced are arranged uniseriately in the mature ascus (Fig. 20-21).

(iii) *Development of paraphyses*

Paraphyses appear to originate from the sub-hymenial disc of the perilocular layer and grow upwards between the tissue of the centrum. These are hyaline, thin-walled, septate, sterile thread-like structures interspersed between the asci (Fig. 17), and are slightly longer than the mature asci when fully developed. In mature condition they show constriction at septa, which are not visible in young stage. No papraphyses were ever observed having taken their position to the lateral walls of the ascocarp.

FORMATION OF PERITHECIAL NECK

The first signs of the neck formation appear at the apex of the perithecium at a very early stage in the form of the epi-hymenial cone in the apical portion of the perithecium which appears to be meristematic. The cells of the epi-hymenial cone are thin-walled, tangentially elongated, smaller than those of the peridial layer and have greater ability to absorb stain (Fig. 22-23). As they divide and enlarge, they create pressure which makes them grow upwards by pushing aside the overlying peridum (Fig. 24). This increasing pressure splits the tissue of the perithecial apex giving way to

the growing cell of the epihymenial cone. The further development of the latter gives rise to a hollow cylinder with an opening at its apex as well as at the base. The cavity of this cylinder or the neck is known as the neck canal. The cells lining the neck-canal extend as a series of delicate, somewhat parallel, more or less obliquely inward and upward directed hyphae on all sides of the neck-canal (Fig. 25). These are known as the periphyses. Cells of the outer layer of the neck become thick-walled and dark-coloured like those of outer peridium. Thus the ostiole is schizogenous in origin.

MATURE PERITHECIUM

The mature perithecium comprises of a peridium, which consists of outer dark-coloured exoperidium of two to four cells and an inner hyaline thin-walled endoperidium of five to six cells in thickness. To the inside of which is the perilocular layer represented by a sub-hymenial disc of thin-walled heavily stained cells in the form of a ridge at the base of the perithecial cavity (Fig. 10).

The centrum consists of the asci intermingled with paraphyses at the base of the ascocarp. Most of the 'nutritive cells' break-down and disappear making way for the developing asci and paraphyses. The neck is lined along the whole inner surface by thin-walled periphyses with an ostiole at the apex.

Discussion

The ascogonial formation in *S. humana* begins when an individual hypha sends out a short side branch. A similar type of ascogonium formation has been described in *S. fimicola* (Carr & Olive, 1958).

In *S. humana*, the ascogonium, before the formation of septum which cuts it off from the main hypha, is multinucleate. It could not be ascertained whether a single nuclear pair enters the ascogonium and then undergoes several rapid mitotic divisions or several nuclei enter it before the formation of the septum. Carr & Olive (1958) think that the latter is true. However, they failed to give any definite reason for their belief.

As regards the possibility of an antheridium being present, the writers have found no sign of any such structure. Ritchie (1937) and Carr & Olive (1958) also never observed a functional antheridium in *S. fimicola*, as reported by Gries (1941, 1942).

There are two possibilities with regard to the origin of hetero-karyotic condition in *S. humana* . It may result from somatogamy or it may be apogamatic in origin. Opposite hyphae were sometimes seen facing each other in *S. humana*, and anastomoses were also quite common (Fig. 24-25). Presumably, the hetero-karyotic condition originates through somatogamy. Perithecia arise presumably through the passage of genetically different nuclei into the young ascogonium from the subtending hypha. The attenuated and elongated terminal cell of the archicarp may represent a non-functional trichogyne. It no more comes out of the perithecial primordium, because it has lost its function.

As described above, the ascogonium becomes enclosed by a few layers of vegetative hyphae, produced from the subtending hyphae of the ascogonium and its branches. They form a sheath of a few layers and produce a spherical mass of pseudoparenchymatous cells, with the archicarp at its centre. Same type of perithecial development has been observed in *S. fimicola* (Zickler, 1952) and in *S. macrospora* (Parguey Leduce, 1967).

In the case of *S. macrospora*, Parguey-Leduc (1967) interprets the basal portion of the helicoid filament as the foot, its middle portion as ascogonium and apical portion as trichogyne. She considers the helicoidal filament which is generally interpreted as archicarp, to be in reality a proarchicarp (Protoperithecium of Goos, 1959), which later develops into an archicarp. The succession of proarchicarp with an archicarp constituting a Woronin hypha has been reported in other Sordariales as well, such as *S. fimicola* (Zickler, 1952).

In *S. humana* as in *S. macrospora* (Parguey-Leduc, 1967), the tissues of the developing perithecium become differentiated into an outer peridium and an inner centrum. The peridium itself later becomes differentiated into two layers, an outer layer of thick-walled dark coloured cells and an inner layer of thin-walled, hyaline and elongated cells. The centrum which consists of all the tissues inside the peridium has an outer layer, the perilocular layer which is thin on lateral sides and several cells thick at the base and at the apex. The basal portion being called as 'disque soushymenial' and the apical as 'Cone sus-hymenial' by Parguey-Leduc (1967). The 'Cone sus-hymenial', by further development gives rise to the ostiolar apparatus. The origin of the wall of the neck as well as periphyses appears to be from the 'Cone sushymenial' and thus form the centrum. This type of development has been described for *S. macrospora* by Parguey-Leduc (1967) however, she believes that in the case of *Gelasinospora calospora*, the ostiolar apparatus originates from the internal wall of the peridium. If true, this would suggest that the origin of the ostiolar apparatus has very little bearing on the classification of Pyrenomycetes above the genus level.

The centrum comprises of the pseudoparenchymatous cells, the ascogenous hyphae and the asci. Later, paraphyses also develop in the case of *S. humana*. The function of the pseudoparenchymatous tissue is generally assumed to be nourishment of asci (Luttrell, 1951). It has been also suggested by Pomerleau (1939) in *Gnomonia*, that this tissue is instrumental in the creation of perithecial cavities within which the asci are formed. It seems probable that this may be its most important function, although it may also be protective and nutritive in fuction.

Many of the species belonging to different genera of Pyrenomycetes (eg. *Xylaria*, *Coronia*, *Chaetomium*, *Cordyceps*) are alike in production of paraphyses, but differ fundamentally in the arrangement of asci. In the first group represented by *Xylaria* and *Coronia*, the ascogneous hyphae spread along the base and sides of the perithecial cavity. The asci are arranged in a continuous wall layer and are interspersed with paraphyses which are usually persistent. In second group represented by *Chaetomium* and *Cordyceps*, the ascogenous system forms a plexus in the base of the perithecial cavity and asci arise in a single aparaphysate cluster. The paraphyses are limited to the sides of perithecial wall and are evanescent (Luttrell, 1951).

Although the presence of paraphyses has been mentioned in reports on numerous species of Pyrenomycetes, their occurrence can be accepted only when a sufficient description is given of their development to justify a condition they represent true paraphyses and not psuedoparaphyses or strands of pseudoparaenchyma On this basis *Xylaria* type appears, to occur much less commonly than is generally supposed.

One of the most important questions, concerning the '*Diaporthe*' type of development is whether it is distinct from the type in which the centrum is composed of paraphyses. In '*Diaporthe*' type both Wehmeyer (1926) and Miller (1949) mentioned the presence of broad, hook-like, evanescent paraphyses in the perithecial centrum.

In *S. fimicola*, Dangeard (1907) reported that after the perithecial cavity is formed by the disintegration of the pseudopararenchyma some of the cells lining the resulting cavity produce hair like outgrowths corresponding to paraphyses. Page (1939) likewise mentioned the presence of paraphyses in this species. Ritchie's (1937) illustrations of the same species, however, indicate that the perithecial centrum is pseudoparenchymatous and paraphyses are lacking. If so, those types appear to be quite different in their development from the typical paraphysate centrum described as "*Xylaria*' type by Luttrell (1951).

In the light of above discussion, it is obvious that the genus *Sordaria*, though a representative of '*Diaporthe*' type as asccepted by Parguey-Leduc (1967) and with some misgiving by Luttrell (1951), shows some affinities with Xylariales. This makes the whole picture somewhat confusing and the classification of Sordariaceous fungi difficult. Variation in centrum structure in all the types of centrum depend to a great extent upon the difference in the sterile tissue within which the asci develop. The essential feature of '*Xylaria*' type of centrum according to Luttrell (1951) is that it is composed of paraphyses, which are at the base as well as at lateral walls of perithecium without many pseudoparenchymatous cells in between. According to him paraphyses are absent in case of '*Diaporthe*' type of centrum and instead the so-called 'nutritive cells' are present. The difficulty with the genus *Sordaria* is that paraphyses as well as nutritive cells are found in the species of this genus.

Parguey-Leduc (1967) gives more importance to the presence or absence of nutritive tissue than the presence or absence of paraphyses. She believes that in the case of '*Xylaria*' type of centrum nutritive cells are totally absent, whereas, they are always present in '*Diaporthe*' type, at least in early stages. She believes that in more evolved '*Diaporthe*'-type of centrum, paraphyses may also develop in between the nutritive cells. Thus this group ot species according to nature ot centrum is in between '*Xylaria*' type and '*Diaporthe*' type. There are similarities in the ascus types as well (Luttrell, 1951). Luttrell places the genus *Sordaria* in the family Xylariaceae, however, he does realise the difficulties when he says, *Sordaria* poses a special problem because its asci are least related to '*Xylaria*' type. Chadefaud & Nicot (1957) and Parguey-Leduc (1967) are probably closer to reality in placing Sordariaceous fungi in a separate order Sordariales, because apart from the difference in centrum type, the Xylariales, unlike the Sordariales, have distict tendency to have flattened or inequilateral ascospores with germ slits instead of germ pores, and their asci have a tendency to be amyloid at their apices.

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