MORPHOGENESIS AND SYSTEMATIC OF THE FUNGI OF THE NEW ORDER CHAETOTHYRIALES *

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The family *Chaetothyriaceæ* was raised by Hansford, C. G., in 1946, in the light of the studies of Bitancourt, A.A., 1936, on type material of *Chaetothyrium guaraniticum* Speg.

Now the characteristics of this family are reduced to contain only fungi of hyaline mycelium, in accordance with the type, and some genera fragmented into other new genera to maintain the best definition on their taxonomic delimitation, while other genera are transferred to another family.

The first rough idea of taxonomic interest about the relative position of mycelium and perithecia is to be attributed to Theissen and Sydow, discriminating, among the Capnodiaceae family, the tribes Eu-Capnodieae Th. & Syd. and Chaetothyrieae Theiss. (See Ann. Mycol. vol. XV, pag. 471-472, 1917).

A progressive step has been taken by Bitancourt (Arq. Inst. Biol. S. Paulo, vol. VII, pag. 7-12, 1936) who effected a thorough revision of the Spegazzinian type of the genus *Chaetothyrium*, *C. guaraniticum* Speg.

He observed that the perithecia are found beneath the mycelial pellicle, which is adherent to the outer wall of the upper part of these fruiting bodies. The mycelium pellicle is then lying on the leaf surface like a cloth covering a solid body.

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A further step was the full validation of this characteristic made by Hansford (I. M. I. Myc. Pap. N. 15, pag. 139-159, 1946) instituting the *Chaetothyriaceæ* family with five genera: *Chaetothyrium*, *Setella*, *Microcallis*, *Phaeosaccardinula* and *Actinocymbe*.

Bitancourt's revision of the Spegazzinian type of the genus *Chaetothyrium* stated that the mycelium is hyaline, not visible under small magnification. The ascocarp is perithecial, unilocular, ostiolate and paraphysate, but it is surrounded by mycelial reticular tissue, not well defined, made according to up of almost loose hyaline hyphae ("subicle", some authors).

The same kind of hyphae, bound in strands, are connecting the perithecia and some scattered setae, while the remaining mycelium is made up of a thin net of hyphae.

This peculiar characteristic has been clearly expressed by Hansford, in the description of the family as a "thin pellicle on loose network over the host leaf"; but he included in this family fungi having subhyaline to olivaceous hyphae, evidently with the purpose of putting together all the fungi with perithecia developed beneath the mycelial pellicle.

During many years, we had occasion to study abundant material pertaining to the *Chaetothyriaceæ* family, and, in our opinion, the establishment of this family is fully justified.

But, by widening the family to include species with dematiaceous, as a rule fumagineous mycelium, the *Chaetothyriaceæ* include heterogeneous elements, having in common the perithecial development under the mycelial pellicle.

One group of species presents hyaline to subhyaline, relatively thin mycelium, sparingly or confusedly septate, not or very slightly narrow at the septa, as a rule firmly adhering to the leaf surface (the leaf cuticle, in some portion of the vegetative hyphae, may be swollen).

The interperithecial infertile mycelium, may be composed of loose, interlaced hyphae, or of delicate, reticulate--vermiculate strands. It is not evident under small magnification. The same mycelial pseudotissue is found on and around the perithecia, obscuring its true structure, and forming a kind of "subicle" with a mucous-evanescent peripherical portion.

The perithecial wall is relatively thin, composed of an outer part build up by one to a few layers of parenchy-

matous cells and an inner part of more or less differentiated layers of hyphae.

Another group of species possesses full dematiaceous mycelium, brown to blackish in color, relatively thick, clearly and densely septate, as a rule narrowed at the septa up to a full dematioid or toruloid shape. This mycelium never adheres to the leaf surface, also in correspondence to the sterile hyphae. The leaf cuticle is not altered in any way. In addition, this kind of mycelium is not rarely mucous or slimy, and, in any case, apt to absorb moisture. The interperithecial sterile mycelium may be loose or aggregate in strand or forming a pellicle, and, in any case, it is well evident also under small magnification. It is easy to separate this mycelium from the leaf. The same strands or pellicle cover the perithecium, completely obscuring its structure when forming a continuous pellicle; the perithe-

In our opinion, this discrimination fully agrees with

The three species referred by the same mycologists (*P.* the characteristics of the type species of *Chaetothyriaceæ*, particularly with the nature of the development of perithecia, and therefore reaches a more natural subdivision of the fungi the perithecia of which is developed beneath a mycelial pellicle.

So delimitated, the *Chaetothyriaceæ* family includes the genus *Chaetothyrium* as typified by *C. guaraniticum* and the genera *Recifea* Bat. & Cif., *Ciferriomyces* Bat., *Sphaerochaetia* Bat. & Cif., *Microcalliomyces* Bat. & Cif., *Ainsworthia* Bat. & Cif., *Treubiomyces* v. Hoehn. emended and *Batistaella* Ciferri.

The problem of the characterization of the genus *Phaeosaccardinula* P. Henn, is more delicate.

Hansford does not mention the nature of the mycelium, translating the diagnosis of Saccardo's Sylloge, with a few modifications. The type species (*P. diospyricola* P. Henn.) possesses "sub-fuscous, simple hyphae". The second species of the same genus, *P. ficicola* P. Henn., is described as having "hyphis radiantibus ramosis, septatis, hyalinis vel fuscis".

Theissen and Sydow (1. c. pag. 480) considered *Phaeosaccardinula* as having dematioid mycelium, but there is no indication of a personal revision of the type specimen.

The three species referred by the same mycologists (*P. malloti* (Rehm) Theiss.; *P. butleri* (Syd.) Theiss. & Syd. and *P. indiæ* (Syd.) Theiss. & Butl. possess black or dull-brown mycelium.

To sum up, it appears justified to take out from *Chae-*

tothyriaceæ Hansf. all the fungi having dematioid mycelium and transferring them to the new families *Phaeosaccardinulaceae*, with the genus *Phaeosaccardinula* as the type, having perithecia unilocular, and *Euceramiaceae*, with the genus *Euceramia* as the type, having perithecia plurilocular.

A few words about the systematic position of the above quoted families are to be added.

The *Phaeosaccardinulaceæ* as well as the *Euceramiaceæ* families, so defined, fully pertains to the sooty molds group in relation to the complete epiphytic life, notwithstanding the morphology and quite distinct origin of their perithecia. These two families and the *Chaetothyriaceæ* are ascribed, then, to the new order, Chaetothyriales.

In addition, *Phaeosaccardinulaceae* may possess a pycnidial stage, either with elongate or spherical pycnidia, in common with the member of the *Capnodiaceæ* family. Also in common with *Capnodiaceæ*, the *Phaeosaccardinulaceæ* and *Euceramiaceæ* may be associated with insects and their secretions or not.

The systematic position of the order Chaetothyriales, appears to be parallel to the order Capnodiales both orders deriving from Pseudosphaeriales (incl. Dothideales). The evolution line in Chaetothyriales we suppose to come from the hyaline mycelium in *Chaetothyriaceæ* to the subhyaline or dull mycelium in *Phaeosaccardinulaceæ* and *Euceramiaceæ*.

The asci, judging from Bitancourt's revision of *Chaetothyrium guaraniticum* and from the results of our study with several genera of the families *Phaeosaccardinulaceæ* and *Euceramiaceæ*, are normally unitunicate, in accordance with Luttrell's statement; however, as an exception, a few species have bitunicate asci; the discharge of the spores has not been observed.

In conclusion, according to our point of view, the *Chaetothyriaceæ* family, as defined by Hansford, *sensu stricto*, is fully justified to include all fungi with superficial hyaline mycelium, the perithecia of which develop beneath a mycelial network or pellicle.

The new family *Phaeosaccardinulaceæ* will comprehend all sooty-molds fungi with dull, dematioid mycelium, having perithecia unilocular developed beneath the mycelial pellicle.

The new family *Euceramiaceæ* should be distinguished from *Phaeosaccardinulaceæ* for having in common the

perithecia developed beneath a mycelial pellicle; they, however, are plurilocular.

These three families are ascribed to the new order *Chaetothyriales* having the family *Chaetothyriaceæ* as the type.

The affinity of the order with Pseudosphaeriales results from the strands of pseudoparaphyses (namely, the remaining of hyphae filling the perithecia, after the differentiation of ascogenous hyphae), having a tendency to grow from the top to the bottom of the ascocarp.

DEVELOPMENT OF THE PERITHECIA OF *CHAETOTHYRIALES*

The development of the perithecia has been followed not on one, but on several species of the Chaetothyriales order, so that the present exposition summarizes the average cycle for the entire order.

There is only the difference of color of the mycelium in the three families, being hyaline in *Chaetothyriaceæ* and dark in *Phaeosaccardinulaceæ* and in *Euceramiaceæ*. . .

At first is the intensive growth and branching of the superficial mycelium, forming on the leaf surface a network with centers of more active development. The result is a more or less defined and scattered formation of flat strands of mycelium, like ribbons or irregular patches. At this stage, the pellicle is thin and more or less incomplete.

The continuous growth and branching of hyphae leads to some thickening in certain points, which are the foci of future perithecia development.

In transversal section, the thickened areas are sublenticular in shape, and the space between the lower surface of the pellicle and the upper surface of the leaf is empty, but crossed by scattered hyphae. This mycelium beneath the pellicle is loose and more or less regularly distributed throughout the flattened cavity.

The following stage is the gradual widening of the lenticular cavity, interlaced by more rarefied hyphae, from the top to the bottom. These hyphae are derived in part from new outgrowth of the lower face of the pellicle, and in part from branching of persistent, vertical hyphae.

The mechanism for the increased widening of the cavity appears to be simple. The pellicle is quite well fixed on the surface of the leaf all around the cavity, so that the increase in length of the pellicle leads to a greater elevation

from the leaf surface. It is possible that the vertical growth of hyphae in the cavity accelerates this process, while at the same time, the pellicle is secured on the leaf surface.

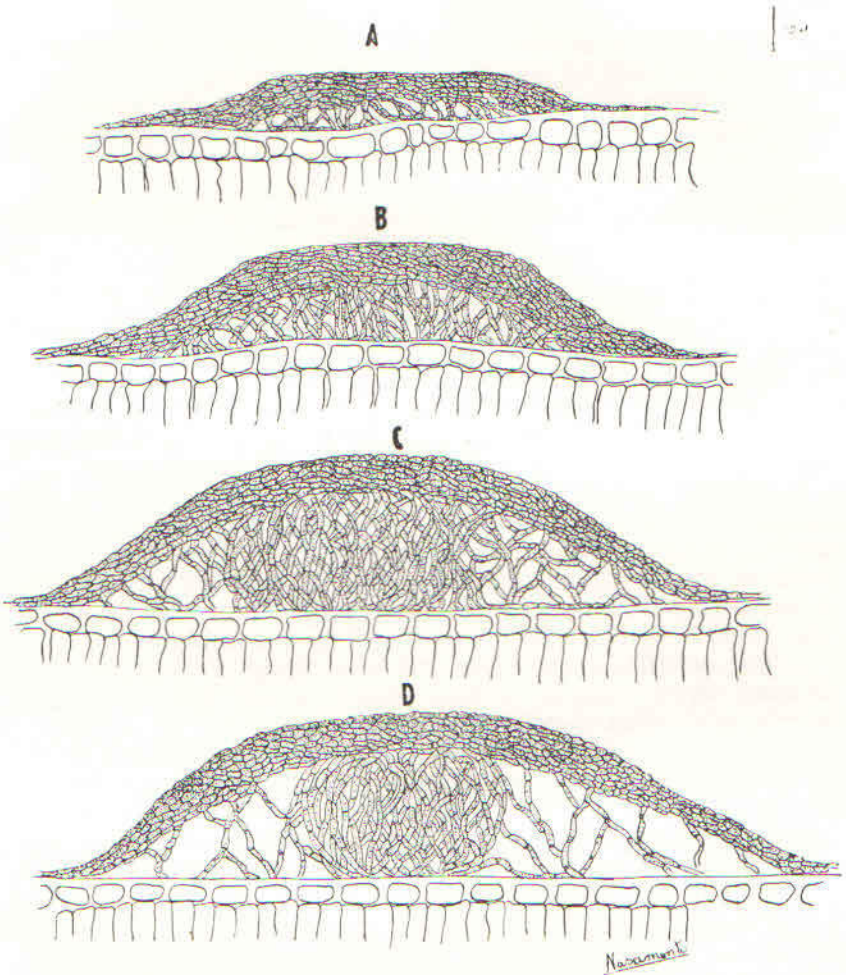

Fig. 1 — Evolutive stages of the development of perithecium of a PHAEOSACCARDINULACEÆ.

A) Beginnings of organization of a perithecium under the mycelial pellicle;
B) Intense growth and branching of the pellicle;
C) A nettle-work of the mycelial hyphaæ more active on the center;
D) Apparent differenciation of the center. Orig.

Gradually the density of vertical hyphae crossing the cavity increases, but not uniformly. There is a clear tendency

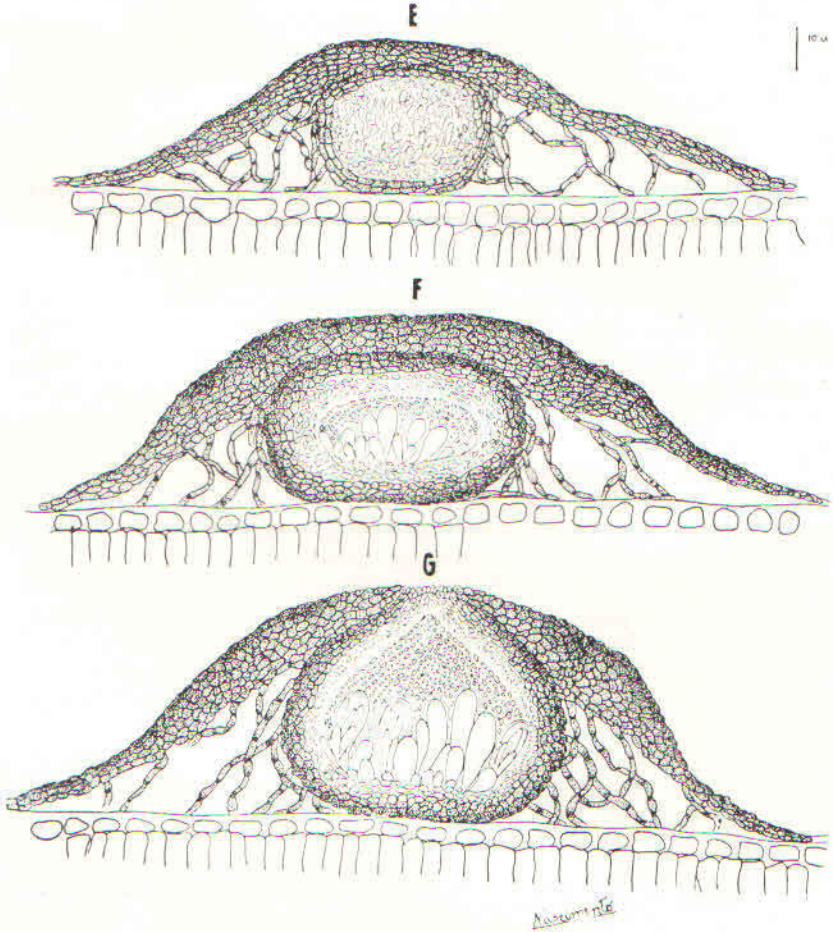

Fig. 2 — Evolutive stages of the development of perithecium of a PHAEOSACCARDINULACEÆ.

E) Development of the perithecial wall, and first step for the organization of the archicarp;
F) Individualization of the perithecium cavity; first sketch of the asci;
G) A full ripe perithecium, with the mature asci and an apical thining for opening. Orig.

to a much active growth and a more intense branching in the central portion, increasing as time passes. There is

also a formation of a thin, then a little thicker bottom layer of hyphae, just above the leaf surface, and stuck to it.

The result is the confused delineation of a spheroidal, tangled mass of hyphae which represents the first sketch of the perithecium. At this stage the shape of the cavity changes from lenticular to almost hemispheric, with narrowed borders. The side hyphae are — or appear — rarefied, probably because to the increased wideness of the cavity does not correspond an active branching of hyphae.

The following stage is a clearer individualization of the perithecium in a relatively thick, dense and dull wall and a less dense content, lighter in color.

The initiation of the archicarp is obscured by the presence of some mucillaginous matter, permeating the cavity up to the maturity of asci. Apparently, it is formed at the bottom of the cavity by some hyphae enlarged at the top, derived from the branching of the mother hyphae descending from the inside of pellicle. The basal, ascogenous hyphae, appears to be enlarged, densely but confusedly septate, and full of protoplasm strongly stained by Cotton Blue. After a reduced, horizontal growth, they originate some upstanding branches that evolve into asci. The primary hyphae are, then, covering most of the bottom, and in some cases a portion of the lateral wall. As the branching is directed toward the center of the ascocarp, the final disposition is that of parallel-convergent bundle of asci.

This situation appears to be quite variable from a species to another. In some instances the growth is limited to the very central part of the bottom, and the asci are divergent-fasciculate, fan-shaped. In other cases the growth interests all the bottom, and the resulting asci are more

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Fig. 3 — Evolutive stages of the development of perithecium of a **PHAEOSACCARDINULACEÆ.**

A) Lying of the mycelial pellicle and the first step of the ascocarpi evolution;

B) The perithecium almost fully developed, beneath the mycelial pellicle;

C) The ripe perithecia entirely covered by the mycelial pellicle; to be compared with the following picture;

D) The ripe perithecium apparently superficial; remains of the mycelial pellicle may be observed at the sides of the fruiting body. Original.

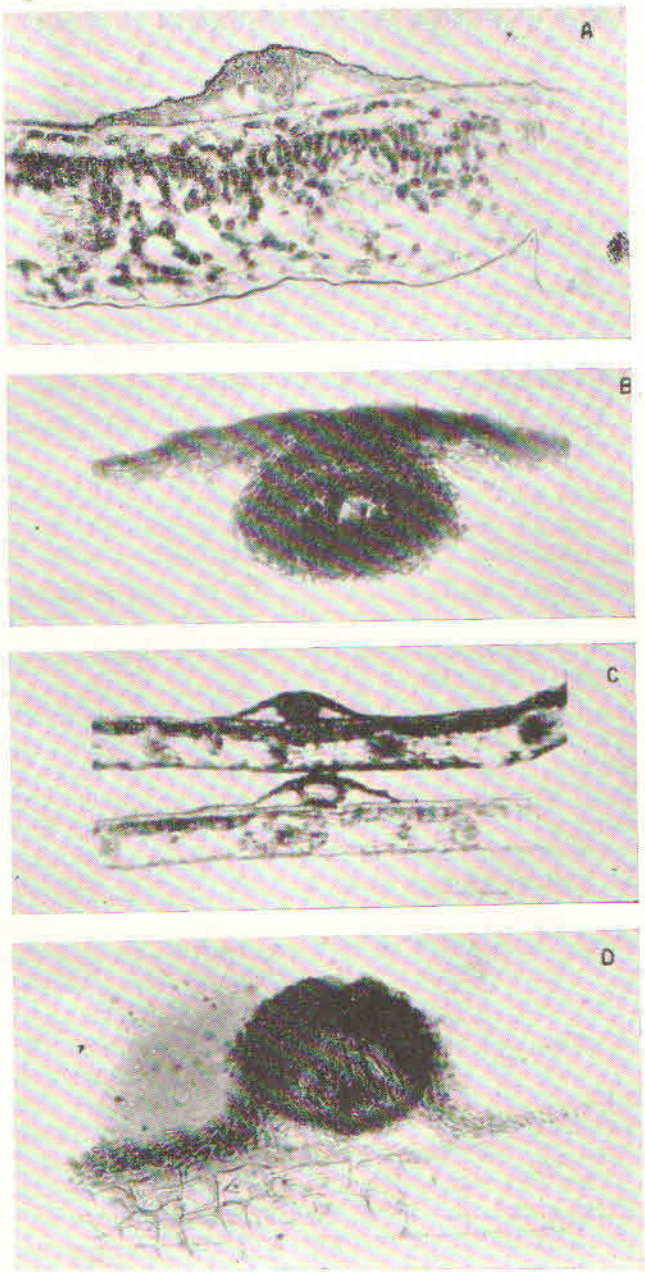

Fig. 3

or less parallel. In a few cases the ascogenous, secondary hyphae grow also from the lateral wall so that the asci are, at last, radiate-convergent to the center. The different arrangement may be related, up to a certain point, to the shape of the ascocarp, from spheric to rounded-flattened.

The young asci are immersed in an irregularly stained, dense mass of tangled hyphae, individually indistinct, and at the ripeness transformed entirely or in part into a mucilagenous substance. In our opinion, the presence of paraphysoids is caused by the remains of old hyphae.

The last phase is the individualization of the ascospores as a rule 8 for each ascus. During this differentiation, very frequently — but not always — an apical pore is opened by the thinning of the perithecial wall at the top.

We are not in position to indicate how the pore is formed, nor the conditions of the opening.

The asci are usually not diffluent, and relatively thick, mostly having a 1-layered wall. It is possible that, at least in many cases, the ascospores are discharged with the asci.

In our opinion, the pushing out of asci is provoked by water imbibition of the mucilage contained in the perithecium. This may explain why the discharge is effected during rainy or moist periods.

Overripe perithecia are frequently collapsed, and the pellicle is more or less completely flaked away. But the interperithecial pellicle, if any, or the mycelium, remains on the leaf. The developing waves are, probably, related to the exhaustion of the nutritive substance (and then with the presence of the insect, if any) as well as with some metereological factors of the environment, chiefly moisture.

In the families *Chaetothyriaceæ* and *Phaeosaccardinulaceæ* the perithecia are always unilocular. In opposition, in the family *Euceramiaceæ* the perithecia are plurilocular.

The development of plurilocular perithecia runs the same process of evolution as the monolocular perithecia; there are meanwhile two or more parallel centers of active development from which evolve two or more neighbor lenticular cavities beneath the mycelial pellicle; these locules are separated from one another through a dense mass of vertical hyphae, from the top to the bottom, while the mycelial pellicle, suffering distinct elevation for each locule may offer a peculiar ondulating aspect (Figs. 1, 2 and 3).

RESUMO

A análise da evolução dos peritécios de diversas espécies de fungos *Chaetothyriales* permite-nos considerar aplicáveis os seus resultados na interpretação da morfogênese dessas frutificações em tôda a ordem que ora apreciamos. Guardada a distinção de côr do micélio, que é hialina na família *Chaetothyriaceae* e marrom-negra nas famílias *Phaeosaecardinulaceae* e *Euceramiaceae* o mecanismo de produção de peritécios é o mesmo para todos os fungos *Chaetothyriales*, sempre por debaixo e às expensas de uma película micélica, que os reveste e que também poderá originar setas ou não.

O micélio desenvolve-se intensivamente à superfície da matriz, mas de modo superficial, compondo uma película, no seio da qual se observam centros de mais ativo crescimento que originarão primórdios periteciais. Em tais centros a histologia revela um esbôço de câmara lenticular entre as faces superior e inferior da película miceliana, na qual algumas hifas se acham distribuídas em tôda a cavidade formada.

Progressivamente distende-se essa câmara graças ao crescimento vertical das hifas que se situam no seu interior enquanto a película de micélio se torna firmemente aderente à superficial foliar. Dentro em pouco individualiza-se o primórdio peritecial como um adensado esferóide de massa hifal, quando então a câmara intrapelicular se torna hemisférica, de margens suaves. O início do arquicarpo é obscuro ante a presença de substâncias mucilaginosas que aí se conservam até quase à maturidade dos ascos. Contudo, principia o desenvolvimento de hifas ascógenas basais, ricas de protoplasma, que crescem no sentido horizontal e logo emitem ramos verticais que se transformarão em ascos. Os ascos jovens acham-se envolvidos nu'a massa frouxa de hifas que se modifica em substância mucilaginosa; antigas hifas podem conservar-se como parafisóides.

Constituídos os ascos, vem a diferenciação dos ascosporos, em número de 8, geralmente, e os ascos, que não maioria das espécies de *Chaetothyriales* são 1-tunicados, mas não evanescentes, aparentemente libertam-se através de um pseudo-ostíolo peritecial que se define por ocasião da maturidade dos peritécios, através de pequena fratura da parede superior dêstes aparelhos de proteção. Não observamos mecanismo próprio dos ascos para a independenciação dos ascosporos e interpretamos a expulsão dos ascos como pro-

vocada pela água de embebição da mucilagem mantida nos peritécios; isso explicaria a freqüente descarga dos ascos de *Chaetothyriales* nos períodos úmidos ou chuvosos.

Completamente maduros, os peritécios colapsam e se destacam fàcilmente da matriz, deixando remanescência ou não da película micélica o que seria uma conseqüência do esgotamento de alimentos ou efeito da umidade do ambiente.

Nas famílias *Chaetothyriaceae* e *Phaeosaccardinulaceae* os peritécios são uniloculares.

Na família *Euceramiaceae* os peritécios são pluriloculares tendo entretanto o mesmo mecanismo de evolução que os uniloculares, apenas os lóculos separados por densas massas de hifas verticais, da base para o tôpo da cavidade peritecial e a película de micélio, por elevações diversas, em correspondência ao desenvolvimento de cada lóculo apresenta-se ondulante.

Do ponto de vista da morfogênese a ordem *Chaetothyriales* tem então a seguinte Sistemática:

A — Micélio peliculoso, hialino
B — Peritécios uniloculares . . . *CHAETOTHYRIACEAE* Hansf. (senso estricto)
AA — Micélio peliculoso — marrom-negro
BB — Peritécios uniloculares . . *PHAEOSACCARDINULACEAE* nobis
BBB — Peritécios pluriloculares . *EUCERAMIACEAE* nobis

Segue-se a chave analítica dos gêneros aceitos ou propostos, de acôrdo com a revisão geral que os Autores realizaram para êsse grupo de fungos, inclusive farta documentação fotomicrográfica e fotográfica sôbre o assunto.

KEY TO THE GENERA OF THE FAMILY CHAETOTHYRIACEAE

HYALODIDYMAE

Spores 1-septate, hyaline

A — Mycelium not setose
BB — Perithecia setose . . *RECIFEA* Batista & Ciferri

AA — Mycelium setose
BB — Perithecia setose . . *MICROCALLIOMYCES* Bat. & Cif.

HYALOPHRAGMIAE

Spores 2 or more transversally septate, hyaline

A — Mycelium not setose
B — Perithecia not setose . *CIFERRIOMYCES* Batista

A — Mycelium not setose
BB — Perithecia setose . . *SPHAEROCHAETIA* Bat. & Cif.

AA — Mycelium setose
BB — Perithecia setose . . *CHAETOTHYRIUM* Speg. emend. Bitanc. (loc. cit.)

B — Perithecia not setose . *ALMEIDAEA* Cif. & Bat.

HYALODICTYAE

Spores muriform, hyaline

A — Mycelium not setose
B — Perithecia not setose . *AINSWORTHIA* Bat. & Cif.

AA — Mycelium setose
BB — Perithecia setose . . *TREUBIOMYCES* v. Hoehn. emend. Bat. & Cif.

PHAEODICTYAE

Spores muriform, dark

A — Mycelium not setose
B — Perithecia not setose . *BATISTAELLA* Ciferri

HYALOPHRAGMOSCOLECOSPORAE

Spores scolecoidal (ratio 10:1)

A — Mycelium not setose
B — Perithecia not setose . *LIMACINIELLA* Stevens Bern. P. Bish. Mus. Bul. 19: 58, 1925.