PERSOONIA
Published by the Rijksherbarium, Leiden
Volume 4, Part. 1, pp. 1–7 (1965)

STUDIES IN ASCOSTROMATIC LICHEN-FUNGI—I *

The problem of Ascohymeniales and Ascoloculares

P. GROENHART †
Rijksherbarium, Leiden

The hyphal elements in the hymenium of the mature ascocarp do not provide a reliable means of distinguishing an ascohymenial ascocarp from an ascostroma. The nature of these hyphal elements is determined by neither their shape nor their tips but solely by their origin. Furthermore, since it has not yet been proved that there is any relation between the structure of the ascus and the type of development of the ascocarp, the kind of ascus is of relatively little value as a means of determining the developmental type of the ascocarp. Moreover, it is often practically impossible to decide whether an ascus is bitunicate or not.

The author does not know of any other feature that is a reliable indicator of the true nature of the ascocarp. Therefore, he sees no other means of determining the group to which the ascocarp is to be referred except to study its mode of development.

When in 1955 I started a revision of the Cryptotheciaceae I was struck by the peculiar nature of their ascocarps which were neither perithecia nor apothecia. Taxonomically, therefore, they should be referred to a group other than Pyrenolichenes or Discolichenes. Santesson (1952: 54) had already reached the same conclusion, referring the Cryptotheciaceae, along with genera of Arthoniaceae and Opegraphaceae, to a group of lichens with ascolocular (ascostromatic) fruitbodies. However, he added that the Cryptotheciaceae do not produce true ascocarps (p. 59), as the asci occur scattered in ascigerous parts of the thallus and are separated by hyphal tissue. Although at first sight Santesson seemed to be right, I was not satisfied. I had

* Shortly before his death, on the 3rd November 1965, our colleague Pieter Groenhart submitted three manuscripts on ascostromatic lichen-fungi. Although he said that he could very probably improve on them, he must have been aware that this would no longer be possible. It is especially regretted that he has not had the opportunity to evaluate the most recent literature on his subject.

Even though his papers are, therefore, not without shortcomings it was decided to publish two of them without delay. The third will appear shortly.

Groenhart, a dedicated lichenologist, gradually came to realize that to understand the construction of the lichen-fructification it is indispensable to follow its development while no real insight in this process can be gained without knowledge of the steadily growing literature on the development of the apothecium in the Ascomycetes since the time Nannfeldt and Luttrell had published their papers.

Groenhart's great merit lies in his endeavour to make lichenologists familiar with recent developments in mycological literature while at the same time leaving it to the mycologists to find out for themselves that they would do well not to ignore lichen-fungi.

R. A. MAAS GEESTERANUS

often observed that the so-called scattered asci, which are usually surrounded by a hyphal tissue, arise from a cluster of what are apparently to be considered ascogenous cells.[1] In my opinion these structures, consisting of ascogenous cells, asci, and enveloping hyphal tissue, represent some kind of ascocarp. This gave birth to a plan to determine the nature of this particular ascocarp. As a consequence I extended the revision of the Cryptotheciaceae with a comparative study of the development and structure of other ascocarps, especially of those in lichen-fungi. From this investigation it has appeared that various types of ascostromatic ascocarps are represented in lichen-fungi.

It would seem rather natural for closely related fungi to show strong resemblances in the development and structure of their ascocarps, as well as in the shape and structure of their asci and spores. Therefore, I have tried to detect groups of lichen-fungi that are characterized by such resemblances. As these groups are based on purely mycological criteria, taxa of the mycological system, in so far as these are based on the same criteria, should be comparable to them. As a result these groups of lichen-fungi can be inserted in the mycological system more correctly than has thus far been possible. The results of these studies will be reported in subsequent papers.

A special debt of gratitude is due to Prof. Dr. H. J. Lam who showed his interest by giving me the opportunity to carry out my work at the Rijksherbarium. I am also very much indebted to the Netherlands Organization for the Advancement of Pure Research (Z.W.O.) which, at the instigation of Prof. Lam, decided to subsidize my work, in order to enable me to spend all my time on these studies. I would like to express my sincere appreciation to the curators of the following herbaria for the loan of specimens: BM, BR, FH, FI, G, GLAM, H, LISU, M, PC, S, TUR, UPS, US, W, WRSL, WU,[2] and to Prof. Dr. C. W. Dodge and the late Dr. A. H. Magnusson for sending specimens from their private herbaria.

A review of the definitions of the three main divisions in Nannfeldt's classification of the so-called higher Ascomycetes (1932) shows that they represent three fundamental types of development; these are characterized (i) by the sequence of initiation of the ascogenous system and of the auxiliary tissues of the ascocarp, and (ii) by the number and origin of the auxiliary tissues.[3]

[1] With this term nothing is implied about the cytology: no particular nuclear condition is postulated.

[2] These standard abbreviations are in accordance with Lanjouw & Stafleu, Index herbariorum, Part I, 5th Ed., 1964 (Regn. veg. 31).

[3] The present author is of the opinion that the ascogenous system represents the true (sexual) reproductive portion of the fungus. The more or less differentiated tissues that clothe and protect this system are considered of secondary importance. The ascogenous system and the auxiliary tissues together constitute the body which is called the ascocarp.

In the Ascohymeniales the ascogenous system initiates unprotected on a hypha.[4] This is followed by the development of two tissues of different origin: (i) a paraphysogenous tissue that arises from the supporting cell of the ascogon to produce true paraphyses; (ii) a tissue that originates from adjacent hyphae to constitute the excipulum.

In the Ascoloculares a stromatous tissue is formed first; within this the ascogenous system later initiates.

The basic criteria determining these two groups are clear-cut and should give no trouble in assigning a fungus to its proper group. However, neither the sequence of initiation and development of the ascogenous system and the auxiliary tissues nor the origin of the latter are usually known. Because of the lack of ascocarps in their earliest stages of development they cannot as a rule be observed in the material to be examined. The only way to obtain the data needed is to culture the fungus. This, however, is not always easy to do and it is often even quite impossible in the case of lichen-fungi. In the practically unique case in which a lichen-fungus was cultivated successfully by Anderson & Ahmadjian (1962), they failed to give particulars about the development of the ascocarps. As a result, mycologists trying to classify the fungi involved used characters of the asci and the auxiliary tissues to determine to what group their fungi belonged. It would seem that hitherto no one had questioned the reliability of the features taken into consideration.

Before Nannfeldt published his new classification, all hyphal elements in ascocarps among which the asci develop were called paraphyses and they were either simple, or branched, or branched and interconnected paraphyses. According to their origin, Nannfeldt distinguished interthecial filaments and true paraphyses. The former were remnants of the original tissue of the ascolocular stroma and were connected with the lower as well as with the upper layer of the stroma; they acquired free tips only in those ascocarps of which the upper layer had crumbled away. True paraphyses were produced by the paraphysogenous tissue in ascochymenial ascocarps; they had free tips from the beginning of their development. It was further pointed out by Nannfeldt that thick-walled asci with a dome-shaped extension of the lumen into the thick top were common in ascolocular ascocarps. Since then hyphal elements with free tips in the hymenium have been considered to be true paraphyses and a proof of the ascohymenial nature of the ascocarp. The origin of these hyphal elements was not even taken into account. The dome-shaped extension of the lumen in the top of thick-walled asci came to be regarded as an inherent attribute of bitunicate asci. Thick-walled asci without this attribute were considered unitunicate. In accordance with these views and ignoring their origin, Nannfeldt

[4] As pointed out by Nannfeldt, the ascogons of ascohymenial ascocarps may also initiate in a stroma like, for instance, in *Xylaria* and *Hypoxylon*. These stromata, however, are bodies which contain complete ascocarps. They are thus not comparable with the ascostromata of the Ascoloculares which contain only asci or groups of asci. For the type of stroma as it is known in *Xylaria* and *Hypoxylon* the present author proposes to use the term c a r p o s t r o m a.

referred the Graphidaceae (sensu stricto) to the Ascohymeniales because of their unbranched hyphal elements with free tips in the hymenium. Nevertheless, the ascocarps of the fungi belonging to this family are truly ascostromatic.

This way of determining the nature of an ascocarp may lead to curious results; this is illustrated in a paper by Mme Letrouit-Galinou (1962), who gave an account of the development of *Lecanora subfuscata* Magn. The development of the ascocarp starts with the formation of a stromatic tissue. Correctly and without exception she called the ascocarp an ascostroma. Moreover, she stated that the "paraphyses" originate not from the base of the ascogon but from the surface of the "réseau paraphysogène." This réseau is the original stromatic tissue within which, after the pseudoparaphyses have started their development, the ascogenous system later originates. All this is perfectly in accordance with an ascostromatic development of an ascocarp but, without motivating her conclusion, the author ends by stating that the ascocarp of *L. subfuscata* is a gymnocarpous ascohymenial apothecium. This unexpected conclusion was obviously induced by the paraphyse-like structures having free tips.

Luttrell (1951) pointed out that the asci in ascostromatic ascocarps are not always enclosed singly in loculi separated by original stromal tissue. Many stromata become differentiated into a hyphal centre and a peripheral part constructed otherwise. The ascogenous system, initiating at the bottom of the central tissue, produces the asci that grow into that tissue, while the peripheral part forms the wall of the stroma. The hyphal elements of the central tissue, which Luttrell called pseudoparaphyses, are either simple or branched. Ascostromata may be divided into two groups according to their development: (i) true ascolocular stromata in which the asci are separated by original stromal tissue (*Myriangium*) and (ii) ascostromata in which the asci are surrounded by pseudoparaphyses (*Pleospora*). In the second group asci and pseudoparaphyses constitute a hymenium similar to the hymenium in an ascohymenial ascocarp. If the pseudoparaphyses grow free tips at an early stage of development, as in Graphidaceae, or if the tips are free from the beginning of their development, as in *Lecanora*, there is no longer a difference between the hymenia of an ascostroma and an ascohymenial ascocarp. The only means, then, of determining the nature of the hyphal elements in the hymenium is to discover their origin.

According to Luttrell (1951) bitunicate asci are correlated with the ascostromatic character of the ascocarp. That bitunicate asci are frequently found in ascostromata may point in that direction but this is nevertheless no absolute proof.

Bitunicate asci are characterized by thick walls, a thick top with a dome-shaped extension of the lumen, and failure to react to iodine. This certainly holds true for the bitunicate asci in *Cryptothecia* Stirt. and related genera. In the bitunicate asci of other genera these characters are often less evident or even absent. If endoascus and exoascus cannot be separated it then becomes very difficult to decide whether the asci are bitunicate or not. In *Aglaothecium saxicola* Groenh. (1962) the ascocarp is an ascostroma. The asci are thin-walled and there is no evidence that the wall is composed of two separable layers. Hence the author called the asci unitunicate.

Because he had at that time not yet studied that type of development he called the ascocarp an apothecium in the conventional sense of the word. Although it now appears that the ascocarp is an ascostroma and the asci show no reaction to iodine he is not as yet prepared to regard the asci as bitunicate. At best a question-mark may be placed after the word unitunicate. If, to the contrary, the asci should really prove to be unitunicate, it would be absurd to call the ascocarp ascohymenial because of that single wall.

As to the iodine reactions in the hymenium, it is questionable whether these have any value other than merely to indicate that the hymenial gelatine and/or the protoplasm of the asci contains a matter that turns wine-red, violet or blue with iodine. It is still more questionable whether these reactions have anything to do with the structure of the ascus wall. The colourable matter is often concentrated close to the inner and the outer sides of the wall of the top of the asci. It is then the question whether the matter is produced by the ascoplasm or in the hymenial gelatine and whether it is transported through the wall from the ascoplasm to the hymenial gelatine or in the opposite direction. As a rule the wall itself is less intensely stained and it is impossible to decide whether it is the wall itself or the matter that is being transported (if any) that is showing the reaction or whether the wall is merely reflecting the colour. To the present author it seems rather hazardous to draw any conclusion about the structure of the ascus wall based on either these vague reactions or an absence of them.

It may be recalled here that according to Nannfeldt's basic characterization the Ascohymeniales possess true paraphyses, that is, paraphyses with free tips. However, while falling definitely within this category certain Ascohymeniales prove also to possess paraphyses without free tips; an example of this is *Ophiobolus graminis* Sacc. The development of that species was described by Jones (1926). The ascocarp initiates with an ascogenous system unprotected on a hypha. This system becomes enclosed by a paraphysogenous and an excipular tissue of differing origins. This is the pattern according to which an ascohymenial ascocarp starts to develop. The mass of paraphysogenous tissue increases by the division and growth of the cells to constitute a core of ovoid cells within the excipular wall. During the course of its further development ascogenous cells come into being in the base of the core to take the place of the desintegrated original ascogenous system. The peripheral cells of the core also desintegrate to form a granular layer close to the inner side of the exciple. The other cells of the core elongate and become arranged into vertical series; these separate in turn to form the paraphyses.

It has been assumed that the characters used as primary ones in the classificatory systems discussed above are fully correlated with others considered secondary. While this may be true in some cases it need not be true in general; in any case the supposition, if not based on specific details, is likely to lead to misinterpretation and confusion. This is exemplified by *Melogramma spiniferum* (Wallr.) De Not. Doguet (1959), describing this species, failed to furnish information about the initiation and

development of the ascocarp. Instead, because of the neatly delimited perithecial wall, the periphyses filling the apical canal, and the paraphyses with free tips, he simply concluded that the fungus was ascohymenial. From this he drew the conclusion that the ascocarp was entirely wrong. According to Luttrell, since the asci are bitunicate, the ascocarp must be ascolocular. To judge from their apical apparatus the asci are nassasceous. Indeed it must be admitted that it seems quite impossible to find more discrepancies combined in a single ascocarp.

Is there any way out of this difficulty to be found? First it should be pointed out that Doguet's conception of the developmental group to which he referred the ascocarp of *M. spiniferum* was based on secondary characters. It has already been shown in the foregoing that without knowledge of their origin it may be impossible to distinguish paraphyses and pseudoparaphyses. It is not known whether a neatly shaped perithecial wall and periphyses are unmistakable indications of an ascohymenial ascocarp. It therefore remains uncertain whether the ascocarp in *Melogramma spiniferum* is ascohymenial or ascostromatic. If it should turn out that the ascocarp develops ascohymenially then it is proved that bitunicate asci are also produced in ascohymenial ascocarps. If the ascocarp should turn out to be an ascostroma then it is certain that hyphal elements with free tips in the hymenium do not provide an infallible means of distinguishing an ascohymenial ascocarp from an ascostroma. The asci in *M. spiniferum*, which are both annellasceous and bitunicate, prove that bitunicate asci, at any rate, may be not only annellasceous but also nassasceous.

From the examples given in this study it appears that (i) the characters considered secondary by the various authors are not always correlated with the primary characters on which they based their systems; (ii) these secondary characters cannot therefore be used to decide correctly to what group a fungus is to be referred; (iii) the use of secondary features as differential characters has led to misinterpretations and confusion.

To avoid these difficulties, the present author sees only one possibility and that is to adhere strictly to the primary characters on which the systems are based, using the secondary characters only in order to distinguish subdivisions.

Nannfeldt's system is based on the sequence of initiation and development of the ascogenous system and the auxiliary tissues of the ascocarp. It would be logical if the main groups of this system were to be subdivided according to the types of development and those structures resulting from this development which may be distinguished within each group. Taxa of lower rank might then be based on the characters of asci and spores.

Luttrell based his system on the structure of the ascus wall. A good basis for the first subdivision of the main groups ought to be the apical structure of the asci, after which taxa of lower rank could be formed according to the types of ascocarps.

In Chadefaud's system the natural sequence logically would be to distinguish the main divisions according to the apical apparatus of the asci, the first subdivisions according to the type of the asci and further subdivisions according to the type of the ascocarp.

Melogramma spiniferum, once a riddle which could not be made to fit into any of the three systems mentioned, could then easily be inserted in each of them in its correct place. In Nannfeldt's system, on account of the development of the ascocarp, the type of the ascus, and the apical apparatus in the top of the ascus, this species would then be an annellasceous, bitunicate ascohymenial (or ascolocular) fungus; in Luttrell's system, an ascohymenial (or ascolocular), annellasceous bitunicate fungus; in Chadefaud's system, an ascohymenial (or ascolocular), bitunicate annellasceous fungus.

REFERENCES

ANDERSON, K. A. & AHMADJIAN, V. (1962). Investigation on the development of lichen structures in laboratory controlled cultures. *In* Svensk bot. Tidskr. **36**: 501–506.

DOGUET, G. (1959). Les bases actuelles de la systématique des Pyrénomycètes et le cas du *Melogramma spiniferum*. *In* C.r. hebd. Séanc. Acad. Sci., Paris **249**: 2605–2607.

GROENHART, P. (1962). *Aglaothecium* Groenh., a new lichen genus from Malaysia. *In* Persoonia **2**: 349–353.

JONES, S. G. (1926). The development of the perithecium of *Ophiobolus graminis* Sacc. *In* Ann. bot., **40**: 607–629.

LETROUIT-GALINOU, M. A. (1962). Recherches sur le développement des apothécies du lichen *Lecanora subfuscata* Magn. *In* Bull. Soc. bot. France **109**: 245–253.

LUTTRELL, E. S. (1951). Taxonomy of the Pyrenomycetes. *In* Univ. Missouri Stud. **24** (3). Columbia, Mo.

—— (1955). The ascostromatic Ascomycetes. *In* Mycologia **47**: 511–532.

NANNFELDT, J. A. (1932). Studien über die Morphologie und Systematik der nicht-lichinisierten inoperculaten Discomyceten. *In* Nova Acta Soc. Sci. upsal., ser. 4, **8** (2).

SANTESSON, R. (1952). Foliicolous Lichens. I. *In* Symb. bot. upsal. **12** (1).