

THE GENUS

*Aspergillus*

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WITH A CHAPTER ON PATHOGENICITY

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# Preface

The genus *Aspergillus* dates from Micheli's *Nova Plantarum Genera* of 1729, but it was not until the middle of the nineteenth century that these fungi began to be recognized as active agents in decay processes, as causes of human and animal disease, and as fermenting agents capable of producing valuable metabolic products. With such cognizance came the first adequately described and illustrated species in the works of Fresenius, Van Tieghem, and De Bary, although Link (1809) had earlier introduced a few species names that are still recognized. The genus began to take definite form with the work of Wehmer (1901). In 1926, Thom and Church attempted to bring together all available material in a monograph, *The Aspergilli*.

Continued study of the *Aspergilli* showed some of their groupings to be inadequate. In addition, large amounts of new material accumulated as the *Aspergilli* became increasingly investigated in the laboratory and utilized in industrial fermentations. The need for a guide to facilitate the identification of the *Aspergilli* led Thom and Raper to publish *A Manual of the Aspergilli* in 1945. This book was based primarily upon comparative study of thousands of strains of *Aspergilli* in laboratory culture, including as many type cultures as could then be obtained. A few species were recognized from published descriptions alone. Upon the basis of morphology, species were arranged into groups which, for the most part, showed related physiological or biochemical activities.

In the years since 1945, scores of new species of *Aspergillus* have been described or newly discovered, necessitating the substantial enlargement of some of the groups then established and the recognition of five new groups. Several of the new species are ascosporic and, in one case, a much delayed ascosporic stage has been discovered in a species previously regarded as strictly sclerotial. Altogether 132 species and 18 varieties are recognized in the present work in contrast to 77 species, 8 varieties, and 4 mutations in the *Manual* of 1945. Parallel with the dramatic increase in the size of the genus has been an even greater proliferation of the published literature relating to the *Aspergilli* as agents of decomposition, as primary or secondary pathogens of animals and man, as tools for physiological and genetical studies, and as agents responsible for the production of a variety of products in industry.

The present book is designed to provide keys and descriptions for the identification of the *Aspergilli*, as well as to present references to the de-

scriptions of species that are not recognized, with our opinions regarding their probable relationships. Species and varieties that duplicate recognized taxa are indicated as synonyms; others are listed as probable synonyms, possible synonyms, or group synonyms, depending upon the adequacy of their descriptions and the degree to which these can be interpreted in relation to the genus as it is known to us.

Named species of *Aspergillus*, and other genera to which these organisms have been assigned, are listed in Part III. For those that are considered in the text, a simple page reference is cited; for those not so considered, a reference to the original description it given, with annotations wherever possible. A separate list of the *accepted* species and varieties is also included.

In their *Manual of the Aspergilli*, Thom and Raper included two types of bibliography: a general bibliography which covered the literature cited in the text, and a topical bibliography of selected papers on particular subjects. Substantial duplications between the two occurred. We have concluded that for the present volume, except for the chapter on pathogenicity, a general bibliography should suffice and may, we believe, be more useful since all of the papers listed therein are cited and often briefly summarized at appropriate positions in the text. At the end of the chapters covering the different groups of the *Aspergilli*, we have reviewed much of the pertinent literature under the general title "Occurrence and Significance." For some groups such literature is minimal; for others (*e.g.*, the *A. niger* and *A. flavus* groups) it is enormous, and we have been forced to select the references to be included.

Because of the increased recognition of the *Aspergilli* as causative agents in animal and human diseases, a specialist in this field, Mr. P. K. C. Austwick, Central Veterinary Laboratory, Weybridge, England, was invited to prepare a chapter on pathogenicity (Chapter VII). We gratefully acknowledge this major contribution.

The cultures cited in the text by number bear the prefix *WB* and are so accessioned in our collection of fungus cultures. Those bearing numbers less than 2000 are the same cultures cited by Thom and Raper in the *Manual of the Aspergilli* (1945) with the prefix *NRRL*, and those between 2000 and 2400 were accessioned at the Northern Laboratory after that date. Cultures bearing numbers above 4000 have been isolated in our laboratory at the University of Wisconsin or contributed by collaborators for this and other investigations since 1953. Lyophilized preparations have been made of all cultures cited in the text, and strains representative of the taxa accepted by the authors have been deposited in the following culture collections: the Centraalbureau voor Schimmelcultures at Baarn, Holland; the Northern Utilization Research and Development Division, United States Department of Agriculture, Peoria, Illinois; the Common-

wealth Mycological Institute, Kew Surrey, England; and the American Type Culture Collection, Rockville, Maryland.

The authors cannot adequately express their indebtedness to Dr. Charles Thom, to whom this book is affectionately dedicated. While the present book has been almost completely rewritten, we have drawn heavily from the previous books by Thom and Church (1926) and Thom and Raper (1945), particularly with regard to the group concept of the genus and in the citation of observations that predate ours.

The authors acknowledge the generous cooperation of culture collections and individuals throughout the world who have collaborated with us in this endeavor. The Fermentation Laboratory of the Northern Utilization Research and Development Division (formerly the Northern Regional Research Laboratory (NRRL)), United States Department of Agriculture, Peoria, Illinois, has been most helpful, and our special thanks are extended to Drs. R. W. Jackson and C. W. Hesseltine for the receipt of many cultures and for permission to use illustrations from their files. We acknowledge also the wholehearted cooperation of the Centraalbureau voor Schimmelfcultures (CBS) and its staff, particularly Amelia C. Stolk and the late directrices, Professor Johanna Westerdijk and Agathe van Beverwijk. Dr. Takezi Hasegawa, Director of the Culture Collection of the Institute of Fermentation (IFO), Osaka, Japan, has sent us many cultures, including type materials of species and varieties described by Japanese mycologists; and we have profited greatly from the full cooperation of Mr. John Elphick, Culture Collection, Commonwealth Mycological Institute (CMI), Kew, Surrey, England. We acknowledge also the helpful cooperation of Mr. George Smith (retired), London School of Hygiene and Tropical Medicine; Dr. J. H. Wareup, Waite Institute, Adelaide, Australia; Dr. G. F. Orr, Dugway Proving Grounds, Utah; and many other collaborators who have provided cultures and information without which this study could not have been completed. Several of the new species described herein were isolated by Mrs. Kyung Joo Kwon during the course of investigations leading to an M.S. degree (1963). We were fortunate in having Mrs. Kwon's help in translating many Japanese papers.

To Dr. Claude E. Vezina, of Ayerst, McKenna and Harrison, Ltd., Montreal, we are especially indebted for preparing the Latin diagnoses of new species and varieties published in this volume.

Illustrations are taken in part from *A Manual of the Aspergilli* (1945), and for many of these we wish to thank Mr. Roland W. Haines, Photographer at the Northern Utilization Research and Development Division. Many others have been made by Mr. Eugene H. Herrling, Department of Plant Pathology, University of Wisconsin. Cultures and photographs, some of which are included in this volume, have been supplied by Messrs. John and Edward Yuill, John E. Sturge, Ltd., York, England.

We are indebted to Dr. S. M. Martin, for having made available to us the extensive file of *Aspergillus* references assembled at the Division of Applied Biology, National Research Council, Canada. The authors acknowledge with gratitude the invaluable help of Mrs. Lillian Karn, who compiled much of the bibliographical material and typed the manuscript in its final form.

The research upon which the book is based was made possible by a Research Grant, "A Comparative Study of the *Aspergilli*", from the National Science Foundation, NSF-G9072, supplemented in part by Research Grants NSF-G24953 and AI-04915, National Institutes of Health, United States Public Health Service.

The Authors

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PART ONE

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*General  
Discussion*



## CHAPTER

# I

## Historical Introduction

The *Aspergilli* have always been a factor in man's environment, but prior to the development of the microscope they were brushed aside as white, yellow, green, red, or black mold without serious attempt at interpretation. Micheli (1729) was the first to distinguish stalks and spore heads. He noted that the spore chains or columns radiated from a central structure to produce a pattern that suggested the aspergillium with which he, as a priest, was familiar; hence he applied the name *Aspergillus* to the molds he observed. He then described his molds in Latin phrases—for example, *Aspergillus capitatus*, *capitulum glauco* for a glaucous green form, *Aspergillus capitatus*, *capitulum pullo* for some member of the *Aspergillus niger* group, etc. Some of these have been cited as specific names by subsequent investigators and attributed to him, e.g. *A. capitulum pullo* (Micheli) Haller.

Micheli's generic description and certain of his illustrations (Plate 91, Figs. 1 and 2) unmistakably represent the genus as it has long been accepted. His other illustrations, however, are less definitive, and at least one of his organisms (*Aspergillus* No. 9; Fig. 4) was clearly different from the above. Interpretation of his broad concept of *Aspergillus* by other early mycologists resulted in the description of representatives of other genera as species of *Aspergillus*; for example, Haller (1742) described what appears to have been a *Sporodinia* as *Aspergillus ramosissimus*.

## GENERAL DISCUSSION

Persoon (1797, 1801) placed the *Aspergilli* in his polyglot concept, *Monilia*, because they produced spores in chains resembling strings of beads. He made no record concerning their origin. Link in 1809 went back to Micheli and based his rejection of *Monilia* upon the fact that these chains of spores have their origin in a definite spore-bearing structure or "head" (capitulum).

Link, in turn, failed to describe the conidial heads in precise terms, but we know that in describing *A. glaucus* he had under his microscope one of that group as it occurs, now as then, upon poorly dried herbarium specimens; and we have every reason to believe that his *A. candidus* and *A. flavus* referred to molds that are still so named. Correct interpretation of the structure of the conidial head appeared first in the work of Corda, who began, about 1828, to publish and illustrate his studies of fresh materials. It appears that, up to about 1850, few workers were able to correctly identify their specimens with the inadequately described and figured species of their predecessors and frequently concluded that each specimen was a new species. Montagne (1856) complained that none of the descriptions written before Corda were identifiable; some of us are equally uncertain of our ability to interpret Montagne.

De Bary and his colleagues, in the early 1350's, seem to have introduced sufficient laboratory cultivation of the molds they encountered to form the basis of a reliable literature, which began with the recognition that the yellow cleistothecia upon herbarium specimens (Fig. 7), called *Eurotium herbariorum* by Link, and the conidial heads of *Aspergillus glaucus* among which they developed were actually borne upon the same mycelium (1854). Fresenius, Cramer, Wilhelm, and Brefeld in Germany followed. In the 1860's, Raulin and Van Tieghem in France developed the gallic acid fermentation from the tannin in gall nuts and studied the associated molds as a corollary. In 1880, in Paris, Bainier began publishing his studies of molds as they appeared in pharmaceutical products. He was followed by Gueguen, the Sartorys, and others in France, and somewhat later by Biourge in Belgium.

Wehmer, in Hanover, Germany, began reporting his biochemical studies in 1891, and this led to the publication of his monograph on the genus *Aspergillus* in 1901. Blochwitz began the development of his "system" early in the new century, but the World War delayed its publication until 1929. Meanwhile, Thom and associates, beginning about 1910, had published a series of reports on different groups of *Aspergilli* important in food microbiology that culminated in 1926 in their taxonomic monograph, *The Aspergilli*. Prior to this time, *Aspergilli* had been reported in a variety of cryptogamic floras, lists, and manuals, as well as in widely scattered reports of molds on particular substrates or in particular lesions of man or animals. Critical discussions were few and limited in scope, however.

In the 235 years since Micheli, an enormous literature on *Aspergillus* has accumulated. Practically all of the early literature was based upon microscopical study of specimens brought in from natural sources. Life histories and comparative examinations of materials from many sources were not undertaken. After De Bary's group began to study molds in comparative culture, the number of publications increased rapidly. By 1929-1930 Tamiya and Morita were able to cite, in their *Bibliographie von Aspergillus, 1729 bis 1928*, 2424 papers which in some way concerned the Aspergilli. A mathematical analysis of this literature by Tamiya followed in 1931. According to Tamiya's Table 1, 71 titles appeared in the 125 years before De Bary's 1854 paper, while 309 appeared in the next 37 years preceding Wehmer's oxalic acid reports in 1891. The remaining 2000 papers, published between 1891 and 1928, represent the pure culture period and mark the beginning of physiological and biochemical studies, which continue to be reported at a progressively accelerated rate.

In *The Aspergilli* Thom and Church (1926) sought to bring together all of the existing taxonomic literature and to present a critical opinion as to the proper relationship of the species described, whether retained in the genus or placed elsewhere. Some 350 names were thus accounted for. The actual number of species accepted, either known in culture or probably determinable from existing literature, was given as 69. These species were considered in 11 groups, within which they were believed to be closely related. Many of the unrecognized forms, whose published descriptions were inadequate for positive identification but complete enough to indicate their affinities, were discussed under one or the other of these groups in order that the many species cited in the older literature might be correlated with more recent studies of the same or related organisms. Of the remaining names, some had to be listed as entirely unidentifiable, and others as belonging to different genera.

Various other interpretations and proposals for grouping have been made. Blochwitz's long-delayed "System und Phylogenie," published in 1929, advocated groupings quite different from those of Thom and Church. Neill (1939) drastically reduced the number of species by recognizing only the best known in each of the larger aggregates, paying little attention to details of head and spore formation. Smith (1938), seeking industrial utility, simplified his descriptions and introduced many photomicrographs. He made no attempt to cover the complete literature but undertook to guide the worker to the larger groups by emphasizing the color and shape of the conidial heads. All of the species whose names had appeared in medical literature up to 1935 were keyed from their descriptions by Dodge, who did not, however, study them in culture.

In 1939, Professor Biourge prepared a manuscript analysis of the genus *Aspergillus* for the Third International Microbiological Congress in New

York. His associate, Dr. Simonart, came to represent him, but left almost immediately because of the outbreak of World War II. The paper was not presented but was transmitted to us because return to the author was impossible. Biourge died somewhat later. His scheme of classification, prepared in his last years, is not presented because it contains many things too bizarre to do justice to a man who, in his earlier years, was a master workman. Certain names that appeared in that manuscript are considered in the text of this work because the cultures to which they were attributed had been widely distributed.

By the early 1940's, the *Aspergilli* were being studied intensively in many laboratories in this country and abroad. Their potential for the industrial production of certain organic acids was already well established; their implication in human and animal diseases was becoming increasingly apparent, as was their role in decomposition processes in soil and elsewhere; and they were being used more and more as research tools for investigating physiological processes and metabolic pathways in microorganisms. The monograph of Thom and Church was no longer available, and the need for a new treatise on this group of molds was obvious. The problems arising from the deterioration of material in the early days of World War II made this need more acute. Thom and Raper undertook a re-examination of the genus that culminated in the publication of *A Manual of the Aspergilli* in 1945. While an attempt was made to consider and correctly allocate all species names published up to that time, the primary emphasis, as the title implied, was placed upon the identification and characterization of recognizable species of *Aspergillus*. Eighty species and 10 varieties, assembled in 14 groups, were accepted as probably valid, and for most of these, descriptions based upon cultures grown in the laboratory under standardized conditions were provided. A guide to the more important literature was presented in the form of a topical bibliography.

The *Manual* of 1945 is now out of print and out of date. Numerous additional species and varieties have been described in the literature or discovered in this laboratory in the years since the *Manual* was published, and an ascospore stage has been encountered in species and groups where none was then known. These developments have necessitated the establishment of four new groups and the modification of some existing groups. The present work is an attempt to summarize as completely as possible current information regarding the interrelationships, activities, and potentialities of the *Aspergilli*.

## CHAPTER

# II

## Classification, Generic Diagnosis, and Synonymy

Because some species of *Aspergillus* exhibit an ascosporic stage, while most do not, considerable confusion exists as to the names which should be applied to members of the genus. As originally used by Micheli (1729), the name was applied to the asexual stage of certain common species. Eighty years later, Link (1809) introduced the name *Aspergillus glaucus* for the conidial heads of a fungus found on herbarium specimens and, not realizing their common origin, applied the name *Eurotium herbariorum* to the yellow cleistothecia formed by the same fungus. The common mycelial origin of the structures to which these names were given was clearly demonstrated by De Bary in 1854 (Fig. 7).

It was the opinion of Thom and Church (1926) and of Thom and Raper (1945) that the generic name *Aspergillus* should be applied to all of these fungi, whether or not an ascosporic stage was produced. Their view was based upon the theses that (1) finding and describing the sexual stage merely completed the characterization of a fungus, usually already known, and (2) this practice would avoid separating the limited number of Aspergilli that develop a sexual stage from the majority of species that do not. The

precedent for this practice had been established by Fischer in his treatment of the *Aspergilli* in Engler and Prantl's *Natürliche Pflanzen Familien* (1897), and by Wehmer in his monograph, *Die Pilzgattung Aspergillus* (1901). The present authors concur in this viewpoint, and in presenting the taxonomy and nomenclature of these fungi we shall key and list the taxa as species of *Aspergillus*, including as synonyms such other names of ascosporic species as may be applicable in the particular case.

Not all mycologists agree with this procedure. Accordingly, to indicate the correct position of the genus *Aspergillus* among the fungi, we will present the suggested classifications of some recognized authorities, showing its placement within both the Ascomycetes and the Fungi Imperfecti.

*Fide* Fischer, in Engler and Prantl, Teil I, Abt. 1, pp. 301-304, Figs. 214-215 (1897)

Class: Ascomycetes  
Order: Plectascineae  
Family: Aspergillaceae  
Genus: *Aspergillus*

*Fide* Lindau, in Engler and Prantl, Teil I, Abt. 1\*\*, pp. 415-416, 430 (1900) (with citation of Fig. 214 in Abt. 1)

Class: Fungi Imperfecti  
Order (?): Hyphomycetes  
Family: Mucedinaceae  
Subfamily (?): Aspergilleae  
Genus: *Aspergillus*

*Fide* Bessey, *Morphology and Taxonomy of Fungi*, pp. 322-326, Figs. 104-105; pp. 586-587 (1950)

Class: Ascomycetes  
Order: Aspergillales (Plectascales)  
Family: Aspergillaceae  
Genus: *Aspergillus* (*Eurotium*)  
Class: Fungi Imperfecti  
Order: Moniliales (Hyphomycetes)  
Family: Moniliaceae  
Genus: *Aspergillus*

*Fide* Alexopoulos, *Introductory Mycology*, pp. 271-278, Figs. 100-102; pp. 407-410 (1962)

Class: Ascomycetes  
Subclass: Euascomycetidae (Series: Plectomycetes)  
Order: Eurotiales  
Family: Eurotiaceae  
Form genus: *Aspergillus* (*Eurotium*, *Sartorya*, *Emericella*)



Form class: Deuteromycetes

Form order: Moniliales

Form family: Moniliaceae

(Form genus): *Aspergillus*

### Generic Diagnosis

Thom and Raper (1945) emphasized the need for broadening the application of the name *Aspergillus* to include organisms whose structures point to close natural relationship. Careful consideration was given to the question whether the whole group should be retained as *Aspergillus* or divided into lesser entities such as *Eurotium* of Link, *Aspergillopsis* of Spegazzini, *Diplostephanus* of Langeron, *Sterigmatocystis* of Cramer, etc. The arguments for keeping these organisms in a single genus seemed to outweigh any advantages that might stem from subdividing them upon the bases of head size, uniseriate versus biseriate sterigmata, or the presence or absence of a sexual stage. As already implied, we concur in this view and present herewith an emended detailed characterization of the genus *Aspergillus*, which is followed by brief considerations of the more significant synonyms.

*Aspergillus* Micheli, in Nova Plantarum Genera, p. 212, Plate 91 (1729).

Compare Link, in Observations, p. 16 (1809); Corda, in Icones Fungorum, IV, p. 31, Table VII, Fig. 94 (1840); Thom and Church, in The Aspergilli, p. 4 (1926); Thom and Raper, in A Manual of the Aspergilli, p. 7 (1945).

Vegetative mycelium consisting of septate branching hyphae, colorless, bright colored, or in a few forms slowly becoming brown or otherwise colored in localized areas. Conidial apparatus developed as conidiophores and heads from specialized, enlarged, thick-walled hyphal cells (the foot cells) producing conidiophores (stalks) as branches approximately perpendicular to the long axis of the foot cell and usually to the surface of the substrata from or upon which they are borne; conidiophores unseptate or septate, usually enlarging upward and broadening into turbinate, elliptical, hemispherical, or globose fertile vesicles bearing fertile cells or sterigmata either parallel and clustered in terminal groups, or radiating from the entire surface; sterigmata in either one series only, or as a double series with each primary sterigma bearing a cluster of two to several secondary sterigmata at the apex; conidia varying greatly in color, size, shape, and markings, produced successively from the tips of the sterigmata (not produced by budding), and forming unbranched chains so arranged that conidial heads are globose, radiate, or compactly columnar. Ascocarps found in certain groups only, unknown in most species, cleistocarpic, usually thin walled and producing asci and ascospores within a few weeks, although sometimes these are borne within preformed sclerotia and require months for maturation. Sclerotia or sclerotium-like structures regularly present in some species, occasionally