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Identifying Moulds

A Practical Guide

J. Cramer
in der Gebrüder Borntraeger Verlagsbuchhandlung

Petrini & Petrini

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with 37 figures und 12 tables

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Pictures on the back cover (from top to bottom):
Melanospora fusispora: Fruiting body; *Ulocladium chartarum*: conidiophore, conidia; *Trichothecium roseum*: conidiophore, conidia; *Mucor plumbeus*: sporangia, columella, sporangiophores.

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Contents

I. Introduction

1. Of fungi and man

For most people, fungi are nothing else but those mushrooms one can pick up in the forest: some are edible, some poisonous, some are more common than others, but all share a similar shape, having a stem and a cap with gills or tubes. Invariably, people are most likely to mention the fly agaric (*Amanita muscaria*) or the button mushroom (*Agaricus campestris*) as "the" fungal representatives. Yet the basidiomycetes, to which both fly agaric and button mushroom belong, are just a small selection of all known fungi and comprise a very large number of species that are extremely diverse – for instance, in addition to the "classical" mushrooms (Agaricomycotina), the Basidiomycota include the plant pathogenic rusts (Puccinomycotina) and smuts (Urediniomycotina), the jelly fungi, the stinkhorns, as well as several less known groups.

The very large and heterogeneous kingdom Fungi is composed of eukaryotic organisms and is clearly separate from bacteria, plants and animals. Fungal cells have mainly cell walls that contain chitin and fungi are physiologically quite different from plants and animals. The Kingdom Fungi includes several taxonomic units, listed in Chapter III. Some fungi are one-celled, others many-celled: in fact, the largest organism on earth appears to be the basidiomycete fungus *Armillaria ostoyae*, a very common, feared tree pathogen: the mycelium produced by one individual has been shown to occupy 965 hectares of soil in Oregon's Blue Mountains!

Most fungi form a vegetative mat called mycelium, composed of thread-like, mostly branching filaments termed hyphae. In the mushrooms, the cap-like structures one sees in the woods are the fruiting bodies, the so-called "carpophores" – comparable to the fruits of a tree: in them, reproductive structures (the basidia) form propagules (the spores) that can be dispersed by wind, water or insects: similarly to plant seeds, they can germinate and produce mycelia that colonise new substrates. Hyphae are usually very small and only the mycelium can sometimes be observed as a whitish or coloured mat covering the surfaces colonised by the fungus. Mostly, however, the vegetative fungal structures are inconspicuous and even the carpophores are microscopic.

Fungi are everywhere: they may grow in soil, on dead organic material or live symbiotically in or on plants, animals or other fungi. They are often noticed only when fruiting or causing disease symptoms on their hosts.

Fungi are known to influence their environment and to induce significant physiological changes in it. In turn, the ecosystem greatly influences fungal growth, exerting a strong selective pressure. Ecologically, fungi play a crucial role in nutrient cycling and exchange. Their importance as a direct source of food is undisputable: mushrooms and truffles are very appreciated; yeasts are used as leavening agents for bread and in the wine and beer fermentation, and several moulds are indispensable for the fermentation of various other food products. In the medical field, antibiotics and many other drugs are extracted from fungi (e.g. penicillin from *Penicillium*; the immunosuppressant Cyclosporin A from *Tolypocladium*; psychotropic compounds and alkaloids from Clavicipitaceae) and many moulds are used in industrial applications, e.g. for the production of enzymes and detergents. Finally, some fungi have found a place as biological pesticides to control weeds and harmful insects.

While several fungi are beneficial, many can be harmful to humans, animals and plants. Some moulds can grow and damage manufactured materials and buildings: high fungal loads can be present in damp buildings that have not been properly restored, particularly after serious water damage. Approximately 300 fungal species are known to cause infections ("mycoses") in humans and animals. Inhalation of mycotoxins or spores can cause severe allergic reactions and asthma in debilitated and predisposed subjects. Allergic reactions caused by fungi have been reported from malt and cheese factories, bakeries, mills, and composting facilities, all sites where high loads of fungal spores may be present.

Economically most relevant, however, are the crop losses caused by some plant pathogenic fungi (e.g. the rice blast disease by the ascomycete *Magnaporthe grisea* or black rot of grape by *Guignardia bidwellii*). Finally, several moulds are involved in food spoilage and mycotoxin production – they, too, are economically important. So far some 400 different mycotoxins, produced by approximately 350 fungal species, have been described. The most prominent toxin producing fungi belong to the genera *Aspergillus*, *Fusarium*, and *Penicillium*.

Approximately 70,000 fungal species are known, but the total number of specific fungal taxa is estimated to be as high as 1.5 millions. Among them, moulds are by far the economically most important.

Moulds grow usually saprobically on organic substrates and may cause modifications of the substrate's appearance, texture, and sometimes (in the case of food) taste. "Mould" is no taxonomic, physiological or biochemical term: moulds are fungi, but not all fungi should be called moulds. Systematically, they belong to several taxonomic groups. The term is not linked to the practical significance of the organism either. Moulds can cause disease or food spoilage, some play an important role in biodegradation, others in the production of food (e.g. *Penicillium roqueforti* in blue cheese), antibiotics (*Penicillium* spp. as penicillin producers), enzymes (cellulases produced by *Trichoderma* spp.), or organic substances (citric acid by *Aspergillus niger*), whereas some may be opportunistic, human and animal pathogens.

2. How to use this book

This laboratory guide is intended to be an introduction to the identification of the most common and important genera of moulds. It considers only fungi that fruit readily in pure culture, are commonly found in the environment, are common plant pathogens or are involved in food spoilage, or may be isolated from human and animal tissues as opportunistic pathogens. It has been written for students and lab technicians and should help them find their way in the complicated taxonomic world of microscopic fungi. Genera not included here are probably rather uncommon; in this case, more specialised monographs will be indispensable for their identification – they are referred to in "Common mould genera" on page 89.

No previous knowledge of fungi is expected in order to use this book. Sections "Fundamentals of mycology" on page 5 and "Taxonomy" on page 15 give some basic information on mycology as well as brief descriptions of the most important taxonomic schemes. The section "The identification of moulds" on page 25 describes briefly the methods used for the identification of moulds and other fungi, whereas Sections V–VII provide practical advises on handling, safety issues and steps to be taken during the identification work. They also contain practical information on culture media and morphological characters typical of the different fungal groups.

Sections VIII and IX are the core of this manual. Section VIII provides a key to the most common mould genera, while Section IX is a catalogue of the genera keyed out in Section VIII, with reference to more specific identification literature.

The mycological taxonomic literature is unfortunately very rich in technical definitions. We provide a glossary in Section X that includes some of the most relevant mycological terms.

Sections XI–XII give a very brief introduction to the ecology of fungi in general and to the significance of fungi for public health.

Those who are interested only in the identification of moulds and already have some working knowledge of mycology will probably use only Sections VIII and IX. The other chapters are intended to provide a very basic introduction to mycology and fungal identification – they are, by no means, in-depth treatises, but contain the necessary bibliographic references to gain additional knowledge on the subjects treated.

II. Fundamentals of mycology

1. What are fungi?

Fungi, formerly considered members of the plant kingdom, have now been partly included in a separate kingdom (Fungi: see section III on page 15). A small number has been transferred to the Protista. Fungi and fungus-like Protista show morphological, ecological and biochemical features that distinguish them from other organisms.

These are briefly summarised in Tab. II.1.1.

Table II.1.1: Features of fungi and fungus-like Protista

Nutrition	Heterotrophic organisms (no photosynthesis). Direct absorption of nutrients.
Thallus	The vegetative part of a fungus; in the Protista mainly a protoplast or a fully developed structure with distinct cell walls, composed at the beginning by several undifferentiated vegetative units that reproduce and absorb nutrients independently. The thallus may later differentiate into distinct organs. In most cases the thallus is not mobile, but some fungus-like Protista can produce zoospores or myxoamoebae.
Cell wall	Usually present, normally containing chitin (cellulose may be present in some groups – regularly in Oomycota and in some Ascomycota).
Nucleus	Eukaryotic (enclosed by a membrane), homo- or heterokaryotic, dikaryotic, haploid or diploid (diploidy is usually limited in time).
Reproduction	Sexual and asexual.
Spores	Non-motile and motile spores (zoospores).
Fruiting bodies	(Also called sporocarps) very small to visible with the naked eye, with limited tissue differentiation.
Occurrence	As symbionts, saprobes, parasites or hyperparasites.
Distribution	Worldwide.

2. Morphology and reproduction

Fungi and fungal Protista share properties that may be found also within other eukaryotes. Not necessarily common morphological or biochemical characters are indicative of a close phylogenetic relationship: the same characters may be present in taxonomically unrelated taxa. The most important morphological characters are presented in Figs. II.2.1–3.

2.1 Vegetative organs

2.1.1 The thallus (Fig. II.2.1)

Spores germinate and produce a germ tube that will eventually elongate and branch to form a hypha. Yeasts usually reproduce by budding and some fungus-like Protista may produce zoospores in closed organs called sporangia.

The thallus is the undifferentiated vegetative tissue of a fungal organism and may have different structural characteristics depending on the taxonomic position of the fungus.

- The mycelium is the vegetative part of a fungus and is composed of a mass of thread-like, often branching hyphae. These are long, branching filamentous structures that may be septate (with cross-walls: Ascomycota and Basidiomycota) or aseptate (without cross-walls: Zygomycota). Hpyhae absorb nutrients from the substrate. Within the hyphae the nutrients are transformed and energy is produced. In septate hyphae, nutrients pass the septa through central pores.Within the thallus, hyphae can differentiate and take up different tasks. Hyphae immersed in the substrate absorb nutrients and metabolise them; aerial hyphae are mainly specialised in rapid growth and substrate colonisation. Reproduction often takes place on aerial hyphae; stolons (runners) are aerial hyphae that are used by the fungus to rapidly colonise new substrates. They often form root-like structures (rhizoids) at the point of contact with the substrate. Rhizoids are special organs that are able to absorb nutrients.

- Yeast colonies are composed of single, totipotent cells, each of them able to absorb nutrients and reproduce by budding or cell fission. Some yeasts (e.g. *Candida*, *Torulopsis*) may produce pseudohyphae and in some cases true hyphae. Pseudohyphae are basically chains of yeast cells that differ from true hyphae by being constricted at the septa. They can be recognised also by their terminal cells that are usually smaller than the other cells.

- Some filamentous fungi (e.g. *Rhizopus oryzae*, Zygomycota) may form budding cells and grow yeast-like under specific ecological conditions. Such organisms are called dimorphic.

- Dimorphic fungi may grow either yeast-like or form hyphae. Switching from the budding yeast to the filamentous form is mainly triggered by ecological factors. Several dimorphic fungi are facultative human or animal pathogens: the parasitic form is mainly yeast-like, while the saprobic phase is filamentous.

Dimorphism may be monofactorial or multifactorial. For instance, the two human pathogens *Blastomyces dermatitidis* and *Paracoccidioides brasiliensis* grow as filamentous organisms at temperatures below 37 °C and yeast-like above, whereas *Candida albicans* grows yeast-like at high sugar concentrations or in the presence of proteins with high sulphide content. In rare cases the switch from yeast-like to filamentous growth is triggered by a combination of factors, as is the case for the human pathogen *Histoplasma capsulatum*, which switches from the mycelial to its yeast form when the temperature reaches 37 °C in the presence of high sugar concentrations and proteins with high sulphide content. Some Mucorales, *Penicillium* and *Aspergillus* species, grow yeast-like under anaerobic or microaerobic conditions.

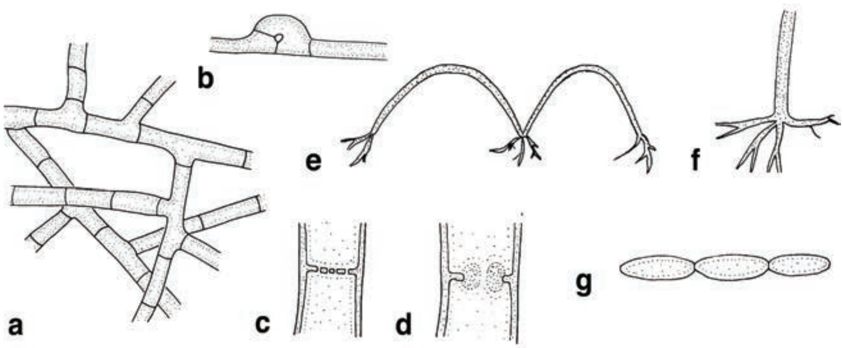

Fig. II.2.1. Vegetative organs

a. Mycelium composed of septate, branched hyphae. **b.** Hyphal septum with clamp connection (only in Basidiomycota). **c, d.** Pores: **c.** Micropores. **d.** Dolipores (only Basidiomycota). **e.** Stolons (runners). **f.** Rhizoids. **g.** Pseudohypha.

2.1.2 Vegetative structures (Fig. II.2.2)

Special organs (Tab. II.2.2.1) are often produced in the thallus following ecological or physiological changes.

- **Aleurospores** are thick-walled, vegetative, resting spores formed at the hyphal tips. They are resistant to adverse ecological and physiological factors (desiccation, high temperatures, etc.), thereby ensuring the survival of the fungus. Morphologically they are almost indistinguishable from chlamydospores (Figs. VIII.5d, VIII.13f).

Tab. II.2.2.1: Vegetative organs of the thallus

Physiological or ecological function	**Fungal structure**
Germination	Germ tubes, budding cells.
Infection	Infection hyphae.
Spread and increase of the fungal mass	Perforating hyphae, stolons, microhyphae, aerial hyphae, rhizomorphs.
Survival	Chlamydospores, aleurospores, sclerotia.

- **Chlamydospores** are thick-walled, often melanised hyphal cells of very variable shape (Fig. VIII.14b). They are resting, long-living and resistant organs able to store nutrients and protect the fungus' genetic material from ecological and chemical damage. They are hardly different from aleurospores, from which they can be distinguished mainly by the site of formation (not on hyphal tips) and their very variable and irregular shapes.
  In the Zygomycota, chlamydospores are mainly formed endogenously within the hyphae or the sporangia; in the Ascomycota and Basidiomycota they may be formed either endogenously or exogenously. Sometimes, Zygomycota may produce rather thin-walled chlamydospores (called **gemmae**) that separate from the mycelium and may in turn reproduce by yeast-like budding.

- **Hairs**, **bristles** and **setae** (bristle-like hairs) are sometimes formed by some mould species. The morphological differences among the three are subtle: hairs are usually slightly thinner than hyphae, whereas bristles and setae are thicker and often tapering; bristles and setae are morphologically almost indistinguishable. Hairs, bristles or setae differ from normal hyphae also by their colour, being usually darker. Their physiological function is not clear – some authors have hypothesised that their purpose is to protect the fungus from grazing by insects. Hairs and setae are often found in or on fruiting bodies of ascomycetes and their anamorphs (e.g. on *Chaetomium* ascomata). They may be characteristic of selected fungal taxa and often help in their identification, as is the case for the plant pathogenic anamorphic genus *Colletotrichum* (Fig. VIII.8d).

- **Infection** or **perforating hyphae** are penetration organs. They are able to perforate the host cell walls mechanically or enzymatically. Plant pathogens, for instance, can digest the host's cuticula and cell walls with enzymes such as pectinases, cutinases, cellulases or other hydrolases. Defensive responses by hosts include callus formation and necrosis in plant infection, whereas in animals and humans mould penetration will elicit inflammation and immunological responses.

- **Microhyphae** are extremely thin, penetrating hyphae (less than 0.5 µm in diameter) formed by some plant pathogenic fungi.

- **Rhizomorphs** are strands of organised hyphae that closely resemble thin roots. They can be up to 1 cm thick and their structure may be already quite differentiated, with an outer cortex-like and an inner mycelial layer. Rhizomorphs are mainly absorption and transport organs, at the same time allowing a rapid substrate colonisation by the fungus. The rhizomorphs of some basidiomycetes (eg. *Armillaria mellea* or *Serpula lacrymans*) are able to penetrate wood and building walls and may reach several meters in length.

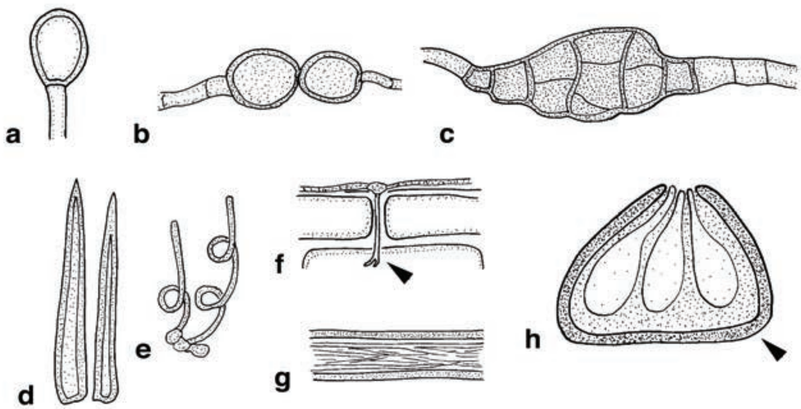

Fig. II.2.2. Organs of the thallus

a. Aleurospore. **b.** One-celled chlamydospores. **c.** Multicellular chlamydospore. **d.** Setae (bristles). **e.** Hairs. **f.** Perforating (infection) hypha (arrow). **g.** Rhizomorph. **h.** Stroma (arrow) with fruiting bodies inside.

- **Sclerotia** are hard aggregations of hyphae, mostly of cellular structure (the "medulla") and with a superficial, melanised rind (the "cortex"), functioning as a resting body (Fig. VIII.12b, c).

- S**tromata** (sing. stroma) are compact structures composed of hyphae, often with a cellular, parenchymatic appearance, in or on which one or more fruiting bodies are seated. Stromata may occur in asexual and sexual fructifications.

2.2 Reproductive organs

Careful study of the morphology of a mould is a crucial first step towards its identification: this includes examining and describing its vegetative and reproductive organs.

Fungi reproduce by means of propagules. These are called spores. Spores may be produced either asexually (no meiosis – only by mitosis) or sexually (meiosis following fusion of nuclei).

Fungi are not only genetically but also morphologically extremely diverse. They may be pleomorphic, meaning that they may assume different forms and shapes, and reproductively form only the sexual or the asexual form, or both. The whole fungus (i.e., all possible morphological and genetic expressions of a taxon) is called the holomorph. This includes its asexual form (the so-called anamorph, from "anatomic morphology") and its sexual form (the teleomorph – "perfect" form). A fungal species reproducing only asexually is sometimes called "mitosporic", whereas those taxa forming a teleomorph are sometimes referred to as meiosporic fungi. To complicate things further, a fungus can sometimes form several anamorphs, all known by different names.

Accordingly, the sexual and the asexual forms of the same fungal species are often known by two different names. One designates the asexual, the other the sexual morph – both together form the holomorph. For instance, the names *Aspergillus hollandicus* and *Eurotium amstelodami* define the same ascomycetous fungus: *A. hollandicus* is the name of the (asexual) anamorph, whereas *E. amstelodami* describes the (sexual) teleomorph. *Aspergillus* is therefore the anamorph of *Eurotium* and, correspondingly, *Eurotium* the teleomorph of *Aspergillus* (Fig. II.2.3). New nomenclatural rules call for the use of only one name, but this will most likely take some time before being fully established.

In the absence of one of the two forms, a taxon is known either by its anamorph or its teleomorph name. Mycologists are currently trying to simplify this confusing situation by using molecular biology techniques – but it will take some time to sort out all known and unknown cases and to call a fungus by a single name.

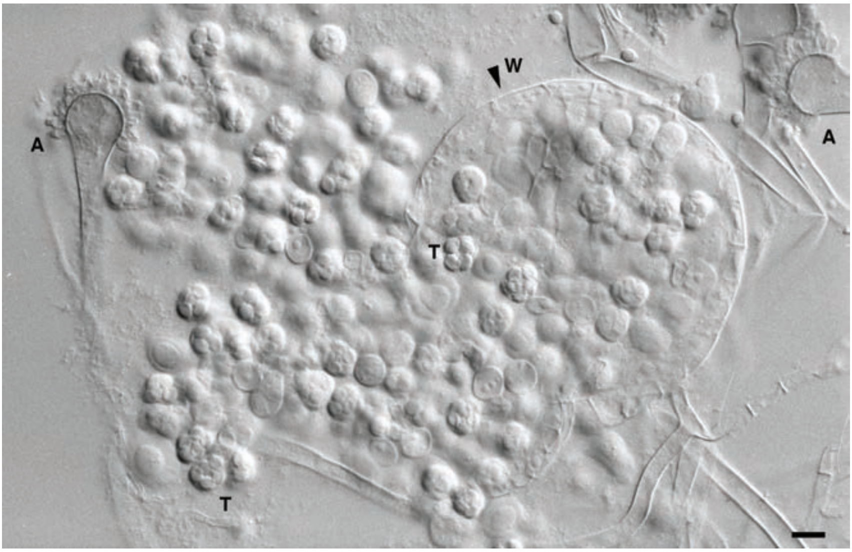

***Fig. II.2.3 Anamorph-Teleomorph connection:* Aspergillus hollandicus *and* Eurotium amstelodami**

Holomorph: **A.** Anamorph (*Aspergillus hollandicus*). **T.** Teleomorph (*Eurotium amstelodami*), asci and ascospores. **W.** Teleomorph – ascoma with ascomatal wall (arrow). Scale: 10 µm.

2.2.1 Asexual reproduction (Fig. II.2.4a–e)

Asexual reproduction allows fast multiplication and spreading of an organism. Spores are produced exclusively by mitosis ("mitospores", "mitotic spores") either endogenously or exogenously.

Fruiting bodies

- **Conidiomata** (sing. Conidioma) are structures in or on which non-motile asexual spores (conidia) are contained. **Pycnidia** (Fig. VIII.9d) are closed, often globose or flask-shaped structures lined inside with conidiogenous (conidia forming) cells, whereas **Acervuli** (Fig. VIII.9a, c, j) are plate-like stromatic layers on which conidiophores (conidia-bearing structures) are seated.
- **Synnemata** (sing. **synnema**; Fig. VIII.15d) are bundles of erect conidiophores, on which conidia are formed mainly apically.

Asexual spores (mitospores)

- **Zoospores** are motile, flagellate spores (planospores), found only in fungus-like Protista. This group is not discussed further here.
- **Conidia** are non-motile spores found predominantly in Zygomycota, Ascomycota and Basidiomycota, as well as in some Oomycota (Chromista).

Fungus-like Protista reproduce usually by endogenous zoospore formation and rarely by forming aplanospores (non-motile propagules). Asexual reproduction in the Zygomycota, Ascomycota, and Basidiomycota, on the other hand, is quite diverse:

- In the **Zygomycota,** spores (sporangiospores) are formed endogenously in sporangia (sing. sporangium; e.g. *Mucor fuscus*, *Rhizopus* spp., Zygomycota). Sporangia are swellings formed at the tip of differentiated hyphae, called sporangiophores, in which sporangiospores are produced by mitosis and are released by breaking of the sporangial wall (Figs. II.2.4b–c, VIII.1–4). Sometimes the hypha forming the sporangium may differentiate into a sterile, dome-like structure within the sporangium called **columella**.

- In the **Ascomycota** and **Basidiomycota**, conidiogenous (conidia-producing) cells are formed either directly on the hyphae or on more or less differentiated, erect conidiophores (conidiogenous cells bearing structures). The conidia are formed **exogenously** by the conidiogenous cells and released at maturity (Figs. II.2.4d–e, VIII.8–18). Several forms of conidiogenesis are known and are described in detail in Section VII. For example, the *Penicillium* anamorph of *Talaromyces* (Ascomycota) is characterised by a conidiophore bearing at its tip a brush-like group of conidiogenous cells from which conidia are formed in long chains.

2.2.2 Sexual reproduction (Fig. II.2.4f–k)

Sexual reproduction is fundamental for the genetic exchange and evolution within a given taxon. Approximately 70% of all known fungi and fungus-like Protista reproduce sexually.

Fruiting bodies

- **Zygomycota** form characteristic **zygosporangia** (sing. zygosporangium), which contain only one sexual spore (Zygospore) each (Fig. VIII.3e–g). Under the microscope however, the zygospore is normally not directly visible because of the thick zygosporangial walls.

- Sexual reproduction of **Ascomycota** normally takes place in typical **ascomata** (sing. ascoma; Fig. VIII.5–7); correspondingly, **Basidiomycota** produce their sexual structures in or on **basidiomata** (sing. basidioma). Ascoma and basidioma shape and their overall morphology are crucial for the taxonomic classification and identification of both groups.

Sexual spores (Meiospores)

- In the **Zygomycota, zygospores** are formed endogenously in a **zygosporangium** (e.g. *Zygorhynchus moelleri*, Fig. VIII.3e–g).
- **Ascomycota** produce their **ascospores** endogenously in an **ascus** (e.g. *Sordaria fimicola,* Fig. VIII.7j).
- **Basidiomycota** (e.g. *Boletus edulis*) form exogenously **basidiospores** on **basidia** (Fig. VIII.8b, c).