Studying the DISCOMYCETES from initiation to advanced levels

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with collaboration of

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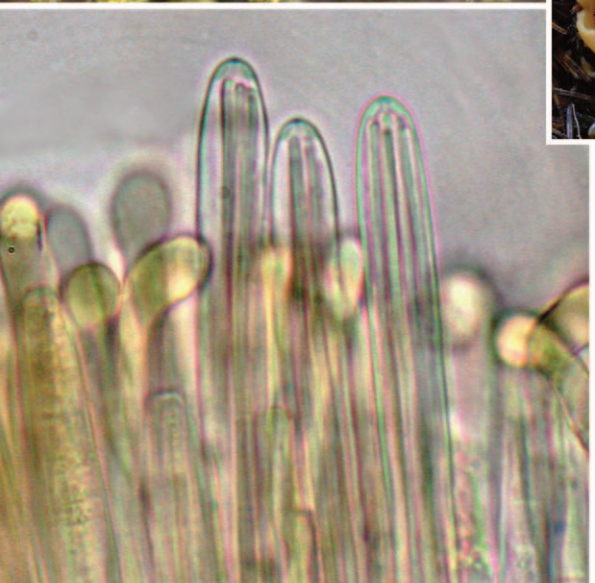
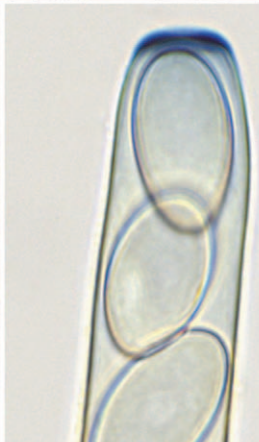
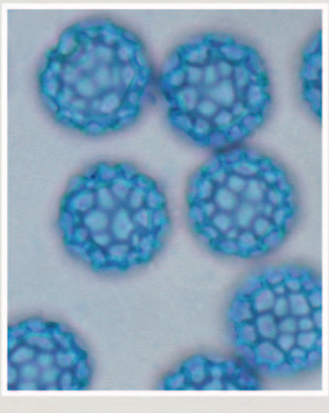
Preface

Michel Hairaud







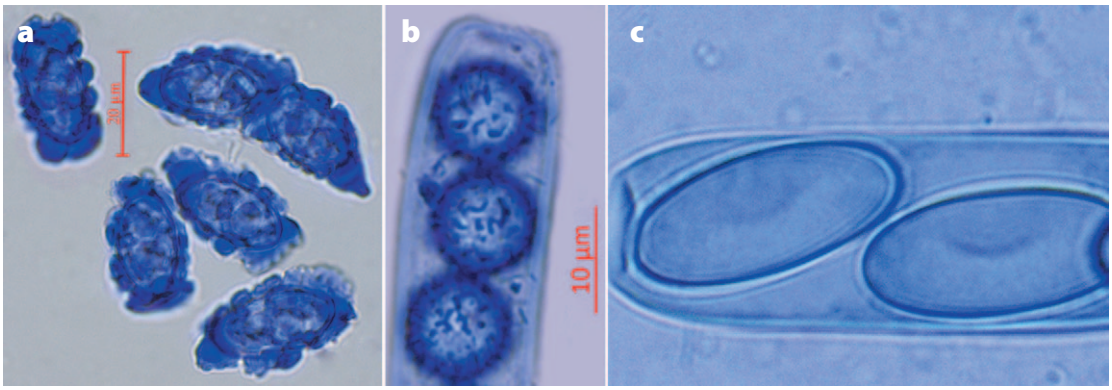


Fig. 10 • Dyeing with the cotton blue. a: spore ornamentation of *Melastiza contorta*; b: ascospores of *Marcelleina rickii*; c: ascus and ascospores of *Peziza* sp.

Both formulations of cotton blue appear to be equally suitable for staining ascospore ornamentation and cell walls. However, CBL gives random results on the dilation of the walls of ascospores of the genus *Cheilymenia* Boud. (Moravec, pers. comm.). CBL is particularly used to study the flesh. Due to the presence of phenol, this dye should be used with caution.

Aqueous cresyl blue = 1 g of cresyl blue, 1 ml of invadin or dishwashing detergent, in 100 ml of distilled water.

Colours some cell walls, including ascospores, in blue to violet. Stains vacuoles and guttules in cells (fig. 11 and 12b), paraphyses (fig. 12b) and ascospores to a greater or lesser extent. Highlights the presence of gelatinised tissue (fig. 12c).

Phloxine = 1 g phloxine, 99 ml distilled water.

Stains the cytoplasm of the cells (fig. 13a).

Congo Red [CR] = 1 g of powdered congo red, 99 ml of distilled water or with a few ml of pure ammonia.

Colours the cell walls. Ammoniacal CR also reconstitutes the cells (fig. 13b).

Aqueous Congo Red = 1 g Congo Red powder, 100 ml of distilled water.

Colours the cell walls.

Congo Red SDS (= Sodium Dodecyl Sulfate) = 1 g Congo Red powder, 100 ml of bidistilled water, 1% SDS.

Colours the cell walls, with a great clarity (fig. 13c).

Acetic carmine = carmine powder to saturation, i.e. 1–2 g per 100 ml of 50% acetic acid (hot).

Highlights the cell nuclei of some species (fig. 14 and 33).

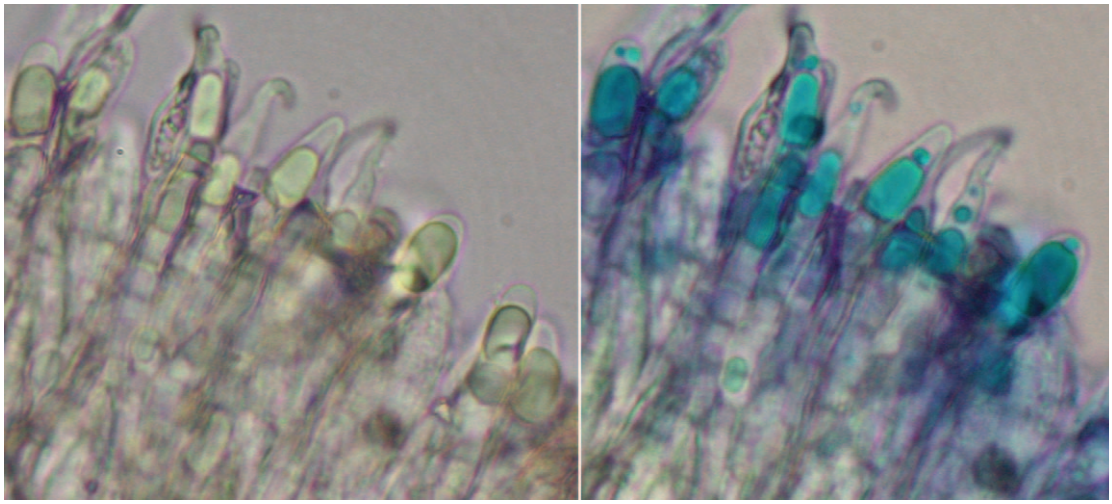


Fig. 11 • Hairs of *Calycellina pseudopuberula*, before and after dyeing of refractive vacuoles with aqueous cresyl blue.

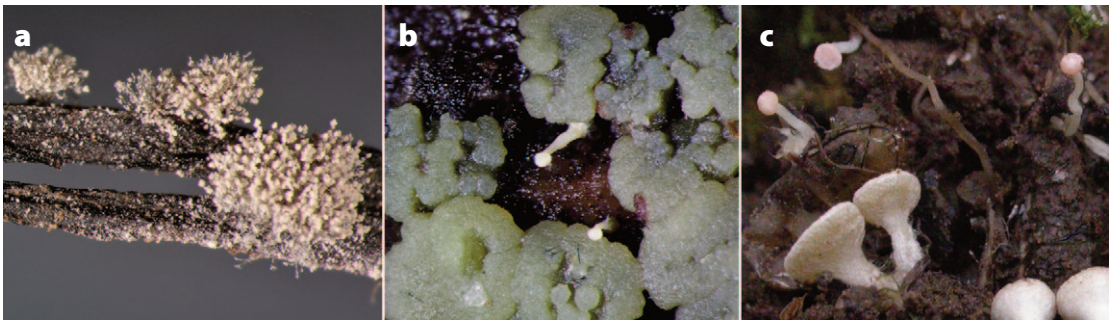


Fig. 16 • a: botrytis-like conidiophores, growing in association with species of the genus *Botryotinia*; b: conidiomata of *Dendrostilbella prasinula* with its teleomorph, *Claussenomyces prasinulus*; c: conidioma of *Symphyosira* (top) with its teleomorph, *Symphyosirinia chaerophylli*, on seeds of *Chaerophyllum*.

bose top, or simply on its periphery. This type of conidiophore or synnemata (also known as coremia) are sometimes visible to the naked eye (fig. 16). In some cases, conidia are formed by hyphal segmentations and are called arthrospores. In other cases, they are formed at the ends of phialides, a flask shaped cell.

The development of other types of anamorphs is concentrated in superficial structures without walls, sporodochia, while other types are confined within spherical bodies covered with a membrane, pycnidia, or within locules, which are small compartments with stromatised internal walls.

Among the other forms of asexual reproduction we can mention chlamydospores, which form short chains around a thickened wall inside sections of mycelial hyphae.

While teleomorphs can produce anamorphs leading to the formation of mycelia, some teleomorphs also produce other asexual forms, microconidia, which do not germinate, but function as spermatia, i.e. fertilising male organs. These conidia are particularly found in the family *Sclerotiniaceae* where, in culture, they are produced on the mycelium, sclerotia and stromata, thanks to the ascospores still in the asci or ejected and often germinated on the hymenium's surface. *Botrytis*, *Dendrostilbella prasinula* and *Symphyosira* are anamorphs that can be regularly found in nature. The former forms tiny whitish to beige bushes, usually close to the apothecia, for example of *Botryotinia* Whetzel, while the latter erects short pale stems with rounded heads, very similar to the teleomorph, *Claussenomyces prasinulus* (P. Karst.) Korf & Abawi, while the third resembles a pink rivet with a hemispherical head, which can be found alongside species of the genus *Symphyosirinia* E.A. Ellis (fig. 16).

On the very mature hymenium of *Sarcoscypha austriaca* (Beck ex Sacc.) Boud, molliardiomyces-like conidia, derived from germinated ascospores, are often found. Dougoud & Moravec (1995) observed on the hymenium of three collections of *Peziza acroornata* Dougoud & J. Moravec, the oedocephalum-like anamorph, formed on germinated ascospores (fig. 17). Typical of many species of *Peziza*, this conidial form had never before been observed in situ, but only in culture. The conidial forms are of fairly relative tax-

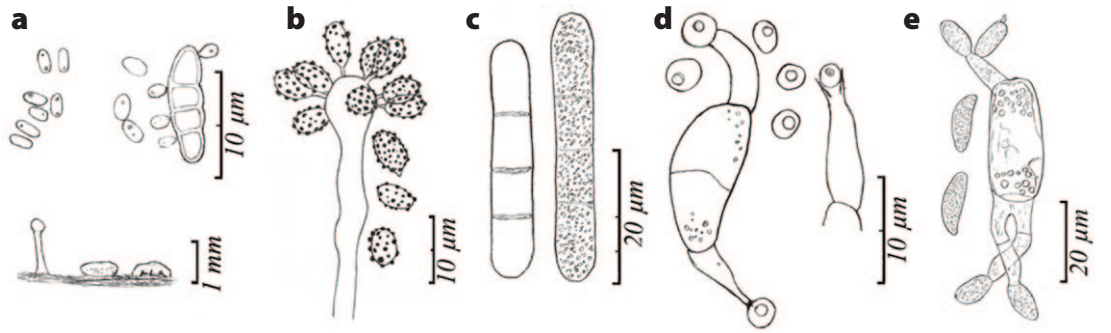


Fig. 17 • a: *Dendrostilbella prasinula* with, its conidia above and one ascospore surrounded by forming and free conidia. b: Conidiophore from a germinated ascospore and verrucose oedocephalum-like conidia. c: symphyosira-like conidia. d: sclerotium-like conidia or microconidia from a germinated ascospore. e: molliardiomyces-like conidia coming from the germination of an ascospore of *Sarcoscypha austriaca*.

Fig. 23 • Apothecia, examples of shapes and colours. a: *Gyromitra infula*; b: *Bryoglossum gracile*; c: *Neottiella rutilans*; d: *Geopora sumneriana*; e: *Ciboria batschiana*; f: *Morchella deliciosa*; g: *Diplonaevia circinata*; h: *Microglossum viride*; i: *Chlorociboria aeruginascens*; j: *Olla scrupulosa*; k: *Delastria rosea*; l: *Lachnum virgineum*; m: *Ascobolus immersus*; n: *Helvella* gr. *lacunosa*; o: *Orbilia luteorubella*; p: *Hymenoscyphus ombrophilaeformis*; q: *Cyathicula coronata*; r: *Hymenoscyphus repandus*.

The introduction of molecular biology as a method for analysing and determining taxa has now become widespread in all areas of life. Molecular biology makes it possible to evaluate new characteristics in the genetic structures of the organisms studied. The main objective is to provide a phylogenetic systematics that enables the evolutionary history of taxa to be retraced and their relationships assessed.

We will not go into detail here on the different methods used to produce a phylogeny, nor their advantages or disadvantages, and we invite the reader to consult works that shed more detailed light on these aspects, such as Lecointre & Le Guyader (2016).

DNA extraction and sequencing

From a practical point of view, the first step is to extract the DNA from a sample of the fungus being studied. The method used is generally specified in the "Materials and methods" chapter of scientific publications and will be communicated to you, if necessary, by the laboratory carrying out this operation. The extracted DNA is then subjected to a technique known as PCR (Polymerase Chain Reaction). The aim is to obtain a sufficiently large quantity of the portions of genomic DNA that we want to use for future analysis, using primers specific to these portions. For eukaryotes (including fungi), it is usual to amplify ribosomal DNA domains such as the ITS1-5.8S-ITS2 fragment (often referred to as ITS) or the 28S fragment (often referred to as LSU), which are repeated many times in the genome and are therefore easier to amplify. It is sometimes useful or necessary to target other regions or 'loci' (locus in the singular) or 'markers', such as the genes encoding RPB1, RPB2, EF1-α, etc., to differentiate between closely related species within a taxonomic group. Their amplification requires separate primers.

For the study of discomycetes, ITS and LSU are frequently used and offer good results at the specific level, used alone or sometimes combined. It should be noted that the ITS1 and 2 domains are much more variable than the 5.8S and LSU domains, and are therefore more suitable for comparing taxonomically close species. Conversely, because it is more conserved between closely related species, the LSU region generally offers a more reliable alternative than the ITS for delimiting phylogenetically distant taxonomic groups. In particular, it is used in combination with coding genes to produce multigene phylogenies for generic or supra-generic classification (see, for example, Hansen *et al*., 2013).

This sequencing phase sometimes results in total or partial failure. Depending on the condition of the material or how old it is, it may not be possible to extract enough genetic material to carry out the PCR. In some cases, the desired domain is only partially obtained. In some cases, the DNA extracted is that of a contaminating organism (moulds, yeasts that have developed as a result of poor drying or storage of the exsiccata).

Sequence processing

If the sequencing is successful, we have a sequence represented in the form of a series of peaks coloured red, blue, green and black, called a chromatogram (fig. 62), revealing the order of the different nucleotides in the region analysed. The nucleotides, written out as letters, correspond to the different bases that make up DNA: adenine (A, green), cytosine (C, blue), guanine (G, black) and thymine (T, red). By analysing this chromatogram, it is possible to detect certain "anomalies" and correct them.

One example is the superimposition of two peaks in a region of the chromatogram that is 'clean', i.e. made up of single, well-isolated peaks (fig. 61). This type of irregularity, often referred to as SNP (Single Nucleotide Polymorphism), is due to the diploid nature of amplified genomic DNA, at least in Basidiomycetes. This property means that any sequence produced from these fungi is actually a mixture of the paternal and maternal alleles of the locus amplified by PCR. Most of the time, these two versions of the gene are identical and a single peak is visible, but certain positions of the

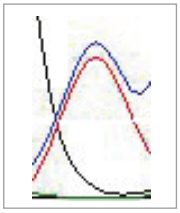


Fig. 61• SNP

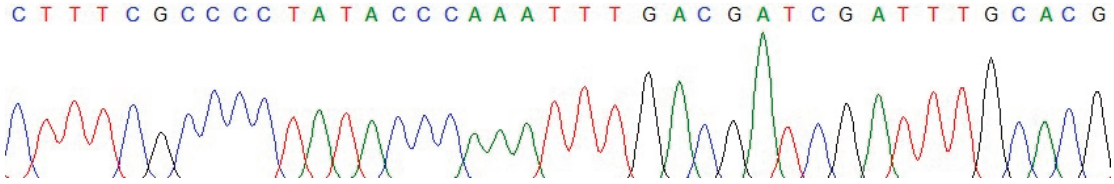


Fig. 62 • Example of sequence chromatogram