## Extracts from



Aqueous cresyl blue $=1 \mathrm{~g}$ of cresyl blue, 1 ml of in vadin 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 $=1 \mathrm{~g}$ phloxine, 99 ml distilled water. Stains the cytoplasm of the cells (fig. 13a).

100 ml of distilled water.
Colours the cell walls.

Congo Red SDS (= Sodium Dodecy (Sulfate) $=1 \mathrm{~g}$ 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.
$1-2 \mathrm{~g}$ per 100 ml of $50 \%$ acetic acid (hot).
Highlights the cell nuclei of some species (fig. 14 and 33).


Fig. 11 - Hairs of Colycellino pseudopiberuio. before and after dyeing offlefractive vacuoles with aqueous cresyl biue.
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Fig. 32 - Types of paraphyses. A: Cheilymenia raripila; B: Aeruginoscyphus sericeus, on the left, with the resorbed protoplasm; C: Pezizo depresso, connected by epithelial material; D: Scutellinia hyperborea; E: Cloussenomyces prasinulus; F: Calycellina alniella, on the right, in Melzer's reagent; G: Ploettnera exigua; H: Velutarina rufo-olivacea; I: Hymenoscyphus ombrophilaeformis; J: Trichophaea abundons, with hooded top.


Fig. 16• a: botrytis-like conidiophores, growing in association with species of the genus Botryotinia; b: conidiomata of Dendrostilbella prasinula with its teleomorph, Cloussenomyces 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 stro-
mata, 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 froma 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: Gyromitro infula; b: Bryoglossum gracile; c: Neottiello rutilans; d: Geopora sumneriana; e: Ciboria botschiana; f: Morchella delicioso; g: Diplonaevia circinata; h: Microglossum viride; i: Chlorociboria aeruginascens; j: Olla scrupulosa; k : Delastrio rosea; I: Lachnum virgineum; m: Ascobolus immersus; n: Helvella gr. locunosa; 0: Orbilia luteorubella; p: Hymenoscyphus ombrophilaeformis; q: Cyathicula coronata; r: Hymenoscyphus repondus.


Fig. 49-Ascospores of inoperculate discomycetes (examples of shapes and contents): A) Neodasyscypha cerina; B) Stromatinia rapulum; C)Calycina claroflava; D) Albotricho acutipillo; E) Trichopeziza leucophoea; F) Cyathicula culmicola; G) Claussenomyces atrovirens(this type, said muricate is rare in inoperculates); H) Hymenoscyphus scutula var. scutula; I) Lanzia Iuteovirescens: J) Orbilia xanthostioma; K) Orbilio sarraziniana: L) Calycellina lutea; M) Rutstroemio petiolorum; N) Rutstroemio sydowiana; O) Lachellula suecica; P) Pirottoea trichostoma; 0) Arachnopeziza ourato; R) Geoglossum cookeonum.

Muriform ascospores, which have both transverse and longitudinal or even oblique septa, are the exception in discomycetes (fig. 49G).
The presence of septa is sometimes early, in which case the septa form inside the asci where they are already visible and are therefore called septate, and sometimes later, either at full maturity, just before or during germination, in which case they are found mainly on the hymenium, on to which the ascospores have fallen. It is necessary to complete this statement by specifying that when the septa are already visible inside the asci, the ascospores are, apart from rare exceptions, all septate, but when the septa appear later, they may only be partially, or even occasionally, septate. Occasional septation is quite common, for example in the genus Hymenoscyphus. This character is certainly not to be overlooked, but it should be treated with caution and not as a true characteristic. It is in fact possible to recognise a
fragments at the post-hymenial stage, i.e. from very old and mature apothecia on which numerous ascospores have fallen.

## Dimensions

Spore dimensions are not defined by a single method, but by a multitude of more or less personal and empirical methods; the result is that a certain anarchy reigns which is likely to add, in addition to natural differences, measurement differences due to these different methods. Recent authors use statistical methods, which are easy to use on a computer, and the calculation is based on a fairly large number of ascospores, which provides more accurate and reliable data. Generally 20 to 50 ascospores are measured, with the highest number of ascospores measured being reserved for ascospores of the most heterogeneous dimensions. HuHtinen (1989) used a much larger population of ascospores, often several


Fig. 48- Ascospores of operculate discomycetes (examples of shapes, contents and ornamentations, the latter in lactic cotton blue, but represented here in black and white): A) Elaiopezia simplex; B) Sepultariella patavino; C) Trichophaea contradicta, with a De Bary bubble; D) Cheilymenia theleboloides (on the right, after heating); E) Phoeopezia apiculata(with various types of apiculit; F) Thecotheus rivicola(with a mucilaginous sheath); G) Gyromitra gigos; H) Miladino lecithina; I) Trichophaea livida; J) Legoliana badiofuscoides; K) Ascobolus carbonarius (natural colour); L) Plicaria carbonaria (on the left, natural colour).

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 28 S 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' (singular, locus) or 'mark-
cally 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 some "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),

